Multiplexing Cell-Cell Communication

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***Short Abstract* — To coordinate complex behaviors, living cells transmit and receive many pieces of information with one another. However, a small limited number of engineered cell-cell communication systems currently exist, limiting our ability to engineer multicellular behaviors. Here, we overcome this limitation by constructing a genetically-encoded Multiplexer-Demultiplexer (MUX-DEMUX) system that enables *Escherichia coli* to have two separate conversations over a single chemical channel.**

***Keywords* — cell-cell communication, genetically-encoded Multiplexer-Demultiplexer, MUX-DEMUX, multicellular behaviors, transcriptional logic gates, CRISPRi**

I. Purpose

Communication among cells enables complex multicellular behaviors and is employed by many natural systems. Examples include the Notch-Delta systems in animals, which coordinate multiple cellular differentiation processes, auxin systems in plants, which orchestrate growth and development, and quorum-sensing systems in bacteria, which regulate bacterial gene expression based on population density. Several engineered systems have also been presented that utilize cell-cell communication, including a bacterial edge detector [1], multicellular NOR gates [2], and an oscillating microbial consortium [3]. However, the most complex engineered multicellular systems currently use at most two communication channels, due in part to the limited availability of engineered cell-cell communication systems. Expanding the effective capacity of existing cell-cell communication systems overcomes this limitation and enables increasingly complex engineered multicellular behaviors.

Constraints on the transmission of information are also prevalent in electrical systems, where digital logic devices are employed to manage access to finite communication resources. These logic devices, known as multiplexers, enable multiple independent conversations to occur over the same communication channel. A multiplexer (MUX) outputs one of multiple input signals based on a "select" signal. Similarly, a demultiplexer (DEMUX) routes a single input signal to one of multiple outputs based on a select signal. If a MUX and a DEMUX are juxtaposed across a communication channel, they can be used to arbitrate access to the channel. Furthermore, if their select signals are derived from the same source, the MUX-DEMUX system can select and transmit one of multiple simultaneous independent signals, effectively increasing the capacity for communication across the channel. We thus used standard logic design techniques to design a MUX circuit and a DEMUX circuit, and we built on existing techniques for constructing genetically-encoded logic circuits in living cells [4] to implement a MUX-DEMUX system that enables *Escherichia coli* to transmit two independent signals over a single chemical channel.

II. Results

We have constructed a novel library of 9 orthogonal CRISPRi-based NOT and NOR gates, and we optimized 3 small-molecule sensors and 1 quorum-sensing cell-cell communication channel to interface with them. From this library of parts, we assembled a chemically-inducible genetically-encoded 2-to-1 MUX from 3 sensors, 4 CRISPRi NOT gates, and 3 CRISPRi NOR gates and a chemically-inducible genetically-encoded 1-to-2 DEMUX from 2 sensors, 3 CRISPRi NOT gates, and 2 CRISPRi NOR gates. We introduced the MUX and DEMUX circuits into separate *E. coli* strains, validated the performance of every regulated promoter in each circuit, and cocultured both strains to show that two different small-molecule inducers, detected only by the MUX strain, can independently control the activity of two transcription units in the DEMUX strain. We also characterized the dynamics of signal propagation in the MUX circuit and established a gate delay time for CRISPRi logic circuits in *E. coli*.

III. Conclusions

This work represents one of the largest synthetic systems constructed to date with 20 regulated transcription units and 8 layers of logic computation. This work also expands the capacity for communication over existing engineered cell-cell communication systems and enables increasingly complex multicellular behaviors.

References

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