Biological Computing with Synthetic Minimal Cells and Quorum Sensing

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1 Abstract

A novel genetic circuit design was modelled to emulate resistor units to be used with biological op-amps via synthetic minimal cells ("synells"). The synells exploited a positive feedback loop in the quorum sensing architecture in Pseudomonas Putida to regulate the equilibrium concentration of the main signalling molecule (AHL). Delay Differential Equations (DDEs) were used to model the substrate dynamics of a Pseudomonas Putida cell and the effects of the synell were tested using varying degradation rates. The degradation rates modulated the equilibrium concentrations of the signalling molecule with high specificity: degradation rates between 0 to 0.61 resulted in an equilibrium concentration of approximately 0.15 and 0.05 respectively, while degradation rates greater than 1.5 dropped the equilibrium concentration to 0. The synell's specificity enables specific regulation of the main signalling molecule and by extension, the feedback loop, single cell gene expression, and the population's group behaviours. This could be used to create a biological operational amplifier (op-amp) and other analog computing components.

2 Background & Rationale

This paper seeks to apply synthetic minimal cells and genetic circuits to emulate operational amplifiers (op-amps) in cultures of the bacterial species *Pseudomonas Putida*. The specificity in feedback control offered by an op-amp would enable novel devices to approach diseases characterized by protein misregulation in the body with the use of a synthetic minimal cell. Emulated op-amps in bacteria would also allow for novel drug delivery applications, as the feedback control offered by op-amps would maintain the necessary conditions and environment for a medication.

The primary **research question and engineering goal** that guides this paper rests on whether the simulation produces modular steady-state concentrations of the signalling molecule based on the controllable degradation rate of the synthetic minimal cell. A constant, non-zero steady-state concentration of the signalling molecule given

by the DDEs must be attained for the simulation and the synthetic minimal cell to be feasible. The steady-state concentration of the signalling molecule should be dependent on the initial conditions and must vary proportionally to the degradation rate. While it is expected that the steady-state concentration of the signalling molecule will be dependent on the initial conditions, the degradation rate must be able to solely vary that steady-state concentration. The **hypothesis** is that steady-state concentration of the signalling molecule will be dependent on the initial conditions and will vary proportionally to the degradation rate.

This project leverages synthetic minimal cells and quorum sensing to emulate an op-amp in bacteria. Quorum sensing is the cell-to-cell communication mechanism through which single bacteria cells are able to measure the whole population density. This mechanism is based on gene regulatory networks that regulate the expression of certain target genes and their production, which results in a positive feedback loop[7]. In Gram-negative bacteria, communication in quorum sensing is facilitated by signalling molecules (autoinducers), specifically N-Acyl homoserine lactones (AHLs) [7]. AHLs are typically produced by synthases and bind to receptors on RNA Polymerase molecules. The complex formed by the receptor and AHL(repector-AHL complex) typically induces the expression of AHL synthases, thus completing a positive feedback loop. This feedback loop is regulated by Lactonase, a degrading enzyme which has been found to be activated after a delay[1].

Synthetic minimal cells ("synell") are artificial cells to perform certain cellular processes such as translation and transcription[4]. The synell proposed in this paper provides the cell with Lactonase enzymes and is introduced at the start of the simulation.

While this paper focuses on the substrate dynamics of the Gram-negative bacteria species *Pseudomonas Putida*, a root-colonizing, plant-growth promoting bacteria[6], the synell can be easily applied to related bacterial species. In this paper, variables dependent on time are denoted by x or $x(t)$. First derivatives with respect to time are denoted by $\dot{x}$, respectively by $\dot{x}(t)$. In this paper, a system of DDEs in [1] is extended to model the effects of a synell that produces a lactonase enzyme from the aiiA gene[5].

3 Methods

Given that this paper explores simulations, the safety precautions followed were primarily related to safe computer handling and use.

3.1 Compartmental Models

The substrate dynamics for quorum sensing in a single cell and the culture can be modelled using a compartmental model, in which compartments of the model are differential equations that represent the dynamics of bacterial population density or the concentration of some substrate. In this paper, the substrate dynamics of a culture of *Pseudomonas Putida* are modelled by extending the reduced model proposed in [1].

3.2 Regulatory Network of a Single Cell with the Synell

The regulatory network in a single cell is modelled using the simplified single cellmodel proposed in [1] and has similar assumptions: the basic production of the recep-tor PpuR is considered to be constant since feedbacks from PpuR appear to have noappreciable effects [5] and mRNA equations are in quasi-steady state[3].

The delayed activation of Lactonase, the main degrada-tion enzyme for AHL, provides negative feedback and requires the use of delay differ-ential equations to model the AHL concentration in the cell.

Since AHL diffusion into and out of the cytoplasm is passive and fast in comparison to other measurements[1], we can assume that the AHL concentrations inside and outside a cell are in equilibrium. Via the steady-state assumption, we consider that the AHL concentration in the intracellular and extracellular space is equal[1].

