

FIVE HARD TRUTHS FOR SYNTHETIC BIOLOGY

Can engineering approaches tame the complexity of living systems? **Roberta Kwok** explores five challenges for the field and how they might be resolved.

To read some accounts of synthetic biology, the ability to manipulate life seems restricted only by the imagination. Researchers might soon program cells to produce vast quantities of biofuel from renewable sources, or to sense the presence of toxins, or to release precise quantities of insulin as a body needs it — all visions inspired by the idea that biologists can extend genetic engineering to be more like the engineering of any hardware. The formula: characterize the genetic sequences that perform needed functions, the ‘parts’, combine the parts into devices to achieve more complex functions, then insert the devices into cells. As all life is based on roughly the same genetic code, synthetic biology could provide a toolbox of reusable genetic components — biological versions of transistors and switches — to be plugged into circuits at will.

Such analogies don’t capture the daunting knowledge gap when it comes to how life works, however. “There are very few molecular operations that you understand in the way that you understand a wrench or a screwdriver or a transistor,” says Rob Carlson, a principal at the engineering, consulting and design company Biodesic in Seattle, Washington. And the difficulties multiply as the networks get larger, limiting the ability to design more complex systems. A 2009 review¹ showed that although the number of published synthetic biological circuits has risen over the past few years, the complexity of those circuits — or the number of regulatory parts they use — has begun to flatten out.

Challenges loom at every step in the process, from the characterization of parts to the design and construction of systems. “There’s a lot of biology that gets in the way of the engineering,” says Christina Agapakis, a graduate student doing synthetic-biology research at Harvard Medical School in Boston, Massa-

chusetts. But difficult biology is not enough to deter the field’s practitioners, who are already addressing the five key challenges.



Many of the parts are undefined

A biological part can be anything from a DNA sequence that encodes a specific protein to a promoter, a sequence that facilitates the expression of a gene. The problem is that many parts have not been characterized well. They haven’t always been tested to show what they do, and even when they have, their performance can change with different cell types or under different laboratory conditions.

The Registry of Standard Biological Parts, which is housed at the Massachusetts Institute of Technology in Cambridge, for example, has more than 5,000 parts available to order, but does not guarantee their quality, says director Randy Rettberg.

Most have been sent in by undergraduates participating in the International Genetically Engineered Machine (iGEM) competition, an annual event that started in 2004. In it, students use parts from a ‘kit’ or develop new ones to design a synthetic biological system. But many competitors do not have the time to characterize the parts thoroughly.

While trying to optimize lactose fermentation in microbes, an iGEM team from the University of Pavia in Italy tested several promoters from the registry by placing them in *Escherichia coli*, a standard laboratory

bacterium. Most of the promoters tested by the team worked, but some had little documentation, and one showed no activity. About 1,500 registry parts have been confirmed as working by someone other than the person who deposited them and 50 have reportedly failed, says Rettberg. ‘Issues’ have been reported for roughly another 200 parts, and it is unclear how many of the remaining parts have been tested.

The registry has been stepping up efforts to improve the quality by curating the collection, encouraging contributors to include documentation on part function and performance, and sequencing the DNA of samples of parts to make sure they match their descriptions, says Rettberg. Meanwhile, synthetic biologists Adam Arkin and Jay Keasling at the University of California, Berkeley, and Drew Endy at Stanford University in Stanford, California are launching a new effort, tentatively called BIOFAB, to professionally develop and characterize new and existing parts. Late last year, the team was awarded US\$1.4 million by the National Science Foundation and is hiring staff, says Arkin. Endy, moreover, has proposed methods to reduce some of the variability in measurements from different labs. By measuring promoter activity relative to a reference promoter, rather than looking at absolute activity, Endy’s team found that it could eliminate half the variation arising from experimental conditions and instruments².

