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Cell

Synthetic Biology Looks Good on Paper

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Tremendous progress has been made in the design and implementation of synthetic gene circuits, but real-world applications of such circuits have been limited. Cell-free circuits embedded on paper developed by Pardee et al. promise to deliver specific and rapid diagnostics on a low-cost, highly scalable platform.

In an age of rapid biomedical advancements and during a push to move from bench to bedside, how can synthetic biology keep up with the expanding clinical need for high-throughput, accurate, and translational point-of-care diagnostics? Pardee and colleagues have a simple answer: paper (Pardee et al., 2014).

Over the last 15 years, numerous gene circuits have been engineered to program behaviors of individual cells or cell populations. In addition to addressing fundamental biological questions (Riccione et al., 2012; Cheng and Lu, 2012), these circuits and the lessons learned from designing them could have practical applications in medicine and biotechnology (Church et al., 2014). Despite impressive progress, however, our ability to program cellular behavior in a predictable manner remains limited. Furthermore, the use of engineered living cells for therapeutic applications faces substantial challenges in overcoming public safety concerns and regulatory hurdles.

These challenges can be partially overcome by cell-free systems (Hockenberry and Jewett, 2012). These platforms consist of either purified gene expression machinery or cell extracts, an energy source (Sun et al., 2014), and the desired DNA constructs of interest. Bypassing the need to deal with complex cellular dynamics allows cell-free systems to be used for rapid iterative prototyping of gene circuits. Also, cell-free systems provide a more controlled environment, which can act as a powerful foundation for increasingly complex synthetic biology applications, such as biosensors. However, solution-based reactions of typical cell-free systems must follow strict protocols such as proper freeze-thaw, temperature regulation, and sample preparation to achieve reproducible results. To overcome these difficulties, Pardee and colleagues demonstrate how paper can be used as a vehicle to embed, store, distribute, and operate diverse gene circuits in a robust manner (Pardee et al., 2014), which lays the foundation for low-cost yet accurate diagnostics.

Their key innovation is to embed the gene expression machinery along with well-defined gene circuits in filter paper by freeze-drying. Doing so renders the operation of gene circuits less constrained by typical laboratory conditions and the complexity of cellular environments. The methods used to reconstitute the reactions from paper and run the experiments involve only a few simple steps. Using this platform, the authors demonstrate the storage and operation of a wide variety of gene circuits. These span constitutive and inducible gene expression, a set of sophisticated RNA-based circuits (toehold switches), and gene expression cascades (Figure 1A). In fact, engineering of the toehold circuits represents a major innovation in its own right (Green et al., 2014 in this issue of *Cell*). As with riboregulators, toehold switches contain a transducer RNA with its ribosome-binding site (RBS) sequestered in a hairpin, preventing target gene expression. Gene expression is activated by a trigger RNA that exposes the RBS by unwinding the hairpin. However, in the toehold switch, the RBS is moved to the loop region of the hairpin, allowing the trigger RNA to take virtually any sequence. This design greatly expands the diversity of target sequences that RNA circuits can sense. Indeed, these switches exhibit precise and orthogonal control of target gene expression in both living cells (Green et al., 2014) and the paper-based platform. Underscoring the modularity and flexibility of these circuits, the authors use the toehold circuit as a component to build more sophisticated circuits to sense different target genes or to realize logic operations.

It is remarkable how this apparently simple platform enables such versatile operation of gene circuits with reliable performance. The robustness of the platform opens the door to diverse applications in environmental monitoring and diagnostics. The authors demonstrated this idea by developing and embedding 24 mRNA sensors, again built upon the toehold module, for rapid detection of Ebola viruses in less than 1 day with high specificity; they included sensors that can effectively distinguish between two different Ebola strains. They further developed colorimetric readouts to enable detection of circuit outputs in minutes by inspection or by measuring absorbance for quantification. This capability can facilitate the use of paper-based diagnostics in resource-limited areas.

