Activation-inactivation intercellular signaling in one- and two-dimensions
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General Description

Imagining a sensor composed of a lawn of bacteria, which under normal conditions are all fluorescing at a steady state. However once the system is exposed to a foreign substrate (substance to be detected), the dynamics of the system suddenly switch from steady state to oscillatory, which can be observed and noted by a technician. We investigated the possibility of such a sensor through mathematical modeling and designed a biological system in order to experimentally observe the system at work.

Abstract

Intercellular signaling constitutes the foundation of many disparate research fields such as neurophysiology, endocrinology, cancer research, and many others. We investigated a simple representation of intercellular signaling network, where a population of cells secretes and activates a molecule of which the production is feedbacked and inhibited. The production of the activating molecule (Figure 1). This is known as an activation-inhibition system. We began by using a partial differential equation model of the system to explore the effect of varying the separation distance of the two populations of cells. We found that three types of dynamics were present: steady states, periodic oscillations, and quasi-periodic oscillations. We further designed two strains of E. coli capable of interacting with each other as an activator-inhibitor system and endeavored to validate our modeling results in a biological system.

Mathematical Modeling

In order to explore the dynamical dependence on separation distance we developed a partial differential equation (PDE) based model, which was an expansion of a model initially presented by Shymko and Glass. The equations are as follows:

\[ \frac{\partial n_1}{\partial t} = D_1 \frac{\partial^2 n_1}{\partial x^2} - \frac{n_1}{b_1} - \frac{n_1^2}{b_1 + n_1} \]

\[ \frac{\partial n_2}{\partial t} = D_2 \frac{\partial^2 n_2}{\partial x^2} + \frac{n_2}{b_2} - \frac{n_2^2}{b_2 + n_2} \]

The above system was solved numerically using a forward Euler scheme in time and a centered difference scheme in space. Cyclical boundary conditions were assumed; as a result, the system is a centered difference scheme in space. The resulting numerical solution is periodic as the solution is periodic in space. The system was solved for the following parameters:

- \( D_1 = D_2 = 1 \)
- \( b_1 = b_2 = 1 \)
- \( n_1 = n_2 = 1 \)

1D - Four Sites (Two Oscillator) Simulations

We next looked at a system consisting of two oscillators, where each consists of an activation and an inhibition. The distance between the two oscillators was varied while the distance between the two sites within an oscillator was held fixed at 6 intervals. This value was chosen for demonstration purposes, however the dynamics to be described have been observed at various separation distances.


1D - Two Sites (One Oscillator) Simulations

The separation distances 474, 473, and 472 resulted in similar dynamics and are not shown for brevity. We further wanted to classify these dynamics as either periodic, quasi-periodic, or chaotic, and we turned to the Poincare Map associated with each oscillation as shown in Figure 7a. We observed chaotic-like behavior at these separation distances.


Wetlab Experiments

In order to observe our abstract signaling network in a real biological system we designed the following two gene constructs.

**Construct 1**

- **Inhibitor (I)**

**Construct 2**

- **Activator (A)**

The lux pl promoter is ON in the absence of inhibitor 3OC6-HSL. It drives the transcription of a biotinric sequence containing the genes for RhlR and the EFPYF reporter. The RhlR+4 promoter is OFF in the absence of activator C4-HSL, which drives the transcription of LUXI and the ECFP reporter. C4-HSL is synthesized by RhlI while LUXI produces C4-HSL. They do not interact with each other's promoters.

Degradation tags (AAV or LVA) were added to ECFP, EFPYF, LuxI and RhlI to limit accumulation inside the cell and allow us to detect changes in fluorescence over time. Synthesis of the constructs was carried out by GenScript Inc. During synthesis, it was noted that the terminators contained nucleotide repeat sequences that destabilized the DNA in ordinary plasmids and E. coli strains. For stability, the copy-number inducible plasmid pCC1 and the E. coli strain EP300 (both from Epicentre) were used to deliver our constructs.

Preliminary Results and Future Directions

Our modeling efforts have confirmed that activation-inhibition signaling possess diverse dynamical modes and has given us ideas of how a novel biosensor could be developed. However, we recognize the faults of our modeling approach, particularly the phenomenologically undefined parameters. Thus we engineered a signaling network in order to experimentally observe the potential dynamics. After incubation and induction of the pCC1 plasmid to high copy numbers for 3-5 hours, we observed fluorescence as expected from the ON promoter lux pl promoter (Construct 2). However, fluorescence was also observed in cells containing the OFF promoter RhlR+C4. This leads us to believe that the RhlR+4 promoter is leaky, and our long incubation period has allowed ECFP to accumulate in those cells. Future experiments will be focused on characterizing the lux pl and RhlR+C4 promoters before testing the system as a whole.

2D Simulations

Will only be discussed during oral presentation at 2009 iGEM Jamboree.

References