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

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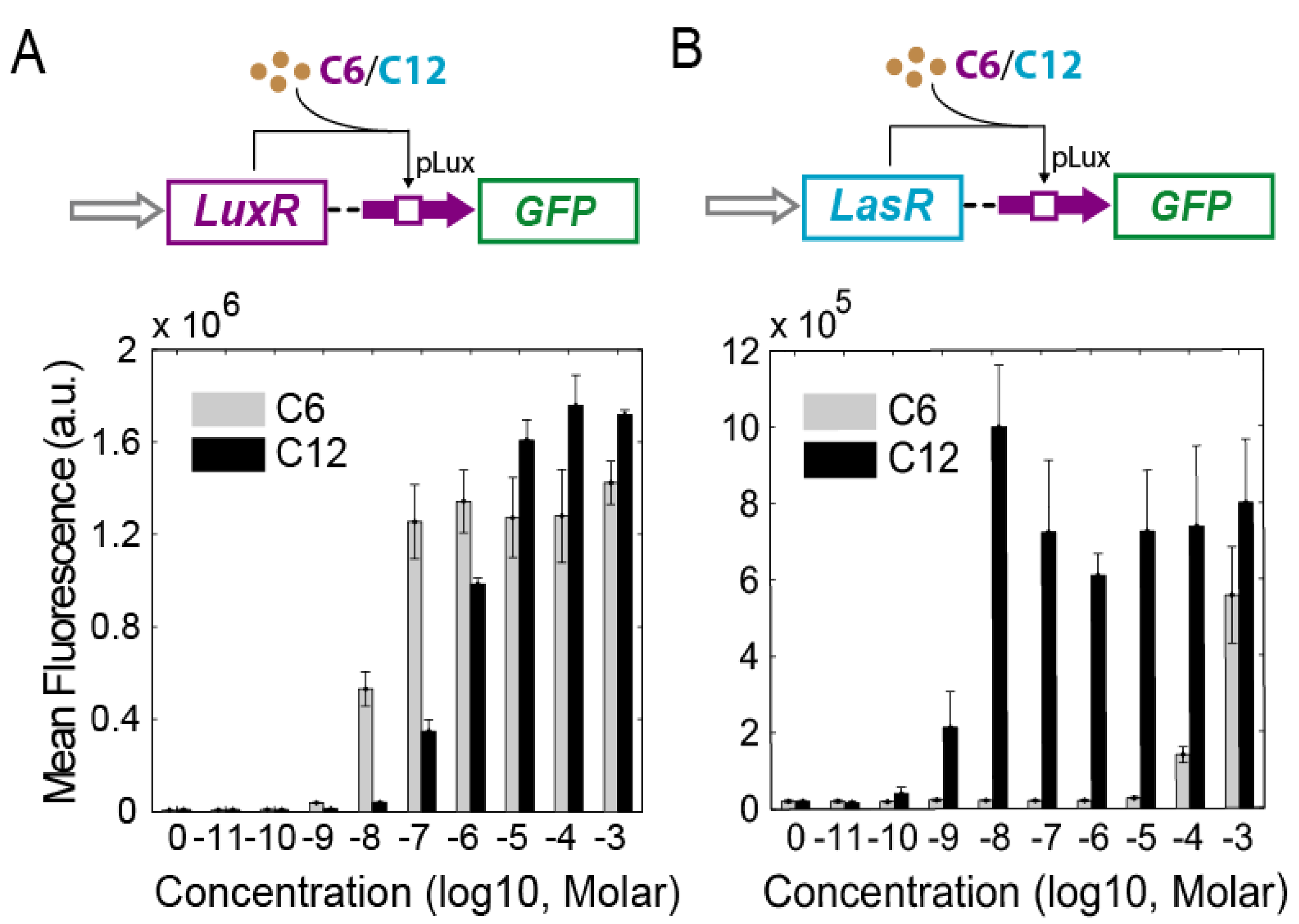
Abstract

