FROM THE LAB BENCH

"WHAT I CANNOT CREATE, I DO NOT UNDERSTAND." - RICHARD FEYNMAN

SYNTHETIC BIOLOGY OR SCIENCE FICTION?

MAKING LIFE BETTER, ONE PART AT A TIME – SYNTHETICBIOLOGY.ORG

Imagine if in your freshman biology course, instead of being given an outlandishly long list of cellular organelles and signaling pathway components to memorize (which made you wish you'd gone into the arts, not the sciences), your first assignment was to design a biological system from its fundamental pieces, like legos, to perform a particular function, for example synthesize a vaccine, complex enzyme, or new type of biofuel? All of a sudden you find yourself a goal-oriented engineer, creatively building biological circuitry and life forms from the bottom up, with a specific application in mind. Would you have avoided Cell Biology 502 as an elective if you had realized a career path in biology could lend itself to the more creative aspects of the scientific process?

Craig Venter describes synthetic biology as a process of 'digitizing' life, where the possibility for designing new life forms is limited only "by biological reality and our imagination."[1] Synthetic biologists look to forward engineer life based on novel metabolic pathways, letting nature perform the chemical synthesis of complex medicinal or industrial molecular species, the synthesis of which may otherwise be extremely difficult or nearly impossible using standard laboratory techniques. In the tune of a science fiction novel, bacteria with synthetic genomes are now 'working for us.' Synthetic biology is has become possible as the work of traditional biologists, piecing apart native biological systems to discover their components and understand their function, has led to a fundamental understanding of the basic 'pieces' that make life work. This understanding permits forward engineering of synthetic systems, which can help in the screening, discovery, design, and production of new drugs[2]. Cells engineered to help scientists screen for new anti-cancer drugs[3], discovery of small molecules that make tuberculosis-causing bacteria more vulnerable to treatment[4], designing bacteria for 'search and destroy' missions in the treatment of cancer cells[5], using bacteria as microscopic 'factories' of complex drug compounds currently in shortage, like antimalarial drugs[6]… these are the possibilities become realities by synthetic biology studies.

> 'People who think that evolution is just one gene changing at a time have missed much of biology'
> –CRAIG VENTER

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From the standpoint of engineering a new life form for commercial or medical applications, we might wish to know what genes, which encode downstream cell signaling and metabolic pathways, among the other molecular components of the cell, are absolutely essential for 'life'. Starting from these fundamentals and building up, the synthetic biologist has the tools to design complex biological circuitry or 'software' which may then be implanted into a biological platform as the hardware. Although genome engineering, as a significant scientific method employed by synthetic biologists, can be accomplished by 'partial' modifications of natural genomes in the form of mutations or gene insertions, in order to achieve the creation and optimization of entirely new life forms, the golden standard might become the successful transplantation and expression of a chemically synthesized chromosome in a 'bare bones' recipient cell. The Craig J. Venter group seems to have done just that in a recent (2010) publication in Science[7]. Their latest work provides "a proof of principle for producing cells based on computer-designed genome sequences". Gibson et al. report the creation of a new synthetic bacterial cell (M. mycoides JCVI-syn1.0) based on the transplantation of an entire synthetic genome (1.08 mega-bas-pairs) into a M. capricolum recipient cell. The recipient cells are a type of 'skeleton' into which the synthetic DNA is placed, creating a new type of cell with a phenotype based on the inserted genome, capable of self-replication in the laboratory. The transplantation of a whole genome into another cell essentially accomplishes the addition of thousands of new traits, the conversion of one species into another, within a seconds as the new genome is expressed. The new genome, with watermark sequences in place to delineate it from non-synthetic DNA, was synthesized by the Venter group without the crippling high error rate that has classically thwarted the rapid sequencing and recreation of whole working genomes in the laboratory. This was accomplished with the help of yeast, where overlapping pieces of the final genome containing specific restriction enzyme sites (which are like jagged cut sites which only one type of molecular 'scissors' can work on) were combined via homologous recombination. Again, the synthetic biologists have devised ways to make nature work for science, where the cell is used as a factory to successfully perform complex multistep chemical reactions.

