Point of VIEW

ACS chemical biology

Evolving Sensitivity

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De novo engineering of gene circuits with well-defined functions is at the heart of the nascent field of synthetic biology (*1–7*). Such engineered systems may offer insights into underlying design principles of biological control and lead to innovative applications in biotechnology, computing, and medicine.

However, engineering gene circuits with desired, nontrivial properties is challenging. When assembled, a circuit may not function as designed because of improper or unbalanced interactions among its components (DNAs, RNAs, and proteins). Multiple rounds of circuit revision and characterization are common practice. To this end, modeling is often used to explore how qualitative and quantitative behaviors of the circuit depend on its parameters. Guided by modeling, one may choose circuit components with specific kinetic properties. If existing components do not satisfy the design criteria for optimal circuit performance, one may choose to modulate their properties by structure-based rational design. However, rational design is often limited by the lack of detailed knowledge of the structure–function relationships of individual circuit components.

Directed evolution serves as a powerful alternative that complements the rational design approach. It entails the generation of a large pool of mutant components *via* random mutagenesis and subsequent screening and selection of mutants with desired function. It relies less on detailed knowledge about the structure–function relationships of the components to be optimized. Traditionally, directed evolution has proved highly efficient in optimizing the function of a wide variety of biomolecules, without the need to resort to detailed understanding of their structures (*8*). In recent years, however, applications of directed evolution for the optimization of a circuit or its individual components have increased (*9–14*). An important element of this development is that components evolved in one context may expand the design scope of gene circuits in a different context (*15*). In this issue, Sun and co-workers (*16*) present an elegant study on optimizing positive feedback loops (PFLs) by directly evolving an individual component. The evolved circuits exhibit significantly reduced activation thresholds in response to *N*-(3-oxo-hexanoyl)-homoserine lactone (OHHL), an inducer. The improved response sensitivity of these circuits may be helpful for potential applications in metabolic engineering and gene therapy.

In the first PFL (PFL1) (*16*), upon binding the OHHL inducer molecule and P_{luxI} promoter, the LuxR transcription factor taken from *Vibrio fischeri* is autoactivated by its own expression in *Escherichia coli*, forming the PFL. A *gfpuv* gene upstream of *luxR* acts as a reporter for the circuit dynamics. The second circuit (PFL2) is the same as PFL1, except for the incorporation of a constitutively expressed LuxR. Both wild-type PFL1 and PFL2 exhibit ultrasensitivity in their response to OHHL. OHHL stimulation above a threshold value ($OHHL_{50}$, where the output GFPuv level is at half of its maximum) can elicit a significant amplification of

ABSTRACT Engineering gene circuits with novel functions holds promise for broad applications in biology, engineering, and medicine. Directed evolution complements rational design as an important strategy for optimizing gene circuits and circuit elements.

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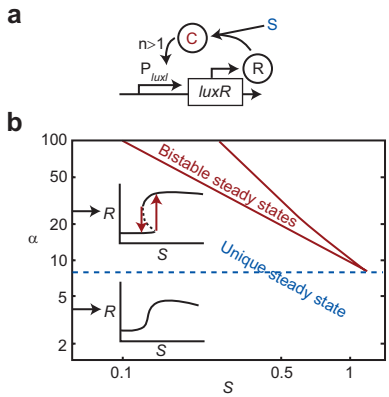

Figure 1. Mathematical modeling suggests the possibility of generating bistable behavior with the starting and evolved positive feedback circuits. a) Schematic of the PFL under the control of a signal OHHL (S). The transcription factor LuxR (R), upon binding with S, forms an activated complex C that forms a homodimer and binds to promoter P_{luxI} to activate LuxR expression, effecting positive feedback. This system can be modeled by two dimensionless equations: $dR/d\tau = \alpha(C^n/1 + C^n)) - \beta R - kSR + \alpha_0$; $dC/d\tau = kSR - C$. Here, *S*, *R*, and *C* in the equations represent concentrations of OHHL, LuxR, and complex C, respectively. Synthesis of R is modeled by a single Hill function, lumping transcription and translation together. The Hill coefficient is set to be 2 to reflect fast dimerization of C and binding of the dimer to P_{luxI}. α is the synthesis rate constant of R due to feedback. α_0 is the basal or constitutive synthesis rate constant of R. β is the degradation rate constant of R. *k* is the binding constant of R with S. To make the model dimensionless, time is scaled with respect to the decay rate constant of C, and concentrations are scaled with respect to the half activation threshold of C. Biologically feasible parameter values are used for bifurcation analysis, with base values of $\beta = 5$, $\alpha_0 = 1$, and $k = 1$. b) Dynamic behaviors of the PFL are determined by α. When α is larger than the critical value (blue dashed line), the system exhibits bistability (upper inset). For each α, the two red lines define the boundaries of the bistable region in terms of *S*.

GFPuv expression. To further improve the sensitivity of these circuits, the authors created a LuxR mutant library by directed evolution. Favorable LuxR mutants with improved circuit behavior were subsequently screened and identified. They hypothesized that the identified LuxR mutants are most likely to increase the binding interaction with OHHL or the promoter P_{luxI}, thereby increasing the strength of the PFL.

PFLs play essential roles in diverse cellular functions, including tissue development, cell fate decision, and long-term memory (*17, 18*). They can result in either graded or "all-or-none" bistable responses to an external cue. For the work by Sun and colleagues (*16*), one may wonder how the evolved parts may impact the overall dynamics. To gain insight, we developed a simple kinetic model to analyze the switching behavior of the systems (Figure 1). When the positive feedback is weak (*i.e.*, the α value is smaller than the value of the blue dashed line, Figure 1, panel b), the circuit can only demonstrate monostable behavior, exemplified by a monotonic dependent "*R vs S*" curve (lower inset, Figure 1, panel b). For sufficiently strong feedback regulation, however, the circuit may demonstrate bistability (see the bistable "*R vs S*" curve, upper inset, Figure 1, panel b).

Bistable gene switches controlled by positive feedback were experimentally examined in a few cellular contexts (*19, 20*). The current data in ref 16, which were measured at the population level, cannot indicate whether the system is bistable. Additional analyses by examining single-cell behavior and detecting the presence of hysteresis are needed to elucidate these interesting dynamics. If this becomes the authors' design goal, further rounds of evolution on LuxR, other circuit components, or the circuit might help.

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