Widespread quorum-sensing (QS) enables bacteria to communicate and plays a critical role in controlling bacterial virulence. However, effects of promiscuous QS crosstalk, functional interactions between QS components that are not naturally paired, remain unexplored. Such crosstalk is prevalent and evolutionarily conserved, and yet its implications for gene regulation and cell decision-making are still largely unknown. Here we systematically studied the crosstalk between LuxR/I and LasR/I systems and found that QS crosstalk can be dissected into signal crosstalk and promoter crosstalk. Further investigations using synthetic positive feedback circuits revealed signal crosstalk significantly decreases circuit's bistable potential while maintaining unimodality. Promoter crosstalk, however, reproducibly generates complex trimodal responses resulting from noise-induced state transitions and host-circuit interactions. A mathematical model that integrates the circuit's nonlinearity, stochasticity, and host-circuit interactions was developed, and its predictions of conditions for trimodality were verified experimentally. Combining synthetic biology and mathematical modeling, this work sheds light on the complex behaviors emerging from QS crosstalk, which could be exploited to disrupt or utilize QS for therapeutics and biotechnology.



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Summarization of the crosstalk between LuxR/I and LasR/I signal systems.

	LuxR-3OC6HSL	LuxR-3OC12HSL	LasR-3OC6HSL	LasR-3OC12HSL	LuxR-LuxI	LuxR-LasI	LasR-LuxI	LasR-LasI
pLux	+	+	–*	+	+	+	–	+
pLas	–	–	–*	+	–	–	–	+

1: Black "+" indicates the original pairs; red "+" signifies pairs showing crosstalk.
2: "*" indicates LasR could only activate the promoter at high 3OC6HSL concentration.

Fig.1 Dissecting QS crosstalk between LuxR/I and LasR/I systems: Signal crosstalk and promoter crosstalk.

Materials and Methods

Circuit Assembling

All the individual components including genes, promoters, ribosom binding site, terminators were from iGEM Registry (www.parts.igem.org). Standard molecular cloning and BioBrick assembly method were employed to construct all the genetic circuits and devices.

Hysteresis Experiment

For OFF→ON experiments, initially uninduced cells (OD600~0.1) were distributed evenly into new tubes and induced with various amounts of autoinducer. For ON→OFF experiments, initially induced cells were Cells were collected, washed, resuspended with fresh medium, and inoculated into fresh medium with varying inducer concentrations. Flow cytometry analyses were performed at 6, 12, and 21 hours to monitor the fluorescence levels.

Microfludics, Fluorescence Microscopy, and Image Processing

The use of microfluidic devices coupled with fluorescence measurement allowed us to measure gene network dynamics in single cells. The chip temperature was maintained with an external microscope stage. Images were taken every 5 minutes for about 28 hours in total. The pixels in all images are normalized to 0 – 1 range before analysis.

Mathematical Modeling

Ordinary differential equation models were solved and analyzed by MATLAB. Stochastic simulations were written in MATLAB and run on a standard personal computer.

