Spatial Patterning of Gene Expression in E. coli Using Zinc Finger Proteins and Small RNAs

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Introduction

Objective: To design a synthetic gene network that generates spatio-temporal patterning, specifically using the mechanism of diffusion-driven instability as described by Alan Turing [1]

Diffusion-Driven Instability: A system that incorporates diffusible molecules and is stable in a single cell spontaneously becomes destabilized in the presence of diffusion in an ensemble of homogeneous cells.

Turing patterns hypothesized in nature:

1. Essential structural property for Turing phenomenon is an unstable subsystem
2. A stabilizing subsystem makes the whole system stable
3. With the proper parameters and diffusion coefficients, some spatial modes become unstable, generating patterning

Quenched Oscillator Networks

1. Oscillator circuit serves as unstable subsystem
2. Quenching loop “quenches” the oscillations
3. Diffusion can weaken the strength of the quenching loop

Conditions for Turing Pattern Formation

1. Essential structural property for Turing phenomenon is an unstable subsystem
2. A stabilizing subsystem makes the whole system stable
3. With the proper parameters and diffusion coefficients, some spatial modes become unstable, generating patterning

Previously Proposed Implementation

1. Oscillator subsystem based on the repressilator [3]
2. Quenching loop uses quorum sensing molecules
3. Diffusible molecule is AHL

New Proposed Implementation

1. Oscillator built using zinc finger proteins and small RNAs
2. Oscillator inverters assumed nearly identical
3. Quenching loop produces sRNA1 instead of zfp1 mRNA
4. Diffusible molecule is still AHL

Zinc Finger Proteins (ZFPs)

- Modularity in length, specificity, and affinity
- Each finger binds to 3-4 bases of double-stranded DNA
- Can be used as monomeric repressors
- Can create large sets of orthogonal promoter-ZFP pairs [4]

Small RNAs (sRNAs)

- Non-coding RNAs that bind to mRNA and can regulate translation
- Sense and antisense regions match and bind
- Can increase effective Hill coefficient of transcription factors

Small RNAs (sRNAs)


Inverter Comparison

Comparison of ZFP-sRNA inverter against standard repressors (n = Hill coefficient):

Gain:

Square Noise Margin:

1. ZFP-sRNA Inverter:

2. ZFP-sRNA Oscillator:

3. ZFP-sRNA Quenched Oscillator: (Implementation shown in 2nd column)

Summary

- Turing patterns are awesome
- No synthetic biological demonstration, but we’re on it
- 3 conditions are sufficient for generating these patterns
- Need to identify feasible parameter sets

New architecture – quenched oscillator networks

- Use oscillators as unstable subsystem
- Brings feasible parameter sets closer to experimental values

New biological parts improve our design

- ZFPs and sRNAs are versatile and modular synthetic parts
- We are constructing the compound parts for use in a quenched oscillator system

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References:


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