VIEWPOINT

Realizing the potential of synthetic biology

George M. Church, Michael B. Elowitz, Christina D. Smolke, Christopher A. Voigt and Ron Weiss

Abstract | Synthetic biology, despite still being in its infancy, is increasingly providing valuable information for applications in the clinic, the biotechnology industry and in basic molecular research. Both its unique potential and the challenges it presents have brought together the expertise of an eclectic group of scientists, from cell biologists to engineers. In this Viewpoint article, five experts discuss their views on the future of synthetic biology, on its main achievements in basic and applied science, and on the bioethical issues that are associated with the design of new biological systems.

An increasing number of publications and institutions are dedicated to synthetic biology. From the initial focus on the design of synthetic genetic circuits, how has the field expanded and evolved? And how does synthetic biology today relate to other disciplines such as systems biology and mathematical modelling?

Ron Weiss. Synthetic biology was seeded when bacterial cells were programmed with basic circuits — a 'toggle switch', an oscillator and cell–cell communication. The focus has evolved from small transcriptional regulatory networks into complex multicellular systems that are embedded in a variety of organisms such as yeast, mammalian cells and plants, using non-transcriptional logic, including microRNAs, protein phosphorylation and DNA editing. Detailed and precise characterization and predictable part composition have become essential for the efficient creation of sophisticated multi-input logic functions, composite states and analogue circuits. This has led to varied and improved interfaces for cellular sensors and actuators along with advancements such as subcellular compartmentalization of logic operations.

Synthetic biology complements systems biology: the former is based on forward engineering and the latter on reverse engineering. For instance, insight gained from systems biology investigations of natural processes leads to improved designs of synthetic systems, and the creation of small artificial networks helps to analyse hypotheses on the function of natural ones. Computational modelling tools have become essential for the design of artificial networks and are also finding application in other areas of biology that require advanced observation and correlation. Finally, synthetic biology researchers are developing an ever-growing appreciation for biological complexity, which requires interdisciplinary research, new circuit design principles and programming paradigms to overcome barriers such as metabolic load, crosstalk, resource sharing and gene expression noise (and sometimes actually utilize these barriers to create more robust systems).

> "the creation of small artificial networks helps to analyse hypotheses on the function of natural ones."

George M. Church. In my view, synthetic biology was never focused on 'genetic circuits', but rather on biology rapidly maturing as an engineering discipline, including computer-aided-design (CAD), safety systems, integrating models, genome editing and accelerated evolution. Synthetic biology is less like highly modular (or 'switch-like') electrical engineering and computer science and more like civil and mechanical engineering in its use of optimization of modelling of whole system-level stresses and traffic flow.

Michael B. Elowitz. At the most general level, synthetic biology expands the subject matter of biology from the (already enormous) space of existing species and cellular systems that have evolved to the even larger space of non-natural, but feasible, species and systems. Although we started with circuits to carry out the simplest kinds of dynamic behaviours, synthetic approaches can be applied broadly to all types of biological functions from metabolism to multicellular development. Synthetic biology allows us to figure out what types of genetic circuit designs are capable of implementing different cellular behaviours, and what trade-offs exist between different designs, by building and testing these circuits in living cells. We can thus use forward design instead of (or rather in addition to) more traditional reverse engineering approaches, effectively 'building to understand'[34]. Beyond the many important immediate applications, I think it is this transformation in the way of thinking about, and working with, biological systems that stimulates the imagination of so many people and explains the rapid growth of the field.

The fundamental questions that are at the heart of synthetic biology overlap considerably with systems biology, as both fields seek to understand principles of genetic circuit design, and I expect that these fields will cross-stimulate each other and become increasingly difficult to disentangle in the future.

Christina D. Smolke. Both synthetic biology and systems biology represent fundamental shifts in approaches from the fields they grew out of. Synthetic biology emphasizes engineering principles and methodology in designing, constructing and characterizing biological systems from traditional genetic engineering research;

systems biology represents a shift in studying integrated components from the more traditional reductionist approach taken in biological research. Computational modelling is an important tool in both fields but used to achieve different objectives. In systems biology, computational models are used to make predictions about the behaviour of a system, whereas modelling is used to direct design in synthetic biology.