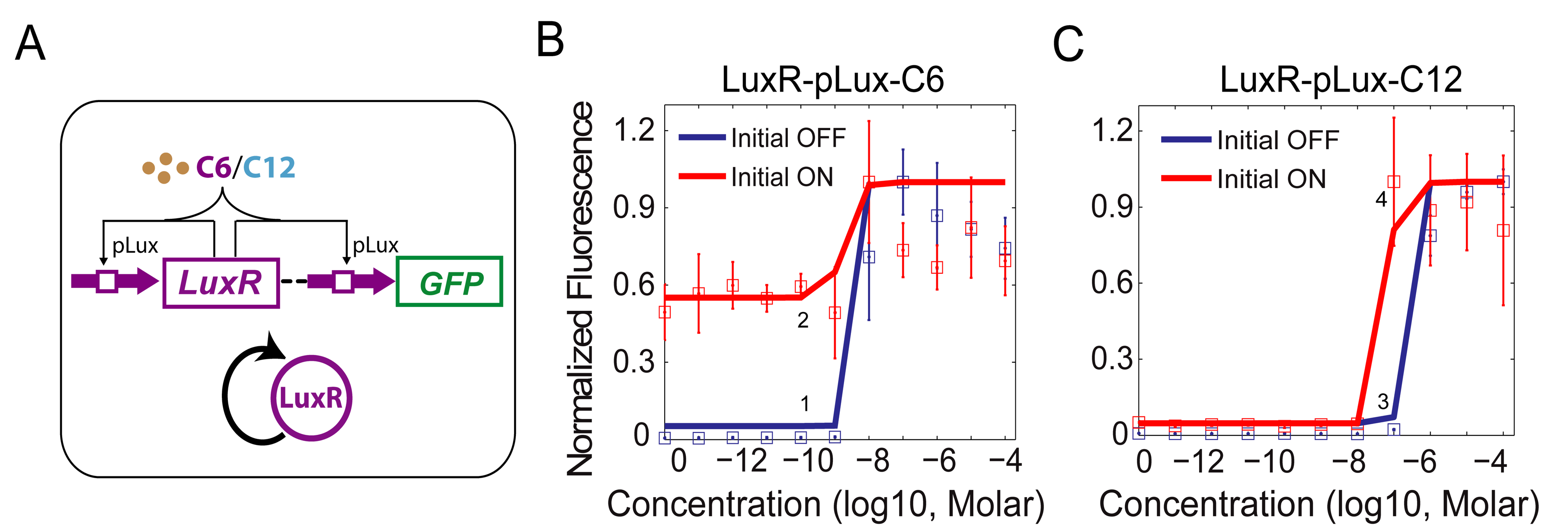

Fig. 2 Signal crosstalk significantly decreases bistable potential of positive feedback circuits.

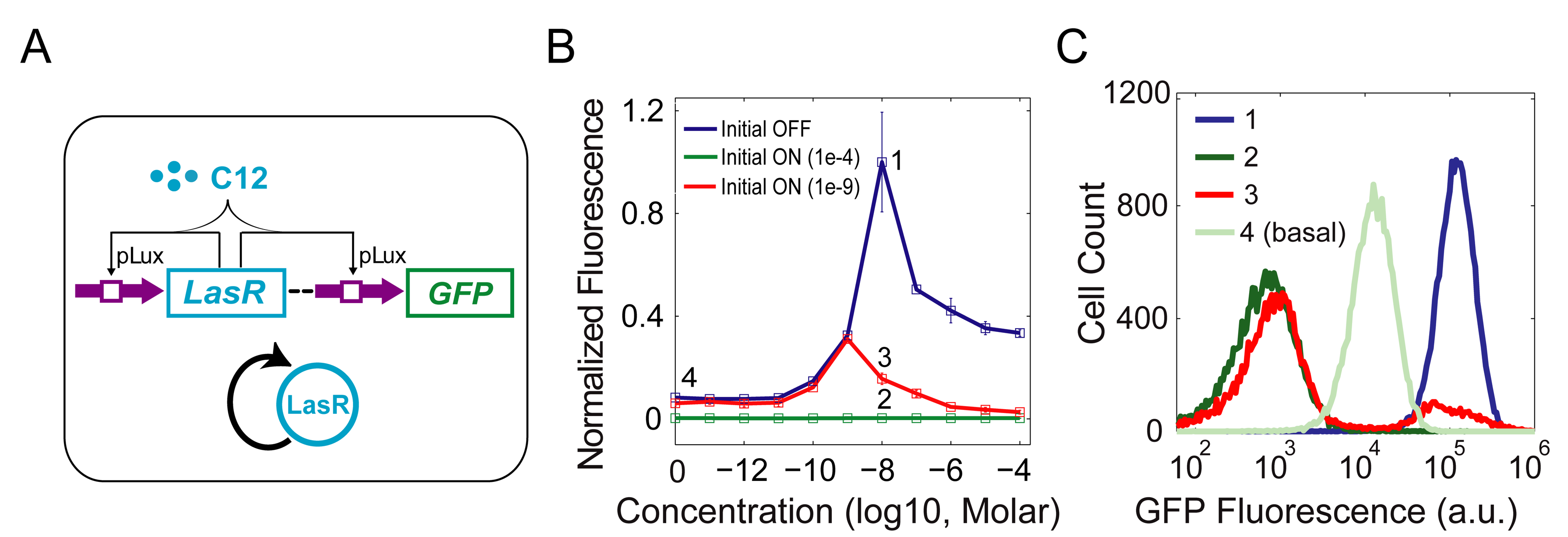

Fig.3 Promoter crosstalk induces unexpected and complex bimodal responses, which resulted from property of positive feedback circuit and host-circuit interactions.

