Synchronization of genetic oscillators in *E. coli*

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We previously constructed a synthetic *E. coli* gene network which produces oscillations due to positive and negative feedback loops. After a few cell divisions, the oscillations of proteins between neighboring cells lose synchrony and produce a wide variability in oscillation phase across a population. We experimentally construct intercellular quorum sensing circuits that enable a population of cells to synchronize. The designs allow for cells to communicate their phases via the luxIR system from *Vibrio Fischeri*. The luxI synthase produces a small molecule, AHL, which can diffuse to neighboring cells and induce the luxI promoter. We discuss modeling and experimental results and spatio-temporal dynamics of synchronization designs for genetic oscillators.

***Keywords* — AHL – acyl-homoserine lactone**

I. Purpose

One of the goals of synthetic biology is to design and construct synthetic networks and understand their dynamics and function. To this end, we previously constructed a synthetic genetic oscillator in *E.coli* containing has positive and negative feedback loops. Each cell's oscillations are essentially independent, and within 2-3 cell divisions oscillations between cells drift out of phase. Previous theoretical work[1,2] has modeled quorum sensing circuits which couple genetic oscillators together to achieve synchronization. In this work, we experimentally construct a circuit design that can achieve synchronization of genetic oscillators and produce interesting spatio-temporal dynamics.

The coupling mechanism of genetic oscillators is based on the luxIR quorum sensing circuit from *Vibrio Fischeri*[3]. The luxI synthase can produce a small molecule acyl-homoserine lactone(AHL), which diffuses across the cell membrane and into neighboring cells. AHL then binds to a luxR protein and activates transcription of the luxI promoter, which can be coupled to one of the genetic oscillator components.

II. Results

One design which can achieve synchronization is a system where the luxI protein is one of the oscillator components. Here, the luxI protein is under control of the native luxI promoter, producing a positive feedback through AHL production. The luxI promoter also drives AiiA, an enzyme which degrades AHL and adds a negative feedback to the system. Modeling this design has shown that the system can produce oscillations.

We experimentally constructed this circuit in *E. coli* using molecular biology techniques. The luxI promoter driving the luxI gene is built on a higher copy colE1 plasmid while the repressor is built on a lower copy p15A plasmid. Cells are monitored in microfluidic devices which can continually supply media to cells for long periods of time and allow for AHL to be externally flushed away at a given flow rate. We find that the synchronization design requires a critical density of cells to produce oscillations. As a small number of cells grow outwardly from their starting point, they produce spatially periodic waves of fluorescence due to the propagation of AHL, and we discuss the experimental properties of these waves. Preliminary modeling results for this design are also discussed.

III. Conclusion

We experimentally constructed a synthetic network which can produce synchronous oscillations in *E. coli* cells. The design has the luxI and AiiA proteins supply a positive and negative feedback and act as oscillatory components. We find that rich spatio-temporal behavior is observed which can be tuned by external controls. Modeling results for this system are also shown.

References

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