Predictive Control of an Engineered Optogenetic Signaling Pathway in *E. coli*

Evan J. Olson[1], Raghav Shroff[2], Andrew Wu[2], and Jeffrey J. Tabor[2]

***Short Abstract* — We have exploited the millisecond-time scale photoreversbility of the cyanobacterial CcaS/CcaR two component system to achieve precise, quantitative control of gene expression levels in real-time in *Escherichia coli*. We have constructed a programmable array of light emitting diodes that allows dosing of light of different colors in up to 64 standard tubes of growing cells. By shining mixtures of activating and deactivating light in a time-varying sequence, we can drive cells to move between desired analog expression levels. Finally, we have constructed a phenomenological model of this system, allowing for prediction of light control sequences that will generate a desired gene expression time-course.**

***Keywords* — Synthetic Biology, Optogenetics, Predicitive Modeling, Optimal Control**

I. Background

Signal transduction networks underlie many cellular decision making processes. Among the simplest of these networks are two component systems (TCSs), which comprise a signal-sensing histidine kinase protein and response regulator protein with which it interacts. When the sensor kinase detects signal it phosphorylates the response regulator which then typically binds to DNA. DNA binding modulates the transcription of one or more target genes, providing a means for the cell to respond to its surroundings. The relative simplicity of TCSs makes them ideal targets for synthetic control of cellular behavior. Previously, the EnvZ/OmpR and CcaS/CcaR TCSs have been engineered to reprogram E. coli to sense the color and intensity of light in the environment [1,2].

II. Methodology

Here, we have exploited the millisecond-time scale photoreversbility of the CcaS phytochrome to dramatically reduce the gene expression response time, and to achieve precise, quantitative control of gene expression levels in real-time. First, we have constructed a programmable array of light emitting diodes (LEDs) that allows the quantitative dosing of different colors of light in any desired temporal pattern in up to 64 standard tubes of growing cells. We demonstrate that the application of different activating to inactivating light ratios allows us to set a desired analog gene expression level. By shining light ratios in a time-varying sequence, we can then drive cells to move between desired analog expression levels without adjusting the growth media. Gene expression is reported by GFP fluorescence and single-cell data is acquired from each culture via flow cytometry.

III. Results

Our observations of cells grown in a variety of time-varying light sequences are well described by a five-parameter two-state promoter model including asymmetric k_{On} and k_{Off} timescales, the transcription rate of both activated and basal promoter states k_H and k_L, and finally the growth rate of the culture. This model of the light input and gene expression output of this system allows for prediction of light control sequences that will generate a desired gene expression time-course.

IV. Conclusion

This level of quantitative, temporal control of gene expression would be extremely difficult to achieve with traditional modes of gene regulation. As it has in neurobiology, the precise perturbative nature of optogenetic tools therefore stands to contribute significantly to systems and synthetic biology [3].

References

[1] Levskaya A, et al. (2005) Engineering Escherichia coli to see light. *Nature* **438**, 441-442.

[2] Tabor JJ, Levskaya A, Voigt, CA (2011) Multichromatic control of gene expression in Escherichia coli. *J Mol Biol*. **405**, 315-324.

[3] Toettcher JE, Voigt CA, Weiner OD, Lim WA (2011) The promise of optogenetics in cell biology: interrogating molecular circuits in space and time. *Nature Methods* **8**, 35-38.

[1]Applied Physics Graduate Program, Rice University, Houston, TX. E-mail: ejo1@rice.edu

[2]Department of Bioengineering, Rice University, Houston, TX. E-mail: jeff.tabor@rice.edu