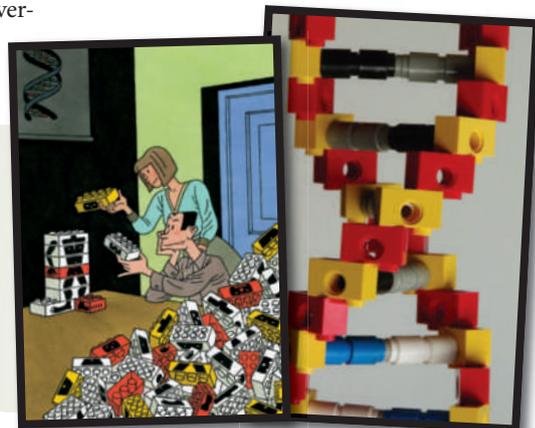
“We are still like the Wright Brothers, putting pieces of wood and paper together.”

— Luis Serrano

THE HYPE

The ‘parts’ work like Lego

Images such as these run in magazines *The New Yorker* (left) and *Wired* portray synthetic biology as simple design and construction. The truth is that many of the parts are not well characterized, or work unpredictably in different configurations and conditions.



Measurements are tricky to standardize, however. In mammalian cells, for example, genes introduced into a cell integrate unpredictably into the cell's genome, and neighbouring regions often affect expression, says Martin Fussenegger, a synthetic biologist at the Swiss Federal Institute of Technology (ETH) Zurich. "This is the type of complexity that is very difficult to capture by standardized characterization," he says.

SLIM FILMS



The circuitry is unpredictable

Even if the function of each part is known, the parts may not work as expected when put together, says Keasling. Synthetic biologists are often caught in a laborious process of trial-and-error, unlike the more predictable design procedures found in other modern engineering disciplines.

"We are still like the Wright Brothers, putting pieces of wood and paper together," says Luis Serrano, a systems biologist at the Centre for Genomic Regulation in Barcelona, Spain. "You fly one thing and it crashes. You try another thing and maybe it flies a bit better."

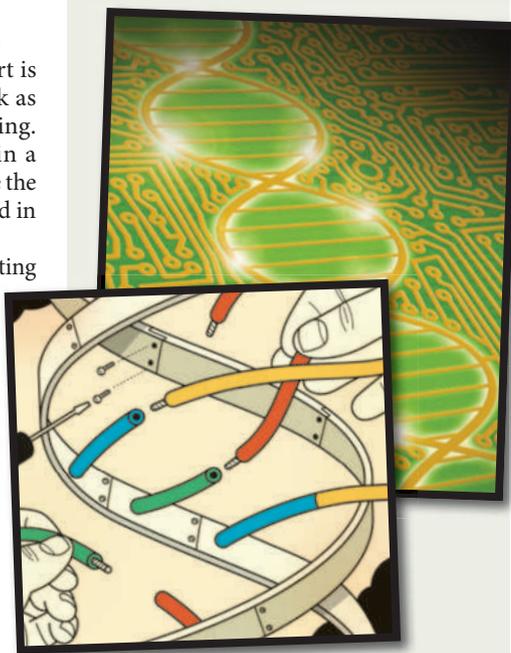
Bioengineer Jim Collins and his colleagues at Boston University in Massachusetts crashed a lot when implementing a system called a toggle switch in yeast. His lab built one roughly ten years ago in *E. coli*³: the team wanted to make cells express one gene — call it gene A — and then prompt them with a chemical signal to turn off A and express another gene, B. But the cells refused to express B continuously; they always shifted back to expressing A. The problem, says Collins, was that the promoters controlling the two genes were not balanced, so A overpowered B. It took about three years of tweaking the system to make it work, he says.

Computer modelling could help reduce this guesswork. In a 2009 study⁴, Collins and his colleagues created several slightly different versions of two promoters. They used one version of each to create a genetic timer, a system that would cause cells to switch from expressing one gene to another after a certain lag time. They then tested the timer, fed the results back into a computational model and predicted how timers built from other versions would behave. Using such modelling techniques, researchers could optimize computationally rather than test every version of a network, says Collins.

