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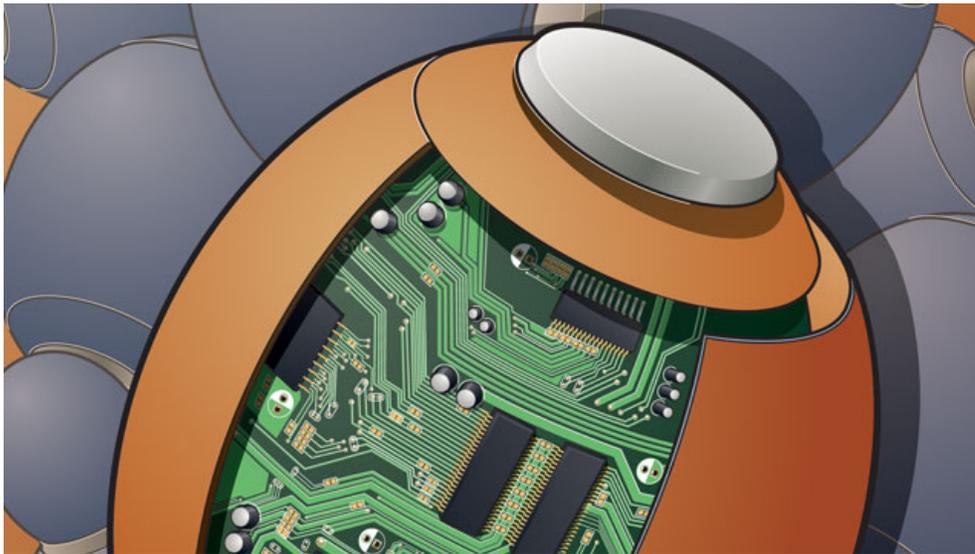
Engineering Life

Cellular “tinkering” is critical for establishing a new engineering discipline that will lead to the next generation of technologies based on life’s building blocks.

By Ahmad S. Khalil, Caleb J. Bashor, and Timothy K. Lu | August 1, 2013

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Engineering began as an outgrowth of the craftwork of metallurgical artisans. In a constant quest to improve their handiwork, those craftsmen exhaustively and empirically explored the properties—alone and in combination—of natural materials. The knowledge accumulated from this exploration and experimentation with natural building blocks eventually led to today’s modern technologies. We can now readily build things like super-lightweight cars and electrical circuits containing billions of transistors that encode highly sophisticated functions, using reliable design and manufacturing frameworks—a vast leap from artisanal craft.

Today, there is a parallel progression unfolding in the field of synthetic biology, which encompasses the engineering of biological systems from genetically encoded molecular components.¹⁻⁷ The first decade or so of synthetic biology can be viewed as an artisanal exploration of subcellular material. Much as in the early days of other engineering disciplines, the field’s focus has been on identifying the building blocks that may be useful for constructing synthetic biological circuits—and determining the practical rules for connecting them into functional systems. This artisanal tinkering with cells is necessary for arriving at a rigorous understanding of subcellular construction material and for determining the extent to which it can be manipulated.

Unlike other engineering disciplines, however, synthetic biology can—and should—be guided by the natural blueprints and organizational principles of evolution, the ultimate “tinkerer” at the cellular level. As a result, physical intuition, which has played such a central role in developing other engineering fields, may be less helpful in guiding this exploration, and we should always question whether we are using the best construction techniques. By following natural design principles, can we build better systems?

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Current Issue

Will the field of synthetic biology progress from a modest group of skilled artisans to a thriving industry on par with modern mechanical and electrical engineering? Will it ever fulfill its many promises to reprogram natural organisms and create new organisms for addressing a range of applications in human health, energy, and the environment?

