

Synthetic microbial consortia enable rapid assembly of pure multi-protein translation machinery

BACKGROUND

Cell-free synthetic biology is a rising field that aims to construct synthetic gene circuits *ex vivo* using artificial chassis, to finally create and control, for example, artificial cells, microfluidics biosensors and paper-based diagnostic systems. A critical part of all cell-free systems is the transcription/translation machinery, comprised of 34 proteins. Two main sources of translation machinery are available: i) crude, whole cell extracts, containing not only the translation machinery but also all the rest of the cellular components, and ii) a mixture of individually purified translation machinery proteins. The first is cheap and easy to obtain, but the presence of multiple proteins from the source can affect reproducibility, difficult data interpretation and hinder numerous applications. On the other side, the construction of pure multi-protein cell-free system relies on laborious and costly purification of the proteins using independent bacterial strains, making its use prohibitive for high-scale applications.

OBJECTIVE

To implement a novel method for one-pot assembly of the pure 34-protein system using an engineered synthetic microbial consortia consisting of 15 to 34 bacterial strains.

RESULTS

The design of the consortium was based on a mathematical model that quantifies bacterial growth and protein expression in a consortium. By using a 4-strain consortia strategy (expressing fluorescent proteins), we demonstrate control of protein yields based on strain densities, RBS sequence, and plasmid copy numbers. The expression rate was also correlated with the level of the proteins following purification.

We applied this knowledge to create synthetic bacterial consortia, using synthetic genetic modules to produce the translation machinery proteins at the correct ratios, in a single culturing, lysis, and purification procedure, considerably reducing time and cost for its production. We show that the pure multi-protein system is functional, has low protein contaminants and is reproducible. We also demonstrate its applications in the screening of synthetic promoters and protease inhibitors. Furthermore, we showcase the reproducibility and robustness of our synthetic consortia, which address two major challenges in synthetic biology.

This work establishes a novel strategy for the production of pure translation machinery, which may be extended for the production of other multi-protein complexes.