Unraveling dynamics of reconfigurable network motifs using a synthetic biology approach

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***Short Abstract* — Gene networks are commonly studied using the classical paradigm of fixed network topology. In contrast, we create and investigate a new class of reconfigurable gene networks that can be switched between different topology without genetic changes. We show that a feedforward motif can be reconfigured to produce three distinct expression patterns. Furthermore, we demonstrate the motif's capacity to control the ratio between three proteins with only two inducers. Our work will impact the understanding of dynamical genetic networks and create a new class of synthetic systems for biotechnological applications.**

***Keywords* — reconfigurable, network motifs, synthetic gene circuits**

I. Introduction

RECONFIGURABILITY refers to the ability of natural systems to dynamically change their properties, including spatial distribution of cells, cellular structures, and organization of cellular networks. While cells achieve such reconfigurability with relative ease, synthetic biological systems are primarily created and studied using the classical paradigm of engineered systems, in which circuit components are connected through static biochemical wiring [1]. However, natural gene networks are fundamentally reconfigurable and could potentially give rise to versatile, emergent dynamics. Can we take advantage of reconfiguration mechanisms of natural cells to create a new class of reconfigurable synthetic systems? What are the tradeoffs between versatility and fidelity of reconfigurable gene networks?

II. Results and Discussion

A. Motif Search and Mathematical Modeling of A Reconfigurable Network Motif

To start, we used a library of three node networks with interactions represented by Michaelis-Menten kinetics to identify network motifs [2] capable of producing multiple dynamics given a fixed range of inducer concentrations. We found the most robust reconfigurable architectures, and derived the analytical solution of one motif composed of a feedforward loop with competing positive and negative regulations at one of the nodes. The analytical solutions guide our subsequent experimental perturbations, as well as interpretation of experimental results.

B. Experimental Validation and Perturbations of the Reconfigurable Motif

To assemble the motif, we first characterized dynamics of each individual component, incorporating their respective kinetic parameters into our model. Next, based on the mathematical model, we assembled a reconfigurable circuit and quantified its dynamics using both population and single-cell assays. We demonstrated that the circuit is capable of reconfiguring its topology to produce three distinct dynamics: linear, band-pass, and inverter. We further perturbed the circuit by changing promoter strengths, copy number of genes, and levels of catabolite repression. Through the perturbations, we showed that network perturbations control the range of circuit reconfigurability.

C. Exploiting Reconfigurability of the Motif to Control Concentration Ratios of Proteins

The mathematical model predicts an intriguing, novel feature of the reconfigurable circuit, which controls the ratio between three proteins using only two inducers. We first showcased the ratio-control mechanism of the reconfigurable circuit using fluorescent reporters, then enzymes of a metabolic pathway to illustrate the modularity of the system.

III. Conclusion

We present a generic mechanism that can reconfigure dynamic behavior of a feedforward loop in response to an inducer. This motif can be applied to study reconfiguration of natural gene networks, and could shed light on mechanisms of cellular decision making. Our results represent a fundamental shift in how biological networks are understood, moving from a fixed-topology to a flexible-topology paradigm.

References

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