

# Synthetic Biology

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If I were a microbe, my favourite novelist would be Mary Shelley. In her most famous novel, she terrifies people with a creature cobbled together from spare human parts. I like scaring people too with tales from my microscopic world. I know it is considered very bad form among human scientists to use that word, dare I say it, but where I come from, microscopic Ff..., 'Frankenbugs', are almost a banality. So far, nature and evolution have been the sole authors of the rapid succession mutants, but something very different is happening today.

Classical biology has traditionally tried to understand existing life. But in the last five years, a brand new science has emerged that turns the paradigm on its head. Synthetic biology, as this new branch of science is called, tries to create new life by cobbling together artificial microorganisms from a 'Lego set' of off-the-shelf genetic building bricks. Each brick has a pre-designed function and contributes to a new metabolic pathway.

Synthetic biology is as multi-disciplinary as a science can get. It brings

together biochemistry and genetic engineering, and surprisingly, finds inspiration in electronic engineering, as an article below explains. Pioneers in the field are as likely to be computer scientists, electronic engineers or physicists as they are to be biochemists. Traditional wet-bench biologists are oddly absent from the ranks.

'Genetic engineering' is a catch-all expression, but broadly it is a toolkit of methods for cutting and pasting genes together, analysing gene sequences and their functions with computers, and modelling how they behave at the level of a single cell. Synthetic biology is at the top of the genetic engineering hierarchy. It uses the tools of genetic engineering to create genetic building bricks endowed with a designer function. Those bricks can then be assembled into whole new artificial biological systems.

Every century brings about its own revolution. The 18<sup>th</sup> century brought us the humanist ideals of Voltaire and Rousseau, the 19<sup>th</sup> gave us the mechanised revolution, steam trains, ships, factories. Physics defined the 20<sup>th</sup> century,

yielding the secrets of the atom, electronics and computers. Making predictions is at best an educated guess, but one thing that seems clear is that the 21<sup>st</sup> century too will leave its mark, and biology appears poised on the cusp of momentous discoveries.

In the following set of articles we explore the principles behind synthetic biology, the expectations and fears it raises. The field holds out the promise of revolutionary impact, such as replacing the fossil fuel economy with clean hydrogen power, insulin-producing cells grafted directly into diabetics, 'sentinel' microbes actively patrolling our bodies and repairing tissues, or even blisteringly fast computers made from DNA strands.

But every new science also raises deep-rooted and healthy apprehensions about its dangers and unintended consequences. The race for patents could drive some research under wraps, whereas transparency and a genuine public dialogue could go a long way towards ensuring the best outcomes.

Tristan Farrow

# Engineering Meets Biology

Biology is full of fascinating marvels, performing complex processes at the nanoscale and with low energy demand. A new generation of engineers is now adding design and intention to biology by crafting newly built genetic parts into microorganisms and living cells. They promise a vast range of applications, from biomedicine to energy production, and the emergent field—synthetic biology—has the potential to bring about a major revolution in the technological landscape of the future.

The most popular aspiration of synthetic biology is the production of microbes with custom-made genomes that synthesize natural products of commercial interest. Engineered yeast cells that produce a highly effective anti-malarial compound known as artemisin, which is only found in small quantities in plants, are their current trophy. The challenge now is to broaden the range of products, the set of genetic instructions inserted into host microorganisms to code new biosynthetic pathways and fine-tune the efficiency of the processes. The principle could then be applied to the cheap production of biopharmaceutical compounds and more general industrial chemicals, or even pave the way to the development of new biomaterials with useful properties. One of the most exciting possibilities is the potentially positive impact on the environment by enabling sustainable energy production—engineered bacteria may well be turned into powerhouses of the future, producing biofuels or even filling the ranks of a green army designed to degrade toxic waste.

The radical approach of this new discipline is to build from scratch organisms from a biological Lego set. And what better way to start than rewriting the code of life? By adding artificial nucleotide bases to the existing four letter alphabet (A, T, C, G) scientists are creating an artificial genetic code with potentially interesting applications. To date this innovative idea has been exploited in healthcare and in the development of more sensitive diagnostics of infectious agents. But the possibilities are expanding as new types of DNA—called TNA and xDNA—are more stable and thus are attractive raw materials for laying out the genetic information to be delivered into microorganisms and living cells.

