

Engineering synthetic killer circuits in bacteria

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Abstract

I will discuss our efforts to engineer suicidal bacteria, by using cell-cell communication to regulate cell killing. Lessons learned from these studies may provide insights into precise programming of bacterial dynamics for diverse applications.

Categories and Subject Descriptors

B.6 [Logic design]; J.6 [Computer-aided engineering]

General Terms

Design, Theory, Reliability, Performance

Keywords: Synthetic biology, Gene circuits, Mathematical modeling.

Synthetic biology, which often entails *de novo* engineering of gene circuits with well-defined function, has shown great potential to impact diverse areas. One appealing application is the reprogramming of bacteria as therapeutic agents, for example, to deliver drugs or to selectively kill tumor cells. To realize this goal, however, we must be able to precisely control bacterial dynamics, including growth, death, and aggregation, under diverse conditions. Combining modeling and experiment, my laboratory has been exploring design strategies to achieve such control by engineering a series of synthetic killer circuits in bacterium *Escherichia coli*. Here I will discuss our recent progress in this direction, focusing on several examples.

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As a prototype example, we have designed and experimentally implemented a population control circuit. Built upon a negative feedback loop, this circuit will lead to autonomously regulated population density. We have further measured the population control circuit dynamics in a microchemostat, a continuously operated microfluidics bioreactor with a reaction volume of 16nL. This device has enabled us to culture and monitor extremely small bacterial populations ($\sim 10^2$ - 10^4 cells) with single-cell resolution for long periods of time (>500hrs). By limiting the population size, the microchemostat provides a fundamental advantage over macroscopic bacterial culturing systems by drastically suppressing the total mutation rate. These experimental observations can be captured by a simple mathematical model that accounts for gene expression and population dynamics. Successful construction of this circuit demonstrates the feasibility of constructing reliable self-regulatory circuits at the population level.

Extending the concept of coupling cell-cell communication with cell survival, we have engineered a *synthetic ecosystem* consisting of two types of *E. coli* populations that communicate through quorum sensing and mutually regulate each other's gene expression and survival. It resembles well-studied predator-prey systems in terms of basic logic and dynamics. Mathematical modeling has predicted diverse types of population dynamics, including extinction of one population, predator-prey co-existence with a steady state, or population oscillations, depending on circuit parameters. These projections have been confirmed by long-term culturing of the system in an improved microchemostat. Approaches such as this, will allow

us to explore the dynamics of interacting populations in well-defined experimental frameworks.

An intrinsic challenge for engineering synthetic killer circuits is to deal with mutations. By definition, these circuits will kill the bacteria under certain conditions and thus create selection pressure to allow mutants to take over. Indeed, we frequently observe this phenomenon when characterizing these circuits. For instance, for the population control circuit, mutants would become dominant between 20 and 150 hrs in the macroscopic cultures (3ml to 50ml), depending growth conditions and cell strains. This phenomenon is a critical limitation for our efforts to engineer therapeutic bacteria, as they may pose threat to human health and the environment. To address this issue, we are developing basic understanding of mutational dynamics in synthetic gene circuits. A central hypothesis is as follows: there exists a tradeoff between the *killing efficiency* and the *genetic stability* of each synthetic killer circuit. Our goal is to quantitatively define the tradeoff by modeling and experiments. Based on this understanding, we are also exploring circuit designs to modulate the tradeoff between killing efficiency and circuit stability as a major strategy to improve circuit performance. Use of synthetic killer circuits is the key for quantitatively defining the tradeoff characteristics in controlling bacterial population dynamics. Their implementation, testing, and refinement in well-defined conditions will provide fundamental insights into precise containment of engineered bacteria for therapeutic or environmental applications.

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