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Searching for parts

Synthetic biology is a direct product of the genomics era—a period encompassing the past several decades, during which recombinant DNA and DNA-sequencing technologies have generated an explosion of information about entire genomes. The molecular building blocks of living systems are now being sequenced and decoded at a rapid pace. Despite these advances, it is still largely unclear how these molecular constituents work in concert or how they give rise to cellular behaviors. Simply dissecting a cell’s genome bit by bit hasn’t been sufficient to answer these questions, so in the late 1990s, researchers began to explore an alternate approach, assembling a small number of biomolecular “parts” into systems that perform desired functions, as an engineer would. Thus synthetic biology was born: the idea of constructing biological circuits, devices, and systems from so-called molecular parts lists. But a primary limitation of how we construct synthetic biological circuits is the small number of well-characterized parts. Fortunately, nature may provide us with a number of strategies for overcoming this deficiency and for generating libraries of unique parts from which to assemble new and interesting systems.

Some researchers have returned to the field’s roots, mining the genomes of many different organisms for more useful parts. Mining is a particularly apt analogy for this strategy, as it requires digging through our current catalog of the world’s organisms to find new molecular components that may prove desirable in synthetic systems. This has been facilitated in recent years by ever faster and deeper DNA-sequencing technologies, which have led to efforts to catalog the vast genetic diversity of organisms on the planet. For example, since sequencing the human genome, J. Craig Venter and his team have been attempting to sequence the DNA of all microorganisms inhabiting the world’s oceans and seas. Marine organisms are extraordinarily diverse, and the microbes and viruses in marine ecosystems play a key role in sustaining life on Earth. (See “[An Ocean of Viruses](#),” *The Scientist*, July 2013.) By sampling the genetic material that has been evolutionarily shaped to accomplish important functions, we may yet discover new molecular parts for the purposeful engineering of living systems.

In turn, the growing power of DNA synthesis enables scientists to rapidly write these cataloged DNA sequences into designed biological constructs. As an example, researchers are taking clues from proteins coded by bacteria-infecting viruses (bacteriophages)—enzymes that can bind, excise, and flip DNA sequences. The viruses naturally use these proteins, known as recombinases, to shuffle and physically manipulate their host’s genome. By expressing these recombinases in bacterial cells, the researchers can turn specific bacterial DNA sequences into digital memory devices that store information based on the orientation of the DNA. Researchers have used such a system to demonstrate integrated universal logic and memory in living cells, where the bacteria could perform all possible Boolean logic functions, such as AND and OR, and store the results permanently.⁸ It is the natural diversity of recombinases found in bacteriophage genomes that enables this feat of molecular engineering.

A second source of new parts comes from elaborating upon evolutionarily conserved molecular “armatures” that can be engineered to perform functions beyond their natural repertoire. Like the framework around which an artist constructs a sculpture, these molecular species can serve as adaptable starting points for engineering diverse cellular functions. The beauty of this strategy is that common molecular armatures have likely been naturally selected because of their functional versatility, thus making them great substrates for engineering. Zinc fingers are one example; they recognize and bind specific DNA sequences, enabling transcription factors to find and turn specific genes on or off. Zinc fingers are found in a wide range of organisms, from yeast to humans, and are believed to be the most abundant domains encoded in all eukaryotic genomes. This suggests that they have emerged as a preferred natural solution to the difficult challenge of DNA sequence discrimination and binding. Recently, researchers have shown that it is possible to artificially reprogram the specificity of these domains. We can now engineer designer zinc-finger proteins that bind virtually any DNA sequence of interest. (See illustration.)

Mutable motifs such as these present endless possibilities for synthesizing useful new genetic constructs. For example, we recently used artificial zinc-finger building blocks to generate a library of new, synthetic transcription factors.⁹ Each transcription factor controls gene expression of a uniquely designed DNA sequence, providing an extensible resource for new biological circuit connections in virtually all eukaryotic organisms. Furthermore, the design of these parts is inspired by the design of natural, eukaryotic transcriptional systems, which are highly modular, meaning that they can be decomposed into functional components, and then reassembled in different ways to perform different cellular tasks. Similarly, our synthetic transcription factors are constructed from separate functional protein domains that can be readily swapped and tuned to perform various functions. Indeed, as proof of principle, we used our zinc finger transcription factors to program into yeast a variety of natural genetic behaviors, such as the integration of multiple signals at a single promoter to control transcription.