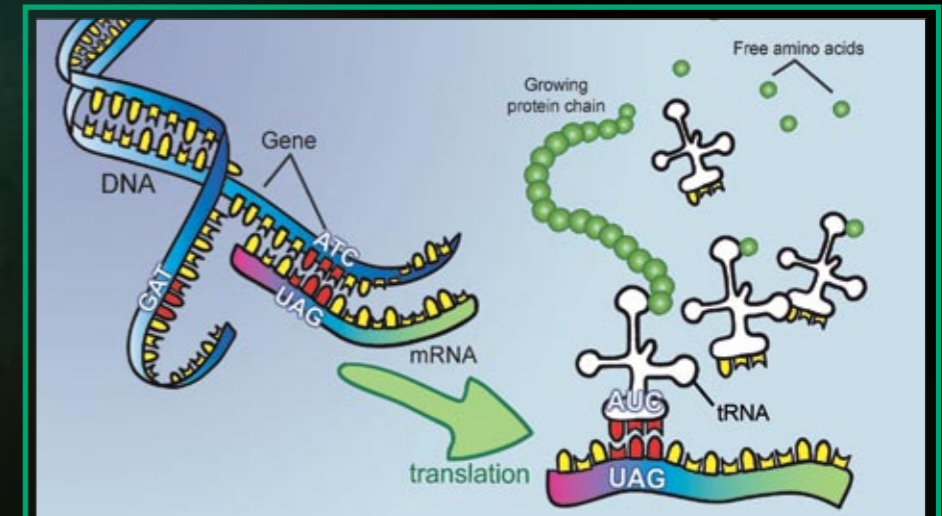
But scientists are not content with adding new characters to the alphabet but are also trying to translate forbidden words of the natural genetic code into new amino acids, the building blocks of proteins (see diagram). The aim is to increase the repertoire of proteins, for example enzymes, inside the cell and explore new useful properties that could for instance make them more effective as drugs.

And if you think that is pushing biology too far, be prepared for more tricks. The holy grail of the field is to endow engineered devices with life, to create machines in symbiosis with living cells. The idea is to make cells programmable through a device integrated within the cell at a molecular level. As complex as it may seem, the first steps taken towards this goal have been fairly simple and are inspired from electronic components. Scientists are creating DNA components—nicknamed BioBricks—that perform logic functions, such as turning a gene on or off, and that could be combined into more complex genetic circuits to produce a final output. The potential outcomes of applying this approach borrow from science fiction. One can imagine devices built

into human cells that perform 'surveillance' functions: a mechanism counting the number of times a cell divides and instructing it to self-destruct at a set number to prevent tumours. Another would sense damaged tissue and repair it. A new generation of drugs consisting of a synthetic molecular assembly could sense molecules associated with certain diseases and make diagnoses by activating the drug. Perhaps more ambitious still is the promise of achieving a more efficient programming of human stem cells to manufacture insulin-producing 'beta cells' to be transplanted into the liver of diabetic patients. But, the manipulation of biological systems is complicated and this challenge will keep scientists busy in decades to come.

Apart from satisfying the curiosity of tinkering with life, the new technologies based on DNA are also appealing because they are cheap. Moreover, industries based on them can be easily implemented locally, in the developing world, regulatory and patenting framework permitting.

Alexandra Lopes



Transcription is the process by which genetic information from DNA is transferred into RNA. The protein-encoding section of the DNA strand is unwound enzymatically, and copied to produce a complementary messenger RNA strand (mRNA). The information in the mRNA is made into a protein chain (a linear string of amino acids) via transfer RNA (tRNA). Codons in the DNA sequence are three bases long, and there are therefore 64 possible variations of C, T, A and G. In nature, 61 of these are used to produce amino acids, and the remaining three are non-coding (termed 'stop' codons). The work of Jason Chin (see next page) has made use of the TAG stop codon, and forced it to code for a novel amino acid, the first time this has been done.

