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.

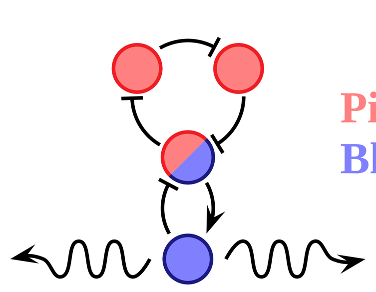
Turing patterns hypothesized in nature:

[1] A. Turing. *The Chemical Basis of Morphogenesis.* Philos Trans R Soc London, 1952.

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

Quenched Oscillator Networks



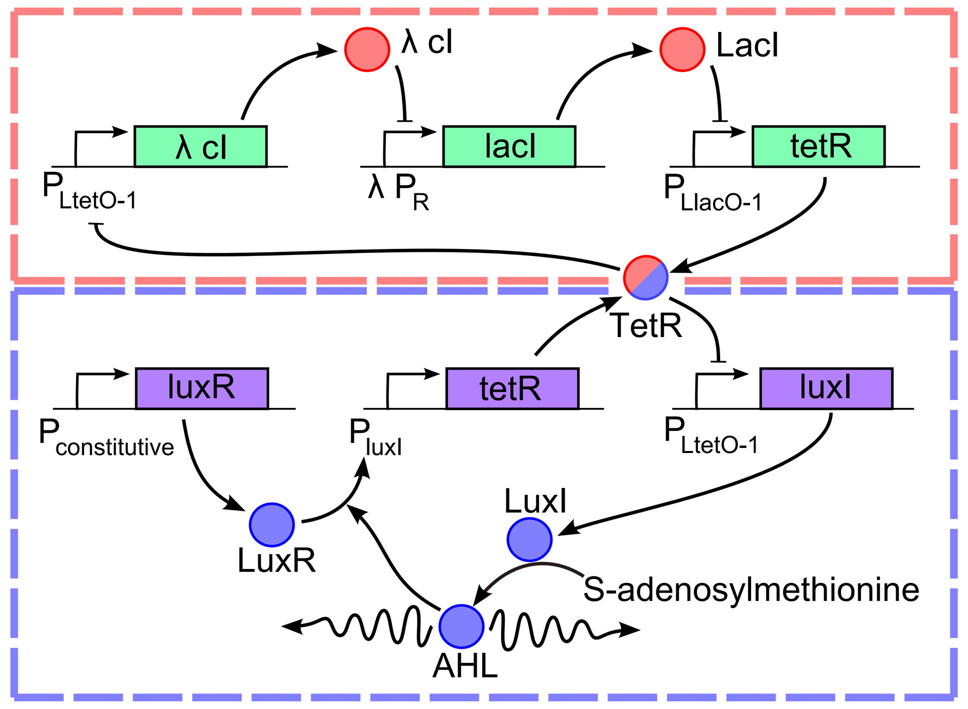
Pink: oscillator loop
Blue: quenching loop

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

- No diffusible species in the oscillator
- Have been shown to be capable of exhibiting Turing patterning in simulation [2]
- Here we propose improvements to our previous design

[2] J. Hsia, W.J. Holtz, D.C. Huang, M. Arcak, and M.M. Maharbiz. *A Feedback Quenched Oscillator Produces Turing Patterning with One Diffuser.* PLoS Comput Biol, 2012.

