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Bottom-Up Synthetic Biology: Engineering in a Tinkerer's World

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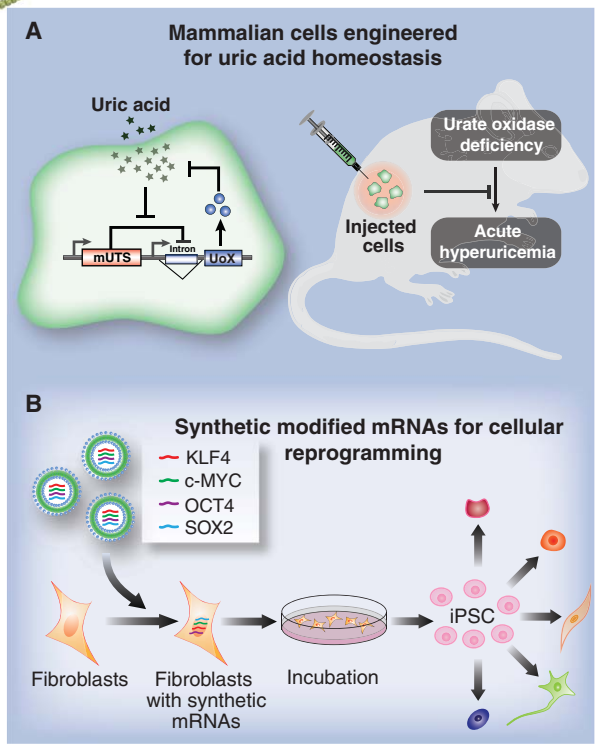

Fig. 4. Diseases can be targeted with new synthetic biology methods for cell therapy and regenerative medicine. **(A)** Urate homeostasis was restored in vivo by the delivery of cells with a synthetic circuit. Uric acid induced the derepression of an engineered urate oxidase, which then lowered uric acid levels in mice. **(B)** Synthetic modified RNAs encoding the KMOS transcription factors were delivered to mammalian fibroblasts to induce pluripotency upon translation. The RNA-induced pluripotent stem cells could be driven down numerous cell lineages.

translational medicine. These and related thrusts will benefit from emerging efforts to integrate synthetic biology with systems biology (*37*, *38*). These developments will aid in the understanding of potential immune responses to synthetic constructs in the body and help identify approaches to ameliorate such responses. These efforts will be critical for developing safe and effective synthetic biology therapies.

Ultimately, we envision synthetic constructs that can sense and seek out aberrant conditions, remediate clinical insult, and restore function. Clearly, there is much to do before synthetic biology can realize its full clinical potential, but the examples discussed here provide insight into the field's exciting potential for helping to prevent and treat disease.

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Supporting Online Material

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Figs. S1 to S3

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PERSPECTIVE

Bottom-Up Synthetic Biology: Engineering in a Tinkerer's World

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How synthetic can "synthetic biology" be? A literal interpretation of the name of this new life science discipline invokes expectations of the systematic construction of biological systems with cells being built module by module—from the bottom up. But can this possibly be achieved, taking into account the enormous complexity and redundancy of living systems, which distinguish them quite remarkably from design features that characterize human inventions? There are several recent developments in biology, in tight conjunction with quantitative disciplines, that may bring this literal perspective into the realm of the possible. However, such bottom-up engineering requires tools that were originally designed by nature's greatest tinkerer: evolution.

An important feature of "synthetic biology" is that it draws on expertise from diverse disciplines; however, these disciplines have not converged on what the new field encompasses. Biotechnologists view it mainly as a new way to organize and structure the art of genetic engineering. To them, synthetic biology enforces the traditional engineering concepts of modularity and standardization and adapts logical operator structures from information processing (*1*). Nevertheless, the assembly of new biological systems for a variety of applications is still carried out in an existing organism; for clinical examples, see the review by Ruder *et al.* [see (*2*)]. Perhaps a more daring view comes from chemists and physicists who take the words literally and focus on the construction of biological systems from the bottom up. They suggest that synthetic biology could follow the tracks of synthetic organic chemistry and open up a new understanding of biology (*3*). This is not to suggest that something as complex as a eukaryotic or even a prokaryotic cell—end

