Competitive Incoherent Feed-Forward Control of Winner-Take-All Resource Competition

Austin Stone[1], Jordan Ryan[2], Xun Tang[2] and Xiao-Jun Tian[1,*]

Abstract — **Resource competition (RC) is the phenomenon where genes in circuits compete over limited resources for expression; this impacts the forward-engineerability of gene constructs by introducing indirect couplings between otherwise orthogonal genes, which may have deleterious effects on circuit function. Previously, we proposed a novel negative feedback topology that utilized synthetically introduced "negatively competitive behavior" via repressive CRISPRi moieties to combat resource competitive effects, which we dubbed a negatively competitive regulatory (NCR) controller. Here we extend our analysis of NCR-type controller systems to the incoherent feed-forward loop (IFFL) network motif and demonstrate NCR IFFL controllers can not only efficaciously combat resource-competitive effects, but also outperform more traditional controller architectures such as global and local IFFL controllers.**

Keywords — **Synthetic biology, computational biology, resource competition**

I. Background

RECENT years have seen an increasing emphasis in synthetic biology to view synthetic gene constructs in the context of their environment rather than stand-alone frameworks. Context-dependent effects have been demonstrated to deviate circuit behavior from intended function through a variety of modalities, including resource competition (RC) and growth feedback, and potentially result in complete collapse of circuit function [1-3]. RC is the phenomenon wherein limited transcriptional/translational resources are available to a circuit, and consequently genes in the circuit are forced to compete for expression. This competition introduces indirect repressive links between otherwise orthogonal genes, as the increase in expression in one gene leads to a deprivation of resources for other genes.

Previously, our lab proposed a novel regulatory schema that used competition to fight against competition, which we coined a negatively competitive regulator (NCR) [4]. In our design, each gene in the network expresses a guide RNA (sgRNA) which repressively targets its own promoter. sgRNAs from individual genes are required to compete over a limited resource, dCas9, to initiate repression. In this fashion, higher gene expression will not only be dampened by the negative feedback from its sgRNA, but its overproduction of sgRNA will also pull dCas9 away from the lower active modules, conferring them an additional benefit.

Here we extend the analysis of NCR-type controller systems to IFFL motifs. We demonstrate that NCR IFFL controllers outperform more traditional control architectures such as global and local IFFL controllers.

II. Results

A. Steady-state & parameter sensitivity analysis of coactivation fraction

Applying our controllers to a system with two orthogonal bistable switches in the presence of RC, we find that limited resources impede the system's ability to sustain both switches in the on state simultaneously. We find that NCR and local, but not global, IFFL controllers can re-expand the portion of the parameter space for which the switches can be coactivated within biologically relevant parameter space. We also find NCR's ability to outperform local controllers is strictly linked to the level of competition over the synthetic resource (dCas9) mediating NCR.

B. Saddle node shift

We discover that IFFL controllers have a unique property that negative feedback controllers do not, which we have dubbed the "saddle-node shift" (SNS) effect. The SNS effects result from connections between a controller for one node and the switch for the contralateral node. NCR IFFL topologies demonstrate an interesting negative SNS that other controller systems do not. We also discover that taking resource consumption of the controller into account adds a predictable positive SNS regardless of the system.

C. Construction of orthogonal switches in presence of RC

We find that an almost perfectly orthogonal system of switches can be generated even in the presence of RC by coupling the NCR IFFL controller with an adequate level of controller resource consumption to offset the negative SNS of NCR-type systems.

References

[1] Qian, Y., Huang, H. H., Jiménez, J. I., & del Vecchio, D. (2017). Resource Competition Shapes the Response of Genetic Circuits. *ACS Synth. Biol., 6, 7, 1263.*

[2] Zhang, R., et al. (2020). Topology-dependent interference of synthetic gene circuit function by growth feedback. *Nat. Chem. Biol. 16, 695.*

[3] Zhang, R., et al. (2021). Winner-takes-all resource competition redirects cascading cell fate transitions. *Nat. Comm., 12, 853.*

[4] Stone, A., Zhang, R., & Tian, X.-J. (2021). Coupling Shared and Tunable Negative Competition Against Winner-Take-All Resource Competition Via CRISPRi Moieties. *American Control Conference, 1882-1882.*

This project was supported by NSF grant 2143229 and NIH grant R35GM142896. A.S. was also supported by the Arizona State University Dean"s Fellowship.

[1] School of Biological and Health Systems Engineering Arizona State University. E-mail: xiaojun.tian@asu.edu

[2] Cain Department of Chemical Engineering, Louisiana State University, Baton Rouge.

[*]Corresponding Author