Light-triggered release of drugs in vivo: amplification strategies, response to new wavelengths, and application to a clinical challenge

Awardee: Adah Almutairi
Award: New Innovator Award
Awardee Institution: University of California, San Diego

Co-authors: Carl-Johan Carling, Jason Olejniczak, Viet Anh Nguyen Huu, Noah J.J. Johnson, Sha He, Arnold Garcia, Jing Luo, Kang Zhang
Co-author Institution: University of California, San Diego

This presentation will cover several recent advances in the development of light-degradable polymers as tools for biological research and drug delivery, including a strategy for chemically amplifying the light signal to accelerate degradation, a polymer that degrades upon single-photon absorption of red light, novel upconverting structures enabling efficient conversion of more biologically compatible wavelengths, and application of a previously reported polymer to the treatment of disease. The chemical amplification strategy relies on phototriggered unmasking of acidic groups that hydrolyze adjacent ketals, which overcomes ketals' requirement of low pH for efficient degradation. Particles composed of the photocaged-acid/ ketal polymer degrade rapidly upon brief irradiation. The red light-degradable polymer incorporates a photocage not previously used in responsive materials, which cleaves in hydrophobic environments (unlike coumarins). Particles composed of this polymer, when subcutaneously injected and irradiated through tissue, release sufficient drug to significantly reduce carrageenan-induced paw inflammation in mice. Our advance in the upconversion field is the application of uniform shell deposition to overcome dopant concentration quenching, allowing unprecedented upconversion efficiencies at 800 nm. Absorption of this wavelength rather than the 980 nm employed by current structures avoids the potential for tissue heating, as water's absorption of 800 nm infrared is much lower. Finally, we have evidence that a UV-degradable polymer (Fomina et al., *J Am Chem Soc* 2010) may be useful for the delivery of anti-angiogenics in the eye to treat macular degeneration. This strategy would preserve clinician control over dose timing while reducing the frequency of intravitreal injections. UV-degradable particles are stable in the eye for months and release a therapeutically effective dose of a small molecule anti-angiogenic; the irradiation required for release is well-tolerated by the eye.

Engineering direct control of synthetic protein-RNA interactions for synthetic biology and functional genetics applications

Awardee: Jacquin Niles
Award: New Innovator Award
Awardee Institution: Massachusetts Institute of Technology

Increasingly, the ease with which RNA and its direct interactions with proteins can be engineered is being exploited in design-oriented, biotechnology applications and the basic life sciences. Using principles inspired by nature, our lab has developed a generalizable framework for achieving direct, small molecule-mediated regulation of protein-RNA interactions. We have demonstrated the use of this approach to directly control several fates of cellular RNA, including its translation and subcellular localization in model organisms (*E. coli* and yeast) and in the human malarial pathogen, *Plasmodium falciparum*. Our application of this technology in the poorly understood and non-model *P. falciparum* context has been valuable in two key ways. First, we have emphasized engineering increased integration of synthetic protein-RNA interactions with native cell regulatory mechanisms as a means of harnessing evolutionarily conserved and optimized mechanisms for regulating cellular RNA processes. Through this approach, we can achieve improved functional robustness and reduced system noise arising from leaky expression during control of translation, for example. This easily generalizable strategy, while counter to the conventional approach of designing regulatory systems agnostic to host cell regulation, should prove broadly useful in synthetic biology and life science applications. Second, we have leveraged our regulatory framework in *P. falciparum* as a novel and much-needed functional genetics tool. Traditionally, few sufficiently robust tools have been available to conditionally perturb parasite gene expression to assess basic biological function or probe the molecular basis for antimalarial drug resistance. By taking advantage of the inter-organism transferability of our system's foundational framework and the principle of host cell integration, we have validated our system as a flexible and robust tool for successfully doing functional genetics in a human pathogen that has been challenging to study.

Single domain antibodies as tools to perturb protein interactions

Awardee: Hidde L Ploegh
Award: Pioneer Award
Awardee Institution: Whitehead Institute for Biomedical Research

The proposed use of single domain antibodies (VHHs) from camelid derived heavy chain only immunoglobulins was geared towards phenotypic screens in yeast and possibly other eukaryotes as a novel means of perturbing the host cell proteome. We have now conducted such phenotypic screens in yeast as a means of inhibiting pyruvate decarboxylase to redirect production of ethanol to that of other higher alcohols, with the first indications of success. We have also conducted phenotypic screens to identify VHHs capable of interfering with the influenza virus life cycle by cloning inducible versions of VHHs in lentiviral vectors, followed by exposure of the transductants to a lethal dose of virus. We thus identified VHHs capable of interfering with the function of flu NP, yielding a near complete blockade in virus replication. We have obtained co-crystals of the inhibitory VHH with recombinant NP, the structure of which will help us identify the essential functionally relevant features of NP that lead to its inhibition by the relevant VHHs. More generally, we have generated several VHHs that have assisted in the crystallization of otherwise difficult to crystallize proteins and thus helped arrive at their structures. This same pipeline was used to generate VHHs against proteins of immunological interest, ultimately with the goal of providing new imaging modalities to explore host-pathogen interactions. We can now image immune cells non-invasively using PET imaging. These approaches demonstrate the ease of manufacture and utility of camelid single domains in experimental approaches where conventional antibodies are less useful or fail altogether.

From sperm to stroke: the science of tethering enzymes with applications from nanoscale energy production to handheld diagnostics for neural injury

Awardee: Alexander Travis
Award: Pioneer Award
Awardee Institution: Cornell University

The ability to attach functional enzymes to nanostructures could enable a number of future medical applications, ranging from devices traveling through our bloodstreams to deliver drugs at a specific tissue, to handheld devices diagnosing disease within minutes from a few drops of blood. However, multiple challenges exist. Being bound to a surface can interfere with enzyme activity by limiting diffusion of substrates, blocking substrate binding sites, or interfering with conformational changes. Mammalian sperm have overcome these challenges by having specialized glycolytic enzymes, designed to function when tethered to a cytoskeletal structure in the flagellum. Through this "solid-state" design, sperm produce energy locally where needed. This inspired us to pursue a biomimetic strategy of oriented immobilization, tethering enzymes by modifying or replacing their biological targeting domains.

Using this approach, we demonstrated several coupled glycolytic reactions, including the first demonstration of co-tethered, sequential steps of any biological pathway. We've shown that when attached using oriented immobilization, enzymes representing several enzyme classes had much higher specific activities than if attached by random adsorption or by chemically-specific but non-oriented immobilization (e.g. carboxyl-amine binding). These improvements have enabled us to achieve our goal of tethering all eleven enzymes of glycolysis to nanoparticles in series and generating the end product of lactate from glucose. This is the first time any complete biological pathway has been reconstituted on a scaffold, and is a significant advancement in tethered reactions over the previous high of three enzymes. Although overall glucose utilization was low, the efficiency of flux through the tethered pathway was higher than when the enzymes were in solution. These data provide proof of principle that tethered glycolytic enzymes could serve as an energy-generating platform technology. Toward this goal, we are working to achieve net ATP production.

Our tethered enzymes enable us to investigate fundamental relationships such as the effects of nanoparticle size and composition on enzyme function and multilayer formation. We are also pursuing immediate applications for tethered enzymes including developing point-of-care diagnostic devices for time-sensitive pathologies such as stroke. This application capitalizes on several advantages of tethered enzymes, including speed, sensitivity, ability to be incorporated into microfluidic devices, and portability. Moving beyond our Pioneer Award, we now show in rat models of stroke and samples from human patients that our tethered enzymes can detect physiological and pathological levels of blood-borne biomarkers within 5-10 minutes, in comparison to hours typically needed for existing technologies (e.g. ELISA).