Paper-based systems have several advantages as a diagnostic tool. They can be produced, stored, and operated at a low cost, and they are highly portable for distribution. Paper can be patterned into different regions that allow spatially segregated distribution of samples in small

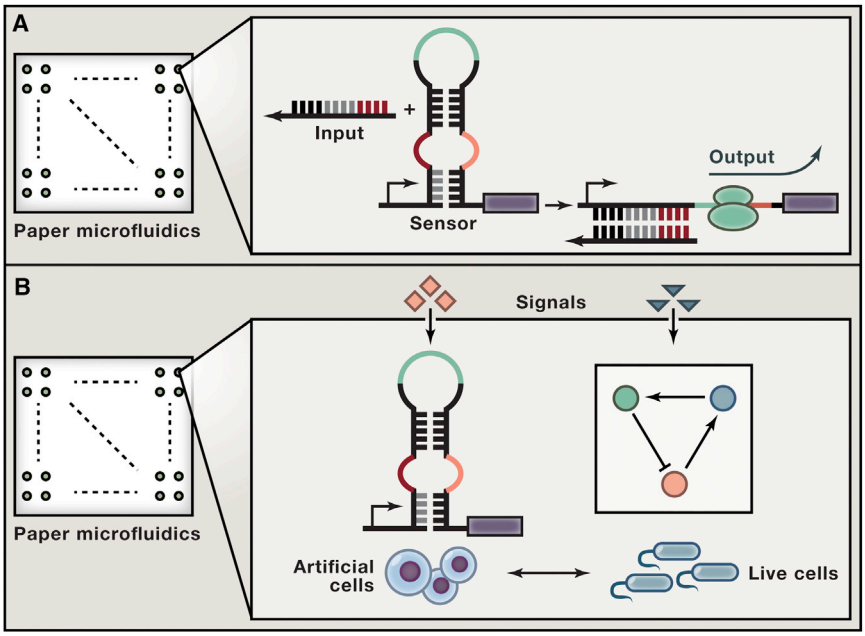

Figure 1. Integration of Synthetic Gene Circuits with Paper Microfluidics

(A) Cell-free systems with paper microfluidics. Cell extracts containing gene circuits are freeze-dried, embedded on filter paper, and later reconstituted to realize diverse functions. These functions range from inducible gene expression to sophisticated input/output functions. Panel shows a typical RNA-based toehold switch, which is used as the core module for an array of biosensors to detect diverse targets, including antibiotic resistance genes and Ebola viruses.

(B) Multiscale, hybrid systems can be built by integrating cell-free and cell-based systems, as well as microencapsulation and paper-fluidics. Such systems can enable division of labor between different components in carrying out sophisticated sensing, processing, and actuating tasks.

volumes (Yetisen et al., 2013). This feature enables multiplexed screening of samples on one device (Pardee et al., 2014). Moreover, the paper-based systems can be designed and optimized in a modular manner. The embedding process, the core circuit function design, and the adoption of a circuit for different applications can each be individually optimized before being assembled into the integrated system. Finally, their simple format can allow mass production in a streamlined manner. One can envision the entire fabrication process carried out by computer-aided circuit design, robotics-mediated assembly of circuits, and printing onto paper. Toward this vision, Pardee and colleagues used standard software (Adobe Illustrator) to design and print patterns of their circuits onto paper, which exhibited full functionality.

Most cell-free systems focus on generation of input/output functions, which are suited for direct biosensing applications. To achieve more complex functions, these systems can benefit from integration with other platforms or techniques. In comparison, cell-based platforms can allow implementation of more complex sensing and processing functions, sometimes in a manner that is also compatible with the paper format. To this end, a recent study demonstrated the use of paper-embedded engineered bacteria to detect quorum-sensing signals produced by bacteria in saliva (Struss et al., 2010). Furthermore, cell-free systems can be engineered to interface with cell-based systems. Indeed, Lentini and colleagues engineered artificial cells containing cell-free circuits to sense and interpret environmental signals and then "translate" these signals to ones that can be interpreted by living cells (Lentini et al., 2014).

All of these strategies can potentially be integrated in a common, paper-based platform, leading to the creation of multiscale, hybrid systems with sophisticated sensing and processing functions, each complementing the other (Figure 1B). The interactions between artificial and living cells can allow a division of labor between the two components. One example would be to incorporate the idea by Lentini and colleagues onto paper, where one component relays information to the other for downstream processing. Further, live-cell encapsulation and feedback control can be introduced to allow more sophisticated control over the system dynamics. One could even see the continuous integration of the artificial and living cells, where each component communicates dynamically with the other to maximize processing efficiency. That the toehold switches work equally well on paper and in cells both reflects effective engineering design and serves as an indicator for the feasibility for such hybrid integration.

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