Since the discovery of zinc fingers, potential new DNA-binding molecular armatures have been identified



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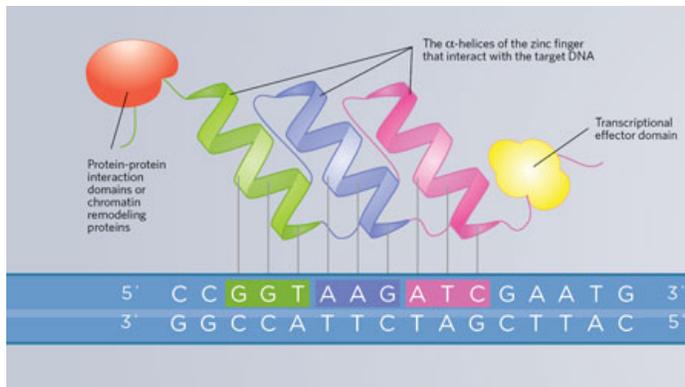
that also use relatively simple codes to recognize DNA sequences. These include transcription activator-like effectors (TALEs)¹⁰ and the CRISPR/Cas system,¹¹ both of which are derived from pathogenic organisms. Like zinc fingers, these systems can be reprogrammed to target virtually any DNA sequence of interest, and thus may also make potentially powerful building blocks for creating new parts.

Making connections in context

In cells, gene and protein interactions are based on chemical specificity, protein localization, and molecular diffusion, among many other properties that we are still trying to comprehend quantitatively. As a result, it's no surprise that engineering living systems requires an entirely different mind-set than the one used, for instance, to wire electrical connections. Various platforms for engineering connectivity using diverse parts have begun to emerge in synthetic biology. Programming networks using DNA-binding transcription factors is the most widely used approach, largely because of the intuitive ease with which new connections can be made. Our new zinc-finger-based transcription factors are a good example of this type of connectivity. By contrast, posttranslational, protein-based signaling networks represent a largely unexplored frontier, though a few labs have begun to try their hand in this arena.³

Because synthetic biologists are largely preoccupied with understanding how to make synthetic network connections, one issue that has been largely overlooked is that of how a synthetic circuit, once introduced into a host, interacts with the native cellular environment—what we like to call the circuit's context. A critical question for synthetic biology will be: How do we engineer for context? Issues of context pervade nearly every engineering discipline. In civil engineering, choice of building material is determined as much by external environment as by functional requirements, such as the load the structure must bear. Electrical engineering is similarly limited by contextual constraints, since packing too many circuit components into a small space, such as a laptop, may result in the accumulation of excess heat. In both examples, the task of engineering the structure or system can be viewed as a separate problem from the engineering of the function itself.

But engineering for context in cellular systems will not be easy. (See illustration.) For one thing, the interior of a cell can be viewed as a moderately well-stirred solution containing thousands of distinct molecular entities. Circuit components may diffuse through the cytoplasm and freely associate with native molecular components, resulting in unforeseen interactions that can adversely impact the circuit's intended function. At the same time, the presence of new synthetic components may disrupt the life of the cell—by imposing a drag on the cell's resources, for example, or by interacting with native regulatory networks—in a way that, in turn, can reciprocally constrain the performance of the circuit. This presents a complex challenge.



DNA CIRCUIT DESIGN: Zinc fingers are modular DNA-binding domains that exist as part of many natural transcription factors. They can be engineered to bind desired sequences of DNA and, when fused to transcriptional effector domains that help recruit RNA polymerase to initiate transcription or that inhibit transcriptional machinery, can act as artificial transcription factors that regulate the expression of target genes. On top of this, additional components can be added to either end of the zinc finger, including protein-protein interaction domains (for recruiting other proteins) and chromatin remodeling proteins, to enable more complex forms of gene regulation.