Transformative but not the way we planned: new approaches to centromere biology

Awardee: David M. Markovitz
Award: Transformative Research Award
Awardee Institution: University of Michigan

Our work on Human Endogenous Retroviruses (HERVs), funded by a Transformative R01, led to our discovery of a previously unknown family of HERVs (termed K-111) that have spread throughout 15 centromeres in modern humans through a process resembling homologous recombination. More recently, we have discovered a second such virus, termed K-222, which has spread among pericentromeric sequences in distinct human chromosomes. The sequence of the human genome is not yet complete, and importantly, major gaps remain at the centromeric region of each chromosome. It would be naïve to believe that centromere sequences are functionally indolent, considering the fact that the centromere is responsible for the faithful segregation of chromosomes, and destabilization of this process results in genomic instability and aneuploidy. Therefore, the sequence of the centromere remains a last frontier of human genomics and genetics. The structure of each human centromere is, however, organized by specific alpha repeats that are distinct to the centromere of each individual human chromosome. We are now using the sequence information from alpha repeats and endogenous retroviruses to develop molecular, qPCR-based assays capable of specifically detecting and quantitating the centromeric material of each human chromosome. To initially address the applicability of these centromere assays to detecting aneuploidy, we have screened the DNA of humans with genetic defects. Using DNA from healthy humans as a control, our centromere assays were capable of detecting aneuploidy in autosomal chromosomal disorders such as trisomy 8 and trisomy 18. We were also capable of detecting sex-linked disorders and aneuploidy in the sex chromosomes. We are currently improving these rapid and reproducible quantitation methodologies to screen for genetic disorders in single cells and maternal plasma. In addition, the potential for centromeres to drive the pathogenesis of cancer has, surprisingly, remained very understudied, and our new methodology will allow us to directly assess whether specific patterns of centromeric evolution are seen in particular malignancies, as preliminary data would suggest. These tools can also be exploited to begin to examine whether centromere biology might directly guide malignant transformation. Thus, advances derived from our work on HERVs has led us to apply new technologies to begin to understand the mysteries of centromeres in health and disease.

Multiplexed DNA repair assays for multiple lesions and multiple doses via transcription inhibition and transcriptional mutagenesis.

Awardee: Leona D. Samson
Award: Pioneer Award
Awardee Institution: Massachusetts Institute of Technology

The capacity to repair different types of DNA damage varies widely among individuals, making them more or less susceptible to the detrimental health consequences of environmental exposures. Current methods for measuring DNA repair capacity (DRC) are relatively labor intensive, often indirect, and one is usually limited to measuring a single DNA repair pathway. We have developed a fluorescence-based, multiplexed, flow-cytometric host cell reactivation assay (FM-HCR) that simultaneously measures the ability of human cells to repair plasmid reporters, each bearing a different type of DNA damage or different doses of the same type of DNA damage. FM-HCR can simultaneously measure DNA repair capacity for four of different repair pathways, and the pathways that can be measured include the following: nucleotide excision repair, mismatch repair, base excision repair, nonhomologous end joining, homologous recombination, and direct reversal by MGMT. We have shown that FM-HCR can measure inter-individual DRC differences in a panel of 24 cell lines derived from genetically diverse, apparently healthy individuals, and that FM-HCR can be used to identify inhibitors or enhancers of DRC. We further developed a next-generation sequencing-based HCR assay (HCR-Seq) that detects rare transcriptional mutagenesis events due to lesion bypass by RNA polymerase II, providing an added dimension to DRC measurements. FM-HCR and HCR-Seq provide powerful tools for exploring relationships among global DRC, disease susceptibility, and optimal cancer therapy.

Imaging the genome with CRISPR

Awardee: Bo Huang
Award: New Innovator Award
Awardee Institution: University of California, San Francisco

The spatial architecture and temporal dynamics of the genome play critical roles in regulating its function. However, visualizing endogenous DNA sequences in living cells remain challenging due to the lack of imaging tools. We developed such a tool by repurposing the bacterial CRISPR system, previously engineered for RNA-guided gene editing and regulation. We utilized an EGFP-tagged endonuclease-deficient Cas9 protein and a structurally-optimized small guide (sg) RNA to enable robust imaging of both repetitive and non-repetitive DNA sequences in the nucleus. The target flexibility of the CRISPR system allows us to simultaneously track multiple genomic loci, helping to elucidate chromosome structure change during the cell cycle. Our study defines a new class of genome imaging tool and highlights its potential to exploit genomic organization and dynamics in living cells.

A Collaborative Chronic Care Network (C3N) is a Peer Produced Learning Health System

Awardees: Peter Margolis and Michael Seid
Award: Transformative Research Award
Awardee Institution: Cincinnati Children's Hospital Medical Center

What if we could create a vastly better chronic care system by harnessing the inherent motivation and collective intelligence of patients, clinicians and researchers? What if this system allowed patients, physicians and researchers to share information, collaborate to solve problems, and learn from every clinical encounter so that this knowledge could be applied at the point of care? Over the last 5 years, we used commons-based peer production to design and develop such a peer-produced learning health system - a Collaborative Chronic Care Network (C3N). We worked with a network of care centers for children and youth with Crohn's disease and Ulcerative Colitis (the ImproveCareNow Network) to improve outcomes, transform health care delivery, spawn innovation, and increase the capacity for research.

A C3N is not an intervention – it is a platform for creating and testing interventions. We have demonstrated:

- Ongoing outcomes improvement. Since 2007, the proportion of patients in remission (inactive disease) increased from 59% to 79% without new medications.
- Exponential growth in the number of care centers (from 19 to 71) a registry of >19,500 patients (~40% of children in the country with the condition).
- A robust community of over 100 innovators contributing to the model
- Partnership with patients and families collaborating with one another and with other stakeholders in all aspects of the system (governance, improvement, research).
- A "data-in-once" informatics architecture enabling data to be easily captured during clinical care and re-purposed for care planning, quality improvement, and comparative effectiveness research.
- Capacity to use registry data to conduct simulated trials replicating studies not possible in children, because of time, cost, and ethical concerns (withholding an efficacious treatment) demonstrating a comparative benefit of anti-TNFα versus placebo and thiopurines.
- Novel use of mobile health technology to enable individuals to collaborate with their clinicians to track symptoms and use those data to guide shared clinical decision-making.
- A pipeline of innovations and a design and development process to manage innovation development.
- Policies that promote data sharing and regulatory approaches (e.g., common IRB) to reduce the "transactional" costs of participating while maintaining patient privacy and ethics.

Results to date suggest that this model of a peer produced learning health system has significant potential to transform care delivery, reduce the cost of research by creating a reusable infrastructure, and accelerate the speed at which new knowledge is implemented.

Insights into macrophage migration in tuberculosis from the zebrafish

Awardee: Lalita Ramakrishnan
Award: Pioneer Award
Awardee Institution: University of Washington

We have developed the zebrafish as a facile, genetically tractable and optically transparent model for the study of host-pathogen interactions in tuberculosis. The ability to observe in unprecedented detail the steps of tuberculosis in a single animal and to perturb these through genetic manipulation has proved to be powerful. We have had surprising insights that suggest entirely new approaches to TB treatment, which are now in clinical studies. I will focus on the very earliest steps of the host-pathogen interface when mycobacteria first infect the host. Our findings suggest that they manipulate host macrophage migration so as to avoid microbicidal macrophages and instead use permissive macrophages to traverse host epithelial barriers. We have uncovered the details of these host evasion strategies and their mechanism which I will present. These findings provide an explanation for longstanding observations about human TB that have been puzzling.

