

Synthetic Biology

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At a glance

Strictly speaking, the term “Synthetic Biology” implies the application of the synthetic principle to biological, or living, systems. This means that entities are being assembled purposefully to form something entirely new that is not simply the sum of its parts. The concept of synthesis is, thus, the backbone of engineering. After a long glorious history in the humanities and the natural sciences, its appearance in the context of biology is relatively recent, in spite of several decades of successful biotechnology and genetic engineering. “Synthetic Biology” means both, the actual synthesis of biological systems from scratch, or the synthesis of new biological systems from elements of existing ones, assuming a general compatibility of essential biological modules. The latter is the logical but ambitious continuation of cutting edge genetic and molecular engineering, moving from simple to more complex modifications of the genetic code, towards “writing” and cloning entirely new gene networks. It is thus, the vision of a new generation of biotechnology, armed with today’s enormous genomic information. The former is much more fundamental, with strong foundations in the basic sciences, as it refers to the investigation of the origin of life as such. Last but not least, any major synthetic effort will require the adaptation of engineering concepts, in particular functional modularization as well as standardization of modules and interfaces, and utilize design concepts based on system-theoretical fundamentals. Thus, it appears that Synthetic Biology will probably be one of the most interdisciplinary fields of research in the coming years.

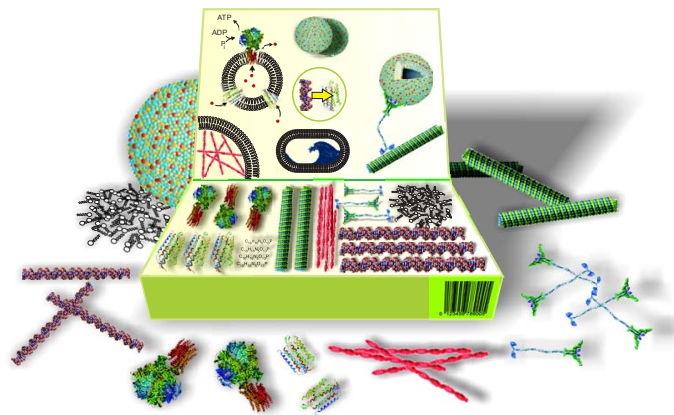


Figure 1: The vision at a glance - toolkit of functional biological parts for a strictly synthetic approach towards living systems [1]

Lessons from Engineering – Modularisation and Standardization

The most dramatic technological revolution of the past decades is certainly the rise of information technology, accompanied by the continuous downscaling and integration of ever more functional elements, aided by the nanosciences. Both, the general concepts of

information processing, and the possibility of understanding and manipulating ever smaller functional units, do also apply to the biosciences. Our today's knowledge and understanding of large genomes and their regulation of massively integrated biological systems is strongly influenced by IT and computer science, not only through the need of enormous storage and processing units, but also conceptually. This can go as far as drawing analogies between biological modules that regulate information production and flow, and electronic circuit diagrams, and designing metabolic pathways from known genetic elements through computer aided design. But there is one significant difference between electronic circuits and biological ones. This is the much easier standardization of technical parts than of biological ones. No cell is like another, and mastering a genetic tool in one organism does not imply that it would be easily mastered in another one. Thus, the task of standardization of functional modules will be of major relevance for the coming years in the post-genomic era [2].

Top-down vs. Bottom-up: *In vivo*, *in vitro* and *ex-vivo*

Engineering living systems is obviously quite different from traditional engineering, because of the high variability and the rather ill-predictable nature of the subject. To date, there is no generally accepted definition of what life is, nor is there, on basis of our today's knowledge about molecules, cells, and organisms, a comprehensive scientific concept able to describe the vastly complex dynamic structures and processes in living systems from first principles. Instead, bioengineering usually starts with existing and thus, already functioning biological systems - mostly microbial organisms that are relatively easy to culture - and manipulates particularly well-understood genetic modules in the context of an otherwise unaltered cellular system. In technical terms, one would call such an approach **top-down**. The top-down approach has become quite successful, aided by the steadily increasing knowledge about new genetic modules and functions, and the availability of ever more sophisticated methods and technologies to analyze and manipulate cellular pathways.

