

Synthetic Biology: Approaches and Applications of Engineered Biology

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Synthetic biology is expected to change how we understand and engineer biological systems. Lying at the intersection of molecular biology, physics, and engineering, the applications of this exploding field will both draw from and add to many existing disciplines. In this perspective, the recent advances in synthetic biology towards the design of complex, artificial biological systems are discussed.

THE RISE OF SYNTHETIC BIOLOGY

Several remarkable hurdles in the life sciences have been cleared during the last half of the 20th century, from the discovery of the structure of DNA in 1959, to the deciphering of the genetic code, the development of recombinant DNA techniques, and the mapping of the human genome. Scientists have routinely tinkered with genes for the last 30 years, even inserting a cold-water fish gene into wheat to improve weather resistance; thus, synthetic biology is by no means a new science. Synthetic biology is a means to harness the biosynthetic machinery of organisms on the level of an entire genome to make organisms do things in ways nature has never done before.

Synthetic biology, despite its long history, is still in the early stages of development. The first international conference devoted to the field was held at M.I.T in June 2004. The leaders sought to bring together “researchers who are working to design and build biological parts, devices, and integrated biological systems; develop technologies that enable such work; and place this scientific and engineering research within its current and future social context” (Synthetic Biology 101, 2004). The field is growing quickly, as evidenced by the rapidly increasing number of genetic discoveries, the exploding number of research teams exploring the field, and the funding from government and industrial sources.

Akin to the descriptive-to-synthetic transformation of chemistry in the 1900s, biological synthesis forces scientists to pursue a “man-on-the-moon” goal that demands they discard erroneous theories and compels scientists to solve problems not encountered in observation. Data contradicting a theory can sometimes be excluded for the sake of argument, but doing the same while building a lunar shuttle would be disastrous. Synthetic biology comes at an important time; by creating analogous “man-on-the-moon” engineering goals in the form of

synthetic bioorganisms, it is similarly driving scientists towards a deeper level of understanding of biology.

APPLICATIONS OF ENGINEERED ORGANISMS

It is expected that advances in synthetic biology will create important advances in applications too diverse and numerous to imagine. Applications of bioengineered microorganisms include detecting toxins in air and water, breaking down pollutants and dangerous chemicals, producing pharmaceuticals, repairing defective genes, targeting tumors, and more. In 2008, genomics pioneer Dr. Craig Venter secured a \$600 billion grant from ExxonMobil to develop hydrocarbon-producing microorganisms as an alternative to crude oil (Borrell 2009).

Scientists are engineering microbes to perform complex multi-step syntheses of natural products. Jay Keasling, a professor at the University of California, Berkeley, recently demonstrated genetically engineered yeast cells (*Saccharomyces cerevisiae*) that manufacture the immediate precursor of artemisinin, a malarial drug widely used in developing countries (Ro et al, 2006). Before, this compound was chemically extracted from the sweet wormwood herb. Since the extraction is expensive and the wormwood herb is prone to drought, the availability of the drug is reduced in poorer countries. Once the engineered yeast cells were fine-tuned to produce high amounts of the artemisinin precursor, the compound was made quickly and cheaply. This same method could be applied to the mass-production of other drugs currently limited by natural sources, such as anti-HIV drug prostratin and anti-cancer drug taxol (Tucker & Zilinskas, 2006).

The most far-sighted effort in synthetic biology is the drive towards standardized biological parts and circuits. Just as other engineering disciplines rely on parts that are well-described and universally used — like transistors

and resistors — biology needs a tool box of standardized genetic parts with characterized performance. The Registry of Standard Biological Parts comprises many short pieces of DNA that encode multiple functional genetic elements called “BioBricks” (Registry of Standard Biological Parts). In 2008, the Registry contained over 2000 basic parts comprised of sensors, input/output devices, regulatory operators, and composite parts of varying complexity (Greenwald, 2005). The M.I.T. group made the registry free and public (<http://parts.mit.edu/>) and has invited researchers to contribute to the growing library.