We can then assume that a reduction in the AHL concentration in the extracellular space from Lactonase in the extracellular space will correspond to a similar reduction in AHL concentration inside a cell. Consider a synthetic minimal cell ("synell") in the extracellular space that contains *aiiA*, a homologous gene that encodes AHL-Lactonase enzymes [3]. This synell produces Lactonase enzymes that degrade extracellular AHL and, via the steady-state assumption, the AHL inside a single bacteria cell. The synell's effects are proportional to the AHL concentration and can be modelled using a degradation rate, q. The model, displayed in the table below, uses a system of DDEs to simulate thedynamics in AHL and Lactonase concentrations. The values for the initial conditions of the system are denoted in the table in 4.

$$\begin{aligned} \dot{x}(t) &= \alpha - \gamma x(t) - \delta x(t) y(t) + \beta \frac{x(t)^n}{x_{th}^n + x(t)^n)} - q x(t) \\ \dot{y}(t) &= \rho \frac{x(t-\tau)^m}{y_{th}^m + x(t-\tau)^m} - \omega y(t) \end{aligned} \tag{1}$$

4 Methods for Data Analysis

The numerical simulations were run with 100 evenly spaced values from 0 to 3.0 for the degradation rate for the AHL concentration caused by the synell. The model was simulated using Scipy's odeint function with the Python programming language. The exact values used as initial conditions for the simulation is summarized in the table below, adapted from [1, 2]. The data collected for this project was sourced or estimated from previous research.

Symbol	Description	Value	Comments/Source
α	Basic AHL production rate	$1.0564 \times 10^{-7}(mol/(lit^2h))$	[1]
β	Feedback-regulated AHL production rate	$1.0564 \times 10^{-6}(mol/(lit^2h))$	[1]
ρ	Lactonase production rate	$5.0521 \times 10^{3}(mol/(lit^2h))$	[2]
γ	AHL decay rate	0.105 (1/h)	[2]
ω	Lactonase decay rate	0.105 (1/h)	[2]
τ	Delay in the release of Lactonase	2 (h)	[2]
n	Hill coefficient for x	2.3 (dimensionless)	[2]
m	Hill coefficient for x	2.5 (dimensionless)	[2]
$x_0(t)$	Initial AHL concentration	$5.4044 \times 10^{-7}(mol/lit)$	[1]
$y_0(t)$	Initial Lactonase concentration	$5.2 \times 10^{3}(mol/lit)$	[1]
x_{th}	Critical threshold for positive-feedback in x	$3.597 \times 10^{-13}(mol/lit)$	[1]
y_{th}	Critical threshold for positive-feedback in y	$3.597 \times 10^{-13}(mol/lit)$	[1]
q	AHL decay rate (Synell)	$0.0 - 3.0(1/h)$	chosen

5 Results

The equilibrium AHL concentrations inside the cell with the synell's effects significantly differ from the AHL concentrations inside the cell with no external influences. Degradation rates of 0 to 0.61 appear to produce equilibrium AHL concentrations between 0.05 M and below, which would be viable for future experimentation. Degradation rates greater than 1.5 produce a final AHL concentration equilibrium of 0 M, effectively preventing a cell from communicating to other cells in the extracellular space.

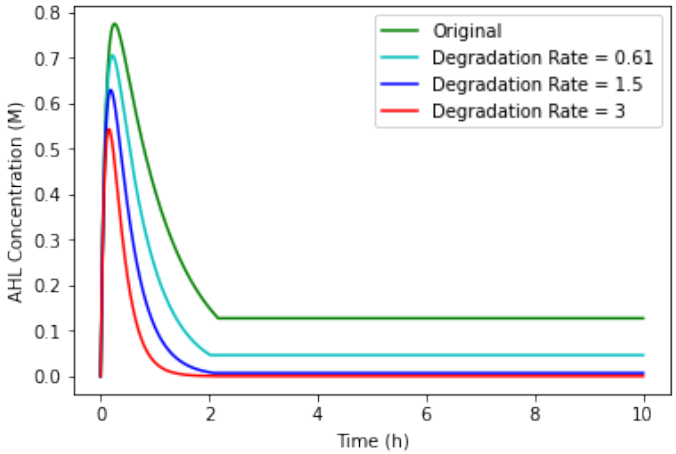

Figure 1: Numerical simulation of the mathematical model in (1). The cell's AHL concentration reaches different equilibrium concentrations depending on the degradation rate approximately 2h from the beginning of the simulation.

6 Discussion

In this paper, a system of two ODEs have been created and adapted to simulate the effects of a synell containing a gene that would interrupt the positive feedback loop in *Pseudomonas Putida* by modulating the equilibrium concentration of the sin galling molecule, AHL.
The synell's degradation rate appears to modulate the equilibrium AHL concentration, which would enable specific control over the feedback loop, single cell gene expression, and the population's group behaviours. The specific control the degradation rate possesses over the equilibrium AHL concentration could allow for processes (such as drug delivery) that require specific extracellular conditions to take place in a stable environment.
The synell's effects on the AHL concentration also mirror those of a resistor's effects on voltage; the AHL concentration is decreased from an initial high concentration to a stable lower concentration. This could be used to emulate circuits, specifically an operational amplifier in analog circuits.
The specific control the synell offers over the feedback loop enables future experimentation to control single cell gene expression and population group behaviours of *Pseudomonas Putida*. The system of ODEs presented enables analysis of the basic qualitative behaviour of the substrate dynamics of a single cell.

References

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