The identity thief: Silencing of B lymphocyte commitment gene PAX5 is coincident with gene methylation in common variable immunodeficiency

Awardee: Julia B. Felippe
Award: New Innovator Award
Awardee Institution: Cornell University

Co-authors: Rebecca L. Tallmadge, Ute E. Schwab, and Balu Reddyjarugu
Co-authors Institution: Cornell University

Common variable immunodeficiency (CVID) is a late-onset humoral deficiency characterized by B lymphocyte dysfunction or loss, decreased immunoglobulin production, and recurrent bacterial infections. Although CVID is the most common human primary immunodeficiency, variability in phenotype, genetic background and age at onset have precluded rapid definitive diagnosis and understanding of its etiology. Causative genetic mutations have been described for <10% of CVID patients; furthermore, family members of CVID patients are usually unaffected. Our laboratory diagnosed CVID in 30 equine patients; these cases manifest with a natural impairment of B lymphocyte differentiation in the bone marrow, and serve as a unique model to identify mechanisms of disease. Several independent lines of evidence revealed the loss of genes and proteins indicative of the pro-B cell differentiation stage in equine CVID patients, including fluorescent immunocytochemistry, bone marrow transcriptome analysis, and immunoglobulin recombination joint quantification. PAX5 expression is a signature of the pro-B cell stage and is essential to B lymphocyte identity. PAX5 expression is significantly decreased or absent in the bone marrow of equine CVID patients.
We hypothesized that aberrant epigenetic regulation caused PAX5 gene silencing, resulting in the late-onset and non-familial manifestation of CVID. Both genome-wide reduced-representation bisulfite sequencing and bisulfite PCR followed by sequencing methods revealed a significant increase in methylation of the PAX5 enhancer region in equine CVID patients ($p=0.000$).

The reversible nature of epigenetic modifications facilitates *in vitro* investigation of their relevance in B lymphopoiesis of both healthy controls and CVID patients. In recent years, our laboratory developed a protocol to differentiate equine hematopoietic stem cells into B lymphocytes *in vitro*. At present, the consequences of demethylation agent azacytidine on B lymphopoiesis are being examined. We further plan to assess effects of histone deacetylase inhibitors, such as valproic acid, on B lymphopoiesis. Successful reversal of PAX5 gene silencing would provide means for rescuing B lymphopoiesis, and significantly, new avenues for patient treatment.

Thus, integrating gene expression with epigenetic status may be a key insight to understanding the onset of CVID and investigating novel therapeutic strategies.

Interactomics: Computational Analysis of Novel Drug Opportunities

Awardee: Ram Samudrala
Award: Pioneer Award
Awardee Institution: State University of New York at Buffalo

We have developed a Computational Analysis of Novel Drug Opportunities (CANDO) platform funded by a 2010 NIH Director's Pioneer Award (http://protinfo.org/cando) that analyses compound-proteome interaction signatures to determine drug behaviour, in contrast to traditional single (or few) target approaches. The platform uses similarity of interaction signatures across all proteins as indicative of similar functional behaviour and nonsimilar signatures (or regions of signatures) as indicative of off- and anti-target (side) effects, in effect inferring homology of compound/drug behaviour at a proteomic level. We have created a matrix of predicted interactions between 3,733 human ingestible compounds (including FDA approved drugs and supplements) × 48,278 proteins using our hierarchical chem and bio-informatic fragment-based docking with dynamics protocol (from over one billion predicted interactions total). We applied our compound-proteome signature comparison and ranking approach to 2030 indications with one approved compound and yielded benchmarking accuracies of 12-25% for 1439 indications with more than approved compound. We are prospectively validating "high value" predictions in vitro, in vivo, and by clinical studies for more than forty indications (http://protinfo.org/cando/collaborations), including dental caries, dengue, tuberculosis, ovarian cancer, cholangiocarinomas, among many others. 57/162 prospective predictions across eleven studies covering nine indications have shown comparable or better activity to an existing drug, or micromolar inhibition at the cellular level, and serve as novel repurposeable therapies. We were able to make predictions against the Ebola virus within minutes and are currently working with collaborators to test them in vitro. Our approach is applicable to any compound beyond those approved by the FDA, and also include can readily consider mutations in protein structures to enable personalisation based on genotype, foreshadowing a new era of faster, safer, better and cheaper drug discovery.

Interrogating co- and post-transcriptional gene regulation at single neuron resolution

Awardee: John Calarco
Award: Early Independence Award
Awardee Institution: Bauer Fellows Program, FAS Center for Systems Biology, Harvard University

Recent transcriptome-wide analyses of multicellular organisms have identified that a significant fraction of messenger RNAs (mRNAs) are subject to tissue-specific regulation of their abundance and/or diversity. Our group is currently exploring the mechanisms governing differential alternative splicing and translation in the nervous system at single cell resolution. Using a fluorescence microscopy-based genetic screening approach in the nematode *C. elegans*, we have recently identified a pair of RNA binding proteins that coordinate differential splicing patterns between GABAergic and cholinergic neurons, the two major classes of motor neurons in the animal. I will discuss our ongoing efforts towards characterizing how these factors establish this neuron-specific regulation. I will also present results suggesting that these proteins play a critical role in fine-tuning the physiological properties of these neurons. Finally, I will describe the adaptation of a method for isolating mRNAs from specific cell types in *C. elegans*, and the future use of this genome-wide approach to uncover tissue and neuronal-subtype specific splicing and translation regulation in the context of animal development and behavior.

The Transposon Storm Hypothesis: Collatoral Damage in the Brain

Awardee: Josh Dubnau
Award: Transformative Research Award
Awardee Institution: Cold Spring Harbor Laboratory

Retrotransposons are inherited virus like repetitive elements that are capable of replicating and re-inserting into *de novo* locations within the genome. As a whole, retrotransposon sequences contribute a vast fraction of the genome, up to 40% in humans. To date, retrotransposition has been largely studied in germline where new insertions produce heritable genetic variants. In fact, the germline is the main battlefield of an evolutionary conflict between our genomes and retrotransposons. Plants and animals have evolved elaborate and effective mechanisms to silence retrotransposons, and they are largely effective. But transposons also are capable of mobilizing in somatic tissue. An emerging literature establishes that some retrotransposons are NORMALLY active at a low level in neurons during development. We have demonstrated, however, that in *Drosophila* the silencing mechanisms begin to falter with advancing age and collapse in genetic models of amyotrophic lateral sclerosis (ALS). This leads to accumulation of *de novo* mutations in neurons. We also have shown that genetically activating LINE-like and gypsy transposons results in accelerated effects of aging on neurophysiological decline. This leads to rapid age-dependent memory impairment, defects in locomotion, and to shortened lifespan. We also have found signatures of this transposon storm at the expression level in deep sequencing datasets from human subjects and in rodent models of ALS. Our findings have implications for the mechanisms of neurodegeneration seen in ALS and frontotemporal dementia.

How neural circuits orchestrate the magic of human cognition

Awardee: Gabriel Kreiman
Award: New Innovator Award
Awardee Institution: Harvard Medical School

The magic of cognition arises from the complex interactions of dynamical ensembles of neural circuits in neocortex. Deficits in these cortical networks lead to debilitating conditions and brain disorders have proven to be particularly resilient to treatment. In collaboration with neurosurgeons, we have developed neurotechnologies to enhance our ability to directly and invasively listen to and stimulate human cortex. These tools have enabled us to interrogate the dynamics of neural responses orchestrating cognitive functions including visual recognition, learning and decision making at unprecedented spatiotemporal resolution.

