Measuring transcription from a single gene copy in live *Escherichia coli* cells

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***Short Abstract* — Single-cell measurements of transcriptional activity inform our understanding of stochastic gene expression, but these measurements coarse-grain over the individual copies of the gene, where transcription and its regulation stochastically take place. In this study, we follow the transcriptional activity of individual gene copies in live *E. coli* cells, by labeling the gene locus of interest and measuring its transcriptional activity at the same time.**

***Keywords* — Transcription kinetics, individual gene copies, two-color labeling, live-cell imaging.**

Gene expression and its regulation are traditionally studied by averaging over large cell populations. These "bulk" measurements mask the heterogeneity in gene expression between individual cells. Single-cell measurements have informed us on the inherent stochasticity of the reactions involved in gene regulation [1]. However, the whole-cell measurement is still limited in many ways: i) It typically represents the summation over the contributions from multiple copies of the same gene, each of which is independently regulated, and whose number doubles during the cell cycle [2, 3]; ii) It fails to distinguish RNA molecules that are being transcribed from those already completed.

In this study, we set to follow the active transcription of an individual gene copy within an individual *E. coli* cell in real-time. We hypothesized that active transcription can be quantified by measuring the amount of RNA that is localized to the transcribed gene [4]. We therefore developed a two-color labeling system to simultaneously mark the gene locus and the RNA produced from the gene. The gene locus was labeled using the Fluorescent Repressor Operator System (FROS), which combines fluorescently-tagged DNA binding proteins with a synthetic array of cognate binding sites [5]. The RNA produced from the gene was tracked using an analogous method, where a fluorescently-tagged MS2 bacteriophage coat protein labels an array of MS2 binding sites [6]. By taking time-lapse movies, we are able to track the transcriptional activity of a single gene copy over multiple cell generations. Using automated image analysis, we can directly measure the kinetic parameters of transcription (e.g., the probabilistic rate of promoter state switching). Moreover, this method allows us to assess the dependence of gene activity on the instantaneous activity of other copies of the same gene in the same cell, and on the event of gene replication during the cell cycle.

Acknowledgements: This work was partially supported by the Center for Theoretical Biological Physics sponsored by the National Science Foundation (PHY-1427654). This research was also funded by the National Science Foundation (PHY-1430124 and PHY-1147498), National Institute of Health (R01 GM082837), Welch Foundation (Q-1759) and John S. Dunn Foundation.

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