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After years of tinkering with cellular circuits, we have built up a rudimentary understanding of component connectivity, but we have yet to decipher the rules that govern how circuits can be insulated from cellular context. While daunting, the task of engineering cellular circuits for both function and context would appear to be critical for maximizing synthetic biology's real-world applications. For example, synthetic circuits programmed to control biofuel production in a bioreactor could support the large-scale production of a target compound, but the circuits could also interfere with microbial growth. Engineering

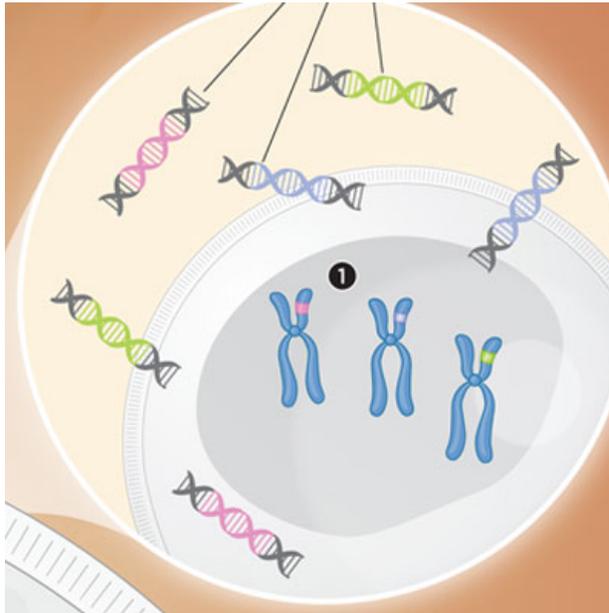
Because of the preoccupation with understanding how to make synthetic network connections, one issue that has been largely overlooked is that of how a synthetic circuit, once introduced into a host, interacts with the native cellular environment.

strains that minimize the interference imposed by the circuit while maximizing yield could require extensive manipulation of both the circuit and the host.

One solution might be to use parts that not only allow for connectivity, but are also selected for their intrinsic properties to minimize host interactions. This will require much experimentation because, unfortunately, researchers can't write a mathematical model that reliably predicts why one strategy for building circuits is better than another. Also, our group continues to advocate drawing inspiration from natural solutions. What systems has nature converged on to carry out regulatory tasks, such as signal transduction and gene regulation? The functions of these biological systems and their building blocks have been optimized within a cellular context by millennia of evolution and should thus serve as good blueprints for making engineered connections within cells.

New rules for new cells

Historically, a focus of synthetic biology has been the construction of molecular logic circuits in bacteria, including switches, oscillators, logic gates, filters, analog circuits, and many other electronics-mimicking genetic devices.^{1,3,4,7,12} (See ["Tinkering with Life,"](#) *The Scientist*, October 2011.) Researchers are now attempting to integrate these small computational circuits to enable more sophisticated information processing and computational power within cells. An exciting new focus is using synthetic approaches to understand the organizational principles of eukaryotic cells and higher-order cellular systems. For example, researchers at the University of California, San Francisco, recently used a synthetic biology approach to interrogate the phenomenon of cell polarization, or the ability of cells to asymmetrically organize their components.¹³ From a toolkit of engineered signaling proteins known to be involved in this process, the team constructed synthetic circuits and looked for the circuit motifs that were capable of producing strong, sustained cell poles in yeast.



Cellular Engineering in Context
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Other synthetic biologists have assembled similar metabolic pathways in cells in hopes of turning microbes into living factories for the production of biofuels and other chemical goods. Another field that now seems poised to undergo a revolution by the forward engineering of cells is biomedicine.¹⁴ Cells naturally perform therapeutic tasks in the body—immune cells identify and remove pathogens, for example—and unlike drugs or molecules, cells can perform complex functions, such as sensing their environments or proliferating. Indeed, patient-specific immune cells are already being genetically engineered with receptors called chimeric antigen receptors (CARs) that allow them to target and destroy tumors in the body. Synthetic circuits and approaches could be used to further enhance these cancer-fighting functions and/or make these cell-based therapies safer.

