A Feedback Quenched Oscillator Produces Turing Patterning Using Hybrid Zinc Finger Protein-sRNA Inverters

Justin Hsia[1], William J. Holtz[2,3], Murat Arcak[1], and Michel M. Maharbiz[1]

[1] Department of Electrical Engineering and Computer Sciences, University of California, Berkeley

[2] California Institute for Quantitative Biosciences (QB3), [3] The Synthetic Biology Engineering Research Center (SynBERC)

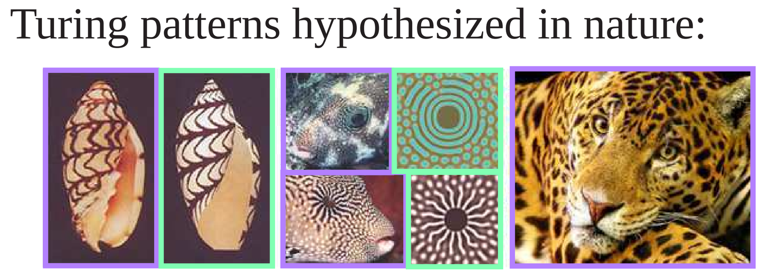
Introduction

Motivation: The production of patterns in gene expression is a phenomenon central to the development of multicellular organisms. What is specifically lacking in the community is an experimentally tractable model system that can break symmetry and spontaneously generate predictable gene expression patterns. Such a system would catalyze the engineering of complex cellular ensembles.

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.

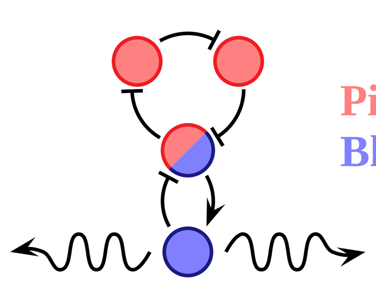
Turing patterns hypothesized in nature:



□ = Reality
□ = Simulated

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

Quenched Oscillator Networks



Pink: oscillator loop
Blue: quenching loop

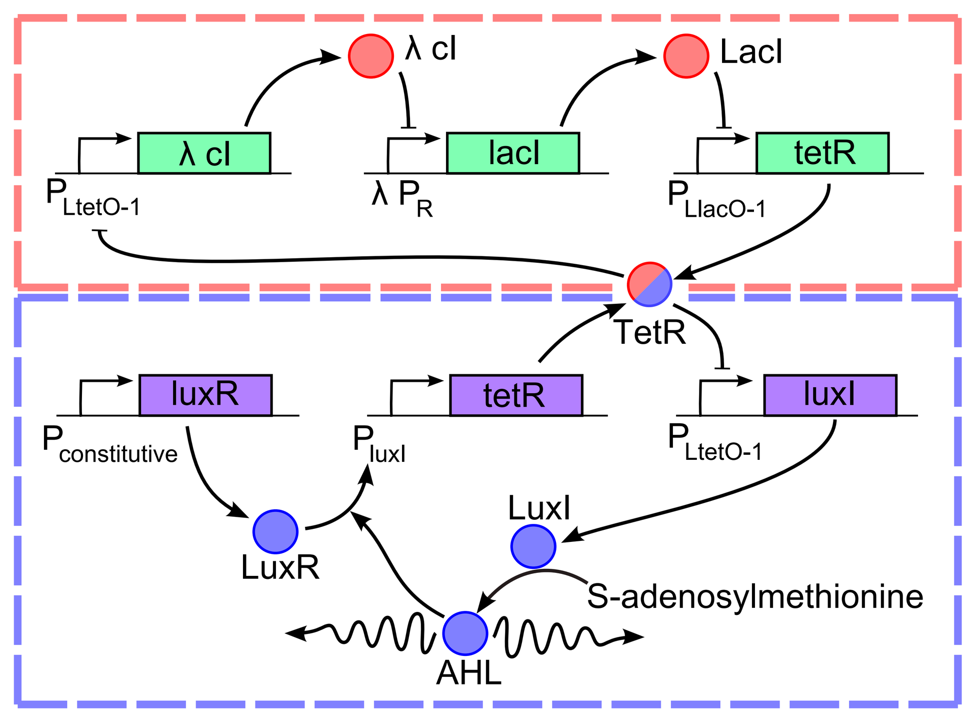
1. Oscillator circuit serves as unstable subsystem
2. Quenching loop "quenches" the oscillations
3. Diffusion can weaken the strength of the quenching loop

- No diffusible species in the oscillator
- Have been shown to be capable of exhibiting Turing patterning in simulation [2]

See [2] for details about the design, modeling, and analysis of quenched oscillator networks.

