A Synthetic Multicellular System for Programmed Pattern Formation

Basu, Gerchman, Collins, Arnold, and Weiss (2005)
Princeton and Caltech

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Goals

- “Design and construction of an artificial multicellular system capable of programmed pattern formation” (p. 1133)
- Simple parts that can be configured in a variety of ways
- Proof of concept-practical applications in 3D tissue engineering, biosensing, and biomaterial fabrication

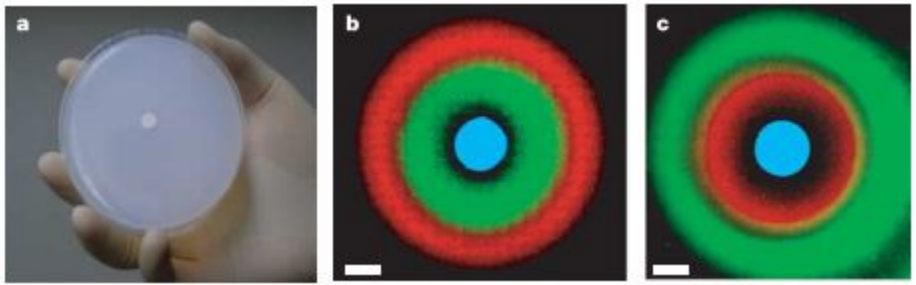


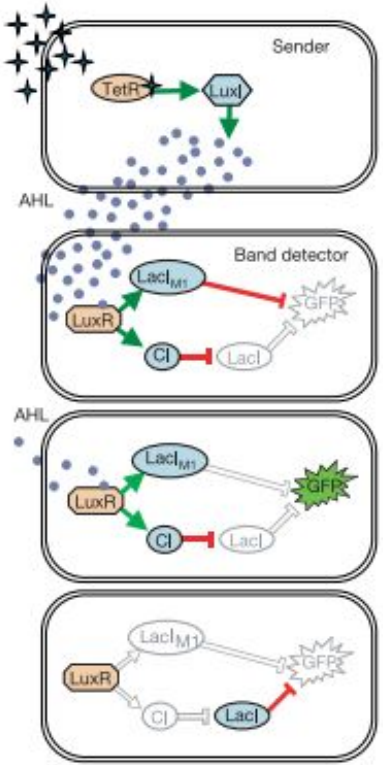
Figure 3. Experimental solid-phase behavior of band-detect networks.

Logic

- GFP (green fluorescent protein) only regressed when $LacI_{M1}$ or LacI is high

Inputs		Output
CL	$LacI_{M1}$	GFP
high	high	off
high	med	on
high	low	on
Med	high	off
Med	med	on
med	low	on
low	high	off
low	med	on
low	low	on

Figure 1a. Band-detect multicellular system. Receiver cells only fluoresce at intermediate distances from sender



Close

Medium

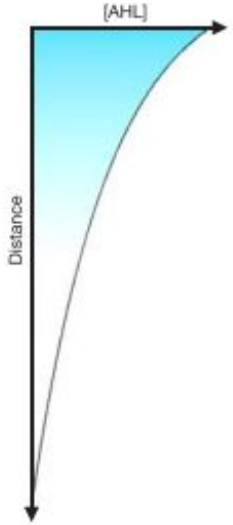
Far

Protein activities of cells at different distances from sender

Protein expression

- GFP (green fluorescent protein) only regressed when $LacI_{M1}$ or LacI is high
- AHL activates expression of CI and $LacI_{M1}$
- Receivers close to senders=high concentrations of AHL
- Receivers far from senders=low concentrations of AHL

Figure 1a. Band-detect multicellular system. Receiver cells only fluoresce at intermediate distances from sender

