Brandeis University
Waltham, MA
Susan Birren, Sacha Nelson
$1,000,000

Homeostatic plasticity describes the capacity of a neuron to increase its synaptic density or excitability under conditions of low activity and to decrease them under conditions of high activity. It allows brain circuits to adapt to changes in the internal and external environment while maintaining brain function within a normal range. Now four collaborators at Brandeis University will test for homeostatic plasticity in the autonomic nervous system, which regulates the physiological function of the peripheral organs. The team will genetically manipulate the activity of rat sympathetic neurons to study plasticity mechanisms and their role in controlling peripheral functions such as blood pressure. They will identify gene expression patterns associated with different plasticity states and use imaging and electrophysiological recordings in intact sympathetic ganglia to test their models for this simple, but crucial neural circuit.

Weill Cornell Medicine, Cornell University
New York, NY
Simon Scheuring
$1,000,000

A research team composed of biologists, engineers and physicists at Weill Cornell Medicine will develop the next generation high-speed atomic force microscope (NG-HS-AFM) to take single molecule movies of rare and transient conformational states of proteins at unprecedented spatiotemporal resolution. The temporal resolution of the planned instrument aims to outperform commercially available instruments by roughly two orders of magnitude, accessing the millisecond regime where many essential biomolecular processes take place. Current structural biology methods (Xray, cryo-EM) solve the structures of major conformational states of proteins through ensemble-averaging of thousands of molecules. Consequently, these techniques are blind to detect individual, rare and short-lived conformations. The new instrument is expected to reveal currently inaccessible and poorly understood areas of structural biology: How fast do interconversions between states occur, what are the pathways between states, and what are the conformational intermediates? Insights into transient states may allow researchers to design new drugs that interrupt or stabilize molecular transitions. The research team will first apply the NG-HS-AFM to pain-sensing ion channels involved in inflammation and chronic pain, but the capabilities of NG-HS-AFM are widely applicable and will open new avenues for interrogating a variety of biomedically important proteins.

University of California, Irvine
Irvine, CA
Chang Liu, Ahmad Khalil
$1,000,000

Two investigators at UC Irvine (CL) and Boston University (AK), will develop a platform to create biomolecules that enable the systematic study of the basis of biased signaling by G-Protein Coupled Receptors (GPCRs). GPCRs are the largest family of cell membrane-bound proteins in humans and function to receive and transduce signals like hormones, neurotransmitters, and immunomodulatory molecules into the cell. In recent years, the classical on/off model of GPCR signaling has been fundamentally expanded through the discovery of biased GPCR ligands that do not simply activate or inhibit all signaling at a target GPCR but rather selectively modulate downstream pathways, in effect turning a black and white signaling paradigm into "color." However, our ability to probe this dimension of GPCR signaling is limited by the lack of specific and potent GPCR ligands capable of inducing biased signaling in desired directions. The investigator team will combine their work on establishing versatile reporters for GPCR signaling with their novel continuous evolution technologies to derive new ligands that induce user-defined signaling bias profiles for human GPCR targets. The new ligands will be used to trap medically relevant GPCRs in distinct, biased, or previously unseen conformations, enabling structural and biochemical studies to uncover the molecular mechanisms of biased GPCR signaling and the detailed interrogation of multidimensional signaling of many human GPCRs.

University of Kansas
Lawrence, KS
Jingxin Wang, Yang Li
$1,200,000

By allowing multiple protein isoforms to be encoded in the same gene, alternative RNA splicing drastically expands the diversity of the human proteome, which is critical for human development and disease. Yet, owing to multiple technical challenges, very little is known about the functional RNA sequences (i.e., cis-regulatory elements) that regulate the selection of splice sites. An investigator at the University of Kansas (JW) and his collaborator at the University of Chicago (YL) will attempt to transform our understanding of RNA splicing, by creating a genome-wide map of splicing cis-regulatory elements. They will develop a new chemical biology tool called alternative splicing analysis with chemical perturbations, or "ASCEPT," which combines chemical probing experiments with bioinformatics algorithms to predict splicing regulatory elements. Then they will apply ASCEPT to a large number of genes with unannotated splicing sequences for validation and optimization of this new technology. Finally, based on their experimental results, they will develop and train a deep learning model, ASCEPT-AI, to predict splicing cis-regulatory elements genome-wide. Importantly, their approach could be used to provide critical insights into RNA splicing mechanisms for all eukaryotic genes and in all cell and tissue

types. The researchers are particularly interested in predicting splicing regulatory elements in disease-driving genes that are conventionally considered “undruggable.” Their splicing map could facilitate the development of new and improved gene-specific splicing modulators, which could revolutionize the treatment of many intractable genetic disorders.