But designs might not have to work perfectly: imperfect ones can be refined using a process called directed evolution, says Frances Arnold, a chemical engineer at the California Institute of Technology in Pasadena. Directed evolution involves mutating DNA sequences, screening their performance, selecting the best

THE HYPE Cells can simply be rewired

The magazines *Scientific American* (top) and *IEEE Spectrum* portrayed synthetic biology as being similar to microchip design or electrical wiring. Although computational modelling may help scientists to predict cell behaviour, the cell is a complex, variable, evolving operating system, very different from electronics.



candidates and repeating the process until the system is optimized. Arnold's lab, for instance, is using the technique to evolve enzymes involved in biofuel production.



The complexity is unwieldy

As circuits get larger, the process of constructing and testing them becomes more daunting. A system developed by Keasling's team⁵, which uses about a dozen genes to produce a precursor of the antimalarial compound artemisinin in microbes, is perhaps the field's most cited success story. Keasling estimates that it has taken roughly 150 person-years of work including uncovering genes involved in the pathway and developing or refining parts to control their expression. For example, the researchers had to test many part variants before they found a configuration that sufficiently increased production of an enzyme needed to consume a toxic intermediate molecule.

"People don't even think about tackling those projects because it takes too much time and money," says Reshma Shetty, co-founder of the start-up firm Ginkgo BioWorks in Boston, Massachusetts. To relieve similar bottlenecks, Ginkgo is developing an automated process

to combine genetic parts. The parts have pre-defined flanking sequences, dictated by a set of rules called the BioBrick standard, and can be assembled by robots.

At Berkeley, synthetic biologist J. Christopher Anderson and his colleagues are developing a system that lets bacteria do the work. Engineered *E. coli* cells, called 'assembler' cells, are being equipped with enzymes that can cut and stitch together DNA parts. Other *E. coli* cells, engineered to act as 'selection' cells, will sort out the completed products from the leftover parts. The team plans to use virus-like particles called phagemids to ferry the DNA from the assembler to the selection cells. Anderson says that the system could shorten the time needed for one BioBrick assembly stage from two days to three hours.



Many parts are incompatible

Once constructed and placed into cells, synthetic genetic circuits can have unintended effects on their host. Chris Voigt, a synthetic biologist at the University of California, San Francisco, ran into this problem while he was a postdoc at Berkeley in 2003. Voigt had assembled genetic parts, mainly from the bacterium *Bacillus subtilis*, into a switch system that was supposed to turn on expression of certain genes in response to a chemical stimulus. He wanted to study the system independently of *B. subtilis*' other genetic networks, so he put the circuit into *E. coli* — but it didn't work.

"You looked under the microscope and the cells were sick," says Voigt. "One day it would do one thing, and another day it would do another thing." He eventually saw in the literature that one of the circuit's parts dramatically disrupted *E. coli*'s natural gene expression. "There was nothing wrong with the design of the circuit," he says. "It was just that one part was not compatible."

Synthetic biologist Lingchong You at Duke University in Durham, North Carolina, and his colleagues found that even a simple circuit, comprising a foreign gene that promoted its own expression, could trigger complex behaviour in host cells⁶. When activated in *E. coli*, the circuit slowed down the cells' growth, which in turn slowed dilution of the gene's protein product. This led to a phenomenon called bistability: some cells expressed the gene, whereas others did not.

To lessen unexpected interactions, researchers are developing 'orthogonal' systems that operate independently of the cell's natural machinery. Synthetic biologist Jason Chin of the Medical Research Council Laboratory of Molecular Biology in Cambridge, UK, and his colleagues have created a protein-production

H. CAMPBELL

THE HYPE

A promise of unprecedented power

Nature has portrayed synthetic biologists as wielding the power to 'hack' life (right), and in its *Guide to Synthetic Biology*, the civil society organization ETC Group even likened their activity to playing God. But in reality, the field has yet to deliver much of practical use.



system in *E. coli* that is separate from the cell's built-in system⁷. To transcribe DNA into RNA, the team uses a polymerase enzyme that recognizes genes only if they have a specific promoter sequence that is not present in the cell's natural genes. Similarly, the system's orthogonal 'O-ribosomes', which translate RNA into protein, can read only 'O-mRNA' that contains a specific sequence, and O-mRNA is unreadable by natural ribosomes.