Synthetic biology has expanded and evolved substantially from its initial rather narrow focus to appreciate and use more fully the diversity of mechanisms found in natural biological systems. For example, early work focused largely on transcription factor-based regulatory networks designed to exhibit dynamic behaviour (such as oscillator and 'toggle switch' behaviours)[1], whereas designs now routinely incorporate other levels of regulation, including RNA-based regulators, post-translational modifications and molecular scaffolds[2]. As another example, mutation and evolution were widely viewed as an obstacle to system performance and something to be minimized, whereas newer approaches are beginning to exploit this unique aspect of biological systems and to design for evolution and adaptation[3].

Christopher A. Voigt. The early ambitions in the field were around the creation of cells that could go through a series of programmed tasks; for example, Adam Arkin envisioned an engineered bacterium that could move through the human body and could identify microenvironments, and carry out therapeutic functions[4]. Doing this requires control over when and under what conditions genes are turned on, which in turn requires synthetic regulation — in other words, circuitry. This was, and remains, one of the most difficult aspects of genetic engineering.

Synthetic biology is an engineering discipline — there is a desire to build things that do not yet exist[5,6]. Systems biology is a basic science, where the goal is to better understand natural biology. The relationship is similar to biological engineering and biology, or chemical engineering and chemistry. There can be similar language, but the motivation is different. For example, both synthetic and systems biology are interested in modularity[7]. For natural cells, genetics and regulatory networks may or may not be modular, perhaps depending on how you frame the question. By contrast, engineers can continue to strive to create synthetic genetics and circuits that are increasingly modular.

Mathematical modelling is a tool that enables quantitative predictions or the understanding of data. It is applicable to both areas, as are other tools such as mass spectroscopy to measure protein levels or transfection methods to move DNA into cells.

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Q *What have been the main achievements of synthetic biology so far in basic research, and what are the challenges to be met?*

R.W. Over the past decade or so, synthetic biology has helped to transform the biological sciences into a true engineering discipline. Notable achievements include the creation of a registry of composable parts, revolutionary advances in gene synthesis technologies and faster and more efficient modular DNA assembly methods. By integrating these rapidly developing technologies with computational modelling approaches, a synthetic biologist can now engineer biological systems top-down in a (somewhat) predictable manner.

What is critically lacking, however, with respect to mammalian synthetic biology (an area that my laboratory is currently most focused on), are 'real-world' applications. We must now make an effort to transition from developing 'toy' applications to creating actual applications for cancer therapies, vaccination and engineered tissues that society can directly benefit from. Arguably, the main challenge here is how to safely deliver synthetic circuits into mammalian organisms. I predict that the development of RNA-based delivery methods could be a game changer in overcoming the regulatory hurdles and required safety guarantees associated with traditional gene therapy.

G.M.C. In addition to some applications (discussed below), the achievements include new tools enabling rapid exponential improvements in genome sequencing, harvesting DNA from chips for libraries, multiplex-automated genome engineering (MAGE)[8] of natural and artificial chromosomes (mainly in *Escherichia coli*) and Cas9–CRISPR technology for genome editing of nearly any genome. As reading and writing genomes progresses, the new challenges lie in system design and selection for new functions, and efficient replication and production. Additional challenges arise from how to anticipate, simulate and improve highly diverse or personalized technologies and their impact on complex physiological and ecological systems[9] (discussed below).

M.B.E. We have come a long way as a field: we have built several generations of oscillators[35-38] and genetic switches[4,39] that work with diverse cellular components and regulatory mechanisms, and which interact with endogenous gene circuits[40]. Complex metabolic pathways have been engineered to produce useful products, and signalling pathways have been rewired to alter their dynamic behaviours in predictable ways. However, synthetic biology remains extremely primitive owing both to technical challenges and, even more, to fundamental inadequacies in our understanding of biological circuit design.

