Dana-Farber Cancer Institute
Boston, MA
Nicholas Polizzi, Edward Boyden
$1,300,000

Single-molecule in situ protein sequencing via expansion microscopy

Proteins carry out nearly all cellular functions, but scientists lack a method to simultaneously read protein sequences, chemical modifications, and locations within intact cells. Two investigators—one from Dana-Farber Cancer Institute and one from Massachusetts Institute of Technology and the Howard Hughes Medical Institute—are developing in situ protein sequencing, an imaging platform that determines each protein's complete amino-acid sequence and modifications while preserving its precise cellular location. The technology combines expansion microscopy, which physically enlarges samples so standard microscopes can see individual proteins, with fluorescent barcoding that "reads" amino acids one by one. The expansion process anchors proteins to a polymer network, removes other cellular components, and expands the polymer in water, making proteins both visible at nanoscale resolution and accessible for imaging. The team will develop chemistry to sequentially expose each amino acid in the anchored proteins, allowing fluorescent markers to bind and reveal the sequence letter by letter. They are also creating a larger library of fluorescent amino-acid binders using AI-assisted protein design. This technology could generate detailed maps linking protein identity to cellular location, potentially transforming our understanding of normal cell function and disease mechanisms.

Emory University
Atlanta, GA
Monika Raj
$850,000

New chemical tools to restore the function of defective proteins

Many diseases, including cancer and inherited disorders, stem from single amino acid errors in proteins caused by genetic missense mutations. Although most of the protein remains intact, this tiny change can break crucial protein interactions and shut down vital cell functions. Current therapies that edit genes or RNA often cannot fix these defects at the level of the mature protein nor can they readily reconstitute the specific interaction that is lost. To meet this need, an investigator at Emory University is developing Targeted Protein Restoration Technology (PRT). Rather than editing genes or replacing entire proteins, PRT uses customizable chemical probes that work like molecular patches. These

probes could specifically bind to defective proteins and restore their lost connections without affecting healthy versions—offering more precise, reversible control than gene therapy. PRT could help researchers test the role of single amino acids and add chemical modifications at specific sites to give proteins new properties. This could reveal how post-translational modifications change protein function. Because PRT is programmable and reversible, it could bridge a key gap in understanding disease mechanisms and in correcting mutation-driven defects directly at the protein level.

Rice University
Houston, TX
Gang Bao, Caleb Bashor
$1,000,000

High-capacity viral vectors for in vivo gene delivery and genome editing

CRISPR genome editing technology offers unprecedented opportunities to precisely modify human DNA for research and medical applications. However, realizing this potential requires delivering large DNA constructs (over 10 kilobases) containing genome editing machinery to specific cell types in the body. Current delivery methods like viral vectors and lipid nanoparticles cannot carry such large genetic payloads while maintaining cell-type specificity. Two researchers at Rice University are developing baculovirus (BV) vectors to solve this challenge. Baculoviruses have exceptional DNA packaging capacity, do not replicate or integrate into mammalian genomes, and trigger minimal immune responses. However, their clinical use has been limited by three major obstacles: the immune system's complement proteins destroy them, they struggle to escape cellular compartments after entry, and they do not efficiently target specific cell types. To overcome these limitations, the team will use high-throughput screening to test thousands of BV variants per experiment, each engineered with different combinations of surface proteins. By creating barcoded libraries of these variants, they can rapidly identify which designs best evade immune destruction, escape cellular traps, and bind to target cells. The optimized BV vectors will then be tested for their ability to deliver large genetic payloads for genome editing and cellular reprogramming in living organisms. This work could unlock the full therapeutic potential of genome editing by finally enabling efficient, targeted delivery of complex genome editing tools throughout the body.

University of California, Berkeley
Berkeley, CA
Gloria Brar
$1,100,000

How do protein complexes assemble while their components are being synthesized?

