**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.

Tuning patterns hypothesized in nature:

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

**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.


**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

**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

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]

**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


**Simulation Results**

Parameter set available upon request.

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

**Experimental Plan**

1. ZFP-sRNA Inverter:
   - Experimental Plan
   - 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

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