On the technical side, synthesizing genetic circuits and transferring them into cells remains far too slow and idiosyncratic, especially in animal cells. However, several new methods, such as those based on the CRISPR system, are extremely encouraging.

On the fundamental side, we still have little understanding of how circuit designs can function effectively in cells and tissues and much to learn from natural examples. In particular, one of the greatest challenges is to move synthetic biology from circuits operating in individual microorganisms to circuits that function in a truly multicellular fashion, for example, circuits sufficient to implement self-patterning of cells. If successful, we may be able to understand multicellular development from a totally new point of view that could inform tissue engineering and regeneration.

C.D.S. One of the important effects of synthetic biology on basic research is that it has driven the advancement of a variety of methods to support the construction of large-scale genetic programmes and genome engineering, including DNA synthesis. Other advances include elucidating frameworks that support the rational design and precise control over activities, such as gene regulatory and enzyme activities[10–12], as well as early steps in pushing standards in metrology and data reporting[13,14]. One big challenge in the field remains to develop measurement technologies that enable the

high-throughput, non-invasive quantification of activities that are not encoded in fluorescent reporter proteins[15]. As the field has shifted towards favouring design approaches that generate diversity within the genetic programmes, there has not been a corresponding emphasis on the development of technologies that enable the characterization of the resulting diversity of genetic designs. As such, the shift towards a 'design smarter' approach (versus a 'design more' approach) is limited by our inability to learn from current design approaches.

C.A.V. Many recent advances that have emerged from synthetic biology are revolutionizing the ways that we engineer cells. DNA synthesis and assembly methodologies make it routine to build constructs in which the designer has full operational control over every base pair for megabases of DNA[16]. This is not a capability that I had as a postdoctoral researcher in 2003 and it remains a challenge to know what to do with such a capability. There have also been many advances in genome engineering and the transfer of DNA into cells, including MAGE developed by G.M.C.[17], the synthetic genome construction by the John Craig Venter Institute (JCVI), USA[18], and CRISPR-based methods to introduce directed changes into genomes[19,28].

Advances have also been made in design methods to build up to these capacities. A number of laboratories have been rethinking the process of the design of genetic systems to make it possible to build more sophisticated systems involving the connection of many parts[20–22].

Many laboratories, including BIOFAB at the Lawrence Berkeley National Laboratory (LBNL), USA, have built a large number of well-characterized genetic parts that can be incorporated into these designs[23–26]. The design of genetic circuits has also improved. We have built large libraries of transcription factors, have converted them into gates, and better understand how to insulate them so that they can be composed into larger circuits. CRISPR interference (CRISPRi) offers a new way to turn off genes, and the intrinsic orthogonality of the system may allow many more synthetic regulators to be used in one cell.

In terms of applications, many products on the market are produced by genetically modified cells. Rob Carlson of Biodesic, USA, has estimated that it adds up to US$350 billion per year, or roughly 2% of the US economy. The contribution of synthetic biology to the current and next generation of products is large. Some notable examples produced by large companies include insecticides (for example, spinosyn) by Dow AgroSciences, USA, and the antimalarial artemisinin by Amyris, USA, and Sanofi, France. Small companies are developing products; for example, Genomatica, USA, produces butanediol (BDO) and Refactored Materials, USA, produces recombinant spider silk. There are many examples, and I am only highlighting a few.

One of the challenges in applications is harnessing cells to build complex functional materials. Cells are natural atomic architects and we already exploit this, as many in-use materials are from biology. The examples above are all relatively simple chemicals, natural products or individual proteins. Obtaining more complex products will require synthetic gene circuits and the ability to control many, possibly hundreds, of genes simultaneously.

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Michael B. Elowitz is an investigator with the Howard Hughes Medical Institute and Professor of Biology, Biological Engineering and Applied Physics at the California Institute of Technology (Caltech), Pasadena, USA. He created the Repressilator, a synthetic oscillatory gene circuit, and showed how gene expression 'noise' and its functional roles can be analysed in individual cells. His laboratory uses synthetic biology, quantitative time-lapse movies and mathematical modelling to understand the design principles of genetic circuits operating in cells and tissues; in systems ranging from bacteria to mammalian cells. He is a Macarthur fellow and recipient of the Human Frontier Science Program (HFSP) Nakasone prize.

