System to improve stability of protein and peptide in serum, as well as to introduce photo-temperature switch

Dr. Mira Amiram, Biotechnology Engineering Department, Faculty of Engineering Sciences, Ben-Gurion University of the Negev, Israel.

In current invention Dr. Amiram design improve system to incorporate unnatural amino acids (UAAs) Alkynes or Azobenzenes to protein using improved tRNA synthetases. The present invention provides bacterial cells comprising orthogonal translation systems, mutant aminoacyl-tRNA synthetase (aaRS) proteins and nucleic acid molecules encoding the mutant aaRSs. The aaRSs were generated using special evolution technique. The alkynes being incorporated can bind any material that have azide. Material such as fluorophore, fatty acids, PEG, ligands and more can be linked in this way. The interaction can be made *in vitro* or *in vivo*. Moreover, the system improve insertion of Azobenzenes into elastin like polypeptide (ELP). ELP is a known domain that can be synthesis as part of protein's tail. In general, ELP can phase transform from soluble to insoluble in specific temperature. This being define by the ELP sequence – more hydrophobic acid will lower the temperature transition, which will result in insoluble composition, and vice versa. The azobenzenes severe as reversible photoswitchable groups. Upon irradiation in a specific wavelength the azobenze molecule undergoes dramatic switch from *trans* to *cis* configuration, with concomitant change from a hydrophobic to a hydrophilic molecule (UV or blue light). Meaning now there are two switches, one is temperature-depended and the other is light depended to cross between soluble to insoluble states of proteins. In principle, these features can be used to control the active sites or binding site in case of protein-protein/peptide drug interaction. This can be used to activate drugs in sites exposed to the light directly or by optical fiber. Additionally, Dr. Amira demonstrated that the incorporation of p ara-azidophenylalanine (pAzF) to ELP, can extend the half-live time of peptides and protein, that can seved as biologics drugs. Precise lipidation of these pAzF residues generated a set of sequence-defined synthetic biopolymers with programmable binding affinity to albumin without ablating the activity of model fusion proteins, and with tunable blood serum half-lives spanning 5 to 94% of albumin's half-life in a mouse model. These finding present proof of concept to the use of genetically encoded bioorthogonal conjugation sites for multisite lipidation to tune protein stability in mouse serum.

Application

Bacterial system to express protein with programmable chemical and biophysical properties for broad range of applications in medicine, materials sciences and biotechnology. Some of these properties can be switch temperature, light dependent and extent half-life time of proteins and therapeutics.

Advantages

- Platform to introduce new chemical and biophysical properties
- Broad applications
- Extents and tune the half-life of proteins and peptides, including biologics.

Patent

WO2021165968A1