

Real-time optogenetic control of intracellular protein concentration in microbial cell cultures

Justin Melendez¹, Benjamin Oakes¹, Michael Patel¹, Ping Xu¹, Patrick Morton¹, Marcus Noyes¹ and Megan N McClean¹

Short Abstract — The ability to finely control the concentration of a desired protein in cultures of cells would be transformative for applications in research and biotechnology. We developed an optogenetic induction system and culturing apparatus, which in combination with feedback control, allows us to generate defined perturbations in the intracellular concentration of a specific protein in microbial cell culture. We demonstrate the potential for this technology by generating fixed and time-varying concentrations of a specific protein in continuous steady-state cultures of *Saccharomyces cerevisiae*. This technology will allow for quantitative studies of biological networks as well as external tuning of synthetic circuits.

Keywords — Control, Signal Processing, Optogenetics, Chemostat, Microfluidics, Microscopy

I. OVERVIEW

PROPERTIES of biological signaling and regulatory networks can be inferred from studying their responses to perturbation. Phenotypes resulting from static network perturbations, such as protein deletion, can reveal essential network connections. Time varying perturbations are more informative for studying network dynamics [1-2]. Indeed, a carefully chosen perturbation scheme can distinguish relatively similar network models based on network output [3].

While experimentalists have at their disposal a wealth of technologies for measuring biological responses to perturbation, the technology for making appropriate informative perturbations has lagged behind. Typical experiments perturb the biological network with a sudden step increase in ligand and assay downstream responses [4]. Such static “step-shock” inputs stimulate the biological network at the receptor level, leaving many unprobed signaling steps between input and output. Microfluidic technology allows for the generation of time-varying perturbations, however recovery of cell material from these devices is difficult, limiting the assay of network response to imaging of fluorescent reporters [5]. Recent optogenetic methods for controlling protein-protein interactions and protein concentration also utilize microfluidic or small culture volumes and thus suffer similar limitations [5-7]. An

ideal experimental tool would allow for defined perturbations in the concentration of an arbitrary protein in large numbers of cells at physiological steady-state, so that enough material could be sampled to simultaneously take advantage of multiple existing measurement technologies.

In this study, we address the limitations in existing methods by integrating an optogenetic induction system, a cell culturing and microfluidic imaging apparatus and feedback control to generate programmed static or time-varying perturbations in the intracellular concentration of a specific protein in a culture of microbes. Using an optogenetic induction system, gene expression is controlled by pulses of light leading to increased concentration of the protein of interest. Microbial culture is maintained in the culturing device, and samples of cells are rapidly removed from the culture for automated protein quantification in a microfluidic device. A feedback scheme is used to achieve the user-defined protein concentration. We demonstrate the utility of this system by both clamping protein concentration at different levels and generating oscillations in protein concentration in continuous cultures of the model organism *Saccharomyces cerevisiae*. The availability of this device will be a great asset to quantitative modelers and bioengineers seeking to perturb and control biological networks.

REFERENCES

- [1] Hersen, P, McClean, MN, Mahadevan, L, Ramanathan, S. (2008) Signal processing by the HOG MAP kinase pathway *PNAS* **105**, 7165–7170
- [2] Lipan, O, Wong, WH (2005) The use of oscillatory signals in the study of genetic networks *PNAS* **102** 7063–7068
- [3] Apgar, J, *et al* (2008) Stimulus design for model selection and validation in cell signaling *Plos Comput Biol* **4** e30
- [4] Vance, W, Arkin, A, Ross, J (2002) Determination of causal connectivities of species in reaction networks *PNAS* **99** 5816-5821
- [5] Uhlendorf, J, Bottani, S, Batt, G, Hersen, P (2012) Long-term model predictive control of gene expression at the population and single-cell levels *PNAS* **109** 142710-14276
- [6] Miliias-Argeitis, A *et al* (2011) *In silico* feedback for *in vivo* regulation of gene expression circuit *Nature Biotechnology* **29** 1114-1116
- [7] Toettcher, JE, Voigt, C, Weiner, O, Lim, W (2011) The promise of optogenetics in cell biology: interrogating molecular circuits in space and time *Nature Methods* **8** 35-38

Acknowledgements: This work was funded by a Lewis-Sigler Fellowship to M. McClean.

¹Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton NJ 08544 USA. Email: mmcclean@princeton.edu