Naturally, metabolic engineering, driven by the desire to arrive at new drugs, materials, and energy in technologically useful quantities, has been the prime goal of ***in vivo* - based synthetic biology**. It distinguishes itself from traditional biotechnology mainly by the degree of complexity of genetic manipulations, where whole gene networks, rather than simple genes, are being transferred, to the point of actually "reprogramming" cells. New nucleic acid-based technologies, like the use of TALENs and the introduction of RNA scaffolds reminiscent of DNA origami have dramatically enlarged the biotechnological toolkit. Among the most noticeable breakthroughs of this kind of synthetic biology are the production of Artemisinin, an anti-Malarial drug [3], the alteration of photosynthetic pathways [4], the production of biofuels with engineered bacteria [5], and genetic manipulations in biopharmaceutical manufacturing, gene therapy and tissue engineering applications [6]. The medium-term goal of **top-down synthetic biology** is the generation of streamlined living cells with inheritable and specific alterations in the genome, proteome and metabolome. These efforts will eventually allow researchers to design cells *à la carte* for the production of platform chemicals, drugs or smart materials.

The exact opposite of this approach of using and reprogramming existing organisms is the concept of truly designing functional biological systems from scratch. Technically speaking, this is a **bottom-up** concept. The starting point is here obviously not the cell,

sometimes not even complex biomolecules such as nucleic acids and proteins, but molecules that do have the potential of assuming relevant biological functions, such as carrying information, self-assembling, self-organizing, or entertaining metabolism, when brought together purposefully. In contrast to the *in vivo*-based top-down approach discussed above, which belongs mainly to the realm of biotechnology, molecular and cell biology, many different research disciplines contribute with their specific techniques and interests to this purely ***in vitro*-based synthetic biology**. The main biological and biochemical contribution is to elucidate interesting cellular functionalities by purifying the essential components, primarily proteins, and studying them under defined conditions. The chemical sciences contribute to the bottom-up approach mainly through the relatively young discipline of chemical biology, devising new strategies of rational protein or compound design, or enlarging the genetic code by synthesis and introduction of non-natural amino acids [7,8]. The desire of biological physics is to understand, from first principles, the emergence of self-organizing and self-sustaining systems that are able to evade the natural tendency to equilibrate by feeding on energy and negative entropy – a fundamental feature of living systems as proposed by Schrödinger [9].

Naturally, both vistas of Synthetic Biology have their merits and drawbacks. The cell-based *in vivo*-approach of top-down synthetic biology faces the challenge of an incomplete understanding and control of the organism, and thus suffers from the obvious complications of having to rely on the viability of the cells and the absence of cellular defense mechanisms against genetic manipulation. The bottom-up approach, on the other hand, has the fundamental problem that a binding definition of a functional living system is still lacking [10], and therefore, only rather technical milestones, such as the reconstitution of self-assembling or self-organizing minimal systems have so far been defined and accomplished [11,12].

An attractive middle way to these two approaches from the distant ends of our understanding of living systems is provided by a third kind of strategies, which are presently contributing to Synthetic Biology: the bioengineering in and with cellular extracts, which could be termed ***ex vivo* synthetic biology**. Using cellular extracts guarantees the presence of necessary factors to entertain nearly all cellular metabolism and biological functions, however without the prerequisite of guaranteeing cell viability or having to deal with genetic regulation strategies to evade external manipulation [13]. To date, prokaryotic extracts such as the famous PURE system from *E. coli* [14], as well as eukaryotic extracts have become accessible and increasingly sophisticated. Designing new genetic circuits in cell-free systems is thus one of the great hopes of new generations of biotechnology. Also, extracts may provide access to the *in vitro* production of proteins that are otherwise hard to purify. To the downside, *ex vivo* systems are neither truly physiological, nor well-defined in the sense that they allow for a systematic elucidation of biological functions under controlled conditions.