As a paradigmatic example of cognition, we will discuss our ability to rapidly and robustly recognize faces and objects, which is essential for navigation, reading, socialization and pattern recognition in general. Visual recognition happens in a small fraction of a second, even when the stimuli have undergone transformations in position, size, color, illumination and rotation. Using machine-learning algorithms, we demonstrated that we could read out in single trials responses along the human ventral visual stream at millisecond resolution. These signals are selective for faces and objects and show tolerance to scale and viewpoint changes and even to small amounts of clutter. The dynamics of these responses arise within 100 to 200 ms of image onset and are consistent with behavioral constraints for recognition. Natural vision often involves recognizing objects from partial information. The ability to extrapolate and make inferences from partial information is central to intelligence and constitutes a significant frontier for engineering systems that aim to emulate human thinking. Responses along the ventral visual stream retain selectivity despite presenting only a small fraction of information and provide evidence for holistic face and object processing. The delayed dynamics observed in the responses suggest that inference and pattern completion rely on recurrent and feedback signals that form attractors in the representational space. Taken together, the neurophysiological signals and computational models are beginning to shed light on how neural circuits can lead to cognition, on the physical basis for the mind. The high-resolution scrutiny of human cortical circuits provides a path to the inner workings of the brain and a way to read out biological codes, which can be translated into computational algorithms to make machines smarter. Additionally, furthering our understanding of where, when and how cognitive functions are instantiated opens the door to developing brain machine interfaces to alleviate brain disorders.

Neural Systems Approach to Monitoring Brain States During General Anesthesia and Sedation

Awardee: Patrick L. Purdon
Award: New Innovator Award
Awardee Institution: Massachusetts General Hospital / Harvard Medical School, Boston, MA

Over the past several years, with the support of an NIH New Innovator Award, I have been able to make fundamental advances on two of the most challenging problems in medical science: to understand how general anesthetic drugs produce states of unconsciousness, and to develop a means to clinically monitor those states. Historically, the field of anesthesiology has sought out a unitary mechanism—one single mechanism—to explain how all anesthetics produce unconsciousness. In contrast, my team and I found that general anesthetic drugs produce different states of unconsciousness by inducing profound neurophysiologic oscillations that disrupt normal brain function. These oscillations are structured in a way that relates directly to the underlying molecular- and circuit-level neuropharmacology for these drugs, such that each anesthetic drug class has a unique "EEG signature." Our work resolves the decades-long contradiction between the search for a unitary mechanism, and the modern evidence that different anesthetic drugs act at unique molecular receptors. Furthermore, through human intracranial studies and computational modeling studies, my work has established the connection between clinically-observable EEG oscillations under anesthesia, and brain activity at the neuronal and local field levels. These advances represent a quantum leap in understanding that would not have been possible using conventional methods under the prevailing hypotheses of the day.

I have rapidly translated this knowledge into clinical innovations that are already being put into practice. Anesthesiologists now have the ability, for the first time, to precisely monitor the brain states of their patients during general anesthesia. Over the past three years, I have been teaching anesthesiologists how to use the EEG to monitor and manage their patients. This past year, I published a CME-accredited website, AnesthesiaEEG.com, that allows anyone to obtain this knowledge, free of charge. I have gone on to characterize age-related anesthetic effects in children and the elderly, providing fundamental insights on how to care for these uniquely vulnerable patients. I have filed 11 patents related to monitoring or control of consciousness during anesthesia and sleep. Recently, I secured a technology licensing agreement with a major medical device company to implement and make commercially available novel techniques to monitor brain states under anesthesia. A first device employing these technologies is due to be released in the market in 2015. I am also developing novel technologies to control administration of anesthetic drugs, to improve sleep monitoring and sleep therapeutics, and to improve anesthetic monitoring in children.

Detection and functional characterization of prion-like protein self-assembly

Awardee: Randal Halfmann
Award: Early Independence Award
Awardee Institution: University of Texas Southwestern Medical Center

The replication of biological information is no longer the exclusive purview of nucleic acids. Information can also be encoded and replicated solely through the self-templated assembly of certain proteins known as prions. We now know that this process occurs frequently, and in ways that are fundamental to human health and disease. On the one hand, a barrage of recent discoveries implicates prion-like mechanisms for the cell-to-cell progression of protein misfolding in ALS, Alzheimer's, Parkinson's, and Huntington's diseases. On the other hand, 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[1]. 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 have discovered that the mammalian death domain superfamily proteins, MAVS and ASC, form bona fide prions that functionally commit cells to antiviral and inflammatory responses, respectively[2]. We further demonstrated that the principles of prion-driven immune signaling are conserved all the way into fungi. Finally, we have developed a powerful new method that enables high throughput detection and quantification of prion-like self assembly. This method has already revealed new prions and prion modulators, and will dramatically accelerate future such discoveries.

1. Holmes DL, Lancaster AK, Lindquist S, Halfmann R. Heritable remodeling of yeast multicellularity by an environmentally responsive prion. Cell 2013; 153:153-65.
2. Cai X, Chen J, Xu H, Liu S, Jiang QX, Halfmann R, Chen ZJ. Prion-like polymerization underlies signal transduction in antiviral immune defense and inflammasome activation. Cell 2014; 156:1207-22.

Barcoding stem cells: surprises, challenges, and perspectives

Awardee: Fernando D. Camargo
Award: New Innovator Award
Awardee Institution: Boston Children's Hospital
Co-authors: Jianlong Sun, Azucena Ramos, Brad Chapman, Jonathan B. Johnnidis, Linda Le, Yu-Jui Ho, Allon Klein, Oliver Hofmann
Co-authors' institutions: Harvard University, Harvard Stem Cell Institute and Boston Children's Hospital

The basic understanding of how tissues are normally maintained by their resident stem cells is key for pursuing regenerative medicine approaches. Though a great deal of knowledge has been gained through the use of traditional experimental approaches over the past two decades, limitations and drawbacks of these techniques have precluded us from gaining a complete understanding of regenerative processes, particularly in the in vivo setting. The goal of my New Innovator proposal was to develop a novel experimental paradigm for the study of stem cell biology and tissue dynamics. In our model, individual stem cells in a population can be uniquely and genetically tagged *in situ* without any sort of perturbation. These genetic tags, or barcodes, are then used to systematically and quantitatively monitor the dynamics, lifespan, and differentiation of thousands of stem cells at the single cell level in highly complex populations over time. We have initially applied this technology to provide unprecedented insight into the biology of the unperturbed blood- and immune-forming systems. Our results challenge the prevailing stem cell-centric dogma in the field, which indicated that a small number of hematopoietic stem cells (HSCs) drive stable and multi-lineage blood-production. Our data instead demonstrate that long-term hematopoiesis is maintained by the successive recruitment of thousands of clones, derived not from HSCs, but from multipotent progenitor cells, a population traditionally thought to have a very restricted lifespan. Our clonal tracing system reveals that these progenitors can be tremendously long-lived and predominantly produce lineage-restricted progeny. Our data argue for a re-evaluation of typical cellular hierarchies in the hematopoietic tree, and have significant implications for understanding the cellular origin of hematopoietic disease. The modular nature of our system should enable cell-type specific transposition, of multiple other lineage and cell populations, paving the way for future systematic and high-resolution analysis of clonal dynamics during development, aging, and multiple biological processes.

Epigenetic Stochasticity, Phenotype and the Environment

Awardee: Andrew P. Feinberg
Award: Pioneer Award
Awardee Institution: Johns Hopkins University School of Medicine

The overall theme of this Pioneer Award is the idea that natural selection will favor the emergence of genetic loci for epigenetic variation that can occur randomly or in response to environmental signals and affect phenotypes in which the environment changes unpredictably but often enough. We have pursued several avenues that all look promising. The first is a model of native honeybee populations with Brian Herb (JHU) and Gro Amdam (Arizona State). We have been generating a comprehensive genetic and epigenetic map related to foraging behavior and pollen production and identified SNPs that appear to regulate variance in methylation associated with behavioral phenotypes. The second is a model of nutrition-dependent metabolic disease in mouse, with Michael Multhaup and Will Wong (JHU) and Juleen Zierath and colleagues (Karolinska Institute), with epigenetic and genetic conservation over 50 million years to humans, showing environmentally sensitive genetic loci with wide epigenetic variability relevant to glucose homeostasis. The third is a novel stochastic mathematical approach, with Garrett Jenkinson, John Goutsias, and Elisabet Pujadas (JHU), to understanding the nature of epigenetic information and its relationship to environmental exposure and biological function. This has led to several new measures, including normalized methylation entropy, which turns out to be surprisingly relevant to the other approaches under the award.

