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# Rewiring the cell: synthetic biology moves towards higher functional complexity

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**A steady stream of research has fueled excitement in the field of synthetic biology. Logic gates, oscillators, and memory elements constructed using genetic and biochemical components have all been demonstrated. However, the nagging question remains as to how higher levels of complexity can be designed into these synthetic systems. A recent paper from Collins' group provides some answers to this question.**

The intertwined development of a fundamental science and an engineering discipline both centered on information processing in genetic circuits and networks is remarkable. It is true that the fundamental science came first as investigators endeavored to describe regulation and control of cellular processes. But even in these early efforts there was a realization that an engineering discipline lay ready for development as Jacob and Monod opined that gene-regulatory circuits with many different functions could be constructed using networks of simple regulatory elements [1]. Although they are still focused on describing natural genetic systems, many recent efforts have turned to engineering concepts. For example, the competition between the two protein populations that control the selection of lytic or lysogenic pathways in  $\lambda$  phage infection has been likened to critical races between nearly simultaneous electrical signals in digital circuits [2]. The role of stochastic fluctuations in the  $\lambda$  switch has been described using the concept of dithering, in which noise is intentionally added to improve the performance of analog-to-digital converters [3,4] and auto-regulated gene circuits have been analyzed using concepts borrowed from electronic amplifier design [5].

Only a small step from borrowing engineering concepts for description, we are now seeing the emergence of a design discipline that focuses on manipulating information processing and transport within genetic circuits and networks. There have been numerous efforts to create engineered gene circuits that function according to human design using a 'silicon mimetic' approach, in which engineered genetic or biochemical systems are made to emulate the functionality of semiconductor devices such as logic gates, latches, and oscillators [6–14]. Combinatorial logic gates have been realized using approaches that include random assembly followed by functional screening [8], the rational design of synthetic gene logic circuits and networks [15], and directed evolution [16]. Adding to the toolbox of elemental gene circuits, an oscillator [6] and a toggle switch [7] have been reported. To date most efforts in synthetic biology have focused on the development of individual functional components or relatively small networks of these components. However, complex functionality is more a result of interconnection than fundamental component complexity. In other words, complexity arises from the massive interconnection of simplicity, and the development of flexible and parallel ways to interconnect these circuits is a daunting challenge for the next crucial leaps forward in synthetic gene-network design. Recent work from Collins' group [17] demonstrates that one promising approach is to leverage the existing complexity of natural gene circuits and networks while using synthetic circuits to customize function.

## Higher levels of complexity

A major obstacle in generating highly complex function using the synthetic gene circuits described above is the requirement of long ('deep') logic cascades [18]. Many electronic circuits are constructed as deep logic cascades

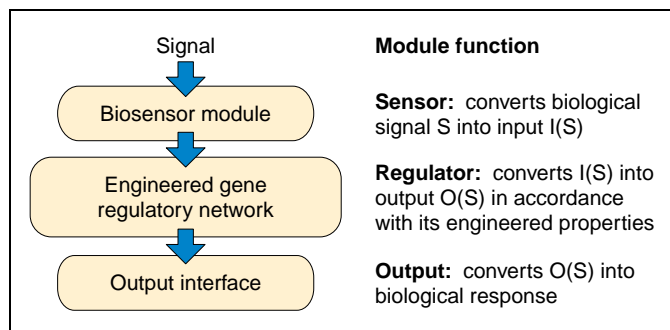
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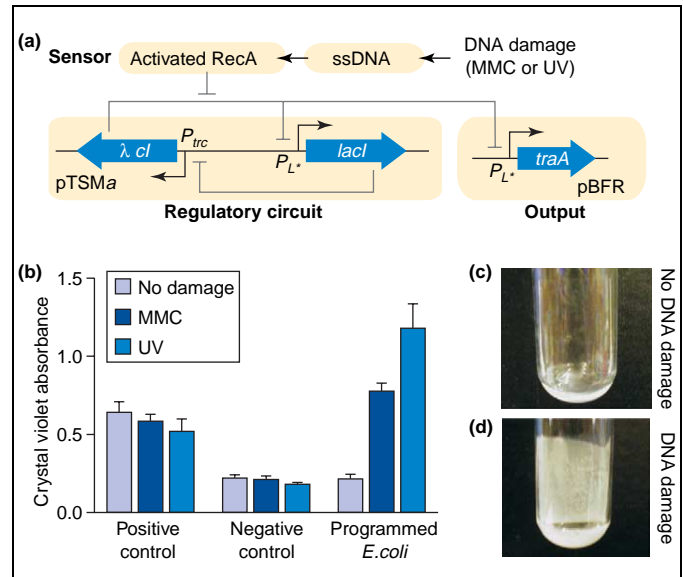
in which signals are serially processed as they pass from the output of one module to the input of the next, and functional complexity grows as the cascade becomes longer. However such architecture is not favored in gene networks, perhaps owing to the very slow dynamics of cascaded transcriptional circuits, which would lead to long transit times for information processed through a deep cascade. Rather, it seems that complex gene networks employ a 'broad and shallow' architecture in which multiple logic functions are integrated into the *cis*-regulatory elements and information is processed in parallel instead of serially [18]. Current synthetic biology circuits have not yet achieved this broad and shallow architecture, but the approach of Kobayashi *et al.* [17] leverages existing complexity by deploying a synthetic circuit as an interface between natural pathways that serve as input (signal detection) and output (response) modules (Figure 1). The role of the synthetic circuit is not the *de novo* creation of complexity, but rather to customize the function of existing complexity by controlling the connection between pathways.