Synthetic biology could also be used in cancer diagnostics. For example, researchers recently engineered into human cells gene circuits that are triggered by microRNA signatures unique to certain cancers.¹⁵ Similar approaches could be envisioned for endowing cells with sense-and-response capabilities to detect and mediate a number of other dysfunctions and pathologies. Promising opportunities for cell-based therapeutics also include patient-specific stem cells for regenerative medicine and microbiome engineering to treat gastrointestinal diseases.

What's more, all of these exciting efforts are occurring simultaneously with our now unprecedented ability to make modifications to the genomes of cells. Using targeting tools, such as zinc fingers, TALEs, and CRISPR/Cas, researchers

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can now edit specific genes within a genome with very high precision. For example, we can—and do, in the form of gene therapy—use these tools to inactivate genes known to be involved in disease progression or in pathogen life cycles. We can also use them to introduce synthetic circuits into precise locations within a variety of genomes, including in human cells—a feat that would have been impossible less than a decade ago. We can even think about de novo designing and sculpting of genomes to have desirable properties.

But we have a long way to go. While engineers of mechanical and electrical systems have common engineering principles (standardization of building components, simplicity and modularity in design, reliability) to guide their designs, no such unifying principles or design frameworks exist for the engineering of biological systems. Although some parts such as zinc fingers have easy-to-engineer, modular designs, many others do not. Furthermore, because a complete systems-level understanding of biology is unavailable, anticipation of interactions between a synthetic circuit and the intracellular environment is impossible.

Ultimately, in considering how to engineer functions in living cells, the only truly unifying design rules may be those gleaned from the blueprints provided by nature. There is evidence that evolution has shaped the design of natural systems through iterative “tuning,” whereby biological parts have been introduced, modified, and assembled over and over again to arrive at systems that are optimized for both function and context. These natural solutions, such as zinc fingers, may be the best starting points for engineering synthetic systems. Similarly, by experimentally tinkering with the existing natural blueprints, we, as synthetic biologists, can find out what works in an engineered context.

In this “preindustrial” era of synthetic biology, it’s hard to know the limitations of engineered biological systems. Yet, it’s important to note that a full physical understanding of how biological parts interact and operate may not be necessary for realizing synthetic biology as an established engineering discipline. In fact, metallurgy became an established craft, allowing us to reliably fashion metals, long before an atomistic picture of the materials was uncovered. Similarly, our understanding and practice of biology has matured to an exciting point: we are poised to establish reliable design rules for building on nature.

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Alexandru

Posts: 36

August 6, 2013

"Similarly, our understanding and practice of biology has matured to an exciting point: we are poised to establish reliable design rules for building on nature."

Please, do not forget to introduce the bio-GPS for a better adaptation of the synthetic creatures in the nature.

Please, read my comment on <http://www.the-scientist.com/?articles.view/articleNo/36881/title/Mapping-Humans--Mental-GPS/>

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Madhu Thangavelu

Posts: 2

August 7, 2013

Engineering began long before metallurgy. It probably has roots in how to move large objects like those Stonehenge pillars, and how to place them in precise formations. Engineering began perhaps when our ancestors, sans Ph.D, learnt how to strike a fire, rather than wait for lightning to cook deer for them. Recorded engineering began in earnest when those Romans, under Vitruvius, undertook the building and service of those aqueducts and roads, not to mention other aspects of prehistoric agriculture, all of which preceded serious metallurgy.

Now, re. life, all that is mentioned here seems icing on the cake. True synthetic biology begins when we create living tissue, living cytoplasm. Sorry, Craig Venter, call us when you make living cytoplasm and then introduce your Synthia nucleus into it....and then we'll all applaud !...hey, this is meant to stir up action at Venter's lab as well as the authors !

Thanks for great article. Keep us all thinking. Keep up the good work.

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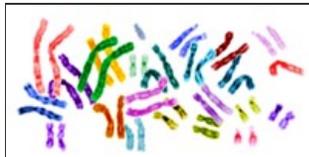
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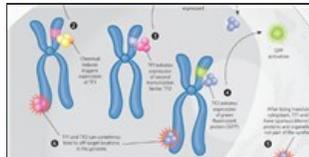
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