Integrative Genomic Studies of Evolution and Adaptation in Africa

Awardee: Sarah Tishkoff
Award: Pioneer Award
Awardee Institution: University of Pennsylvania

Co-authors: Joseph Lachance, Joseph Jarvis, Sameer Soi, Laura Scheinfeldt, Alessia Ranciaro, Jibril Hirbo, Afi Rawlings, Simon Thompson, Benjamin Vernot, William Beggs, Alain Froment, Jean-Marie Bodo, Muntaser Ibrahim, Thomas Nyambo, Sabah Omar, Charles Wambebe, Meredith Yeager, Stephen Chanock, Joshua Akey, Kun Zhang

Co-authors' Institutions: University of Pennsylvania, University of Washington, Musée de l'Homme, Ministere de la Recherche Scientifique et de l'Innovation, University of Khartoum, Muhimbili University of Health and Allied Sciences, Kenya Medical Research Institute, International Biomedical Research in Africa, NCI (NIH), University of California, San Diego

Africa is the ancestral homeland of all modern humans, and it is also a region of tremendous cultural, linguistic, climatic, environmental, and genetic diversity. Despite the important role that the African continent has played in human history, groups residing there have remained critically understudied and are underrepresented in the recent explosion of genomic studies. The goal of this project is to characterize genomic and gene expression variation in diverse Africans and to integrate those data with detailed information about normal variable anthropometric traits, as well as traits that play a role in cardiovascular function, nutrition, metabolism, and immune function. This allows us to explore the genetic and environmental factors that play a role in human adaptation, health, and disease. We seek a comprehensive knowledge of variation in African populations that is critical to a deeper understanding of human genetic diversity, the identification of functionally important genetic variation, and the gene-by-environment interactions that underlie traits of biomedical significance.

To achieve the aims of this project, we conducted three field seasons in Eastern and Southern Africa, obtaining DNA, RNA, frozen plasma and detailed ethnographic, nutritional, and health information as well as anthropometric, cardiovascular, and metabolomic phenotype data from ~2,400 individuals from >40 ethnic groups. Here we present integrative analyses of high coverage whole genome sequence and SNP array data, RNA-Seq data, and bi-sulfite sequencing data obtained from blood to characterize patterns of genomic, transcriptomic, and methylation differences across ethnically diverse Africans. Using these data, as well as detailed phenotype measurements of the same individuals, we explore correlations between genetic ancestry and phenotype, and identify regions of the genome that play a role in adaptation to diverse environments. We show that Africans have high levels of variation within and between populations for genomic, transcriptomic, and methylomic data which is distinct from non-African populations. Additionally, we have identified candidate loci that play a role in

adaptation to infectious disease, diet and high altitude, as well as the short stature trait in African Pygmies. This work provides an unprecedented view of the genomic variation that underlies human health and disease in Africa and also a window into human evolutionary history.

The Reductive Stress Hypothesis and the Antioxidant Treatment Paradox

Awardee: Ivor J. Benjamin
Award: Pioneer Award
Awardee Institution: Medical College of Wisconsin

The reductive stress hypothesis in disease pathology was recently revisited by Benjamin and colleagues who have demonstrated that a protein-misfolding (R120G CryAB) cardiomyopathy was under **reductive stress**, as opposed to oxidative stress, from an over-active antioxidative system. Decreasing the function of glucose-6-phosphate dehydrogenase (G6PDH), which generates the reductant NADPH, "cures" the disease in a mouse model by ameliorating reductive stress, aggresome formation, hypertrophy, heart failure and death. Since this discovery, several laboratories have independently implicated the effects of reductive stress as causal mechanisms in hyperglycemic-induced metabolic syndrome, experimental ischemic injury (e.g., dominant negative Nox4 isoform), cardiomyopathy, and inheritable skeletal and cardiac myopathy. Of direct translational relevance, carriers of the Gd-Mediteranean allele of G6DP deficiency living on coastal island in Sardinia, Italy are remarkably protected against ischemic heart disease, cerebrovascular strokes, retinal vein occulusion (RVO), nonarteritic anterior optic neuropathy (NAION), and perhaps, diabetic retinopathy, underscoring the farreaching implications of this work in humans.

An alternative to the reductive stress hypothesis has been extensively pursued for almost five decades, beginning with the oxidative stress theory of aging, on the basis that free radicals and reactive oxygen species (ROS), the byproducts of oxidative phosphorylation, are deleterious in the setting of inadequate ROS scavenging by the antioxidative system. Oxidative stress has been proposed as a major mediator of vascular dysfunction, and has been proposed as a pathological factor in almost every disease from glucotoxicity in pancreatic β-cells, inflammation in infection, cancer metastasis and survival, liver fibrosis, neurodegenerative disease. Stroke, (not 'classic' neurodegenerative), affects 141 million people worldwide in 2012. Thousands of preclinical and clinical studies over the decades have been inconclusive and failed to show efficacy of antioxidant therapeutics while other trials were prematurely terminated owing increased morbidity and mortality. What has not been clear are what factor(s) might account for such abysmal failure. Both proponents and opponents of oxidative stress have fueled the confusion and controversy—and both sides have overlooked the importance of "reductive stress," as opposed to oxidative stress, as a causal mechanism in disease pathogenesis. What are the factor(s) that might increase the susceptibility for major adverse toxicity and fatal outcomes from antioxidant therapy? We postulate that either pro-reducing redox state among heterogeneous clinical cohorts and/or the pro-reducing effects of antioxidants agents will, either alone or combinatorially, promote life-threatening reductive stress. Antioxidant therapeutics *per se* are not the culprits unless indiscriminately administrated without regard for their biological context and/or consequence across the redox spectrum. Moreover, we think that the rationale for the similar use of current compounds in antioxidant clinical trials are seriously flawed and are unlikely to answer or overcome the existing deficiencies and major barriers in the field. Understanding the mechanism for basic redox reactivity and for biological

redox effects is a *sine qua non* for the rational design of clinical trials using targeted oxido-reductive therapeutics in disease pathology.

The DNA Damage-Control Network: a New Class of Cancer Genes Discovered in Bacteria

Awardee Name: Susan Rosenberg
Award: Pioneer Award
Awardee Institution: Baylor College of Medicine

Cancer genes can be divided into two broad functional classes: the "gatekeepers", which change cellular properties to more cancer-like; and the genomic "caretakers"—DNA repair genes that suppress cancer by suppressing genomic instability, thus reducing mutations in gatekeeper genes. Most DNA damage that caretaker pathways ameliorate arises endogenously in cells. We report networks that identify a third functional class: the "DNA damage-control genes" using *Escherichia coli* with translation to human. We reasoned that genes that affect endogenous DNA-damage levels would be cancer genes because their perturbation could increase DNA-damage levels above repair capacity, causing repair deficiency *without* mutations in caretaker genes, and that these would be highly conserved metabolic and other genes.

Damage-control network discovered: Using high-throughput screens for *E. coli* that fluoresce when they experience DNA damage, we screened an overexpression library of all *E. coli* genes, to model gene amplifications that drive many cancers. We identified 238 DNA-damage-promoting genes. Only 8% are traditional "caretakers". The rest are a new class of DNA-damage-affecting genes.

Human damage-control genes in cancer: We identified 282 human homologs. These are highly significantly overrepresented in cancer-driver genome databases. Only 5% are traditional caretakers, implying a new cancer-gene functional class. We overproduced and have validated 25 as genuine human damage-promoting genes in human cells. Thus, these are genuine human DNA damage-control genes. These represent genes that would otherwise be mis-diagnosed as gatekeepers or cancer-role unknown. These data indicate cancer-promoting roles of many identified and some new cancer genes.