A parallel system gives biologists the freedom to tweak components without disrupting the machinery needed for the cell to survive, says Chin. For example, his team has stripped down the DNA sequence encoding part of the O-ribosome to speed up production. This allows the cell to boot up protein manufacture more quickly, he says.

Another solution is to physically isolate the synthetic network from the rest of the cell. Wendell Lim, a synthetic biologist at the University of California, San Francisco, is experimenting with the creation of membrane-bound compartments that would insulate the genetic circuits. Lim's team is working in yeast, but similar principles could be applied to bacterial cells, he says.

5 Variability crashes the system

Synthetic biologists must also ensure that circuits function reliably. Molecular activities inside cells are prone to random fluctuations, or noise. Variation in growth conditions can also affect behaviour. And over the long term, randomly arising genetic mutations can kill a circuit's function altogether.

Michael Elowitz, a synthetic biologist at the California Institute of Technology in Pasadena, observed the cell's capacity for randomness about ten years ago when his team built a genetic oscillator⁸. The system contained three

genes whose interactions caused the production of a fluorescent protein to go up and down, making cells blink on and off. However, not all cells responded the same way. Some were brighter, and some were dimmer; some blinked faster, others slower; and some cells skipped a cycle altogether.

Elowitz says that the differences might have arisen for multiple reasons. A cell can express genes in bursts rather than a steady stream. Cells also may contain varying amounts of mRNA and protein-production machinery, such as polymerase enzymes and ribosomes. Furthermore, the number of copies of the genetic circuit in a cell can fluctuate over time.

Jeff Hasty, a synthetic biologist at the University of California, San Diego, and his colleagues described an oscillator with more consistent behaviour⁹ in 2008. Using a different circuit design and microfluidic devices that allowed fine control of growth conditions, the team made nearly every monitored cell blink at the same rate — though not in sync. And in this issue of *Nature* (see page 326)¹⁰, Hasty's team reports the ability to synchronize the blinking by relying on cell-cell communication. But Hasty says that rather than trying to eliminate noise, researchers could use it to their advantage. He notes that in physics, noise can sometimes make a signal easier to detect. "I don't think you can beat it, so I think you ought to try to use it," says Hasty. For example, noise could allow some cells to respond differently to the environment from others, enabling the population to hedge its bets, says Elowitz.

Meanwhile, geneticist George Church at Harvard Medical School in Boston, Massachusetts, is exploring ways to make a

bacterial strain more stable. Church says that this might be achieved by introducing more accurate DNA-replication machinery, changing genome sites to make them less prone to mutation and putting extra copies of the genome into cells. Although stability may not be a serious issue for simple systems, it will become important as more components are assembled, he says.

Time to deliver?

Despite the challenges, synthetic biologists have made progress. Researchers have recently developed devices that allow *E. coli* to count events such as the number of times they have divided and to detect light and dark edges. And some systems have advanced from bacteria to more complex cells. The field is also gaining legitimacy, with a new synthetic-biology centre at Imperial College London and a programme at Harvard University's recently launched Wyss Institute for Biologically Inspired Engineering in Boston. The time has come for synthetic biologists to develop more real-world applications, says Fussenegger. "The field has had its hype phase," he says. "Now it needs to deliver."

Keasling's artemisinin precursor system is approaching commercial reality, with Paris-based pharmaceutical company Sanofi-Aventis aiming to have the product available at an industrial scale by 2012. And several companies are pursuing biofuel production via engineered microbes. But most applications will take time.

As the cost of DNA synthesis continues to drop and more people begin to tinker with biological parts, the field could progress faster, says Carlson. "It's a question of whether the complexity of biology yields to that kind of an effort."

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— Martin Fussenegger

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