Salk Institute for Biological Studies
La Jolla, CA
Clodagh O’Shea, Alan Saghatelian
$1,300,000

Life and death decisions are made in the blink of an eye. In our cells, the combinatorial assembly and disassembly of dynamic protein and DNA interactions that decide gene expression and our fates are faster still, flash mobs that dash past our microscope lenses uncaptured and dissolve into the background of a crowded nucleus. Seeing such interactions is hard enough but identifying the proteins, DNA, and RNAs that were there at the time has been impossible. To crack the dynamic spatiotemporal combinatorial nuclear codes of living cells, two investigators at the Salk Institute for Biological Studies have invented a groundbreaking new technology platform called “Dyenamics.” The Dyenamics platform will enable the critical proteins and nucleic acids that assemble in the nucleus to specify individual and global gene activation and silencing to be fluorescently labeled, visualized, memorialized, and identified in living cells for the first time. The team will apply Dyenamics to reveal fundamental new insights into the dynamic 4D combinatorial code and interactions that determine gene expression and is dergulated in cancer and viral infection.

University of California, San Diego
La Jolla, CA
Johannes Schöneberg, Fleur M. Ferguson, Gal Mishne
$1,300,000

Accumulation of misfolded proteins is a hallmark of many degenerative diseases, but it is still unclear if and how protein misfolding and aggregation impacts cellular health in the tissue context. A team of three investigators at the University of California, San Diego plans to study patient-derived induced pluripotent stem cells differentiated into brain organoids, where misfolded mutant proteins have been shown to accumulate in vitro. They want to know whether the rapid, targeted removal of the pathologic aggregates will have a toxic, neutral, or even protective effect on mitochondrial dynamics, an early indicator of health status in cells. By combining novel small molecule targeting agents with advanced microscopy techniques, organoid culture, and machine learning, the team will build an entirely new model of cellular health based on four-dimensional organelle dynamics in patient-derived brain organoids, and image the immediate downstream consequences of

rapid, selective aggregate clearance by small molecule drugs to reveal their direct effects on organismal health. Achieving these two goals could lead to a breakthrough in understanding cellular responses to protein misfolding and treating associated neurodegenerative diseases.

University of Washington
Seattle, WA
Joshua C. Vaughan, David Baker, Xiaohu Gao, Neil King, Justin Kollman
$1,300,000

Recent advances in microscopy that enable biomacromolecules to be studied in their native cellular microenvironment are transforming molecular cell biology. Cells can be imaged at sub-nanometer resolution in flash-frozen cells via cryo-electron microscopy (cryo-EM) and at ≥20 nm resolution in live or fixed cells via super-resolution optical microscopy. However, there is a tradeoff between cryo-EM, which provides very high-resolution structures of all cellular components but not their individual identities, and fluorescence imaging, which provides precise identities of cellular components in live or fixed cells but without structural context. Ideally, identifying specific proteins, determining their structures in situ, and studying their spatial relationships and interactions with other cellular structures should be as simple as immunocytochemistry where specific proteins and even specific epitopes on a protein can be marked by antibodies. A fundamental limitation, however, is that in live cells, antibodies can only be used for cell surface markers and that cell fixation and permeabilization for intracellular labeling leads to loss of ultrastructure. Thus, there is a pressing need for new tools that can identify specific proteins in living cells, for imaging by fluorescence and electron microscopy, while preserving cellular ultrastructure. A team of five investigators at the University of Washington have embarked on a project to leverage new technologies that allow delivery of specific labels directly into live cells, without common artifacts induced by traditional sample preparation and labeling techniques, using both existing and de novo designed labels to enable high-resolution electron and fluorescence microscopy.