Previously Proposed Implementation

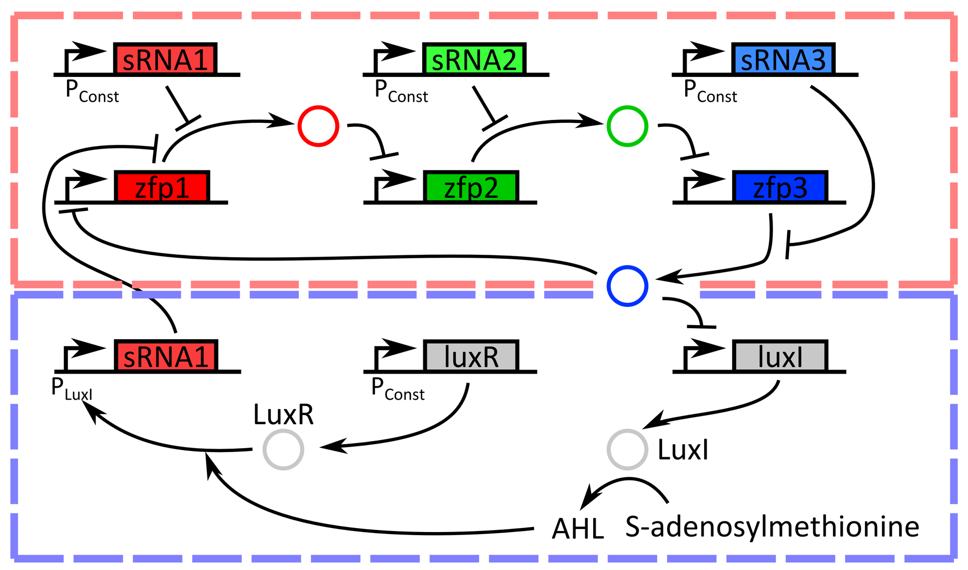


1. Oscillator subsystem based on the *repressilator* [3]
2. Quenching loop uses quorum sensing molecules
3. Diffusible molecule is AHL

- Can generate *feasible* parameter sets that exhibit Turing patterning that sit just outside the realm of experimental plausibility

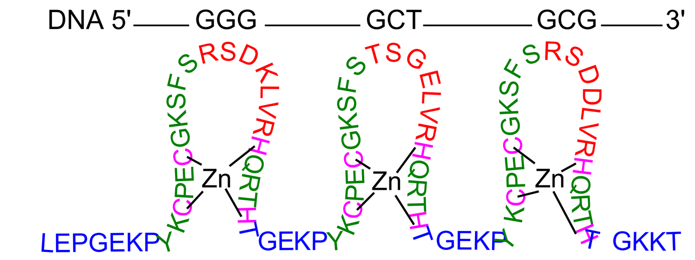
[3] M.B. Elowitz and S. Leibler. *A synthetic oscillatory network of transcriptional regulators.* Nature (2000).

New Proposed Implementation



- Oscillator built using zinc finger proteins and small RNAs
- Oscillator inverters assumed nearly identical
- Quenching loop produces sRNA1 instead of zfp1 mRNA
- 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]

[4] W.J. Holtz. *Engineering scalable combinational logic in Escherichia coli using zinc finger proteins.* Ph.D.

Small RNAs (sRNAs)

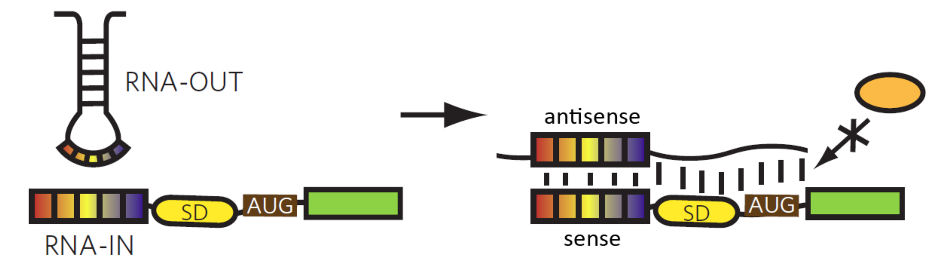
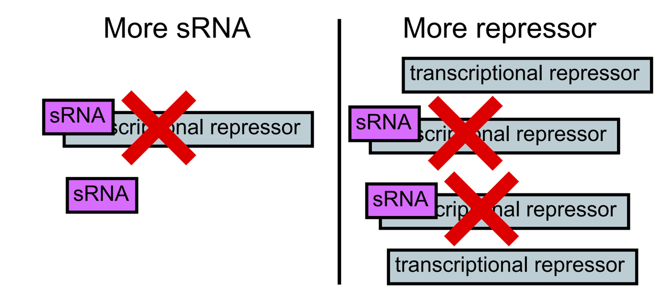