Some genetic parts code for a promoter gene that begins the transcription of DNA into mRNA, a repressor that codes a protein that blocks the transcription of another gene, a reporter gene that encodes a readout signal, a terminator sequence that halts RNA transcription, and a ribosome binding site that begins protein synthesis. The goal is to develop a discipline-wide standard and source for creating, testing, and combining BioBricks into increasingly complicated functions while reducing unintended interactions.

To date, BioBricks have been assembled into a few simple genetic circuits (McMillen & Collins, 2004). One creates a film of bacteria that is sensitive to light so it can capture images (Levskaya et al). Another operates as a type of battery, producing a weak electric current. BioBricks have been combined into logic gate devices that execute Boolean operations, such as AND, NOT, OR, NAND, and NOR. An AND operator creates an output signal when it gets a biochemical signal from both inputs; an OR operator generates an output if it gets a signal from either input; and a NOT operator changes a weak signal into a strong one, and vice versa. This would allow cells to be small programmable machines whose operations can be controlled through light or various chemical signals (Atkinson et al, 2003).

Despite the enormous progress seen in the last five years and some highly publicized and heavily funded feats, the systematic and widespread design of biological systems remains a formidable task.

CURRENT CHALLENGES

Standardization

Standards underlie engineering disciplines: measurements, gasoline formulation, machining parts, and so on. Certain biotechnology standards have taken hold in cases such as protein crystallography and enzyme nomenclature, but engineered biology lacks a universal standard for most classes of functions and system characterization. One research group’s genetic toggle switch may work in a certain strain of *Escherichia coli* in a certain type of broth, while another’s oscillatory function may work in a different strain when cells are grown in supplemented minimal media (Endy, 2005). It is unclear whether the two biological functions can be combined despite the different operating parameters. The Registry of Standard Biological Parts and new Biofab facilities have recently emerged to begin addressing this issue, and a growing consensus is emerging on the best way to reliably build and describe the function of new genetic components.

Abstraction

Drawing again from other engineering disciplines, and specifically from the semiconductor industry, synthetic biology must manage the enormous complexity of natural biological systems by abstraction hierarchies. After all, writing “code” with DNA letters is comparable to creating operating systems by inputting 1’s and 0’s. Levels could be defined as DNA (genetic material), Parts (basic functions, such as a terminating sequence for an action), Devices (combinations of parts), and Systems (combinations of devices). Scientists should be able to work independently at each hierarchy level, so that

