Tuning Incoherent Feedforward Circuit Architectures with Feedback to Rapidly Develop a Modular Toolbox

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I. Abstract

A central goal of synthetic biology is to build sophisticated, precise cellular programs that predictably control gene expression. In our joint experimental and computational work, we synthetically built and tuned a gene regulatory motif called the incoherent feedforward loop with additional feedback for precise and predictable control. Additionally, we leveraged its synthetic implementation with designer proteins to rapidly develop a modular toolbox of eight signal processing circuits in *S. cerevisiae*.

The incoherent feedforward loop (I1-FFL) is an over-represented motif in gene regulation, in which an activator molecule regulates both a gene and the repressor of that gene. The I1-FFL motif is a pulse generator since it responds to a step increase in input with a pulse of output gene expression. In our work, we implemented the I1-FFL motif transcriptionally in *S. cerevisiae* with two inducible synthetic transcription factors, GEM and SynTF, and the previously published de novo protein degronLOCKR system for repression.

While the I1-FFL motif has been hypothesized to return to basal activity after a pulse, a property known as adaptation, our transcriptional implementation did not. Our theoretical work demonstrated that nonlinearities inherent in transcription compounded to prevent adaptation, but that the addition of negative feedback would restore it. Furthermore, we proved that the addition of positive feedback would improve the amplitude of the pulse. We demonstrated these results experimentally by tuning the dynamics of four I1-FFL synthetic circuits via the addition of positive and/or negative feedback.

Our combined work demonstrated the versatile role that layered feedback can play in changing the dynamic properties of systems. Through our joint experimental and computational efforts, we tuned the properties of a gene expression pulse produced by the transcriptional I1-FFL using positive and/or negative feedback with predictable control. By constructing our circuit designs with de novo proteins, we rapidly composed them together into a toolbox of eight circuits with signal processing capabilities - four pulse generators and four concentration filters. Our toolbox of synthetic circuits facilitated non-monotonic control over gene expression dynamically and at steady state. Our computational pipeline of validated mathematical models and Bayesian inference algorithms facilitated the inference and prediction of the eight composed circuits from flow cytometry data.

II. Conclusion

We synthetically built a transcriptional pulse generator circuit and tuned its properties with feedback. To our original pulse generator circuit, we added feedback loops to create a library of eight composed circuits for signal processing - four pulse generators and four concentration filters. The impact of this work is to prove for the first time that we can build a library of robust, modular circuits by composing layers of biological feedback.

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