Dynamics of Bacterial Quorum Sensing in Microfluidic Devices

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Introduction:

Quorum sensing is an essential communication mechanism in bacteria. By transmitting small molecules to each other, bacteria can sense their own population and respond accordingly. Coupling such mechanisms with synthetic biology can lead to a wide range of potential applications, including microbial detection of heavy metals [1]. Although many quorum sensing mechanisms have been characterized at the molecular level, variations in population-level responses are not well understood.

We engineered the *luxI*/*luxR* quorum sensing mechanism from *V. fischeri* into *E. coli* cells such that the cells functioned as fluorescent receivers of a signal molecule, an acyl homoserine lactone (AHL) [2]. The cells were studied in a microfluidic device to allow for constant population density and dynamic media variation, to introduce signal molecules. Finally, we created and extended a deterministic mathematical model to characterize the responses of the fluorescent experiments. Utilizing the model's predictions will allow for the use of quorum sensing in practical applications.

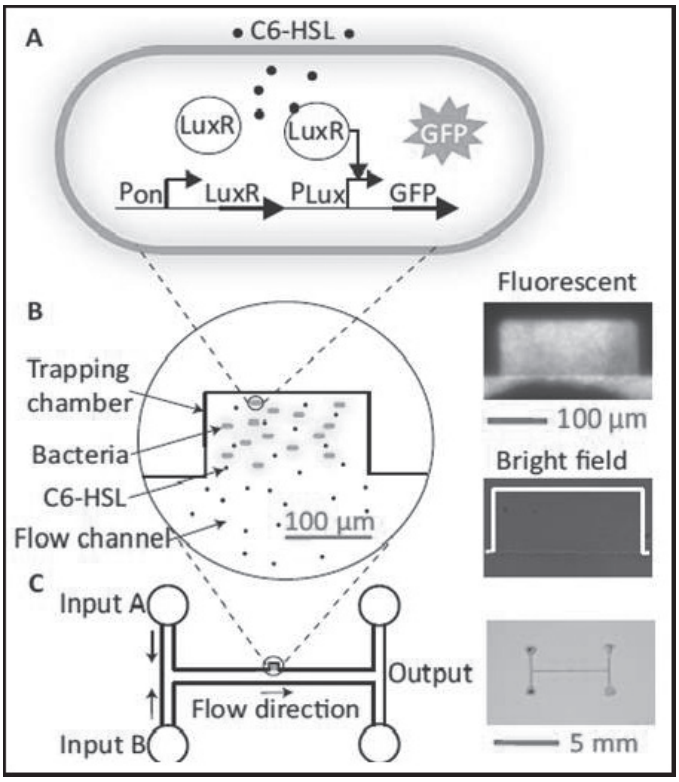

Figure 1: E. coli *cells engineered with the* luxI/luxR *system and the microfluidic device setup in our experiments [4]. C6-HSL is a specific type of AHL.*

Experimental Procedure:

SU-8 master molds were fabricated via a two-step photolithography process in order to produce 10 μm tall channels and 5 μm deep chambers. Wafers were silanized with tridecafluoro-1,1,2,2,-tetrahydrooctyl-1-dimethylchlorosilane in a vacuum dessicator.

The polydimethylsiloxane (PDMS) devices were fabricated by pouring a mix of 10:1 polymer to cross-linking agent (65 g total weight) onto the SU-8 mold then cured for four hours at 50°C, then removed.

COMSOL software was used to create a model of the fluid dynamics and transport processes in the channel and chambers, and a time frame for AHL transport into the chamber from the channel was determined.

Fluorescent microscopy using 2 μM fluorescent bovine serum albumin (Alexa Fluor 488) pumped through the chip using 5 mL syringes and a charge-coupled-device (CCD) camera were utilized to validate the devices. Results were compared against COMSOL simulation, and confirmed the accuracy of the simulation's predictions of mass transfer in an empty chamber, as seen in Figure 2.

The mathematical model was set up to capture the following biochemical interactions: External AHL diffuses into the cells, reversibly binds with *luxR* to form a dimer, which acts as a transcription factor for the *luxI* promoter. Upon binding of this transcription factor, transcription and translation of the *gfp* gene is initiated. The green fluorescent protein (GFP) undergoes chemical maturation before fluorescing. All species degrade over time.