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products of billions of years of evolution—could be constructed from scratch. Our current knowledge of genes and gene products, of networks and feedback systems, make us all too aware of the daunting complexity that is often compared with interlinked hyper-systems like the World Wide Web. On the other hand, biology has long surpassed its mainly descriptive stage, and the questions now asked are increasingly amenable to experimental approaches and theoretical concepts taken from the physical and engineering sciences. This in turn has directed quantitative scientists and engineers toward studying biological phenomena, bringing with them their successful "divide and conquer" rigorous reductionist approach.

From such a perspective, the underlying question of synthetic biology would not be how a biological system actually functions, but rather, how it could in principle function with a minimal set of elements. Unequivocally testing hypotheses, and quantitatively predicting experimental outcomes, is only possible if all parameters of interest can be independently regulated and determined—a formidable task for living systems. However, a quantitative molecular-level understanding has been achieved for reconstituted minimal systems that were stripped of their cell-inherent complexity, such as in the investigation of motor-filament assemblies at the single-molecule level (*4*) or the study of protein-controlled membrane transformations (*5*, *6*), to name but two. Clearly, understanding the biochemical or mechanical features of limited subsystems—although being an important prerequisite for a quantitative understanding of the cell—does not suffice for addressing cellular complexity, let alone the organization and function of whole organisms. But perhaps a reductionist approach can take biological sciences back to their roots: What is life? How did it originate? And how could its very simplest representation, the minimal cell, be envisioned (*7*)?

Many biologists reasonably argue that biology and biological systems can only be understood in the light of evolution and that speculations about how life could be simulated in a minimal chain of causes and effects are pointless. Indeed, it is tempting to suggest that the very features of biological systems that so discourage quantitative approaches—compositional complexity, low hierarchy, and large redundancy of regulatory processes—at the same time impart unparalleled adaptability and resilience. The critical engineer, rather than marveling at the beauty and design of biological systems, may consider some of them suboptimal and inefficient in terms of material usage and energy consumption. Francois Jacob, in his seminal article of 1977 (*8*), compared evolution with a tinkerer rather than an engineer. Rather than designing a tool from scratch, specifically tailored for a certain task, a tinkerer takes what he finds around him and adapts it to his use. The complicated biological solutions to seemingly simple tasks, such as identifying the middle of a cell or budding vesicles from membranes, might call to mind Rube Goldberg's famous Professor Lucifer Gorgonzola Butts (the godfather of all tinkerers) rather than a brilliant engineer. That biology still functions, and functions robustly, may indeed thwart any hopes of arriving at a set of minimal functional elements sufficient to reconstitute a living system.

On the other hand, important human inventions, such as the airplane, have been sparked by biological phenomena without the end products being even close to their sources of inspiration. In this vein, engineering biological molecules and using these as functional units—although its potential for truly understanding living systems may be limited—could yield miniaturized functional elements for sustainable and resource-efficient nanotechnology. Perhaps this is where the disciplines meet; chemists and physicists arriving here from their bottom-up approach come face to face with the biotechnologists who always had cell engineering as their goal. Here, all can agree that synthetic biology goes far beyond the insertion or deletion of single genes. Those practicing synthetic biology are aware that every module added or changed in an already well-evolved system has to be considered in the context of cellular metabolism and growth while also taking into account the host cells' ability to deal with (usually hostile) alien DNA and gene products. Therefore, engineering biology implies the design of whole systems and circuits, along with the standardization and shuffling of protein modules tailored to specific functional tasks. Thus, to be successful, synthetic biology of any kind will have to join forces with systems biology.

