Docket #: S24-413

High Throughput Production of Protein Variants

Researchers at Stanford have developed a novel high throughput system for the addition of amino acids into specific sites on proteins of interest.

Classical biochemical assays yield rigorous kinetic and thermodynamic measurements but are slow, expensive, and inherently low-throughput, limiting their use in large-scale protein variant studies. Microfluidic platforms such as MITOMI and HT-MEK address throughput and reagent constraints by enabling parallelized quantitative analysis of thousands of proteins, yet they depend on costly DNA array preparation and are largely restricted to the canonical amino acids. Genetic code expansion can introduce noncanonical amino acids and broaden protein chemical diversity, but current in vivo and in vitro implementations suffer from scalability, efficiency, and experimental-control limitations. These challenges underscore the need for scalable, integrated approaches that couple high-throughput microfluidics with expanded genetic encoding to systematically probe protein structure–function relationships.

Stage of Development
Research - in vitro

Stage of Research
This invention yields a novel method for high-throughput production of a set of protein variants of protein(s) of interest where each of the set of protein variants independently includes one or more site-specific amino acid substitutions. Briefly, engineered DNA variants encoding a protein of interest with suppressable codons at defined positions are loaded into distinct microfluidic compartments. Subsequently, site-specific amino acid substitutions occur by adding one or more species of suppressor tRNAs that are charged with a pre-selected amino acid to the microfluidic compartments. This platform combines high throughput metholodolgy while maintaining the ability to systematically probe protein structure-function

relationships.

The disclosure allows rapid, programmable installation of canonical or noncanonical amino acids at designated sites, with precise temporal control and reduced reagent consumption.

Applications

- **Programmable Installation of Amino Acids**: By allowing for the systematic dissection of the function of different amino acids at different positions, this method facilitates comprehensive mutagenesis and biochemical characterization in vitro
- **Interrogation of Protein Structure-Function Relationships**: Allows for the global study of how a protein's molecular structure, including its amino acid sequence, three-dimensional fold, and chemical features determines its biological activity, such as binding, catalysis, regulation, or stability.

Advantages

- **Precise Temporal Control**: This method allows for the ability to control precisely which position amino acids are added in any protein of interest.
- **Reduced Reagent Consumption**: Microfluidic compartmentalization allows for reaction sizes to be reduced and therefore allows for the reduced consumption of necessary reagents.

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