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Current Opinion in Chemical Biology

Integration of biological parts toward the synthesis of a minimal cell

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Various approaches are taken to construct synthetic cells in the laboratory, a challenging goal that became experimentally imaginable over the past two decades. The construction of protocells, which explores scenarios of the origin of life, has been the original motivations for such projects. With the advent of the synthetic biology era, bottom-up engineering approaches to synthetic cells are now conceivable. The modular design emerges as the most robust framework to construct a minimal cell from natural molecular components. Although significant advances have been made for each piece making this complex puzzle, the integration of the three fundamental parts, information–metabolism–self-organization, into cell-sized liposomes capable of sustained reproduction has failed so far. Our inability to connect these three elements is also a major limitation in this research area. New methods, such as machine learning coupled to high-throughput techniques, should be exploited to accelerate the cell-free synthesis of complex biochemical systems.

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Current Opinion in Chemical Biology 2014, **22**:85–91

This review comes from a themed issue on **Synthetic biology**

Edited by **Pier Luigi Luisi**, **Pasquale Stano** and **Cristiano Chiarabelli**

For a complete overview see the Issue and the Editorial

Available online 4th October 2014

http://dx.doi.org/10.1016/j.cbpa.2014.09.028

Introduction

The bottom-up synthesis of a minimal cell represents a challenging but conceivable goal for the synthetic biology community [1]. The design of a minimal biological cell from scratch deals with the creation of an out-of-equilibrium system capable of self-reproduction and open-ended evolution [2,3]. Such project consists typically of the assembly of molecular components toward a gradual increase of complexity under the fundamental laws of thermodynamic [4]. The construction of reduced cell analogs in the laboratory increases basic knowledge of unicellular life, its primary goal, but also provides novel platforms for biotechnological and biomedical applications [5–10]. The construction of predictable biochemical systems programmed with genetic information is one of the other major objectives of such ambitious projects. As first stated by Virchow [11], a cell originates from another cell. As a consequence, the logic of self-reproduction, one of the most fundamental features of biological systems, is difficult to break down. An approach to this problem consists of synthesizing cell analogs from its basic natural molecular components.

The molecular synthesis of living entities relies on three features: information, metabolism, self-organization. Each of these parts is made of molecular machineries, each of these parts is indispensable to construct synthetic compartments capable of sustained self-reproduction and evolution [12,13]. Self-organization is needed for the formation of the compartment and for protein macromolecular assemblies; metabolism is needed for the self-maintenance of the system, nutrients synthesis and waste recycling; information is essential for evolution and regulation of cellular functions. Taken separately, considerable work has been done on each of these three topics. However, the integration and the coordination of self-organization, metabolism and information into cell-sized compartments have failed so far.

Many definitions of life have been proposed that could direct or help the construction of a minimal cell in the laboratory. Although certainly useful in defining a context, capturing life in concepts or lists of properties is not enough. The definition of life stays elusive and many different definitions can be written [14] that would satisfy biologists, chemists, physicists and philosophers. The Autopoiesis theory, one of the first conceptual efforts to define cellular life, is a formulation of chemical self-reproduction and self-maintenance [15]. The first synthetic cells genetically programmed to sustain self-reproduction will certainly arise in ideal environmental conditions far from real conditions to be considered as really alive. This is why the definition of unicellular life remains volatile: beyond their biochemical and biophysical attributes, the first biological cell-analogs will be also defined by their external synthesis medium, which can have a wide range of conditions (type of primary source of energy to be exploited, osmotic pressure for mechanical robustness, ionic strength for molecular interactions, among many others aspects).

Most of the credibility in this research area has been provided by the origin of life approach to synthetic cells. The origin of life is still one of the major motivations for the construction of cells from the bottom-up. However,

with the era of synthetic biology and the considerable heritage of soft matter, purely constructive approaches to minimal cells are conceivable. High-throughout methods, lab automation and machine learning algorithms are powerful tools to accelerate the prototyping of cell analogs in the laboratory [16–19]. Whether fully predictable and controllable DNA-programmed synthetic cells can be obtained is a question that cannot be answered yet. The top-down creation of a bacterium with a reduced synthetic genome also supports the construction of a cell from its molecular components, although both projects address different questions [20,21].

