Ribocomputing devices for sophisticated in vivo logic computation

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ABSTRACT
Synthetic biology aims to create functional devices, systems, and organisms with novel and useful functions taking advantage of engineering principles applied to biology. Despite great progress over the last decade, an underlying problem in synthetic biology remains the limited number of high-performance, modular, composable parts. A potential route to solve parts bottleneck problem in synthetic biology utilizes the programmability of nucleic acids inspired by molecular programming approaches that have demonstrated complex biomolecular circuits evaluating logic expressions in test tubes. Using a library of de-novo-designed toehold switches with orthogonality and modular compositability, we demonstrate how toehold switches can be incorporated into decision-making RNA networks termed ribocomputing devices to rapidly evaluate complex logic in living cells. We have successfully demonstrated a 4-input AND gate, a 6-input OR gate, and a 12-input expression in disjunctive normal form in E. coli. The compact encoding of ribocomputing system using a library of modular parts is amenable to aggressive scale-up towards complex control of in vivo circuitry towards autonomous behaviors and biomedical applications.

Keywords
synthetic biology, toehold switch, RNA network.

1. INTRODUCTION
Synthetic biology aims to address challenging problems in the fields of biotechnology and medicine by constructing novel and complex circuits by applying engineering principles. Starting from the first demonstration of synthetic circuits in bacteria more than a decade ago, milestone achievements have demonstrated bistability [4], oscillation [3], feedback [2], and logic processing [7] in living cells. The synthetic biological circuits to date have relied heavily on protein-based regulators that are difficult to scale up due to the limited number of designable parts and significant resource requirements to encode and operate such circuits. More recently, insulation strategies and advanced computer programs were used to assemble 55 sequence elements and 4 computational layers to construct a 3-input consensus circuit [8] -- this circuit required using the equivalent of seven inverter or 2-input logic elements.

A potential route to solve parts bottleneck problem in synthetic biology utilizes the programmability and large sequence space of nucleic-acid-based parts libraries. The advanced understanding of nucleic acid thermodynamics and kinetics allows purely in silico design of novel parts with predictable interaction with existing circuit elements. Thus, we are presented with a great opportunity to exploit the versatile nucleic-acid-based computation in synthetic biology circuits [1,5]. These design strategies can be used in combination with existing protein-based layered circuit architecture for even more complex information processing in cells.

2. RESULTS
Here, we report an integrated molecular computation strategy that employs RNA-only circuits to evaluate complex logic in living cells. Central to our current work is a novel class of in vivo RNA-based devices called toehold switches that activate gene expression in bacteria only when they detect a cognate trigger RNA [5] (Figure 1A). The toehold switches take trigger RNAs without any sequence constraints overcoming the limitations of conventional synthetic riboregulators [6]. Further, these toehold
switches can be forward-engineered to modulate gene expression by several hundred-fold rivaling the best protein-based regulators and provide extremely low levels of crosstalk across large library of components.

4. ACKNOWLEDGMENTS

We thank K. Pardee, X. Chen, and A. Chavez for helpful discussions. This work was supported by a DARPA Living Foundries grant (HR001112C0061) to P.A.S., P.Y., and J.J.C.; NSF ERA SynBio grant (1540214) and Wyss Institute funds to P.Y.; an ONR MURI Program to J.J.C.; and startup funds provided by Arizona State University to A.A.G. J.K. acknowledges the support of Wyss Institute Director’s Cross-Platform Fellowship.

5. REFERENCES