## DNA COMPUTING?

Far from the living world, DNA is equally a contender for a surprising potential technological novelty: biocomputing. Information can be stored very efficiently in the DNA molecule (in quaternary code) and is naturally compact, or 'zipped', due to the tight spatial distribution of its constituent bases. It has been demonstrated over a decade ago that even a complex computational problem can be solved using DNA. At present a molecular computer is little more than a 'soup' of several strands of DNA in which different inputs interact to produce a certain output. Its most appealing features are an astonishing computing speed, several orders of magnitude faster than a supercomputer, and a natural capacity for parallel processing along several different DNA strands.

# Intelligent Design

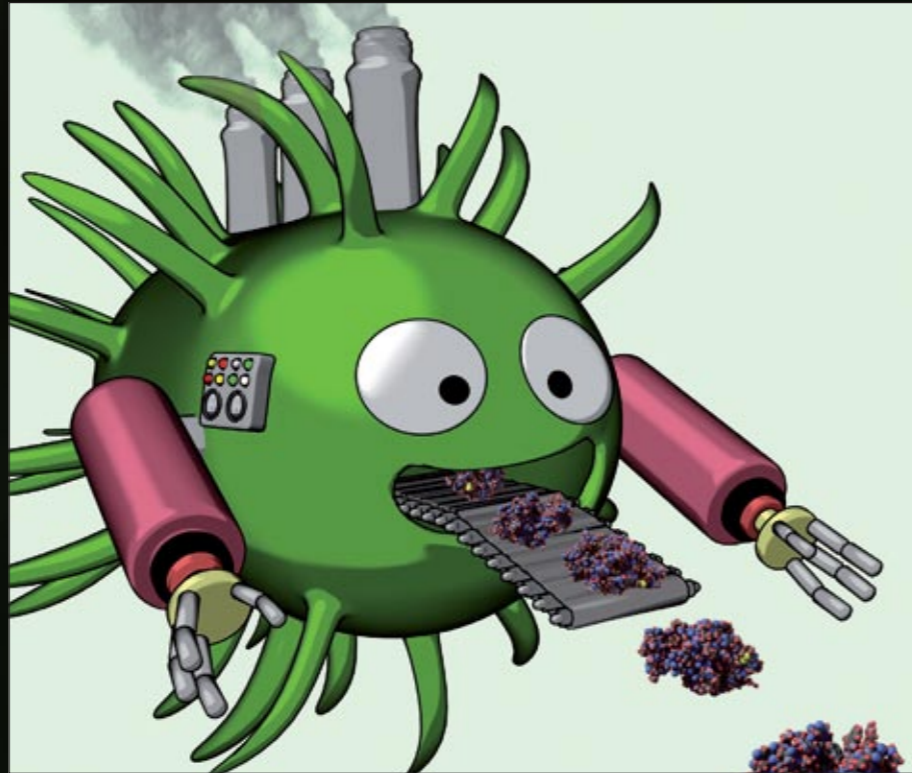
Current research in synthetic biology typically takes one of two approaches: assembling known natural systems into networks to perform specific tasks, and creating artificial modules to expand the range of organism function.

Researchers have, for example, constructed biological logic gates, which take two (or more) biochemical input signals and control the cellular response accordingly. Logic gates form the core of traditional computing, and so would be essential if we were to 'program' the biological networks we create—the first steps toward 'genetic computing'. The AND gate designed by Anderson, Voigt and Arkin at the University of California uses the fact that two inputs—the gene itself, and a 'nonsense suppressor'—are required in order for a cell to express the protein T7 RNA polymerase. Only when both inputs are supplied is the polymerase expressed which activates a chemical output from the cell. The logic gates could have medical applications, too—the same researchers have also designed a version which, when switched on, causes *E. coli* cells to express the invasive gene, and thereby acquire the ability to invade mammalian cells, which they cannot do if the gate is off.

Similarly, by randomly rearranging the genes for three different repressors of transcription, and five promoters these control, Stanislas Leibler and co-workers at Rockefeller University created 512 different networks, which exhibited logical properties including NAND, NOR and NOT IF—with the surprising observation that circuits with very similar connectivity can demonstrate diverse logical properties. This emphasises the fact that in biological networks, the underlying molecular interactions of the components, rather than just the overall setup, is fundamentally important.

