Experimental Validation of Differences in Robustness between Activation and Repression Based Transcriptional Regulation

by

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Abstract
Transcription control is a fundamental process in biology, coordinating and governing animal developments through transcriptional cascades. However, little attention has been given to the principle constituents, events of activation and repression. Rappaport et al. proposed a mathematical model which has not been experimentally tested, claiming an advantage in repression over activation in conferring robust control on timing of response. My work aimed to validate their hypothesis in vivo. To this end, I designed and built synthetic genetic constructs to mimic their stated conditions, using the nuclease deficient CRISPR/Cas system. The optimization process revealed configurations that confer strong repression stability. Two inducible, single-layer activation and repression circuits were constructed. The activation circuit was functional, but its performance could not meet the conditions necessary for a fair comparison. The repression circuit was subjected to a time-lapse image analysis in a microfluidic device. Perturbations were introduced, and the response delays of the circuit were measured. The results and analysis will be presented and discussed in this thesis.