Defining the Roles of Regulatory Elements and Gene Syntax in Mammalian Gene Circuit Design

by

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Abstract

Synthetic gene circuits have the potential to program sophisticated dynamic cell behaviors in application-relevant contexts, such as cell-based therapies. However, many gene circuits have not been implemented beyond the lab scale due to unpredictable and heterogeneous performance in integrated contexts. Variation in copy number, locus of integration, and neighboring genes can introduce transcriptional noise and unintended biophysical coupling that undermine the function of the circuit. As a result, monoclonal selection is often used to generate integrated cell lines with homogeneous performance despite this method not being tractable for application-relevant cells, such as primary cells and stem cells. Towards defining gene circuit design rules that enable robust performance beyond the lab scale, my thesis work addresses two key knowledge gaps: (1) how sequences of synthetic genetic parts impact expression across cell types and expression contexts and (2) how existing gene circuits can be optimized for compact cargo delivery and improved polyclonal performance.

Leveraging long-read RNA sequencing and flow cytometry-based RNA-FISH (Flow-FISH), I systematically interrogated how promoter strength, polyadenylation signal, and coding sequence shape mRNA abundance and translation efficiency. Most notably, I discovered that stronger promoters not only produce higher levels of mRNA but also correlate with higher effective translation rates. This effect is not attributed solely to the 5' UTR associated with the promoter, and similar trends are observed even when paired with other UTR sequences. Therefore, synthetic promoters do not solely tune transcription rate, but also have significant impacts on mRNA processing and transport.

Moving beyond previous work on compact design of inducible gene circuits, I apply these insights to improve the performance of a bistable gene circuit, a complex therapeutically-relevant genetic controller that has not been translated beyond immortalized cell lines. Guided by computational modeling, I identified a circuit design that reduces genetic cargo size by over 80% while maintaining balanced performance comparable to the original implementation. Moving to single-cargo integration, I found that gene syntax (the relative order and orientation of genes) significantly impacts cell-state commitment through biophysical coupling. Finally, I established that modRNA delivery can be used to program precise ratios of cell states in a polyclonal population.

Overall, this work increases our understanding of how to achieve precise gene expression and compose multiple genes for predictable and scalable control. Extending this design framework to new genetic tools and controllers will contribute to improved reliability and safety of engineered cell therapies across cell types.

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