“ Synthetic biologists have been attempting to create life from scratch ”

Excitingly, if the external stimuli are switched off, cell populations have been shown to 'remember' the state set by the last stimulus applied. James Collins at Boston University designed a 'genetic toggle switch' and used it to set the level of protein expression from a cell. When the molecules acting to initiate the toggle were



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removed, after subsequent generations, daughter cells continued to express the same levels of protein as set for the original cell. These developments allow the creation of cell networks having very high sensitivity to large numbers of external conditions and stimuli—important steps toward biosensor devices.

Increasingly, too, techniques of synthetic biology are being applied to enhance and extend the functioning of organisms. In nature, for example, only 20 different amino acids occur in the genetic codes of all life forms—limiting the varieties of proteins that can be assembled from them, possibly limiting the function of the entire organism. In November 2007, however, scientists led by Jason Chin, of the Laboratory for Molecular Biology at the University of Cambridge, succeeded in adding artificial amino acids into the genetic code of the yeast *Saccharomyces cerevisiae*. Inserted amino acids which are metal-binding or photoisomerisable, for example, could confer useful properties such as light-sensitivity on the cells, which could then be incorporated into a synthetic network. Not only this, but, theoretically, the technique used (using a synthetase from *E. coli* bacterium to insert the artificial amino acids) could also be directly applied to enhance function in higher organisms—possibly even humans.

Taking this enhancement to extremes, a number of synthetic biologists—notably, those led by Craig Venter, the owner of Celera Genomics, the private corporate challenger to the Human

Genome Project—have been attempting to create 'synthetic life', building a living organism from scratch.

“ The technique could enhance function in humans ”

Inspired by the fact that, in nature, cells can exchange genetic information—either by 'transduction', in which viruses act as chromosome-carrying intermediaries, or 'conjugation', when DNA is passed directly between cells in contact—Venter's team have performed a full 'genome transplant'. Donor cells of the *M. mycoides* bacterium are suspended in agarose blocks, immobilising the intact chromosomes inside the cell, but digesting the proteins, lipids, RNAs and other cellular components which are then siphoned off. The agarose is then melted away, leaving a solution of naked *M. mycoides* DNA, which is mixed with cells of *M. capricolum* and yeast transfer RNA. The resulting bacteria are incubated and grown into colonies which display all the characteristics (such as antibiotic resistance) of the donor organism.

Though the transplant mechanism is still poorly understood, and highly inefficient (about one success per 150,000 cells), if biologists can eventually initiate processes analogous to transduction

and conjugation, and transmute one species into another, they may ultimately be able to construct microorganisms specifically to perform given tasks in medicine, environmental regulation or energy generation—perhaps even by transplanting the artificial, laboratory-synthesised chromosomes which the Chin group suggests are possible.

Venter's team have also recently been working on developing a 'minimal organism'—stripping organisms down to their very basics to determine how few elements it actually takes to

construct a living cell. Transposons, or 'jumping genes'—sequences of DNA which move around to different positions on the genome within the cell—are inserted in 2500 different sites of the genomes of *M. genitalium* bacteria, creating 'knockout mutant' variants of the bacterium. By identifying which of these mutants survived, the scientists found 100 'dispensable' genes out of *M. genitalium*'s already small total of 470 (around 600,000 base pairs). Though such a minimal organism would only be able to survive under

ideal conditions—surrounded by all the necessary nutrients and free from external stresses, adapting to which might require some of the 'extraneous' genes—it gives an insight into exactly which functions (or phenotypes) are essential to life. For example, no genes coding for proteins in the cytoskeleton of *M. genitalium* were found to be dispensable, suggesting that the membrane is essential for the organism's survival no matter how good the going gets.

Michaela Freeland

## Building with Biology

Genetic engineering is often described as more of an art than an engineering discipline. The difficult and time consuming methods of transferring individual genes between organisms are unpredictable and often rely on luck.

But a revolution could well be under way with synthetic biology, and the standardisation of genetic parts. These standard genetic parts are collectively known as BioBricks DNA segments encoding particular functions. They are designed to be easily assembled, to communicate by a universal biochemical language, and to fit together like Lego bricks to form working systems.

In this respect synthetic biology is very much engineering, and uses these BioBricks to interact with living systems in an analogous way to an electrical engineer assembling components to form a working circuit.

