Understanding, Predicting, and Control of Circuit-Host Interactions

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***Short Abstract* — Failure of modularity remains a significant challenge for assembling synthetic gene circuits with tested modules as they often do not function as expected. Hidden circuit-host interactions, such as growth feedback and resource competition, could significantly impair intended circuit function but are often neglected. Here, I will present our recent efforts on the quantitative understanding of how the functional perturbation of gene circuits by these hidden interactions depends on network topology, host physiological environment, and resource competition, together with modeling frameworks for predicting circuit behaviors and practical control strategies for engineering robust gene circuits.**

I. Purpose

Circuit-host interactions, including metabolic burden, cell growth, and resource relocation/competition, affect behaviors of synthetic gene circuits, thereby adding an additional layer of complexity to already intricate gene regulatory networks. These interactions are often neglected in the design of gene circuits by assuming that gene circuits are orthogonal to host backgrounds. In many instances, however, the impacts of circuit-host interactions are significant. Understanding the mechanisms of the effects of these interactions on gene circuit functions will help us to formulate control strategies for engineering robust gene circuits.

II. Preparation of Abstracts

In the first work [1], we revealed a topology-dependent interference of synthetic gene circuit function by growth feedback. Specifically, the memory of the self-activation switch is quickly lost due to the growth-mediated dilution of the circuit products. Decoupling of growth feedback reveals its memory, manifested by its hysteresis property across a broad range of inducer concentrations. On the contrary, the toggle switch is more refractory to growth-mediated dilution and can retrieve its memory after the fast-growth phase. The difference between these two circuits in response to growth feedback lies in network topologies.

In the second work [2], we unveiled a Winner-Takes-All (WTA) resource competition within synthetic gene circuits. We first built a synthetic cascading bistable switches (Syn-CBS) circuit in a single strain with two coupled self-activation modules to achieve two successive cell fate transitions. Interestingly, we found that the in vivo cell fate transition path was redirected as the activation of one switch always prevailed against the other, contrary to the theoretically expected coactivation. This qualitatively different type of resource competition between the two modules follows a WTA rule, where the winner is determined by the relative connection strength between the modules. To decouple the resource competition, we constructed a two-strain circuit, which achieved successive activation and stable coactivation of the two switches.

In the third work [3], we studied how nutrient level modulates growth feedback and circuit functions and found an unexpected damped oscillation behavior of a bistable switch circuit. The underlying mechanism was demonstrated by a mathematical model, which includes ribosome regulation and allocation. Interestingly, we predicted a counterintuitive dependence of oscillation amplitude on the nutrition level, where the highest peak was found in the medium with moderate nutrients, which was verified experimentally.

In the fourth work [4], we proposed an alternative strategy for controlling resource competition using a shared and tunable system of negatively competitive regulation (NCR) system, which punishes transcriptional modules that take up more than their fair share of resources while having minimal effect on modules operating within normal activity ranges. We compare NCR to global/local negative feedback controllers and demonstrate that NCR can significantly increase the efficacy of controlling WTA resource competition.

In the fifth work [5], we uncovered a surprising double-edged role of resource competition in gene expression noise. We also compared three types of negative feedback controllers and found that both local and NCR controllers with mRNA-mediated inhibition are efficacious at reducing noise, with NCR controllers demonstrating a superior noise-reduction capability.

III. Conclusion

In summary, we have found several interesting phenomena induced by circuit-host interactions, developed quantitative mathematical modeling frameworks to simulate these hidden interactions and to accurately predict gene circuits' behavior, and have developed serval practical control strategies that can be applied to other systems.

References

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