Christina D. Smolke is Associate Professor, Associate Chair of Education and William M. Keck Foundation Faculty Scholar in the Department of Bioengineering at University of Stanford, California, USA. Her research programme develops foundational tools that drive transformative advances in our ability to engineer biology. For example, her group has led the development of a novel class of biological input/output (I/O) devices, thereby fundamentally changing how we interact with and programme biology. Her group uses these tools to drive transformative advances in diverse areas such as cellular therapies, natural product biosynthesis and drug discovery. She is an inventor on more than ten patents and her research programme has been honoured with numerous awards, including the US National Institutes of Health (NIH) Director's Pioneer Award, the World Technology Network (WTN) Award in Biotechnology and TR35 Award.

Christopher A. Voigt has been a professor in the Department of Biological Engineering at the Massachusetts Institute of Technology (MIT), Cambridge, Massachusetts, USA, since 2010, where he co-directs the Center for Synthetic Biology and founded the MIT-Broad Foundry. He serves as the Editor-in-Chief of the American Chemical Society journal, *ACS Synthetic Biology*. Before MIT, he was at a faculty in the Pharmaceutical Chemistry Department at the University of California, San Francisco, USA.

Ron Weiss is a professor in the Department of Biological Engineering and in the Department of Electrical Engineering and Computer Science at the Massachusetts Institute of Technology (MIT), Cambridge, USA. He founded and currently serves as the Director of the Synthetic Biology Center at MIT. He received his Ph.D. from MIT in 2001, and held a faculty appointment at Princeton University, New Jersey, USA, between 2001 and 2009. His research helped to pioneer the field of synthetic biology through the computational design, construction and experimental testing of several fundamental synthetic gene circuits that carry out digital logic, analogue control and intercellular communication. More recently, the Weiss laboratory has also focused on mammalian synthetic biology, with a combined emphasis on the engineering foundation and several therapeutic application areas, including programmed tissue engineering, diabetes and cancer therapy.

*Listed in alphabetical order

Glossary

CRISPR
(Clustered regularly interspaced short palindromic repeats). An adaptive immune system that is found in bacteria and archaea, which is based on an RNA-guided nuclease (Cas9). Components of the CRISPR system are being repurposed to provide powerful, flexible and precise genome engineering, and regulatory systems across diverse species.

Genetic switches
Natural or synthetic systems for regulating gene expression in response to one or more external or internal signals. The output of genetic switches is often a complex logical function of input signals that in many cases can provide a persistent response to transient inputs or other capabilities.

Genomically recoded organisms
(GROs). Changing every instance in a genome of one or more of the 64 codons in the genetic code for higher safety and productivity.

Multiplex-automated genome engineering
(MAGE). Efficient genome editing that is capable of making dozens of changes per genome and billions of genomes by inserting short (90 bases long) single-stranded DNA into the cellular replication fork with one or more DNA changes.

Optogenetics
A technique to control and perturb cellular behaviour using light and genetically encoded light-sensitive proteins. It has been extensively used to precisely control neuronal activity spatially and temporally through light.

Oscillator
Produces oscillations that underlie diverse biological behaviours from neurobiology to multicellular development. Synthetic biology has shown that remarkably simple circuit designs can produce clock-like oscillations of protein levels in individual living cells.

Q Some applications of synthetic biology have steered development of new clinical therapies and of biotechnology for the production of drugs and fuels. What advantages have such approaches brought, and what do you foresee for the future of such applications?