Network deconvolution: We developed tools to deconvolute functions in large protein networks, and parse how damage-control proteins promote damage. Using a 93-gene mutation-network as test-case, we successfully assigned function to >50% with broad function-based screens (*Science* 2012, now 65%, submitted). The human homologs of a DNA-damaging group in this test network are also overrepresented among cancer drivers. We are creating toolkits of synthetic proteins to trap and label specific DNA damages (*eLife* 2013; *Nat Comm* 2013) and their causes (submitted). We report the sorting of the bacterial damage-control network into bins of mechanisms of action with these tools. We also developed tools for forward genomics in *E. coli*—allowing rapid deconvolution of causative mutations from screens after whole-genome sequencing (submitted).

This project discovered a new functional class of cancer genes and created platforms for deep translation across phyolgeny from simple models to human disease.

MEK critically regulates cellular proteome homeostasis via HSF1

Awardee: Chengkai Dai
Award: New Innovator Award
Awardee Institution: The Jackson Laboratory
Co-authors: Zijian Tang, Siyuan Dai, Yishu He, Rosalinda Doty, Leonard Shultz and Stephen Sampson
Co-authors' institutions: The University of Maine and The Jackson Laboratory

The RAS-MEK-ERK signaling cascade is central to biology. ERK, canonically, is perceived as the only substrate for MEK. Herein we report that HSF1, the master regulator of the highly evolutionarily conserved proteotoxic stress response, is a new MEK substrate. Through physical interaction and phosphorylation, MEK mobilizes HSF1 to govern the cellular proteome. Surprisingly, ERK repressively phosphorylates MEK to inactivate HSF1. Beyond mediating cell-environment interactions, this MEK-HSF1 regulation critically impacts malignancy. In tumor cells, MEK blockade provokes protein destabilization, aggregation, and, strikingly, amyloidogenesis. Further, combinatorial proteotoxic insult potently exacerbates this proteomic chaos. Remarkably, amyloidogenesis is tumor-suppressive and evidently contributes to the therapeutic effects of proteotoxic stressors. Importantly, compared to their non-transformed counterparts, malignant cells are particularly susceptible to amyloidogenesis. Thus, our findings unveil a previously unrecognized key biological function of RAS-MEK-ERK signaling in guarding cellular proteome homeostasis. Conceptually, our findings suggest proteomic instability as an intrinsic feature of malignancy and, therefore, that perturbation of fragile tumor proteostasis may be a feasible therapeutic strategy.

Imaging cancer heterogeneity and therapy resistance in real time

Awardee: Tannishtha Reya
Award: Pioneer Award
Awardee Institution: University of California, San Diego

A major challenge in biology and medicine is the ability to visualize and understand normal and diseased processes as they occur in vivo. To begin to address this, we have developed molecular strategies to image cell dynamics within cancers as they grow, progress, and recur in vivo. Based on our discovery that the stem cell determinant Musashi is reactivated in many cancers as they develop and progress, we created Msi1 and Msi2 knock-in reporter mice. In these mice, a fluorescent signal reflective of endogenous Msi expression allows high resolution visualization and tracking of Msi expressing cells. Using these unique tools together with high resolution real time imaging methods, we have found that a rise in Msi reporter activity marks the transition from benign lesions to a malignant aggressive state, and that Msi reporter activity uniquely marks tumor propagating capacity as well as therapy resistance in both hematologic malignancies and solid cancers. By providing a molecular view of the high-risk cells that drive cancer development and progression, our work has significant implications for developing new methods for early detection and targeted delivery of current therapies.

Computational dissection of phenotypic and functional heterogeneity in cancer

Awardee: Dana Pe'er
Award: Pioneer Award
Awardee Institution: Columbia University
Co-authors: Jacob H. Levine, Erin F. Simonds, Sean C. Bendall and Garry P. Nolan
Co-authors' institution: Columbia University

Cells within a single tumor are known to display extensive phenotypic and functional heterogeneity. Many life-threatening features of cancer, including drug resistance, metastasis and relapse, are facets of intratumor heterogeneity. With emerging single-cell measurement technologies, the field is poised to make important strides in understanding and controlling this heterogeneity. However, these technologies require advances in analytical methods to interpret the complex data they produce.

Acute myeloid leukemia (AML) is an aggressive bone marrow malignancy in which the importance of cellular heterogeneity has been well characterized. However, previous studies have only scraped the surface of the heterogeneity in this disease. Using mass cytometry, which measures single cells in ~31 simultaneous proteomic features, we developed novel methods for analyzing phenotypic heterogeneity in cancer. Our approach provides an extensive compendium of surface-marker and signaling phenotypes in AML that extends current boundaries of knowledge.

The heart of our approach is Phenograph, a graph-based representation of single-cell samples. The graph represents the phenotypic structure of the sample and can be partitioned into subsets of densely interconnected nodes, called *communities*, which represent distinct phenotypic subpopulations. Using Phenograph, we deconstructed several AML samples into discrete phenotypes. Comparing phenotypes across patients, we found a striking degree of order. Every identifiable phenotype was discoverable in multiple (but not all) patients, implying a constraint on the space of allowable AML phenotypes.

Our data contain measurements under various environmental perturbations and we designed a method to statistically quantify evoked signaling responses, producing high-dimensional signaling phenotypes for each subpopulation, which we regard as a representation of cellular state and functional potential. We found a tight coupling between surface and signaling phenotypes in healthy cells that is disrupted in AML. We identified a primitive signaling phenotype, derived from healthy stem and progenitor cells, which was not correlated with the primitive surface marker profile typically used to define primitive cells in AML. Using single-cell frequencies to deconvolve existing bulk gene expression data, we identified genes associated with this primitive signaling phenotype. These genes produce a clinically predictive signature that is more powerful than genes associated with the primitive surface profile, validating the utility of our approach and providing a new characterization of primitive cells in AML. Phenograph can be applied to characterize heterogeneity and primitive subpopulations in additional cancers.

Engineering Smarter and Stronger T Cells for Cancer Immunotherapy

Awardee: Yvonne Chen
Award: Early Independence Award
Awardee Institution: University of California, Los Angeles

Adoptive T cell therapy for cancer has demonstrated exciting potential in treating relapsing cancers. In particular, T cells that express synthetic chimeric antigen receptors (CARs) specific for the B-cell marker CD19 have shown impressive results in clinical trials for various B-cell malignancies, prompting avid interest from both scientific and entrepreneurial communities in recent years. However, CD19 CAR-T cell therapy remains the only robustly effective T-cell immunotherapy to date, and several obstacles remain to be overcome before the full potential of adoptive T-cell therapy can be realized. My laboratory is pursuing several strategies for the engineering of T cells with stronger anti-tumor functions and greater robustness against evasive mechanisms employed by cancer cells. I will discuss the design, construction, and implementation of multi-input CARs to increase tumor specificity and decrease the probability of mutational escape by tumor cells. I will present the design of synthetic circuits to reroute signaling pathways triggered by tumor-secreted cytokines, thus negating the immunosuppressive effects of the tumor microenvironment. Finally, I will discuss efforts to engineer a cytotoxic protein that triggers target-cell death upon recognition of intracellular oncoproteins, thus expanding the repertoire of detectable tumor markers beyond surface-bound antigens. These strategies combine to address critical limitations facing adoptive T-cell therapy, providing potential treatment options for diseases that are otherwise incurable with current technology.

NIH COMMON FUND HIGH-RISK HIGH-REWARD RESEARCH SYMPOSIUM
December 15 – 17, 2014
SPEAKER ABSTRACTS – DAY 3 (DEC. 17, 2014)

The dynamics of translation

Awardee: Joseph D. Puglisi
Award: Transformative Research Award
Awardee Institution: Stanford University School of Medicine

Translation of proteins by the ribosome is intrinsically dynamic. Supported by the TRO1 program, we have developed and applied single-molecule approaches to monitor both prokaryotic and eukaryotic translation directly in real time. We have explored dynamics underlying basal translation initiation, elongation and termination, and determined how mRNA sequence and structure, as well as nascent chain interactions with the ribosome, cause translational pausing and recoding. Our results highlight the intricate interplay of mRNA sequence and structure, nascent chain sequence and ribosomal conformational dynamics in translation elongation.