[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

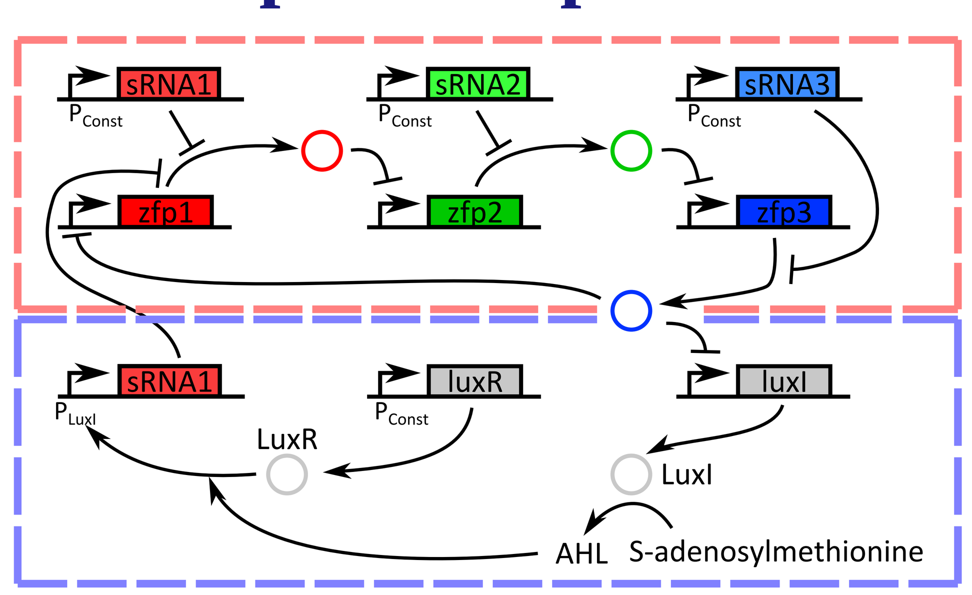


1. Oscillator subsystem based on the *repressilator* [3]
2. Quenching loop uses quorum sensing molecules
3. Diffusible molecule is AHL

- 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).

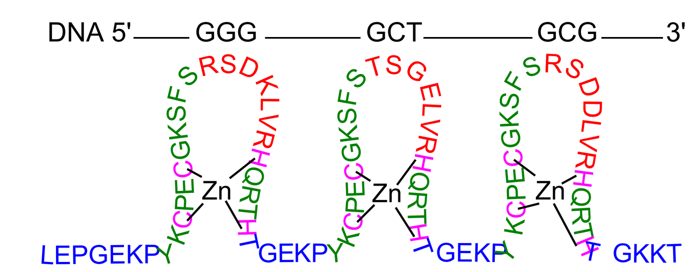
New Proposed Implementation



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

Mathematical model available upon request.

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)

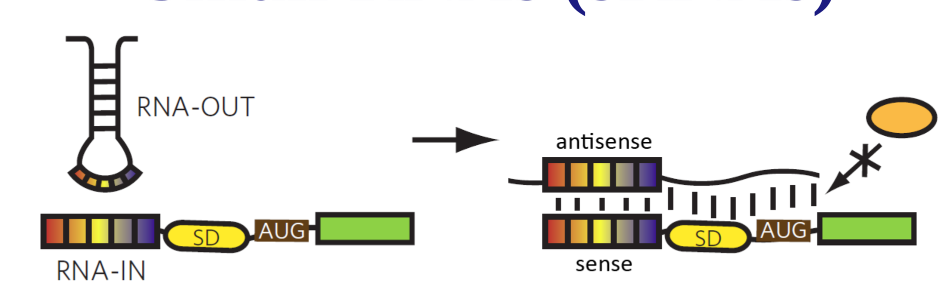
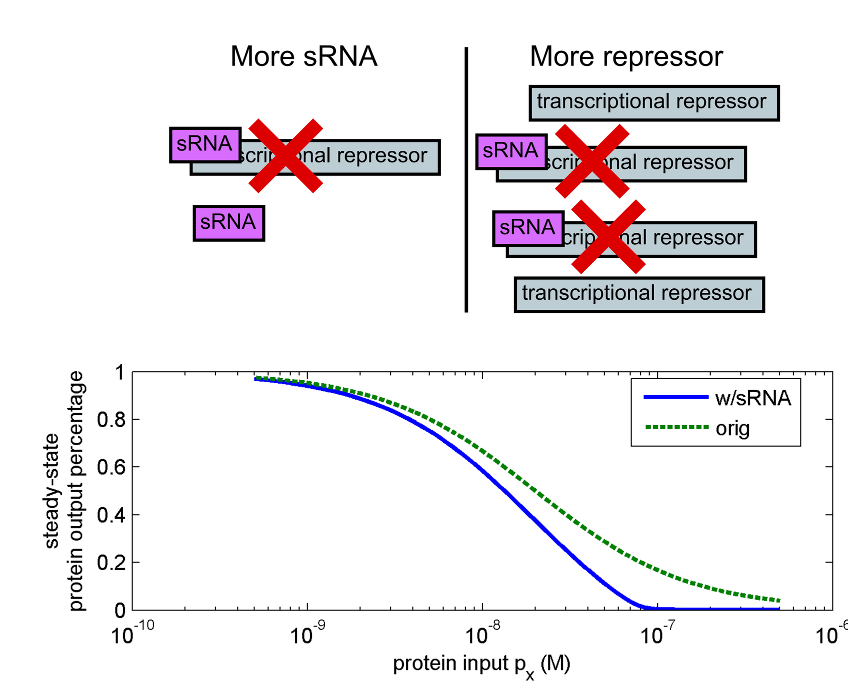


