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A novel *in vitro* metabolic scheme for the construction of a minimal biological cell

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Abstract

The bottom-up synthesis of a minimal biological cell is achieved by integrating and connecting three fundamental modules: metabolism, information and self-organization (Noireaux et al., 2011). The execution and connection of these three parts into cell-sized liposomes should generate a system capable of self-reproduction and ultimately evolution. Each of these molecular sub systems is the result of a forward engineering process where bio-parts, e.g. sugars, proteins, phospholipids and nucleotides are interlocked in a functional way (Mann 2008). The creation of a minimal biological cell is certainly one of the most challenging goals of the synthetic biology community (Porcar et al., 2011). The objective of this research area is to elucidate the fundamental design principles found in biology and to understand cellular functions by applying a reductionist approach (Elowitz, 2010). This work also promotes the development of new technologies based on life's principles (Bedau et al. 2010).

The functionalities of biological cells are dependent on the activity of molecular machineries (enzymes). However, such machineries require energy for their physiological function. Therefore, in order to attempt the construction of wet artificial life is essential to reconstitute an in vitro metabolic network that supports the cellular energetic. In biological systems, the energy requirements are stored in the high-energy molecule adenosine triphosphate (ATP). The conversion from ATP to adenosine diphosphate (ADP) is crucial for energy generation. In particular, the hydrolysis of one chemical bond releases inorganic phosphate (iP) and liberates energy used for life processes.

Noireaux's lab has recently developed a unique cell-free transcription-translation (TX-TL) system for synthetic biology applications, as for instance, the possibility of executing DNA programs made of up to ≈ 60 genes (Shin and Noireaux, 2012a, 2012b). In a cell-free system, the rate of protein synthesis depends exponentially on the adenylate energy charge (Atkinson 1968; Matveev et al., 1996). Protein degradation by AAA+ proteases is also highly dependent on the pool of chemical energy available. Therefore, efficient long-lived ATP regeneration and byproducts recycling is at the heart of a minimal cell construction by allowing the execution of larger and larger DNA programs with interesting dynamical behaviors. We will present our recent efforts to design an in vitro metabolism for efficient protein synthesis.

This new metabolic scheme relies on the catabolism of poly-sugars molecules as energetic resources, and it only exploits the endogenous enzymes present in the cellular extract. Cell-free protein synthesis is improved by addition of maltose or maltodextrin in the reaction mixture. The initial phosphorylation of maltose or maltodextrin produces either glucose or glucose-6-phosphate, which are intermediates of the glycolysis. In turn, this allows for higher level of sustained ATP concentration through recycling of iP, the byproduct of the transcription/translation processes. We will present biochemical experiments that quantitatively measure several system's parameters: concentration of synthesized protein (a reporter gene eGFP), level of ATP and inorganic phosphate, as well as pH fluctuations during *in vitro* protein synthesis. Recently, we reported the highest protein yield ever achieved with an E. coli cell-free expression system with this new metabolism (Caschera and Noireaux 2013). Therefore, compared to others cell-free expression systems used to design a minimal cell (Ichihashi et al. 2010), it represents a more powerful solution in term of adenylate energy charge.

We are now using this system to develop a minimal cell. One of the current bottlenecks in this research area is the encapsulation of the cell-free TX-TL reaction into cell-sized vesicles of complex phospholipid composition.

Further optimization of the reaction mixture for cell-free protein synthesis, as well as its integration into liposome with an active membrane (Noireaux and Libchaber 2004), could be accelerated exploiting a machine learning approach coupled to robotic workstation for liquid handling (Caschera et al. 2010, 2011).

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