R.W. Synthetic biology has certainly revolutionized the field of metabolic engineering, enabling the production of drugs and other small biomolecules inspired by natural products. With respect to mammalian synthetic biology in particular, as I mentioned above, despite the great recent progress in the field, very few applications have actually come to fruition so far. However, this is probably in large part owing to the field being so new. I see largely two areas to which mammalian synthetic biology will contribute applications in the future. The first is the use of numerous recently developed genome engineering tools to create next-generation programmed mammalian cell lines for the production of more tailored advanced biologics. Progress in this area may benefit from a closer connection between the mammalian synthetic biology community and the biopharmaceutical industry. The second is the creation of synthetic gene circuit therapies that match the complexity of biological systems. Systems biology is helping us to decipher the complexity of living organisms by unravelling regulatory networks and crosstalk between these networks. It has also taught us that biological diseases themselves, such as cancer, or metabolic, immunological, neurological or psychiatric disorders, are equally sophisticated and complex. I believe that such complex diseases will be best treated by modulating our bodies with correspondingly sophisticated synthetic gene circuit encoded in therapeutic agents. With its incredible pace of research, mammalian synthetic biology will soon be able to find flagship applications that will win over the support and trust of the general public.

G.M.C. Examples include the efficient bioproduction of the anti-malarial drug artemisinin by Amyris and Sanofi and the 'green-chemistry' replacement of petrochemicals[27] by LS9, USA, and Joule Unlimited, USA. In the future, we will see increased use of synthetic biology for therapeutics: moving from random transgenic insertions to precise Cas9-based genome editing[28]; from antibiotic carpet-bombing to skin and gut microbial therapies; from blunt brain ablations and drugs to optogenetics and other bio-nano neurotherapies. Bio-nano-materials are atomically precise and more than a million times more energy efficient than current electronic circuits for handling information.

M.B.E. The work in all of these areas is extremely exciting. Although we have been successful in engineering microorganisms to produce important products, these platforms are designed to work under well-controlled laboratory conditions. One key challenge is figuring out how to engineer cells that can act as programmable devices and function safely in complex natural environments, including the human body. Recent cell-based therapy results suggest the potential power of such approaches. However, we need the ability to routinely synthesize multi-gene circuits, integrate them precisely in cells and control their behaviour quantitatively. Right now this design cycle remains tediously slow, and this dramatically limits the diversity of designs and ideas that we can explore. Having said that, I think that a lot of these problems are solvable. Just imagine what will happen when researchers can routinely control and read out systems of multiple interacting genes and proteins in living cells and organisms — effectively changing the unit of routine genetic engineering from the gene to the gene circuit!

C.D.S. Synthetic biology is bringing new tools and approaches to applications in clinical therapies and biotechnology. For the production of drugs and fuels, synthetic biology is both advancing the complexity of biosynthetic processes that can be engineered and increasing the diversity of molecules that can be manufactured through biological processes. Harnessing the full manufacturing capacity of biology is a big future opportunity, providing us access to more complex scaffolds and materials. Synthetic biology tools also bring the potential to develop safer and more effective clinical therapies, by enabling control over the delivery and dosing of therapeutic activities in a dynamic temporal and spatial manner within a patient. Current efforts have focused on cell-based platforms such as immunotherapies and probiotics[29]. As the field progresses to tackle spatiotemporal programming and pattern formation, long-term applications of synthetic biology in the clinic should extend to tissue engineering and regenerative medicine.

C.A.V. In the short term, the tools of synthetic biology can be directly applied to exploit natural products, including pharmaceuticals, human performance enhancers, industrial chemicals, energetic materials and agricultural products (for example, insecticides and pesticides). The potential of accessing the natural diversity that is already present in large strain banks and the sequence databases is extraordinary[30]. The DNA sequence information is available, and bioinformatics can predict gene clusters that encode the machinery for producing high value chemicals. DNA synthesis enables the conversion of the sequence information back to physical DNA. However, the host organism is often long lost, cannot be manipulated or the genes are 'silent'. Overcoming this requires synthetic regulation and tools from synthetic biology. We are at the early phase of a gold rush of mining the databases and strain banks for valuable products. There will also be deep enzyme mining (that is, the high-throughput identification, printing and screening of genes from databases) to diversify the chemical structures. This may lead to 'the dream' of having enzymes that can be as flexible as synthetic chemistry in building arbitrary chemical structures.