In many ways, synthetic biology is analogous to electrical engineering, where circuits are assembled from individual parts that shunt back and forth inputs and outputs in the form of electrical pulses. In synthetic biology we have an organic circuit where the signals are chemicals.

Any synthetic biologist is able to search for parts in an online catalogue and pick BioBricks tailored to specification. The designer does not need to understand how each component was synthesised, just how it will perform when pieced

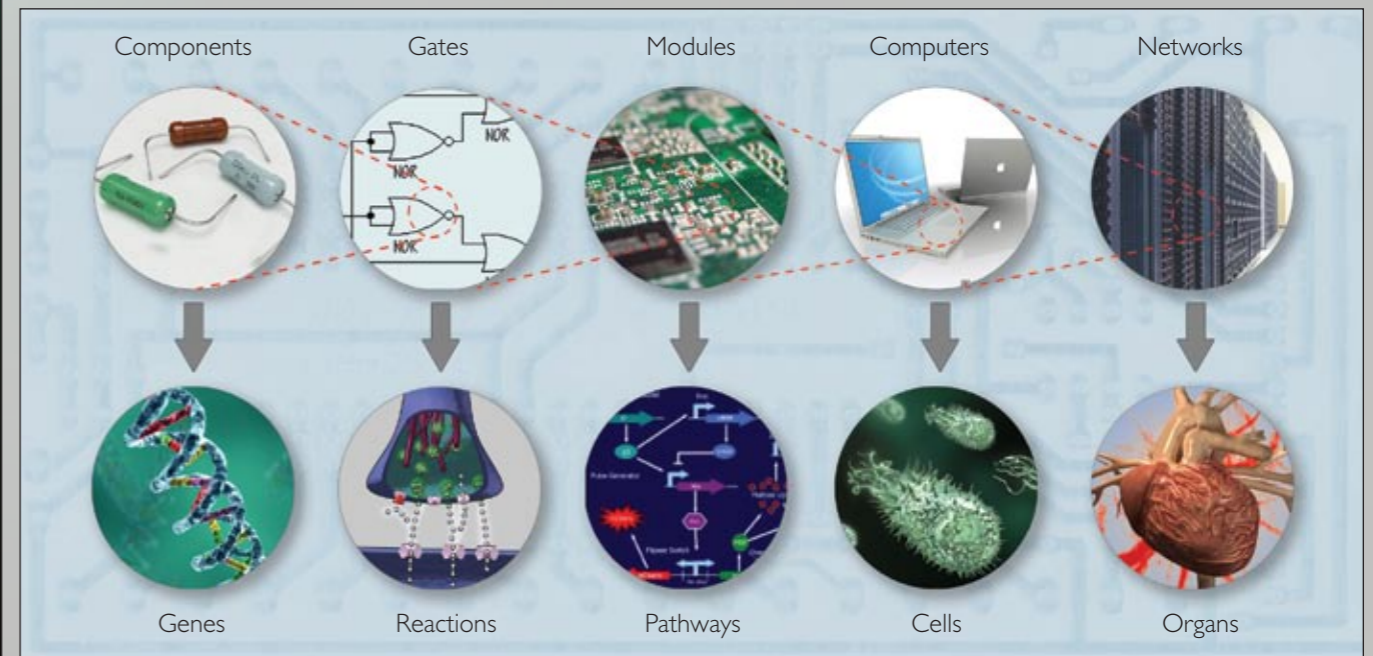
together. In both biological and electrical engineering disciplines there is a hierarchy of complexity with basic parts at the bottom and complex systems at the top. Each level requires specialist knowledge, but without necessarily needing any knowledge of the other levels.

Electrical engineers begin with a toolbox of components. These range from transistors to diodes and include resistors and LEDs. Each performs a certain task, which is of little use in isolation. However, when the components are linked together they form devices which can attend to more complex tasks, such as computations.

A synthetic biologist uses DNA, RNA and proteins as basic parts. Together they form devices able to complete discrete functions, such as a transformation of a chemical regulating a biochemical signal. As these parts are put together, the complexity increases until a system like an integrated circuit is formed.

The genetic part can be regarded as the software of the system, and the cellular host as the hardware. But that is where the analogy ends. The engineering system is able to work independently of its surroundings, but the biological system goes on to interact with the environment, moving, responding and reproducing.

Amy Chesterton



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