The genetic toggle switch mentioned is used for this intermediate layer of synthetic circuitry. The toggle switch is unique among the suite of synthetic gene circuits because it implements a memory function and Kobayashi *et al.* use this property to produce a circuit that implements epigenetic inheritance leading to a persistent phenotypic alteration in response to transient signals. That is, signals of limited duration produce enduring responses at the individual cell level that determine group behavior of a cell's progeny long after the signal has subsided. This is convincingly demonstrated by a circuit coupling the SOS response pathway that is activated by single-stranded DNA (input) and a pathway leading to biofilm formation (output; Figure 2). Transient DNA damage induced by chemical or physical stresses latched the toggle switch into a stable state that led to biofilm formation many cell cycles after application of the input signal.

A second intriguing example demonstrated by Kobayashi *et al.* used the endogenously generated signals of quorum sensing (QS) to create a latched stable state in response to cell population. In contrast to most synthetic gene circuits, this system does not require exogenous input signals, but rather taps into a natural cell-population sensing mechanism to control circuit function. QS has particular appeal



**Figure 1.** Block diagram of the 'plug-and-play' genetic circuit devices scheme reported by Kobayashi *et al.* [17]. The biosensor and output modules are natural pathways that have been coupled using a synthetic gene circuit. Reproduced with permission from [17].



**Figure 2.** Example of programmed phenotype that couples the SOS response pathway that is activated by single-stranded DNA (input) to a pathway leading to biofilm formation. (a) Diagram of the engineered genetic circuitry. The genetic toggle switch module (pTSMa) controls the expression of *traA* from plasmid pBFR in response to DNA damage. (b) Biofilm formation quantified by crystal violet staining in cultures of strain K12/AK4 (positive control), strain K12/AK3 (negative control) and strain A2 (programmed *E. coli*). (c,d) Photographs of microfermentors incubated with untreated cells (c) or cells treated with mitomycin C (MMC) (d). Reproduced with permission from [17].

for practitioners of synthetic biology because it allows gene circuits in separate cells to be coupled, and as demonstrated in natural systems enables greater functional complexity by coordinating cooperative activity. Other recent work also highlights the potential value of QS architectures for synthetic biology applications [19,20].

Looking to the future, Kobayashi *et al.* provide a broad view of the implications of this work by stating that it is '...paving the way for the development of 'plug-and-play' genetic circuit devices.' [17]. Promising as it might be, the authors point out many of the obstacles that hinder the realization of this vision. Perhaps the most significant of these is that interfacing synthetic gene circuits with natural signaling pathways is not a trivial task, but requires system, not just individual component, optimization. The widely varying input-output requirements of natural pathways are not well-suited to the rational design approaches followed in some synthetic strategies [15] and Kobayashi *et al.* suggest that directed evolution techniques [16,21,22] could be of significant benefit for such complex system optimization.

### Future of synthetic biology

Although many parallels exist between electronic and biological circuits, the biological medium is not a blank slate, but rather comes complete with a history of complex circuit implementation. In efforts to simplify the problem, most prior efforts have designed synthetic gene circuits that interact as little as possible with existing gene circuits or pathways. Kobayashi *et al.* break from this tradition to demonstrate that achieving higher levels of functional complexity might require tighter coupling with and greater leveraging of natural complexity. Given this demonstration, expect to see much greater exploration of

this design paradigm in future synthetic biology efforts. In line with much of current synthetic biology thinking, the concept of 'plug-and-play' genetic circuit devices has a decidedly technological orientation. However, it has been suggested that there is an element of convergent evolution between complex engineered devices and biological systems [23], and synthetic biology efforts, such as that reported by Kobayashi *et al.* could take us full circle to arrive back at more fundamental scientific goals. A concept well known in systems engineering is that often more is learned about existing system architecture through an attempted redesign than through analysis alone. One could envision the implications of this work for investigations in areas such as development, in which epigenetic inheritance again leads to a persistent phenotypic alteration in response to transient signals, or in cell-cell communication systems that coordinate the rich complexity of group behavior. Perhaps such a coupling between systems and synthetic biology is the route to a deeper understanding of the subtleties of these natural systems.

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#### Letters

## Can heterologous gene expression shed (a torch) light on protein function?

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Heterologous gene expression is of prime importance for a wide variety of applied and basic biological research areas, including tissue engineering, gene therapy and

biopharmaceutical manufacturing. In functional genomics, the expression of foreign genes *in vivo* is a standard method for disclosing functions of specific genes, in particular in complementation experiments in yeast. Another major application of heterologous gene expression in biotechnology is foreign protein production

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