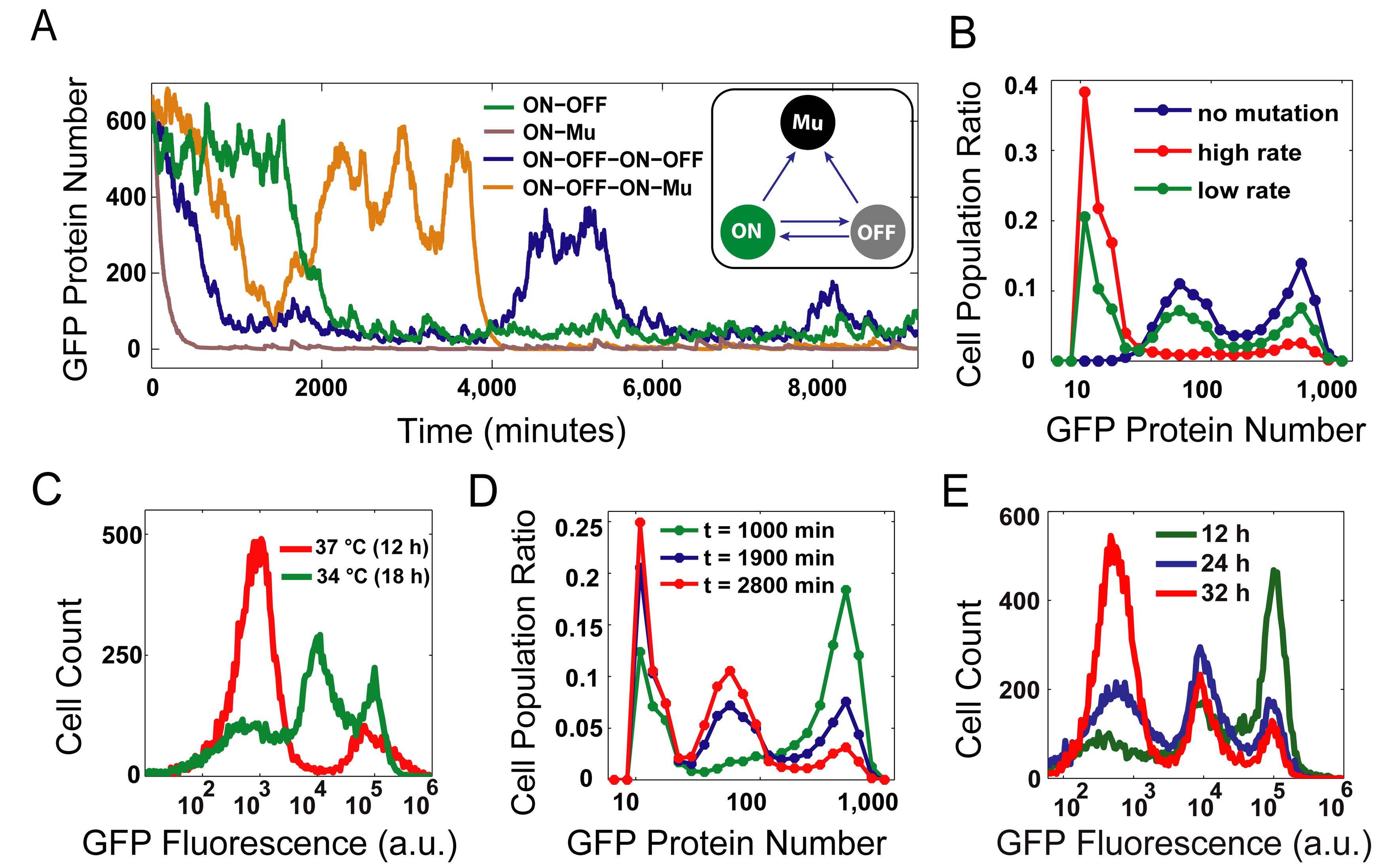

Fig. 4 Trimodality predicted by expanded model and experimental validation.