Another success story published this month in Nature[8] was the successful creation of three synthetic biochemical oscillators, based on transcriptional switches, designed from biological components in cell-free *in vitro* (test tube) reactions. Biochemical oscillators, for example those that govern the circadian rhythm, or 'biological clock' of an organism, are based on feedback loops that guide gene expression and rhythmic processes in biology. Transcriptional switches, made up of genetic material (DNA), regulatory units and promoters that define when the DNA sequence is read, as well as output signals (the transcriptional product RNA), are fundamental components that allow for complex oscillatory processes in life. Kim et al demonstrated a synthetic negative

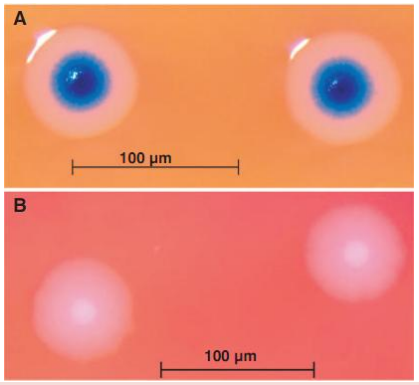

Figure 1. Images of synthetic bacteria M. mycoides JCVI-syn1.0 and wild type (WT) M. mycoides. Three days after plating, the JCVIsyn1.0 colonies are blue because the cells contain the lacZ gene and express b-galactosidase, which converts the X-gal on the agar plates to a blue compound (A). The WT cells do not contain lacZ and remain white. Progeny of the synthetic bacterial will not contain any protein molecules that were present in the original recipient cell[7].

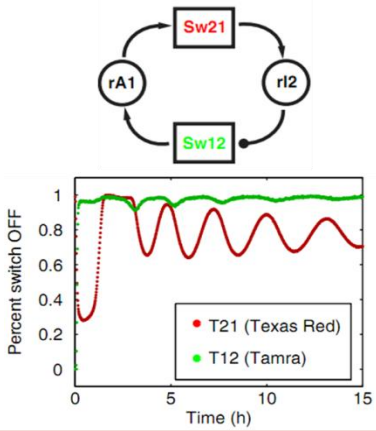

Figure 2. Biochemical oscillator based on synthetic switches (Sw21 and Sw12). Switches are fluorescent in their OFF-state[8].

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feedback oscillator regulated by RNA signals that showed up to 5 complete cycles in vitro, as well as the successful construction of several other more complex oscillators[8]. Synthetic transcriptional oscillators "could prove valuable for systematic exploration of biochemical circuit design principles and for controlling nanoscale devices and orchestrating processes within artificial cells"[8].

Although the promise of synthetic biology seems limitless at this point, especially with the recent scientific breakthroughs and advances in gene synthesis/gene transfer technologies and computer-based modeling of complex biological circuitry in helping to predict the behavior of synthetic systems, some still express their doubts[9]. The biological building parts in synthetic systems have to be extremely well characterized, and their overall function in a different environment, for example in the context of a different cell type then where the 'part' originated, must be predictable. A news feature in Nature Magazine (2010) expressed concerns over the claim of synthetic biology to "tame the complexity of living systems"[9]. "We are still like the Wright Brothers, putting pieces of wood and paper together." The complexity of a mammalian cell genome can make the outcome of specific genetic insertions or mutations very unpredictable. For example, even modifications in reportedly non-coding or 'junk' DNA regions can have physiological impacts. A big concern is whether the inserted components will be compatible with the host cell machinery, without affecting the natural gene expression of the modified cell. "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."[9] The question is, can technology and continued scientific breakthroughs in understanding the fundamental units of life and how they combine to produce extremely complex systems, that yet work harmoniously together, give biological engineers and synthetic biologist the tools to create a wide range of synthetic systems for the advancement of medicine and continued exploration into the question: What is 'life'?

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BioFab : International Open Facility Advancing Biotechnology (BIOFAB)

Initiated by a grant from the NSF (National Science Foundation), and led by bioengineers from UC Berkeley and Stanford University, BioFab is a response to the need for an all-in-one collection of standard biological parts available to academic and commercial users. BioFab facility offers "tens of thousands of professionally engineered, high quality standard biological parts…", as well as software for computer-aided design of standard biological parts, "Bio-CAD". Researchers *can mix and match the available 'parts' in the creation of synthetic organisms to produce new drugs, fuels or chemicals* (UC Berkeley News Center - Robert Sanders, Media Relations | January 20, 2010)

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