# Quorum-sensing crosstalk driven synthetic circuits: from unimodality to trimodality

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***Short Abstract* — Quorum-sensing (QS) enables bacteria to communicate and plays a critical role in controlling bacterial virulence. However, effects of promiscuous QS crosstalk remain unexplored. Here we systematically studied the crosstalk between LuxR/LuxI and LasR/LasI systems and found that QS crosstalk can be dissected into signal crosstalk and promoter crosstalk. Investigations using synthetic positive feedback circuits revealed that signal crosstalk decreases circuit's bistable potential. Promoter crosstalk, however, reproducibly generates complex trimodal responses resulting from noise-induced state transitions and host-circuit interactions. A mathematical model that integrates nonlinearity, stochasticity, and host-circuit interactions was developed, and its predictions of conditions for trimodality were verified experimentally.**

***Keywords* — Quorum-sensing, synthetic circuits, noise, host-circuit interactions.**

Quorum-sensing (QS) is a ubiquitous mechanism in nature, and its regulator-autoinducer pairs, such as LuxR/LuxI and LasR/LasI, have been used in synthetic biology for a wide range of applications [1]–[3]. However, evolutionary pressures from limited resources in a competitive environment promote promiscuous bacterial communication, which takes the form of either different genera of bacteria producing the same types of autoinducers or non-specific regulator-autoinducer binding [4]. As a result, QS regulator-autoinducer pairs are not orthogonal, and there is crosstalk between them. Dissecting the crosstalk is critical for unraveling the underlying principles of bacterial decision-making and survival strategies for both natural and synthetic systems.

In this work [5], we used synthetic biology approaches to dissect QS crosstalk between LuxR/I and LasR/I. By applying engineering principles to construct modular gene networks, we were able to characterize and categorize QS crosstalk into signal crosstalk, where LuxR binds with the non-naturally paired C12 to activate pLux, and promoter crosstalk, where LasR binds with C12 to activate non-naturally paired pLux.

When signal crosstalk is constructed and tested in the context of positive feedback, our results showed a significant shrinkage of the bistable region. On the other hand, promoter crosstalk caused complex ***trimodal*** responses when embedded within a positive feedback circuit. This can only be explained when network bistability, gene expression stochasticity, and genetic mutations are all taken into consideration. We computationally predicted and experimentally verified that the C12-LasR-pLux positive feedback circuit could drive the formation of three subpopulations from an isogenic initial culture: one population expressing high GFP expression, the second showing basal GFP expression, and the third population with no GFP expression. The high and low GFP states are the result of positive feedback enabled bistability and gene expression stochasticity-induced random state transitions.

The third non-GFP population is the result of genetic mutation from IS10 insertion. From an engineer's perspective, the mutation stands in contrast to previously reported host-circuit interactions, which are primarily related to resource limitation and resulting growth defects [6]. Here we were able to illustrate that both the components used and the topology of the network constructed could contribute to resource independent host-circuit interactions. This concept of combining nonlinear dynamics and host-circuit interactions to enrich population diversity expands our understanding of mechanisms contributing to cell-cell variability, and suggests new directions in engineering gene networks to utilize hybrid factors.

Taken together, our studies not only showcase living cells' amazing complexity and the difficulty in the refining of engineered biological systems, but they also reveal an overlooked mechanism by which multimodality arises from the combination of an engineered gene circuit and host-circuit interactions.

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