Figure adapted from [5].

- 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 Effective Cooperativity via sRNAs

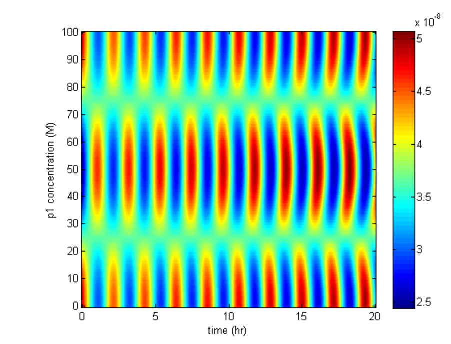


Simulation Results

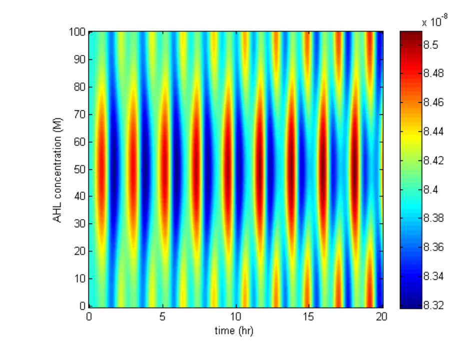
Parameter set available upon request.

Deterministic simulation initialized with specific wave number. High wave numbers grow over time.

Protein concentration:

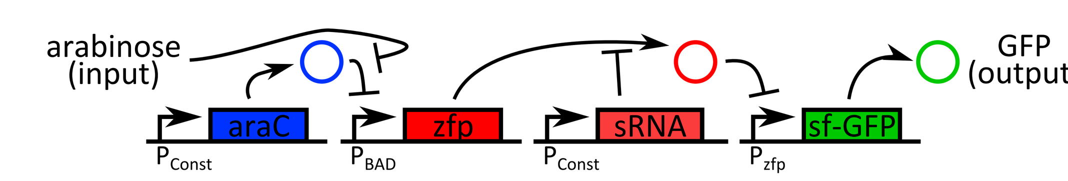


AHL concentration:

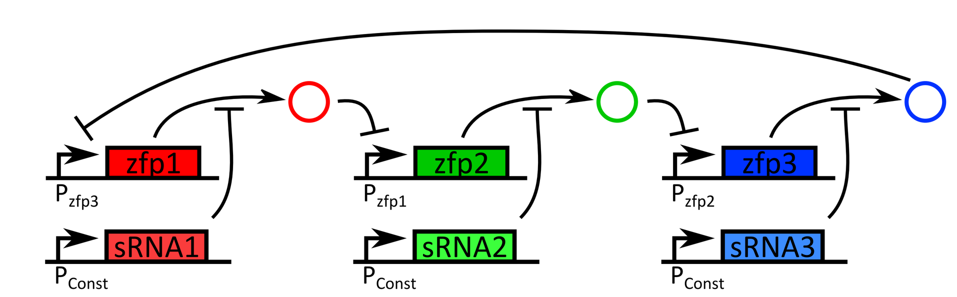


Experimental Plan

1. ZFP-sRNA Inverter:



2. ZFP-sRNA Oscillator:



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

Summary

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

Contact: Justin Hsia, 253 Cory Hall, Department of EECS, University of California, Berkeley, CA 94720; **E-mail:** *jhsia@eecs.berkeley.edu*;

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