On the construction of minimal complex biological systems

The construction of biochemical systems *in vitro* is not just an exercise, it is a forward engineering approach necessary to understand the emergence of complexity in genetically encoded systems, to capture, in isolation, the cooperative link between the molecular machineries making living systems, and to characterize the molecular repertoire and networks found in biology. The purpose of cell-free biology is also to be quantitative and to expand the capabilities of natural systems [22].

Arguably one of the most challenging goals of cell-free synthetic biology is the bottom-up construction of minimal cell systems. It is a multidisciplinary research area, a problem of biology, chemistry and physics. Such projects have only recently become conceivable, but several approaches to assembling self-reproducing minimal cells using the basic molecules of life have been advanced [23–29]. The vocabulary used is often confusing: artificial cell, minimal cell, protocell, semi-synthetic or synthetic cell, reduced cell, coacervates, cell mimicry, partial cell, cell imitation and other jargons reduce the visibility of the work done in this research area.

The *protocell* approach explores the origin of life through the construction of cells from prebiotic components [30]. The most basic protocells do not contain information-carrying molecules, and are solely based on self-assembly and metabolism [13]. Sophisticated protocells, also deprived of complex molecular machineries, use peptides or RNA for information and fatty acids for membranes. The goal is to develop molecular scenarios for the emergence of cellular life on Earth in prebiotic conditions, from the formation of cell-sized compartments to their autonomous growth and reproduction [31,32]. The *artificial cell* approach consists of merging natural and synthetic chemical components to engineer chemical carriers or genetically programmable systems with predictable behaviors and to expand the capabilities of biological systems [33]. Such approach may lead to the design and construction of orthogonal-life. Polymersomes, mechanically more robust than natural membranes, are an example of artificial cells [34]. The synthesis of self-reproducing entities using molecules of real cells, often refers as the *minimal cell* approach, seeks to advance knowledge of biological self-reproduction through the assembly of synthetic cells made of natural components [29,35]. The bottom-up construction of minimal cells seeks to understand the cooperative link between the major molecular machineries and mechanisms making real self-reproducing cells. Although the minimal cell approach is well established in the community, there are potentially as many possible minimal cells as laboratory conditions.

All these approaches revolve around the same challenges: how to integrate and boot up information, metabolism and self-organization to create sustained self-reproduction of a container. Emulsions droplets have appeared as valuable intermediate synthetic cell systems. In particular, water-in-oil emulsions offer an easy way to create cell-sized compartments useful for proto-, artificial and minimal cells work [36•,37].

The bottom-up modular design of minimal cells: container–metabolism–information

A minimal cell would incorporate the machineries necessary for the execution of a small synthetic DNA genome based on bacterial regulation into a liposome supplemented with nutrients and a basic chemical energy regeneration system. The modular design [12], which consists of integrating and connecting three molecular modules (Figure 1), arises as the main strategy.

The three indispensable parts for the bottom-up construction of a biological minimal cell are: self-organization (the physical boundary that makes the container and other self-assembly processes), metabolism (energy processing and regeneration) and information (DNA program) [12,13]. This tenet is also common to the protocell and artificial cell approaches to some extent. Many pieces of this puzzle have been experimentally realized, but they have never been put together successfully, not even closely.

The container makes the linkage genotype-phenotype [28]. The physical boundary of a minimal cell is a closed phospholipids bilayer, namely a liposome. Soft matter and medical research have provided numerous methods to create cell-sized liposomes [38•,39–43]. Despite this considerable heritage, the creation of stable phospholipids compartments is challenging because of the complex reactions that must be encapsulated. All of these methods work well for diluted aqueous solutions. Just adding salts at physiological conditions (≈100 mM) and macromolecules at low concentrations (proteins, ribosomes, DNA, or RNA) dramatically decreases the yield of liposomes formation and their stability. The encapsulation efficiency varies from a method to another, and depends on the composition of the inner and outer solutions. One