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SYNTHETIC BIOLOGY

It's an analog world

The first synthetic genetic circuits to use analog computation have been developed. These circuits involve fewer components and resources, and can execute more complex operations, than their digital counterparts.

HERBERT M. SAURO & KYUNG KIM

The engineering of biological networks, a discipline called synthetic biology, has seen remarkable progress since its inception in 2000. The fact that we can now design simple but functional circuits *in vivo* is probably one of the most important aspects of this progress — it suggests that our biophysical understanding of the cellular milieu is largely correct, even if many details remain to be resolved. The significance of this should not be underestimated, because it means that predictive engineering of new cellular networks and systems is possible. Until now, approaches to synthetic biology have been greatly influenced by the digital computing technology that we use every day. But biological cells do not process information in a solely digital fashion; rather, they carry out many operations in an analog manner. In a paper published on *Nature*'s website today, Daniel *et al.*[1] break the mould and venture into a completely new area of synthetic biology, presenting synthetic circuitry that can perform analog calculations.

Synthetic biology has already led to several notable firsts — from the construction of switches, oscillators and feed-forward networks to the creation of gene regulatory circuits that can carry out Boolean logic. In many ways, digital computing has coloured our approach to synthetic biology. It is a truly remarkable achievement that so much can be done with just the 'ones' and 'zeros' that form the basis of digital processing. After more than 60 years of digital computing, it is no surprise that many modes of thinking have become highly influenced by the digital paradigm. It is often said that a biological cell is just like a digital computer, and that reprogramming a cell is like writing software. But the digital analogy is a misleading one, because many processes that occur in a biological cell have no counterpart in a digital computer. If reprogramming a cell was just a case of writing code, then re-engineering cells would be simple — but it is not.

The reality is that cells use a hybrid approach to information processing. In some cases they use digital yes-or-no decisions, but in many cases cellular signals are analog, with levels of gradation. More exotic, little understood signal-processing techniques involving noise and other forms of signal probably also contribute. And on top of that, a complex chemistry exists that continuously reassembles the cell in real time.

Nevertheless, constructing digital devices is both a useful and an interesting engineering challenge for synthetic biologists, and many advances have been made in mimicking digital systems either *in vivo* or *in vitro* using DNA as components. One remarkable study was the construction of an *in vitro* 4-bit square-root calculator consisting of 130 DNA strands[2]. Another was the construction of an *in vivo* system to detect four different inputs, comprised of four sensors and three logic gates[3].

Although these are significant achievements, such circuits require large numbers of components to perform even the simplest computation. A 4-bit binary adder, for example, might require 30 or more proteins to operate, and at the same time would place a substantial metabolic burden on a cell. More challenging and perhaps more interesting is to attempt to mimic analog computation in a cell, as Daniel *et al.* have done.

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One key advantage of analog over digital is that far fewer devices are needed to carry out a given computation at the moderate precision needed in cells[4], and fewer devices mean lower resource requirements. In addition, the richness of signal processing that can be carried out in analog systems is far greater than what can be accomplished in digital systems using the same number of components. But analog information processing brings with it a new set of challenges. Compared with that of digital systems, the design of analog circuitry requires greater expertise in feedback-system design, circuit theory and signal processing. Daniel and colleagues' work is founded on the close analogy they saw between the exponential thermodynamic electron flow that occurs in transistors and the exponential thermodynamic rates seen in chemical reactions[5]. This similarity led them to realize that analog electronic circuits that operate in the logarithmic domain might be effective mimics of analog biological circuits that operate in the logarithmic domain.

To test this idea, Daniel *et al.* constructed a gene-transcription unit in which the concentration of a transcription factor (a protein that regulates gene transcription) could be logarithmically transformed over a wide range of concentrations, thus enabling fine control of gene expression.

Moving an input signal into the logarithmic domain has many benefits, especially that basic calculations such as division and multiplication can be more easily executed in log space. The core of the authors' logarithmic unit is a positive-feedback loop operating in an analog mode. A shunt is included that removes excess transcription factor so as to increase the range of operation and efficiently implement positive feedback. With this basic logarithmic unit in place, the researchers could then focus on adding higher-order functions. For example, by attaching a repressor module, they created a circuit whose readout was the negative of the log of the input. Another circuit simply summed the activity of two parallel logarithmic circuits to mimic a multiplication operation.

Furthermore, using just two transcription factors, the authors were able to create a negative-feedback analog circuit that scaled the logarithmic function. Especially interesting was their construction of a circuit that computed the ratio of two inputs over four orders of magnitude of input concentration. Computing a ratio might have many applications, such as normalizing or comparing values, or even providing an *in vivo* pH meter.

The ability to process graded information by means of synthetic biological circuits will be of interest to many researchers. For example, most inputs from the environment are graded, and a synthetic circuit with a fluorescent protein readout might be able to represent the rate of change of an environmental input rather than its absolute level. This could be accomplished by having an analog differentiator circuit. Other analog designs might be used to measure the weighted sum of environmental inputs, such that when a particular combination of inputs is reached it triggers a change in the state of the cell. Designing such analog circuitry may also further our understanding of natural systems. The function of many regulatory systems in cells remains obscure, and studies of synthetic biology in the analog domain should lead to new theories on how biological systems process information, and thus allow such systems to be more finely controlled. ■

Herbert M. Sauro *and* **Kyung Kim** *are in the Department of Bioengineering, University of Washington, Seattle, Washington 98195-5061, USA.*

e-mails: hsauro@u.washington.edu; kkim@uw.edu

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