The model was programmed into MATLAB, using generalized mass action to capture the species mentioned above vs. time. Euler's method for 1st order linear differential equations was used, and parameter estimation was coded in order to estimate

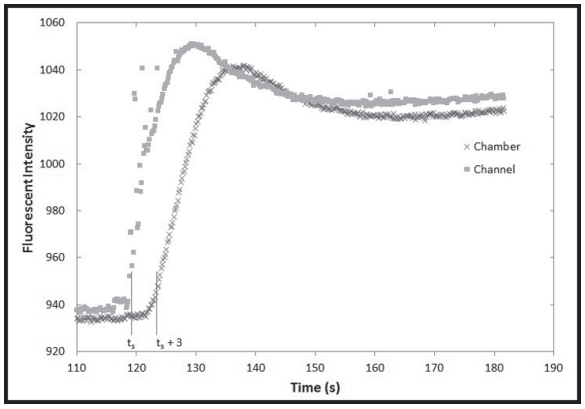

Figure 2: Fluorescent BSA experiment showing the start time of fluorescent increase in the channel (t_s). The 3-second delay between channel and chamber supports COMSOL predictions.

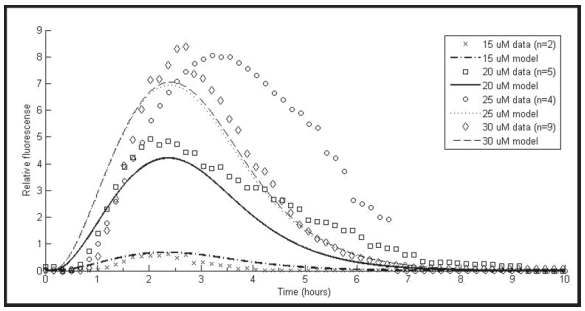

Figure 3: Model fit to experimental data of multiple concentrations, varying only protein production rates.

the rate constants governing each term. The mean-squared-error was minimized to best-fit the model to experimental data.

Results and Discussion:

Figure 3 depicts the model's fit to an AHL pulse length of fifty minutes at varying concentrations, using identical rate constants for all experiments, varying only protein production rates. Our model clearly captured the fluorescent responses for most of our experiments, with some deviations, as the 25 μM curve shows. We believe this is due to the wider peak generated in the 25 μM experiment, for unknown reasons. Finally, Figure 4 applies our model to data taken from literature and directly compares our fit with theirs [3]. Since their experiment setup is different, allowing for unbounded growth, some of the rate constants were adjusted accordingly.

One major improvement that needs to be made includes accounting for the delay in the response in our data. In quorum sensing, transcription levels are basal until a critical concentration of the signal molecule is reached, upon which a burst of transcription occurs [2]. This is likely due to some form of internal feedback control. Still, these fits prove that our model's ability to characterize a variety of experimental setups, and has promising potential to predict future experiments.

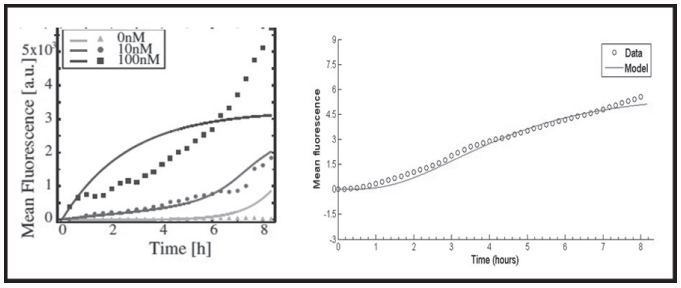

Figure 4: Comparison of literature fit (left) vs. our model fit (right) to the 100 nM concentration data points [4].

Future Work:

Our model will be extended to fit other growth conditions. Once the model has been validated with a sufficiently wide range of experiments, we will begin testing the model by predicting experiments with specific signal pulse profiles and concentrations.

We will use transmitter cells that produce AHL and emit it via diffusion, and run experiments with both transmitters and receivers. These experiments more closely represent quorum sensing in nature. We can then begin moving towards genetically engineering bacteria for practical applications such as the biosensor mentioned earlier for detecting toxic heavy metals in water. Our work will open the door for many applications of quorum sensing in the bioengineering field.

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References:

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