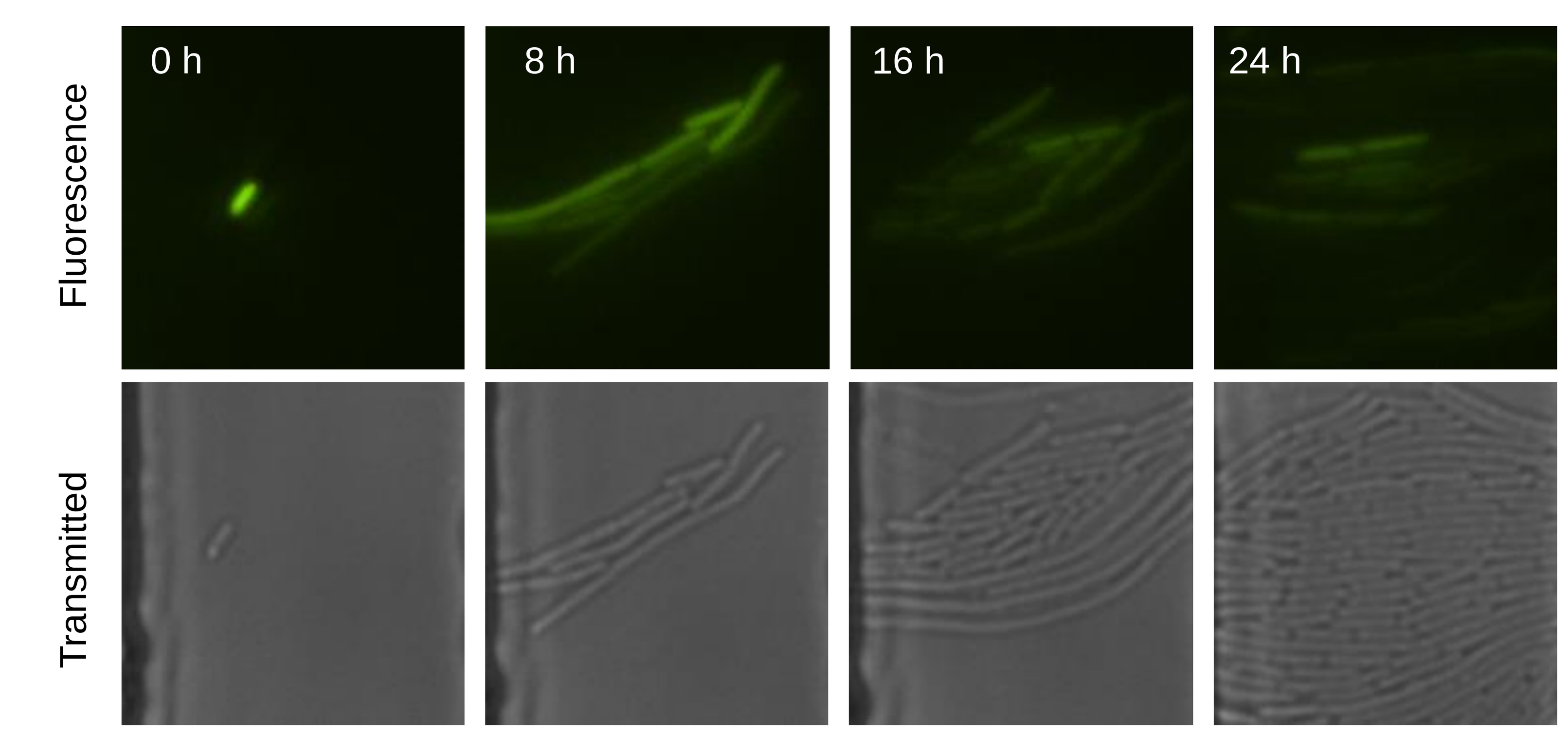

Fig. 5 Microfluidic results further confirmed model predicted trimodality.

Results and Conclusions

- Here we systematically studied the crosstalk between LuxR/I and LasR/I systems and found that QS crosstalk can be dissected into signal crosstalk and promoter crosstalk.
- Further investigations using synthetic positive feedback circuits revealed signal crosstalk significantly decreases circuit's bistable potential.
- Promoter crosstalk reproducibly generates complex trimodal responses resulting from noise-induced state transitions and host-circuit interactions.
- A mathematical model that integrates the circuit's nonlinearity, stochasticity, and host-circuit interactions was developed, and its predictions of conditions for trimodality were verified experimentally.
- Combining synthetic biology and mathematical modeling, this work sheds light on the complex behaviors emerging from QS crosstalk, which could be exploited to disrupt or utilize QS for therapeutics and biotechnology.

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