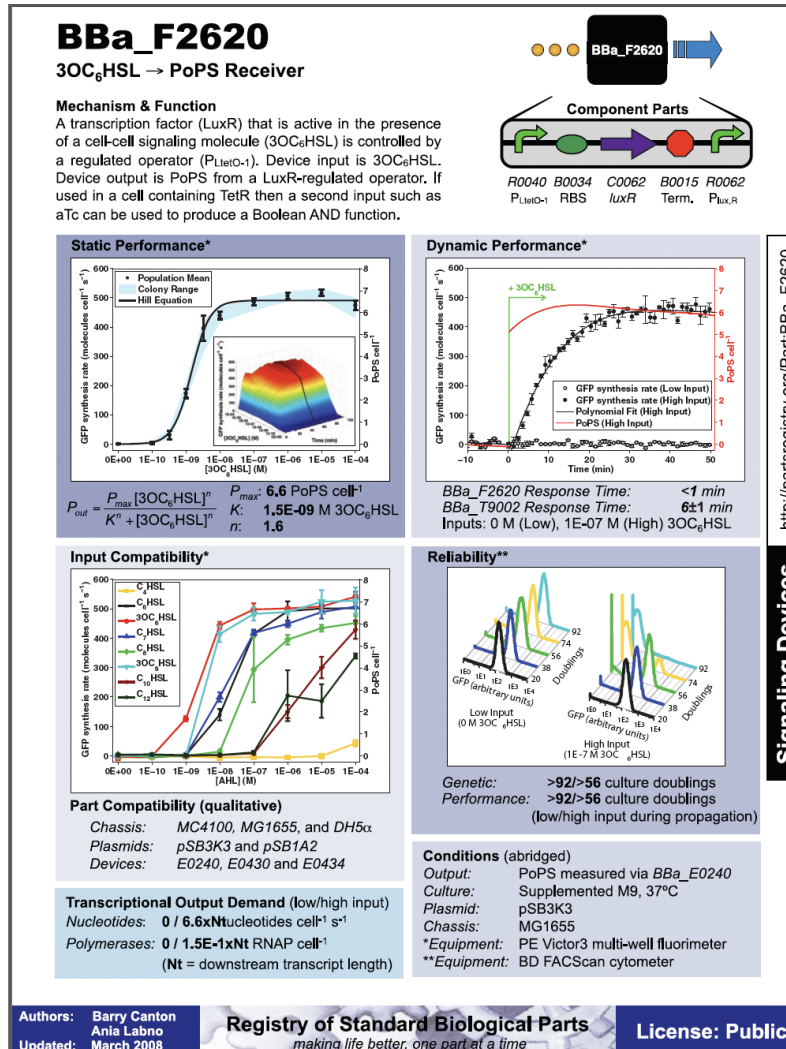


Figure 1. The Registry of Standard Biological Parts. This registry offers free access to basic biological functions that are used to create new biological systems. Pictured is a standard data sheet on a gene regulating transcription, with normal performance and compatibility measurements, plus an extra biological concern: system performance during evolution and cell reproduction. The registry is part of a conscious effort to standardize gene parts in the hopes of creating interchangeable components with well-characterized functions when implanted in cells. The project is open source; anybody can freely use and add information to the Registry.

device-level workers would not need to know anything about phosphoramidite chemistry, or genetic oscillators, etc. (Canton, 2005).

Engineered Simplicity and Evolution

The rapid progress made by mechanical engineering in this century was made possible by creating easily understandable machines. Engineered simplicity is helpful not only for repairs but for future upgrades and redesigns. While a modern automobile may seem complex, the level of complexity pales in comparison to a living cell, which has far more interconnected pathways and interactions. Cells evolved in response to a multitude of evolutionary pressures and mechanisms were developed to be efficient, not necessarily easy to understand (Alon, 2003). A related problem is that other engineered systems don't evolve. Organisms such as *E. coli* reproduce and have genetic mutations within hours. While this offers possibilities to the biological engineer (for instance, human-directed evolution for fine-tuning organism behavior), it also increases the complexity of designing and predicting the function of these new genetic systems (Hasteltine, 2007).

RISKS ASSOCIATED WITH BIOLOGICAL ENGINEERING

Accidental Release

Researchers first raised concerns at the Asilomar Conference in California during the summer of 1975 and concluded that current genetic experiments carried minimal risk. The past 30 years of experience in genetically-manipulated crops demonstrated that engineered organisms are less fit than their wild counterparts, and they either die or eject their new genes without constant assistance from humans. However, researchers concluded that the abilities to replicate and evolve required special precautions. It was recommended that all researchers work with bacterial strains that are specially

designed to be metabolically deficient so they cannot survive in the wild. Still, some have suggested that an incomplete understanding and emergent properties arising from unforeseen interactions between new genes could be problematic. Such dangers have given rise to fears of a dystopian takeover by super-rugged plants that overwhelm local ecosystems.

Bioterrorism

Research in synthetic biology may generate "dual-use" findings that could enable bioterrorists to develop new biological warfare tools that are easier to obtain and far more lethal than today's military bioweapons. The most commonly cited example of this is the resurrection synthesis of the 1918 pandemic influenza strain by CDC researchers (Tumpey et al, 2005) and the possibility of recreating smallpox from easily-ordered DNA (Venter, 2005). There has been a growing consensus that not all sequences should be made publicly available, but the fact remains that such powerful recombinant DNA technologies could be used for harm.

Attempts to limit access to the DNA synthesis technology would be counterproductive, and a sensible approach might include some selective regulation while allowing research to continue. Now, as SARS, bird influenza, and other infectious disease emerge, these recombinant DNA techniques enhance our ability to manage this threat today compared to what was possible just 30 years ago. The revolution in synthetic biology is nothing less than a push in all fronts of biology, whether that impacts environmental cleanup, chemical synthesis using bacteria, or human health.