What are realistic goals for bottom-up synthetic biology in the next five years, and how may it converge with cellular-level engineering? There are some exciting developments that have raised expectations: Several genetic circuits have been successfully constructed in vivo [see the Review by Nandagopal and Elowitz see (*9*)], and solution- and membrane-based protein oscillators (*10*, *11*) have been realized in cell-free minimal systems, pointing the way to the molecular origin of polarization and pattern formation, two important phenomena in understanding the emergence of order from self-organized systems (*12*). Researchers are engaged in the bottom-up assembly of protein-based functional toolboxes for building self-organizing systems (Fig. 1). Ideally, these toolboxes will contain motifs for cooperativity and nonlinearity, feedback loops, and energy-dependent conformational toggle switches for activity and large-scale localization (*13*). Beyond this, it is possible to insert in vivo functional switches that can be addressed by temporally and spatially well-controlled physical rather than biochemical signals, such as light-activated protein modules (*14*) in optogenetic approaches.

From a physicist's perspective, although the ultimate goal may be the design of a minimal cell, the primary goal is to characterize the interactions between hybrid systems of nucleic acids, lipids, and proteins under well-defined conditions. Here, fundamental physical concepts, which are usually rather neglected in complex biosystems (such as surface and line tension in membrane transformations or electrostatic forces between charged residues) can be precisely addressed and compared with activation energy barriers and free energies of key regulatory processes. Since the advent of sophisticated single-molecule methods, tiny quantities such as femtonewton forces or single units of thermal energy are no longer inaccessible. Once these interactions are quantitatively understood, the next task is to assemble a set of key motifs and functions in biomolecules so as to construct minimal analogies of specific

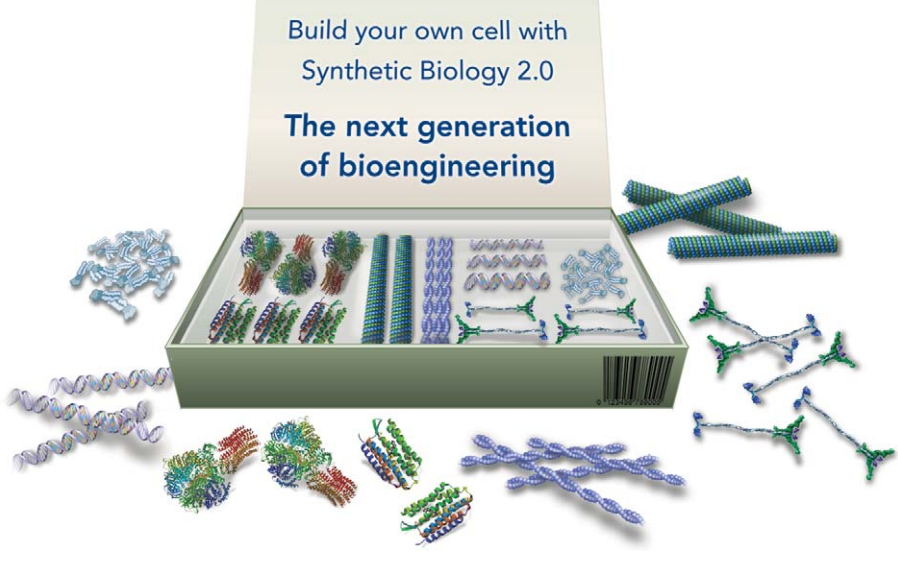

Fig. 1. The biological toolkit: Will it become reality? [Image source courtesy of Jakob Schweizer, BIOTEC/TU Dresden]

cellular tasks and phenomena. In such bottom-up approaches to biological function, there is no need to be constrained to bio-derived molecules. If a synthetic polymer or a piece of DNA origami can do a specific task as well as a lipid or protein module, why not construct bottom-up systems as a molecular "Borg," with biological, bioderived, and nonbiological elements combined for higher efficiency and robustness? Polymersomes made of block copolymers have already been shown to support protein activity in adenosine triphosphate–producing "artificial organelles" (*15*). And, multidimensional RNA structures were successfully designed as scaffolds in vivo to engineer the spatial organization of bacterial metabolism (*16*).

Synthetic biology is benefiting from and contributing to an increasing understanding of biology. The fascination is no longer limited to life scientists but has drawn in polymer chemists, physicists, and lately also engineers. In this exciting time, crossing traditional disciplines may lead us to new bioderived technology and an even deeper admiration of the power of living systems.

