

Optimizing protein expression yield in bacterial vector using UAAs or RTS elements

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Technology

Prof. Alfonta's invention provides a system for integrating unnatural amino acids (UAAs) into proteins expressed in *E. coli*, that leads to optimize the protein yield. Methods for the incorporation of unnatural amino acids have emerged in the recent years as a promising technology to expand the genetic code and engineer novel proteins used in an increasing number of applications. The enormous potential of this technology resides in the site-specific nature of the incorporation and the unlimited variety of unique synthetic amino acids that can be incorporated, allowing novel chemistries. However, the existing methods have constraints that affect the yield and of the recombinant synthetic protein. Current invention comes to reduce these limitations. The current system is very promising as it shows superior results in terms of protein yields as well as in UAA incorporation fidelity and efficiency, compared to the widely used pET system. In general, the research demonstrates that it is indeed beneficial and possible for the user to fine-tune the strength of promoter according to the specific protein expressed. Another invention was focus on the ribosome termination structures, RTS (in collaboration with Prof. Tuller Tamir, TAU) to improved protein yield (without incorporation of UAAs). Mostly the expression is being fine tuned in expression of two proteins together, where the RTS control the kinetics of the process. The invention is the design and utilization of this discovery for the engineering of genomic circuits, networks and whole genome design, and to improve protein expression with precise control over translation re-initiation and insulation. The invention provides nucleic acid molecules and vectors comprising regions of high or low folding energy. Methods of producing coding sequences optimized for protein expression comprising introducing a mutation that increases or decreased folding energy are also provided.

Application

Improve expression of protein in bacterial system. Both with incorporation of UAAs or without. Moreover, a method to control and improve expression of two protein together while using RTS elements.

Advantages

- High protein yield
- Synchronized expression of two proteins
- Improve incorporation of UAAs to protein
- Easy to use
- Lower the cost

Patents

[WO2018/087760A1](#); [WO2021/149062A1](#) (in collaboration with Prof. Tuller Tamir, TAU)