CONCLUSION

At present, synthetic biology's myriad implications can be glimpsed only dimly. The field clearly has the potential to bring about epochal changes in medicine, agri-

culture, industry, and politics. Some critics consider the idea of creating artificial organisms in the laboratory to be an example of scientific hubris, evocative of *Faust* or *Dr. Frankenstein*. However, the move from understanding biology to designing it for our requirements has always been a part of the biological enterprise and used to produce chemicals and biopharmaceuticals. Synthetic biology represents an ambitious new paradigm for

building new biosystems with rapidly increasing complexity in versatility and applications. These tools for engineering biology are being developed and distributed, and a societal framework is needed to help not only create a global community that celebrates biology but also to lead the enormously constructive invention of biological technologies.

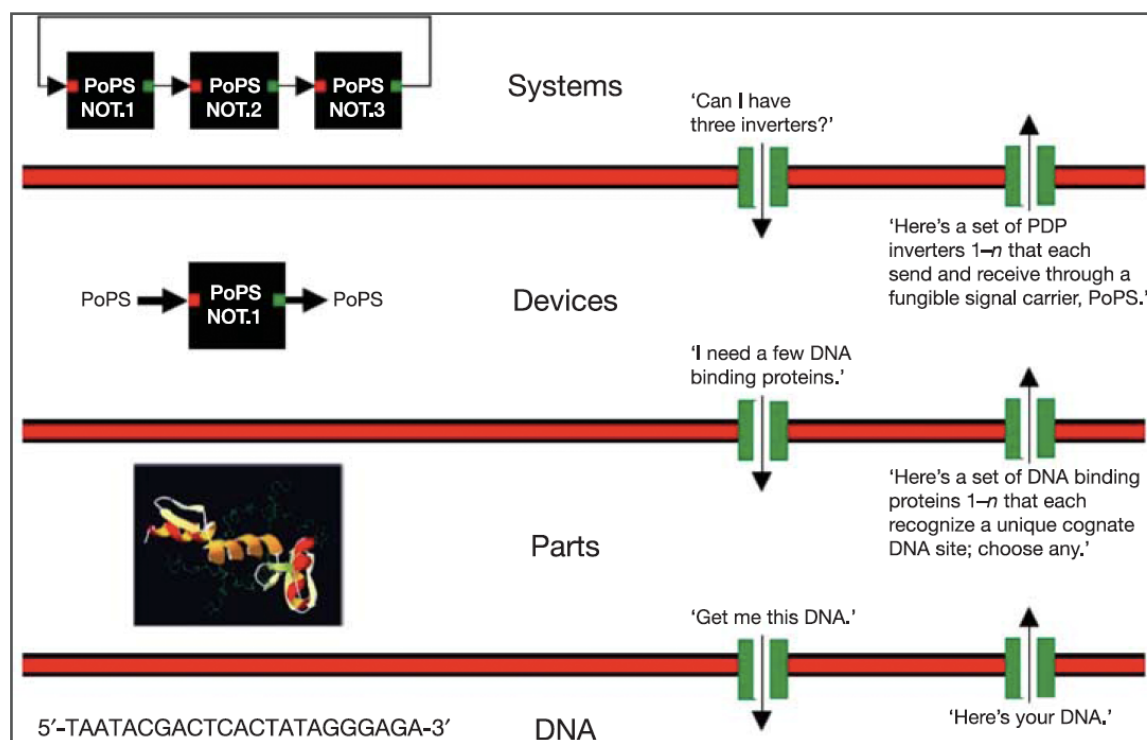


Figure 2. Abstraction Hierarchy. Abstraction levels are important for managing complexity and are used extensively in engineering disciplines. As biological parts and functions become increasingly complex, writing 'code' with individual nucleotides is rapidly becoming more difficult. Currently, researchers spend considerable time learning the intricacies of every step of the process, and stratification would allow for specialization and faster development. Ideally, individuals can work on individual levels, one can focus on part design without worrying about how genetic oscillators work, while others could string together parts to construct whole systems for possible biosensor applications. Image originally made by Drew Endy.

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