The Concept of a Minimal Cell

The cell is indisputably the smallest representation of a living system and captures the essence of biology. Thus, one of the most formidable ultimate goals of Synthetic Biology in general is the construction of a living cell with a genome that comprises a minimal set of necessary functions. From the technological application point of view, such a cell would be the ideal chassis, or production platform, for new bioengineered metabolic

pathways, as it would operate energetically at maximum efficiency, devoid of any functionality that interferes with its biotechnological purpose. But also from the viewpoint of bottom-up synthetic biology, the transition from any possible protocell to a minimal living cell marks the breakthrough toward the understanding of biological systems. There is a clear consensus in the communities of pre-biotic chemistry and Synthetic Biology that compartments are essential for the apparition of life [15-17]. An important property of compartments is to provide boundaries and hence, means to avoid diffusion and dilution of products. With respect to evolution, the compartment provides the concept of identity, and a link between the genotype and the phenotype, when the catalytic molecules (e.g. enzymes) differ from the replicating molecules carrying the information. Such a link is required to guarantee that the encoding molecules are selected when they provide a selective (catalytic) advantage.

Certainly, all our knowledge to date about the stunning complexity of even the smallest and simplest unicellular organisms suggests it to be impossible to actually re-engineer “modern” cells that are essentially the endpoints of billions of years of evolution. Through their continuous cycles of variation and selection, being forced to adapt and survive in various kinds of hostile environments, cells accumulated a plethora of redundant and puzzling features that are almost impossible to fully comprehend, assuming a deductive and modular way of analysis. Thus, the target of Synthetic Biology will not be to synthesize an identical replicate of a “modern” living cell. Instead, one should define suitable model systems of moderate complexity from a limited number of well-characterized functional modules. Here again, the approach can either be top-down or bottom-up, as outlined below.

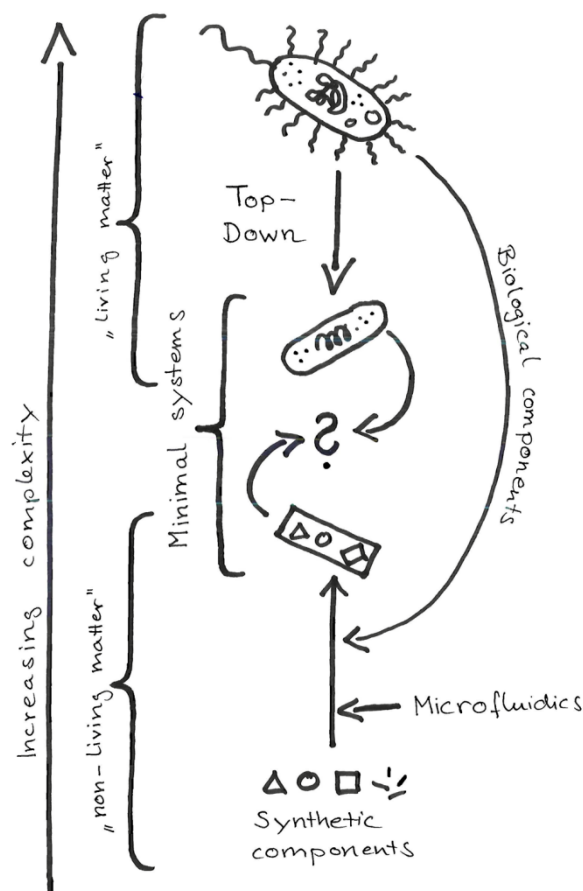


Figure 2: Different concepts of the minimal cell. Following the top-down approach, an existing organism (e.g. bacterium) may be reduced with regard to its genetic program, resulting in a minimal configuration

of a living cell. For the bottom-up concept, individual parts from synthetic or biological origin are assembled together to build a new entity. End products of both approaches can be denominated as “minimal cells”, but they are distinguished by an essential feature: minimal systems obtained by top-down do, without doubt, still belong to the realm of living matter, whereas a possible transition to this regime is still questionable for the bottom-up approach.

The minimal cell approached from top-down

One of the most spectacular enterprises of the recent years with respect to the top-down engineering is represented by the initiative of Venter and colleagues. They were identifying the organism with the smallest known genome of ca. 480 genes, *Mycoplasma genitalium*, and first initiated a genome-wide screen for dispensable genes, to arrive at a proposed minimal genome of necessary genes of ca. 380 [18]. Then they attempted to synthesize the full genome artificially and introduce it back to a “ghost” microorganism void of its own genome. Although the attempt failed with *M. genitalium*, they succeeded a couple years later with a related species *M. mycoides*, whose gene was fully synthesized and successfully transplanted into another *Mycoplasma* species [19]. This marks the so far greatest accomplishment, also in terms of technology manpower, with respect to minimal cells. From an application perspective, however, this may only mark the starting point of a development of minimized chassis.