Most proteins are found in complexes, physically associated groups that act together as molecular machines to carry out the processes that enable life. It has long been assumed that proteins destined to form complexes are made by the ribosome at arbitrary locations in cells and then find their partner(s) by random diffusion within the complex mixture of cellular factors. How specificity of interactions would be ensured by this strategy is unclear, although mistakes in assembly could both reduce important normal cellular functions and lead to undesirable toxic assemblies. Through a global approach, this investigator and her team have found evidence that contrary to traditional models, most protein complexes assemble while multiple components are in the process of being synthesized. In this proposal, they will use innovative methods to map such protein-protein interactions comprehensively. They will also address major fundamental blind spots in our understanding of cellular organization by revealing key features of protein surfaces that seed productive interactions, the factors that enable it, and how specific complex function is ensured through ordered assembly. Their results will constitute an atlas of eukaryotic protein complex assembly, advancing fundamental understanding of protein interactions and cellular organization. They also expect these findings to reveal disease-associated mutations at protein surfaces guiding the co-translational assembly process.

University of California, San Francisco
San Francisco, CA
Roarke Kamber
$1,200,000

Engineering immune activity into non-immune cells as a novel therapeutic modality

Chimeric antigen receptor (CAR) therapies reprogram immune cells to selectively destroy disease-causing cells, but their effectiveness is limited in tissues that lack immune cells or actively suppress them. A researcher at UCSF has developed a platform called “CAR-X,” which extends CAR technology beyond immune cells by giving non-immune cells the ability to perform immune-like functions. His team has demonstrated that compact, single-chain CAR-X receptors equipped with specific intracellular motifs can enable diverse non-immune cell types to engulf and kill targeted cells (a process known as phagocytosis) at rates comparable to professional immune cells. The research program has three main objectives. First, the team will uncover how CAR-X receptors enable killing by conducting genome-wide screens and quantitative imaging across multiple non-immune cell types to identify pathways that enable or restrict phagocytosis. Second, they will determine optimal receptor designs by screening large libraries of barcoded CAR-X variants to establish predictive design rules for different cell types. Third, they will test CAR-X as a treatment for liver metastasis by using viral vectors to deliver optimized CAR-X variants to liver cells called hepatocytes in mouse models, evaluating tumor clearance, cell identity maintenance, organ function, and potential side effects. Hepatocytes are particularly promising for this approach because they constitute the majority of liver cells and are positioned to intercept cancer cells early in metastasis. This project could both validate CAR-X therapy for liver

metastasis and create a generalizable design framework for engineering non-immune cells with targeted immune functions.

University of Texas Southwestern Medical Center
Dallas, TX
Yi Liu
$1,200,000

Synonymous codon usage: the Achilles' heel of viral protein synthesis

Viruses cannot produce their own proteins and must hijack a host's cell translation machinery in order to survive. Although most amino acids can be encoded by multiple synonymous codons during mRNA translation, cells do not use all synonymous codons equally. Instead, they preferentially use certain codons over others, a phenomenon known as codon usage bias. This bias significantly influences how efficiently a gene is translated into protein. Interestingly, most human viruses, including those responsible for pandemics and other widespread diseases, use synonymous codons that differ markedly from those preferred by human cells. Despite this mismatch, viruses are still able to produce their proteins efficiently within human cells. This raises a fundamental question: How do viruses with non-optimal codon usage achieve efficient translation in human cells? The preliminary research of this investigator and his team suggests that viruses can bypass typical codon usage constraints by employing a distinct translation mechanism. They have found that multiple viruses, including SARS-CoV-2, hepatitis A, and hepatitis C, utilize this strategy. In this project, the investigators aim to (1) uncover the mechanism that enables viral RNAs to evade codon usage control of host cells and (2) evaluate how widespread and functionally important this mechanism is across major human RNA viruses. Understanding this process may pave the way for the development of broad-spectrum antiviral drugs, therapies that could be used to combat both emerging and known viral diseases, rather than targeting one virus at a time.