The use of cells as therapeutics offers the advantage of being able to harness the sensing and signal processing capabilities of the cell, as well as the capability to synthesize multiple chemical and protein effectors at a site of interest.

Q *Technical advances have made it possible to write entire synthetic genomes. However, the possibility of creating synthetic forms of life has raised public concerns about the potential development of infectious agents or effects on biodiversity. What challenges lie ahead for this field, and what are the key ethical and regulatory issues that must be addressed?*

R.W. It is interesting to consider entirely new forms of life — and these may eventually be possible. However, the majority of synthetic biology projects are focused on improving existing strains by adding new functionality to bacterial, yeast or mammalian cells that are useful to the biotechnology industry. We seek to increase the diversity and application of standardized, well-characterized cell lines through forward genetic engineering. In mammalian synthetic biology, therapies and treatments will require viruses that are safe but effective at targeted delivery of DNA circuits before any regulatory approval. We have seen promising results with delivery of RNA synthetic circuits as a safer alternative. In addition, programmable 'organs-on-a-chip' may provide a more humane alternative to animal models for drug development and diagnosis. Like other responsible scientific fields of endeavour, synthetic biology has an ingrained culture of seeking to reduce risks with existing technological solutions, while seeking to predict and avoid problems with proposed new solutions.

G.M.C. Examples of synthetic genomes include interleukin-4 (IL-4)-ectromelia-pox virus, human endogenous retrovirus (HERV) and resurrected 1918 influenza[31]. The issues that are raised now for synthetic biology date back to the dawn of recombinant DNA when Paul Berg and Rudy Jaenisch inserted SV40 tumour virus into bacteria and mice in 1973. For decades, molecular biologists focused on single genes with only rudimentary skills in complex biological systems. Synthetic biology is now positioned to embrace systems design and safety engineering with proactive, community-based, scenario planning for ecosystems and physiological nuances (US Environmental Protection Agency (EPA) and US Food and Drug Administration (FDA)). We can finally construct genomically recoded organisms (GROs)[32,33] that cannot exchange functions with natural genomes, and at the other end of the spectrum, genes that intentionally spread to control parasites, disease vectors and invasive species (for example, malaria, mosquitoes, kudzu and carp).

M.B.E. There is a lot we do not yet know about what will or will not become possible with the technologies of synthetic biology. I worry in particular about the potential for misunderstanding, sometimes increased by a misleading hype. Great efforts have been made to improve the information gap, for example by the Sloan Foundation, USA, and the US National Academy of Sciences. Ultimately, we scientists must collectively 'keep it real,' focus on the most critical risks seriously and thoughtfully in a way that is grounded in reality, engage with the larger community and make the most of the unique opportunity we now have for fundamental discovery and understanding.

C.D.S. Advances in the fabrication of genetic systems (through DNA synthesis technologies) have far outpaced advances in the design of these systems. While we can build synthetic genomes, we are nowhere near being able to design novel genome-scale genetic programmes and thus completely synthetic life forms. However, ethical and regulatory concerns still apply to engineered organisms that incorporate some synthetic genetic information in addition to a native genome. The ethical and regulatory concerns depend on the potential uses and applications. For example, if the organism is to be used outside of a contained environment (such as an enclosed bioreactor), then potential effects on the environment and existing ecosystems should be addressed, as well as related issues such as evolution, adaptation, containment and removal. Part of the challenge for the field is being able to design in accordance with quantitative design, performance specifications and tolerance.

C.A.V. The new applications promised by the field require different approaches for the categorization of risk and how the products are regulated. For example, the current regulatory structures of the FDA and US Department of Agriculture (USDA) are not conducive for evaluating the impact of highly engineered organisms as therapeutics into the human body or in a field, respectively. The field has taken a proactive approach to addressing these issues and it is a very active area of discussion and research.

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Competing interests statement
The authors declare no competing interests.

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