The minimal cell viewed from bottom-up

In bottom-up Synthetic Biology, the goal is to design artificial systems with minimal but sufficient functions to serve as artificial biomimetic compartments with certain cell-like properties. Such bottom-up synthesized cellular entities are often called “protocells” (Rasmussen). The starting point is to generate, understand and control individual compartments with well-defined and well-controlled physico-chemical properties and functions. To create a compartment compatible with the minimal requirements of (pre-)biotic systems is actually quite simple. In first approximation, this could simply be droplets generated from two immiscible fluid phases, or, one level higher in complexity, vesicles made from amphiphilic molecules that contain the same kind of fluid on their outside and inside, and imitate the basic structure of natural cell membranes [20,21]. Compartmentalized within, proteins can be up-concentrated locally, and compounds can be continuously produced and released at bioactive surfaces. Spatial restrictions also allow to effectively separate chemical reactions from each other by allocating sets of reactants and catalysts to semipermeable compartments.

Droplets and especially vesicles have recently successfully combined with cell-free protein expression using the PURE extract. It has been possible to equip them with pores to enable energy and small metabolite exchange [22], cytoskeletal elements [23,24], and reconstitute mechanical transformation induced by proteins [25,26]. Importantly, vesicle transformation and division could be coupled to redox reactions mimicking metabolism, or nucleic acid amplification within [27,28]

Perspectives for bottom-up synthetic biology

Among the many exciting perspectives of Synthetic Biology, most of which are concerned with new applications, such as the production of drugs, materials, and renewable energy carriers, one of its most fundamental implications is the quest to

understand biological systems and processes deductively, moving from simple to complex. After taking a mainly descriptive approach through most of its history, fundamental research in the life sciences has reached a level of self-confidence at which the mere admiration of the complexity and wealth of function of living systems has turned into the desire to use our knowledge for constructing entirely new biological features and systems. This is not only exciting through the eyes of an engineer, but also opens up new vistas for the fundamental understanding of biology. One of the greatest nuisances in quantitative studies of living systems, their relatively low reproducibility and poor control of the parameter landscape, may eventually be alleviated when it becomes feasible to reconstitute the essence of a particular phenomenon with a minimum number of components. The key task will, however, be to define or identify the **most fundamental** features and phenomena for living systems in general.

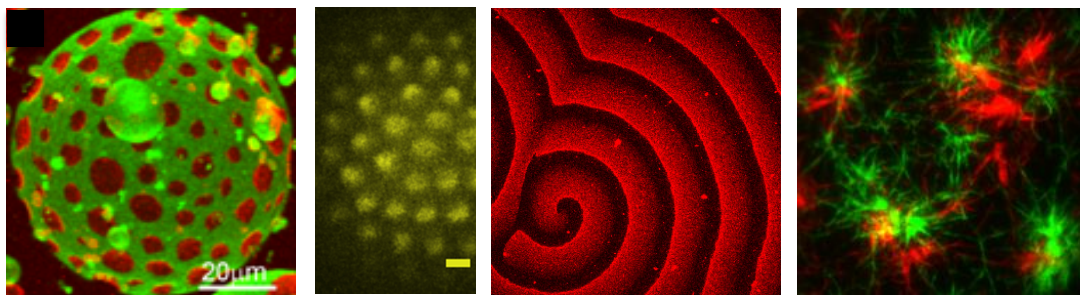


Figure 3: Simple biophysical model systems to reconstitute pattern formation and mechanical transformations of membranes. From left to right: Phase-separating phospholipid vesicle; surface deformations of vesicles by FtsZ of the E.coli divisome; patterns formed by Min proteins [11]; contracting minimal cortex on model membranes [37]

From a very basic point of view, life is based on the self-organization, homeostasis, and self-replication of complex (information-carrying) structures by the continuous flow of energy through these systems [9]. Consequently, many research activities of bottom-up synthetic biology today are aiming at reconstituting the following main features of living systems in cell-like microcompartments which are of overwhelming importance for their homeostatic operation under thermodynamic nonequilibrium conditions, and for their potential to replicate autonomously:

