

Ribosome synthesis and construction of a minimal cell using a cell-free expression platform

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Abstract

The creation of wet artificial life in the laboratory is a non-trivial challenge for biologists, chemists, and computer scientists (1-4). Such a challenge revolves around the modular integration of complex reactions networks to obtain functional biochemical units able of self-replication, self-reproduction, spatial-temporal control and ultimately open-ended evolution, e.g. minimal and artificial cells (1,5-8).

As a step towards building minimal cells, we have developed a cell-free expression system for bacterial ribosome synthesis named iSAT: integrated Synthesis, Assembly and Translation for *in vitro* construction of *Escherichia coli* ribosomes (9). The ribosomal RNA, transcribed from its natural operon, self-assembles with ribosomal proteins added to the reaction mixture. Afterwards, *in vitro* built synthetic ribosomes translate a reporter gene (10,11). Such system is important to design ribosome with new functions, and for the bottom-up construction of a minimal cell.

Ribosome cell-free synthesis is an essential process for building a minimal cell that can maintain itself (1). Indeed, regeneration of encoded DNA molecular machineries, through a compartmentalized reaction network, will be necessary to ensure gene expression after cycles of self-reproduction (12,13).

In this work, we have sought to improve the efficiency of the iSAT reaction to achieve the break-even milestone of ribosomes that are capable of constructing ribosomes (7,434 peptide bonds are needed to make a complete set of r-proteins). To do this, we prepared and optimized the iSAT reaction system using a robot for liquid handling. The open nature of cell-free expression platforms enables precise settings of each component level for an optimal system's configuration. Previously, high-throughput screening and machine learning have been used for the optimization of a cell-free protein synthesis and a liposomal drug formulation (14-16).

Here, I will present the optimization of *in vitro* ribosome construction using a cell-free expression system, and I will

introduce future directions of the project. In particular, I will describe biochemical experimental spaces underlying the cell-free ribosome synthesis. I will show results on the optimization using a liquid handling robot for high-throughput experimentation. Our cell-free protein synthesis platform is the only one enabling *in vitro* ribosome construction, which is relevant to the synthesis of a minimal cell.

Adding effective energy regeneration modules is also important for minimal cell projects. Therefore, I will also present data highlighting the ability to regenerate ATP with a non-phosphorylated energy substrate with the iSAT system. Recently, I developed a novel metabolic scheme for a minimal cell (17). The system is based on the catabolism of polysaccharides and/or a polyphosphate (18-20) to regenerate ATP. Proteins are synthesized using a custom-made amino acids mixture (20).

The system is improved by overcoming a fundamental limitation: the efficient recycling of the orthophosphate (iP), which is the by-product of protein synthesis. As a result, ATP (adenosine triphosphate) is kept at steady state and available for *in vitro* transcription and translation (19). Currently, it represents the most powerful *in vitro* protein synthesis systems (18).

One important feature of a minimal cell is the compartment or container, which more than physically interlock components and sub-systems, confers the necessary genotype-phenotype linkage for evolution (21). The compartment of the minimal cell is based on liposomes (1-3,22), and evolutionary dynamics such as fusion and division, are important for resource feeding and selection respectively (6,8,12).

In summary, the work described is designed to lay the foundation for the construction of a synthetic replicating entity by building up synthetic biological unit operations (e.g. cell-free synthesis of constituent parts) and fine-tune the starting blueprint. Indeed, through the bottom-up synthesis of a minimal cell, we are building and understanding complex biological systems.

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