A Synthetic Gene Circuit with Tunable Expression Level and Dosage Compensation for Mammalian Cells

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***Short Abstract* — The ever-expanding synthetic biology toolbox has seen the fast development of synthetic gene circuits due to their ability to implement diverse functions in living cells. Tunable gene circuits have been given particular attention because they enable the implementation of different output states in response to user-defined input signals through a single circuit. Previous studies have optimized the tunability by achieving linear input-output relationship [1]. The circuit, however, shows compromised tunability and substantial heterogeneity among cells under transient plasmid transfection. Transient plasmid transfection or viral transduction without genomic integration usually results in a broad distribution of DNA copy number, and cells with higher DNA copy number tend to have undesirable overexpression, leading to cytotoxicity and/or impaired transgene function. Combining experiments with computational simulations and mathematical analysis, we developed a second-generation linearly tunable circuit that applies to a broader audience with robust dosage compensation capacity to achieve homogeneous expression across individual cells and better protein localization.**

***Keywords* — synthetic biology, tunable circuit, dosage compensation, expression level, linearizer, localization**

I. Background

TUNABLE circuits are widely applied because they enable the regulation of output through an external input signal. The toggle switch circuit is one of the most well-known tunable circuits with the capability of switching between ON and OFF states depending on whether the input exceeds the activation threshold. The tunability of the toggle switch is low because the output is all or none, and a ON-state optimized for one application may not be applicable for another, so much effort is often required to adapt the circuit for different applications. In contrast, continuously tunable circuit is more user-friendly and adaptable as one can obtain a continuous spectrum of output simply by varying the input intensity. A linear input-output relationship can further improve the tunability by avoiding regions of steep change in output with little change in input level or vice versa. A synthetic circuit named the "linearizer" meets these design criteria [1]. However, the linearizer circuit showed compromised tunability and dosage compensation when transiently transfected (see Results). Therefore, we engineered a new version called Linearizer 2.0 with more robust linear tunability and improved dosage compensation.

There is a broad application for this novel gene circuit. The state-of-the-art gene delivery using viral vector in gene therapy or other applications often results in a non-homogenous expression profile as a function of distance from the injection site: cells closer to the injection site have higher expression level, while expression level decreases further away from the injection site [2]. These variations in expression level are typically undesirable, yielding many cells that exhibit either inadequate or excessive transgene expression. The Linearizer 2.0 circuit can be tuned to achieve the best balance between maximal expression and minimal cytotoxicity. We also demonstrate the potential of using this circuit to improve localization of membrane proteins that formed intracellular aggregation when overexpressed.

II. Results

A. Linearizer 1.0 improves the localization of membrane proteins when genome integrated but showed reduced tunability and no dosage compensation in transient transfection

We observed that chromosomally-integrated Linearizer 1.0 can be tuned to obtain improve plasma membrane localization of multiple membrane proteins including natural and light-gated ion channels. However, transient transfection of the same circuit produced large cell-to-cell variation and compromised tunability.

B. Development of Linearizer 2.0

Combining experiment results with computational simulations and mathematical analysis, we engineered an improved circuit design, Linearizer 2.0, that retains its linear tunability while achieving dosage compensation by combining negative feedback and incoherent feedforward loops. We will present the performance of this circuit under various expression contexts including genomic integration, transient transfection, and viral transduction.

III. Conclusion

Linearizer 2.0 is a versatile synthetic circuit for tunable expression level and dosage compensation. We anticipate it will be of broad utility in applications that benefit from tunable expression levels and reduced cell-to-cell variation.

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