- Self-assembling of compartments to separate different information units
- A machinery to replicate information units by self-organization
- A machinery to replicate compartments by self-organization
- Intrinsic cues to couple compartment transformation and information inheritance
- Supply and exchange of energy and matter [to/from the environment](#), as well as metabolic conversions to sustain growth
- Sensory systems to react to environmental cues

While nearly everything to do with biological information and its replication has been outlined intensively, and *in vitro* reconstitution of DNA/RNA and nucleic acid-processing machineries is quite feasible, much less has been accomplished with regard to functional compartmentation, and particularly, the transformation of compartments along with information transfer. In the past years, the physical and chemical sciences have provided various ways to produce droplets and lipid vesicles at defined conditions of size, composition, and content, often utilizing the vast toolkit of modern micro- and

nanofabrication techniques [29]. Polymer sciences have contributed the attractive alternative of creating polymer-based mimicry of lipid-based biomembranes, so-called polymersomes, to overcome problems of low stability and limited chemical functionality of phospholipid assemblies [30]. The feasibility of functional protein production in water droplets immersed in oil [31], or in compartments confined by artificial membranes [22,32] has already been demonstrated, taking the “*ex vivo*” approach and encapsulating cellular extracts as discussed above.

Further research activities aim at the reconstitution of fundamental self-organization phenomena, like pattern formation and polarization. The simplest representation of self-organization is a reaction-diffusion system, including chemical motifs that couple an [energy-consuming](#) reaction to an [energy-releasing](#) step, e.g., ATP hydrolysis. At least one of these reactions is usually non-linear, to be able to amplify spontaneous thermal fluctuations. Another important ingredient of self-organizing systems is the existence of positive and/or negative feedback, which can involve both physical and chemical cues (by, e.g., mechanical deformations, or signaling molecules). Here, a new level of complexity, involving mechanical force generation from chemical energy is usually required. A beautiful archetypical system that shows the emergence of self-organization and pattern formation is the bacterial clockwork constituted by the Min protein machinery [11], by which dynamic patterns and polarization arise from the antagonistic, energy-consuming reaction-diffusion of only two protein species.

Regarding energy supply, [adenosine](#) triphosphate (ATP) is the main energy carrier while nicotinamide adenine dinucleotide (NAD⁺/NADH) or nicotinamide adenine dinucleotide phosphate (NADP⁺/NADPH) couples are the most abundant redox cofactors in living systems. *In vitro* regeneration of nicotinamide cofactors was mainly studied with respect to potential applications of nicotinamide dependent redox enzymes for the synthesis of chiral compounds [33]. The state of the art method is based on the enzymatic regeneration of these cofactors. Even greater challenges will be posed by the supply of macromolecules that constitute the basis for growth and replication. All of these, nucleic acids, proteins, and lipids, require elaborate enzymatic machineries for their homeostasis that may not easily be minimalized. However, first pioneering work with respect to the design and construction of artificial scaffolds with catalytic activity raises many hopes that essential functionalities such as protein translation and folding chaperonage may even be aided by nanotechnology.

Finally, the fundamental process that needs to occur in the transition from self-organized [to](#) living systems is self-replication. The spatial basis of self-replication, i.e., the transition from one mother into two (equal) daughter compartments, is far from being [fully](#) understood. Cell division is in all known model organisms a highly regulated process of considerable complexity, concealing the fact that at the origin of life, this fundamental process must have been inherently simple and easy to regulate. The key task is obviously, to establish a mechanical force strong enough to induce significant morphogenesis of the compartment boundaries to engage in division. In higher cells, these forces are thought to be regulated by protein assemblies such as motors and cytoskeletal filaments, and may also be exerted by cell wall synthesis. However, simpler mechanisms relying on fundamental physical forces, such as surface and line tension, adsorption, as well as entropic and electrostatic forces may constitute minimalistic alternatives reminiscent of the transforming features early life forms. First attempts to recapitulate fundamental physical transformations of phospholipid vesicles have been

made [34,35], many others, including simple cytoskeletal mimicry will follow [36]. Taken together, we are expecting fascinating insights not only into the minimal requirements of cellular life, but only into life as we know it today.

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