Developing a Pipeline of Bacteria-Specific Imaging Agents

Awardee: Sanjay Jain
Awards: New Innovator Award and Transformative Research Award
Awardee Institution: Johns Hopkins University School of Medicine
Co-authors: Weinstein, E.A. and Ordonez, A.A.
Co-authors' Institution: Johns Hopkins University School of Medicine

Early accurate diagnosis of infection is essential for effective therapy, but traditional diagnostic methods are invasive, labor intensive and time consuming, as well as subject to the uncertainties of incorrect sampling and contamination. CT and MRI detect anatomic changes that occur late in a disease process and are neither sensitive nor specific for the diagnosis of bacterial infections. Moreover, though more sensitive, nuclear medicine imaging (99Tc-tagged WBC or [18F]FDG-PET) have poor specificity in differentiating between sterile inflammation and infection. Therefore, bacteria-specific imaging tracers are required to discriminate infection from other disease processes, and to monitor treatment efficacy.

We hypothesize that small prokaryote specific molecules can be identified and developed for use as radiotracers. We screened a commercial library of over 400 random 14C and 3H radiolabeled small molecules looking for low molecular weight compounds with excellent penetration into diseased tissues and scored these molecules according to our selection criteria: metabolized by prokaryote-specific pathways, evidence for prokaryote accumulation or antimicrobial activity, and absence of known eukaryotic accumulation or metabolism. Compounds of the library that passed all 3 selection criteria and were tested for intracellular bacterial accumulation in model bacteria representing three important pathogen classes: *Staphylococcus aureus* (gram-positive), *Escherichia coli* (gram-negative), or *Mycobacterium smegmatis* (mycobacteria). Intracellular bacterial accumulation was determined by % of cell associated radioactivity measured at different time points using a scintillation counter.

Seven of the eight compounds were accumulated within *E. coli*, with sorbitol and D-xylose selectively retained by this organism. 4-Aminobenzoic acid (PABA) and D-mannitol were noted to accumulate in all species of bacteria significantly and rapidly. Follow up testing of PABA with *M. tuberculosis* revealed 96%±15% cell associated radioactivity at 18 hours of incubation. *In vivo* PET imaging was performed using a sorbitol analog (2-[18F]fluorodeoxysorbitol - FDS) showing a significant difference between infection and inflammation in a murine myositis model. Our findings were extended to models of mixed Gram-positive and Gram-negative thigh coinfections, brain infection, *Klebsiella* pneumonia, and mice undergoing immunosuppressive chemotherapy.

We have developed an innovative approach for screening bacteria-specific imaging tracers with promising results including [18F]-FDS which is a candidate imaging probe for translation to human clinical cases of known or suspected infections owing to Enterobacteriaceae. These tools would be useful in both preclinical and clinical settings for a broad variety of bacterial infections, including tuberculosis in which could be a key component for decision making and appropriate treatment.

Quantitative Imaging of Gut Microbiota Spatial Organization

Awardee: Kerwyn Casey Huang
Award: New Innovator
Awardee Institution: Stanford University
Co-authors: Kristen Earle, Gabriel Billings, Justin Sonnenburg
Co-authors' institution: Stanford University

I will discuss a pipeline for the assessment of intestinal microbiota localization within immunofluorescence images of fixed gut cross-sections. The pipeline includes a flexible software package, BacSpace, for high-throughput quantification of microbial organization, including proximity to host mucus and epithelium. Using gnotobiotic and humanized (colonized with human microbiota) mice, we demonstrate that elimination of fiber from the diet, which is known to increase microbiota utilization of host mucosal glycans, results in thinner mucus in the distal colon, increased proximity of microbes to epithelium, and heightened expression of the inflammatory marker REG3β. We demonstrate that hypotheses of how microbe-microbe metabolic interactions impact spatial organization can be tested using this quantitative method, including the role of mucus processing in the colocalization of a promiscuous polysaccharide utilizer with the pathogen *Salmonella typhimurium*. Furthermore, we quantify the invasion of Helicobacter pylori into the glands of the mouse stomach, illustrating the generalization of this approach. This broadly applicable framework will accelerate the elucidation of the roles of microbiota localization in health and disease.

Biochemical Activity Architecture in Living Cells

Awardee: Jin Zhang
Award: Pioneer Award
Awardee Institution: Johns Hopkins University School of Medicine

The assembly/disassembly and enzymatic activities of protein nanomachines underlie all aspects of cellular functions. The quest of understanding the molecular logic behind cellular functions requires the biomedical field to go beyond our current focus on molecular constituents of the cellular machinery and to start establishing conceptual framework to understand cellular organization of molecular activities. We hypothesize that cellular biochemical activities are spatially organized into an "activity architecture", which together with structural and mechanical architecture of the cell, encodes all the necessary information that drives cellular functions. However, testing this hypothesis is beyond the reach of the current technology because in conventional light microscopy, the diffraction of light hampers direct observation of any minute activity domains at the molecular length scale. We address this challenge by introducing a new molecular ruler, which we term Fluorescent fLuctuation Increase by Nonlocal Contact (FLINC). Exploiting its nanometer sensitivity, we developed a new class of biosensors that enabled the visualization of biochemical activities in living cells at a resolution three fold better than the diffraction limit. This general approach is applied to protein-protein interactions and kinase activities. We further present compelling evidence that cAMP dependent protein kinase (PKA) activity is restricted to distinct domains on the plasma membrane of living cells. We anticipate that this new molecular ruler will lead the way in revealing the currently hidden live-cell biochemistry activity architecture.

Super-Resolution Microscopy across Arbitrary Scales

Awardee: Edward S. Boyden
Awards: Pioneer Award and Transformative Research Award
Awardee Institution: Massachusetts Institute of Technology
Co-authors: Fei Chen and Paul W. Tillberg
Co-authors' Institution: Massachusetts Institute of Technology

Microscopy has facilitated the discovery of many biological insights by optically magnifying images of small structures in fixed cells and tissues. Much effort has been invested, accordingly, in the design and implementation of lenses of increasing refracting power and quality. We here report that physical magnification of the specimen itself is possible.

Polymerizing electrolyte monomers directly within a sample into an electrically charged polymer network, followed by dialysis in pure water, results in expansion of the polymer network into extended conformations, and thus specimen expansion. By covalently anchoring specific molecules within the specimen to this polymer network and proteolytically digesting endogenous biological structure, we found that samples could be expanded isotropically ~4.5-fold in linear dimension.

We discovered that this isotropic expansion applies to nanoscale structures, and thus this method, which we call expansion microscopy (ExM), can effectively separate molecules located within a diffraction limited volume, to distances great enough to be resolved with conventional microscopes. Thus, this process can be used to perform scalable super-resolution microscopy with diffraction limited microscopes.

ExM represents a new modality of magnification, and enables scalable, multi-color super-resolution imaging of fixed cells and tissues. Unlike many other super-resolution methods, ordinary dyes can be imaged, enabling multicolor imaging with conventional dyes. Since the expansion is isotropic in all directions, our resolution improvement applies to axial as well as lateral directions. Since the expanded sample is mostly water, it is transparent. We demonstrate ExM in both cultured cells and intact brain tissue, performing three-color super-resolution imaging of ~10^7 μm^3 of the mouse hippocampus with a conventional confocal microscope, achieving ~70 nm lateral resolution.

