Understanding and Mitigating the Effects of Limited Transcriptional Resources in Mammalian Cells

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Mammalian synthetic biologists seek to design molecular circuits with many potential therapeutic and biotechnology applications. Developing these often complex devices requires being able to reliably compose multiple modular regulatory modules in varying cellular contexts. However, predicting module behavior can be difficult due to "hidden" or unpredictable interactions between circuit parts. Parts competing for limiting cellular factors can be a significant contributor to these hidden interactions. In eukaryotes, *trans*-activation domains of transcription factors can bind and sequester co-activator proteins, causing effective repression of otherwise active promoters in a phenomenon known as "squelching" [1, 2]. Since many synthetic mammalian circuits are based on transcriptional networks, understanding and mitigating the effects of squelching will allow for the design of more reliable and predictable circuitry for many applications.

To understand squelching, we built an extended model of transcription, which includes a pool of conserved general co-activator resources required for transcription initiation at each promoter. We then experimentally characterized the degree of competition between commonly used promoter and activator parts to parameterize the effective repression due to resource competition in the model. The experimental results show that promoters and activators can both compete with each other and among themselves to a significant degree. Further, we have shown the dose-dependence of titrating in competitors for select combinations of promoters and activators.

To mitigate the effects of squelching, we designed a novel phosphorylation-based feedback module, which aims to buffer against resource competition within reasonable competitor input regimes. The circuit is composed of a kinase input that phosphorylates a transcription factor, allowing it to bind DNA and initiate transcription of a target promoter. A phosphatase is co-expressed with the output, and de-phosphorylates the transcription factor. The feedback works on the principle of pseudo-integral feedback, which theoretically allows the target protein concentration to be independent of resource availability. Simulation results show that this feedback module works for buffering out resource competition effects, and we are in the process of experimental characterization. The module parts are implemented with *E. coli* two-component signaling histidine kinases and phosphatases, which have been shown to work in mammalian cells when the response regulator is fused to a eukaryotic activation domain such as VP16 [3]. We have applied conserved mutations [4] and domain rearrangements [5, 6] to convert bifunctional histidine kinases to variants with only kinase or phosphatase activity, rather than both. We have verified that these variants work as predicted in HEK 293FT cells, and have achieved up to 400-fold changes in promoter output between when a phosphatase or kinase variant are present. We are currently pursuing experiments to determine under what regimes the feedback is effective in buffering against resource competition.

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