

## Incorporation of unnatural amino acids into proteins expressed in E. Coli

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### Technology

Site specific incorporation of UAAs into proteins is a rapidly developing and increasingly employed protein engineering technology, that expands the genetic code of an organism to introduce novel and unique chemical and/or physical properties into a protein of choice. Current known methodologies have various constraints that result in low protein yields and low efficiency. Incorporating UAA into proteins can be used for introducing post-translational modifications into proteins, bispecific antibodies where the UAAs serve as the linker sites, antibodies conjugated with a therapeutic agent or a marker, improved affinity of antibodies toward their antigen, increasing protein and peptides stability, a way of binding proteins into surfaces and more.

Current invention provides a highly efficient and reliable system for integrating unnatural amino acids (UAAs) into proteins expressed in *Escherichia coli*. The bacterial cell contains orthogonal translation system, an orthogonal tRNA that had an anticodon that corresponds to a stop codon (o-tRNA) and a UAA specific orthogonal amino-acyl-tRNA synthetase (o-aaRS), and an expression vector that promotes slow transcriptional initiation rate and slow ribosome initiation rate. To optimize the protein expression the system exploits both incorporation of UAA using stop codon suppression, synonymous mutations in the gene of interest and modular tuning of the expression system that control ribosomal traffic. By reducing the strength of the regulatory elements, the expression density decreases, resulting in significantly improved protein yield.

### Advantages

- Novel and efficient technology
- Flexible technology for custom proteins
- May be used for various treatment solutions

### Applications

- Highly efficient and tunable system to incorporate UAAs into proteins expressed in E.Coli.
- May create new features in proteins

### Patent

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