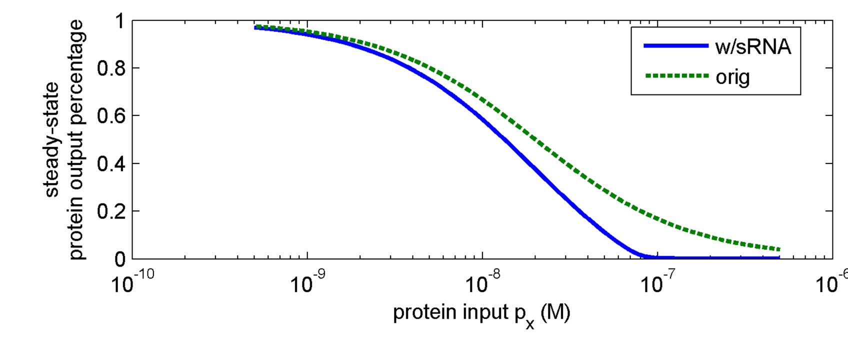
Figure adapted from [5].

- 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

[5] V.K. Mutalik, L. Qi, J.C. Guimaraes, J.B. Lucks, and A.P. Arkin. *Rationally designed families of orthogonal RNA regulators of translation.* Nature Chemical Biology, 2012.

Increased Cooperativity via sRNAs

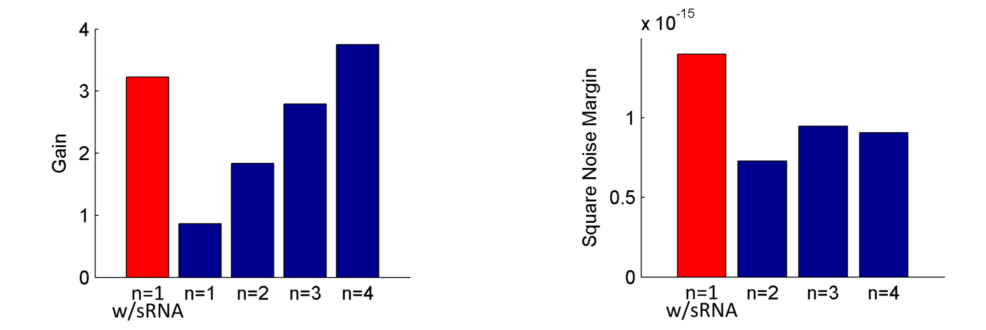




Inverter Comparison

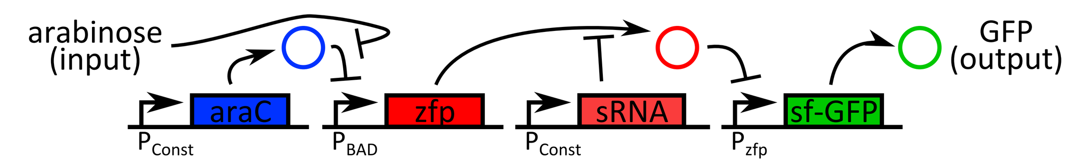
Comparison of ZFP-sRNA inverter against standard repressors (n = Hill coefficient):

Gain: **Square Noise Margin:**

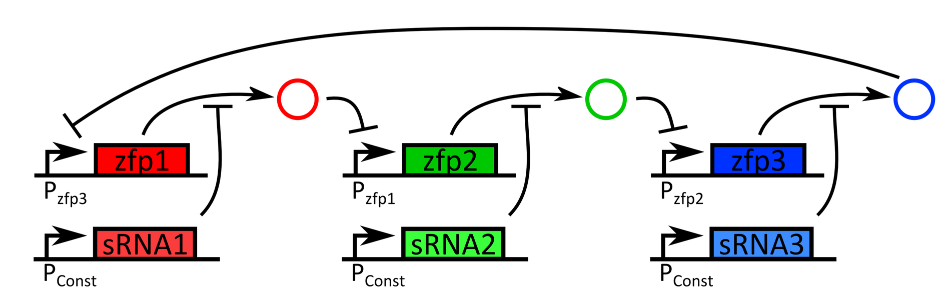


Experimental Plan

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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