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POLICY FORUM

Synthetic Biology: Regulating Industry Uses of New Biotechnologies

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In our view, synthetic biology is an extension of the continuum of genetic science that has been used safely for more than 40 years by the biotechnology industry in the development of commercial products. Examples of synthetic biology use by biotechnology companies illustrate the potential to substantially reduce research and development time and to increase speed to market. Improvements in the speed and cost of DNA synthesis are enabling scientists to design modified bacterial chromosomes that can be used in the production of renewable chemicals, biofuels, bioproducts, renewable specialty chemicals, pharmaceutical intermediates, fine chemicals, food ingredients, and health care products. Regulatory options should support innovation and commercial development of new products while protecting the public from potential harms.

The emergence of synthetic biology into the public's perception has raised some concerns analogous to those expressed at the introduction of genetic engineering in the 1970s, particularly focusing on the potential for developing biological weapons, possible unforeseen negative impacts on human health, the morality of creating artificial life forms, and any potential environmental impact (*1*). Although some nongovernmental organizations have called for "an immediate moratorium on the release and commercial use of all synthetic organisms" or for regulation of the tools used in synthetic biology research, the President's Bioethics Commission "found no reason to endorse additional federal regulations or a moratorium on work in this field at this time" (*2*–*4*). The biotechnology community recognizes that synthetic biology, like other areas of biotechnology, can have both positive uses and negative impacts, and it has responded with guidelines for ethical, self-regulated research (*5*). Beyond that, the current framework for regulation of laboratory research and development of commercial biotechnology products can serve as a basis for regulation of synthetic biology.

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What Is Synthetic Biology?

In our view, synthetic biology is an extension of the continuum of genetic science that has been used safely for more than 40 years by the biotechnology industry in development of commercial products (Fig. 1). For instance, gains in the speed and efficiency of DNA synthesis, sequencing, and recombinant DNA technology combined with cataloging of genomic data permit advanced methods for predictable biological production of commercial proteins and chemicals. Gene shuffling and directed evolution, based on the rapid iteration and sequencing of recombinant proteins, are other outgrowths of the increased efficiency of standard biotechnology techniques and have been safely used for many years. Metabolic engineering—the optimization of microbial fermentation pathways, cellular processes and enzymatic activity for biochemical production—is an outgrowth of the increased knowledge of genomics.

Synthetic biology encompasses a set of emerging tools, including applied protein and genome design, the standardization of genomic "parts" or oligonucleotides, and synthesis of full genomes, that are important to the continued evolution of biotechnology. The continued refinement and capability of metabolic engineering techniques, combined with digitized proteomic and genomic data, are expected to enable increasingly complex, multistep fermentation of organic chemicals and longer gene synthesis. Novel proteins and biological functions are envisioned as tools for advanced metabolic engineering. The BioBricks Foundation is creating a catalog of oligonucleotides that they believe can be certified to perform standardized biological functions when inserted into a microbial system (*6*). Similarly, the Massachusetts Institute of Technology has established a Registry of Standard Biological Parts (http://partsregistry.org/) and the International Genetically Engineered Machine (iGEM) competition (http://igem.org). The J. Craig Venter Institute has achieved initial steps in the design and construction of a simplified genome for a natural, self-replicating bacterium (*7*, *8*).

As often occurs with the introduction of new technology, metaphors that exploit effective, yet still imperfect, similarities in more familiar technologies are used to help illustrate the potential offered in the new field. The BioBricks Foundation, for instance, has consciously sought to leverage "time-honored engineering principles of abstraction and standardization" "to reduce the complexity and cost of producing synthetic living organisms" (*9*). The foundation has established four standards—for assembly, measurement, compatibility and exchange of data—taken directly from the field of mechanical engineering, as requirements for BioBricks listed in its catalog. Metaphors utilized for synthetic biology have often been based on electronic toolkits—i.e., systems that are modular and open to reconfiguration. However, these metaphors can mislead public perception of biotechnology because living organisms are not directly analogous to modular electronics, and therefore, law, policy, and research and development in synthetic biology probably should not be modeled after law, policy, and research and development in the fields of computer science and electronics.