Single Cell Genomic Analyses of Circulating Tumor Cells

Awardee: Sunney Xie
Award: Pioneer Award
Awardee Institution: Harvard University
Co-authors: Xiaohui Ni, Jessica Sang, Zhe Su, Yan Gao, Bai Fan, Jie Wang, Ning Zhang
Co-authors' institutions: Harvard University, Peking University, Third Hospital of Peking University, Beijing Cancer Hospital, and Tianjing Medical University

Cancers are genomic diseases. Point mutation and copy number variation (CNV), which are two major dynamical changes of in cancer genomes, can now be studied at the single cell level by improved whole genome amplification and next generation sequencing [1]. Circulating tumor cells (CTCs) enter the circulation system from primary cancer issues and lead metastasis, which is responsible for 90% cancer related death. We found that CTCs of the same patient exhibit reproducible CNV gain and loss patterns, which are similar to those of the metastatic site. More interestingly, patients of the same cancer exhibit similar CNV patterns, suggesting that the CNV patterns are cancer and tissue dependent [2]. Reproducible for many patients of lung, breast, gastric, prostate and colon cancers, this result provides clues for the genesis of metastasis, as well as the prospect of noninvasive early diagnosis to identify the cancer types.

Systems analysis of human pluripotent stem cells during self renewal and differentiation

Awardee: Ipsita Banerjee
Award: New Innovator Award
Awardee Institution: University of Pittsburgh

Human pluripotent Stem Cells (hPSCs) are an attractive raw material for regenerative medicine due to their unique properties of self-renewal and lineage specific differentiation. Delicate balance of signaling pathways decide on hPSC cell fate. Current experimental efforts have successfully identified the primary signaling pathways maintaining the balance between self-renewal and differentiation. Our objective is to develop mathematical modelsof the signaling pathway along with systems level analysis to identify key sensitive nodes controlling information flow through the pathway. This analysis will result in identification of targeted perturbations to maintain self-renewal or induce lineage specific differentiation.

We have developed a suite of modeling techniques specifically targeting to capture the dynamics of hPSCs. We first described the signaling dynamics of self-renewing hPSCs by mathematical model of the Insulin induced PI3K pathway. Performing global sensitivity analysis of the pathway using a meta-modeling approach we identified negative regulation through PKCζ to be the dominant signaling node controlling expression level of pAKT. This was further experimentally verified by inhibition of PKCζ which resulted in significant enhancement of pAKT levels in self-renewing hPSCs. Further, analysis of noise propagation through the pathway revealed that the negative feedback through PKCζ helps in noise elimination and protects pAKT levels against upstream variations.

While pAKT supports self-renewal, they were observed to be detrimental for Activin induced endoderm differentiation. Analysis of the dynamics of the multiple pathways during Activin induced differentiation by Dynamic Bayesian Network (DBN) analysis identified significant crosstalk between pAKT and pSMAD molecules. Suppression of pAKT by inhibition of the PI3K pathway successfully removed the interactions and enhanced endoderm differentiation. Current modeling efforts are targeted towards representing these interactions between PI3K and TGFβ pathway and identification of key nodes regulating endoderm differentiation.

In addition, hPSCs are known to have a unique cell cycle behavior, with a shortened G1 phase resulting in shorter doubling time. This G1 phase lengthens with differentiation, leading to an overall longer doubling time.We have developed a stochastic cell population model to track the dynamic and heterogeneous cell cycle behavior during self-renewal and differentiation of hPSCs. Thisstochastic model is able to accurately predict the statistical phase resident time distributions from experimentally synchronized hESC. The primary finding of this algorithm is the possible existence of a lag between cell commitment to differentiation and lengthening of G1 phase.

Regulatory roles of mechanical fluctuations in biology

Awardee: Béla Suki
Award: Transformative Research Award
Awardee Institution: Boston University
Co-authors: Harikrishnan Parameswaran, Jasmin Imsirovic, and Erzsébet Bartolák-Suki
Co-authors' institutions: Boston University

Biological processes must obey the laws of mechanics. Consequently, most cell types are sensitive to their physical environment and physiological forces acting on cells play a dominating role in many regulatory cell functions. In the laboratory, such mechanotransduction is invariably studied using monotonous mechanical stimuli; however, cells in the body experience only irregular stimuli. Two salient examples are breathing and circulation in which cycle-by-cycle variations in tidal breath and beat-to-beat variations in blood pressure should influence cell function in all resident cells of the lung and the vasculature, respectively. Evolutionary forces should favor processes and structures that can adapt to and take advantage of such fluctuations. Accordingly, our central hypothesis is that physiological levels of variability in mechanical stimuli that are normally present in the body have fundamental regulatory roles in basic cell functions. If this hypothesis is true then the most basic cellular process, ATP generation by mitochondria, is regulated not by the mean stimulus to which cells can adapt, but the irregularity of the stimulus that maintains the cell under non-equilibrium conditions. We test this hypothesis in energetic vascular smooth muscle cells by exposing them in culture to variability in mechanical stretch. We find that ATP production is downregulated in vascular smooth muscle cells stretched with a monotonous pattern compared to cells stretched with physiological level of cycle-by-cycle stochastic variability in strain. Furthermore, there is an optimum level of variability that maximizes ATP generation and ROS production implying that changes in variability due to disease should also alter cell signaling. We also find that variable stretch enhances ATP production by increasing the expression of ATP-synthase's catalytic domain, cytochrome c oxidase and its tyrosine phosphorylation. These phenomena are mediated by the ability of variable stretch to increase the microtubule network's fractal dimension by altering fission-fusion dynamics and enhance the association between mitochondria and microtubules, which are destroyed during monotonous stretch that typifies current laboratory experimentation. Variable stretch also influences mitochondrial structure and function in other cell types such as fibroblasts and stem cells. Furthermore, stochastic regulation of mechanotransduction appears to be a general theme of biology: secretion of extracellular matrix molecules and cellular contraction are also influenced by the body's natural rhythms. Since such stochastic regulation represents normal physiology in vivo built into cell functions by a billion years of evolution, our results have implications for all ATP-dependent and mechanosensitive intracellular processes both in health and disease.

Harnessing Gene-Expression 'Noise' for Therapy

Awardee: Leor S. Weinberger
Awards: Pioneer and New Innovator Awards
Awardee Institution: Gladstone Institutes
Co-authors: Roy D. Dar, Nina Hosmane, Robert Siliciano, and Michelle Arkin
Co-authors' institution: California Institute for Quantitative Biosciences

Over the past decade, a stubborn debate has persisted in biology regarding the role and importance of stochastic fluctuations (probabilistic 'noise') in gene-expression. The debate roughly parallels the early 20th century "Bohr-Einstein" debates in Physics between the deterministic classical-mechanics view and the probabilistic quantum-mechanical view (emblemized by Einstein's grand indictment of quantum mechanics: "God does not play dice with the universe"). Clearly, quantum mechanics prevailed and is a cornerstone of modern physics, in large part because of its practical utility to atomic-orbital theory and semiconductor applications. In Biology, there have been no analogous applications for 'noise'; the debate will likely continue until practical uses for noise are identified.

I will present a set of newly identified noise-modulating chemicals (Dar et al. ***Science*** 2014). These compounds modulate noise in HIV expression and synergize with conventional activators to reactivate HIV from latency.

By screening a diverse library of bioactive small molecules, we identified over 80 compounds that modulated HIV gene-expression fluctuations ('noise'), without changing mean expression. These noise-modulating compounds would be neglected in conventional screens and strikingly they synergized with conventional transcriptional activators. Noise enhancers reactivated latent cells significantly better than existing best-in-class reactivation while noise suppressors stabilized the latent state. Noise-modulating chemicals synergized with in both cell-culture lines and human primary cells and are all FDA-approved compounds that exhibit minimal cytotoxicity. Noise-modulating chemicals may provide novel probes for the physiological consequences of noise and may provide an unexplored axis for drug discovery, allowing enhanced control over cell-fate decisions in diverse biological systems.