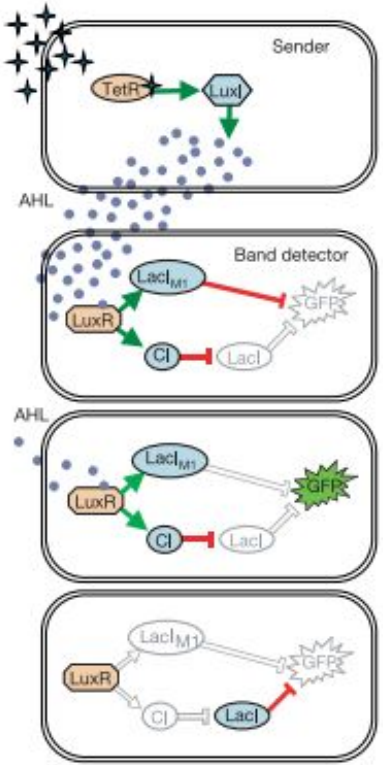


Approximation of AHL gradient as a function of distance from sender

Close

Medium

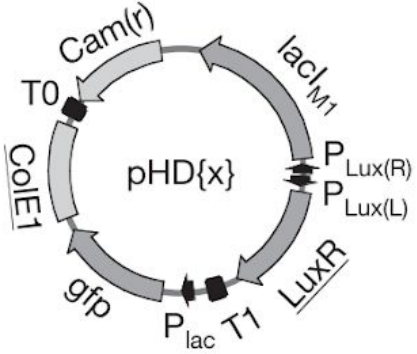
Far

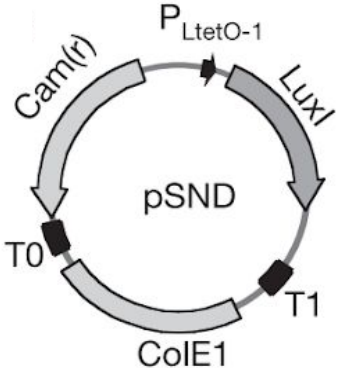


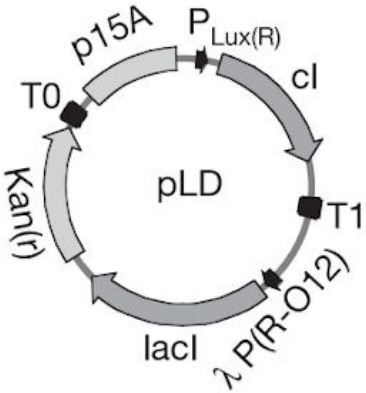
Protein activities of cells at different distance from sender

Plasmids

- pSND
  - Sender plasmid which codes for LuxI
- pHD
  - Sets Upper bound for AHL to turn on GFP (variable parts underlined)
  - pHD1
    - Mutation for hypersensitive LuxR
  - pHD2
    - Wild type LuxR
  - pHD3
    - Mutation causing ~half the copy number due to ColE1 codon swap
- pLD
  - Sets lower bound for AHL to turn on GFP







Model

- System of ODEs with Hill functions of protein synthesis activation and repression
- Five components
  - GFP (G)
  - LacI (L)
  - CI (C)
  - LuxR / AHL (R)
  - AHL (A)
- LuxR and AHL mechanism leads to second order dynamics

$$\frac{dG}{dt} = \frac{\alpha_G}{1 + (L/\beta_L)^{\eta 1}} - \gamma_G G \quad (1)$$

$$\frac{dL}{dt} = \frac{\alpha_{L1}}{1 + (C/\beta_C)^{\eta 2}} + \frac{\alpha_{L2} \cdot R^{\eta 3}}{(\theta_R)^{\eta 3} + R^{\eta 3}} - \gamma_L L \quad (2)$$

$$\frac{dC}{dt} = \frac{\alpha_C R^{\eta 3}}{(\theta_R)^{\eta 3} + R^{\eta 3}} - \gamma_C C \quad (3)$$

$$\frac{dR}{dt} = \rho_R [\mathrm{LuxR}]^2 A^2 - \gamma_R R \quad (4)$$

$$\frac{dA_{x,y,z}}{dt} = \xi(A_{x-1,y,z} + A_{x+1,y,z} + A_{x,y-1,z} + A_{x,y+1,z} + A_{x,y,z-1} + A_{x,y,z+1} - 6A_{x,y,z}) - \gamma_A \quad (5)$$

Model conti

Use of the model allowed for

- Improve design procedure by visualizing change in dynamics when switching HD{x}
- Insight into the real parameters using GraphPad PRISM and regression analysis

Liquid Phase pHD *Band Detect pHD{x} and pLD*

Model

Experiment

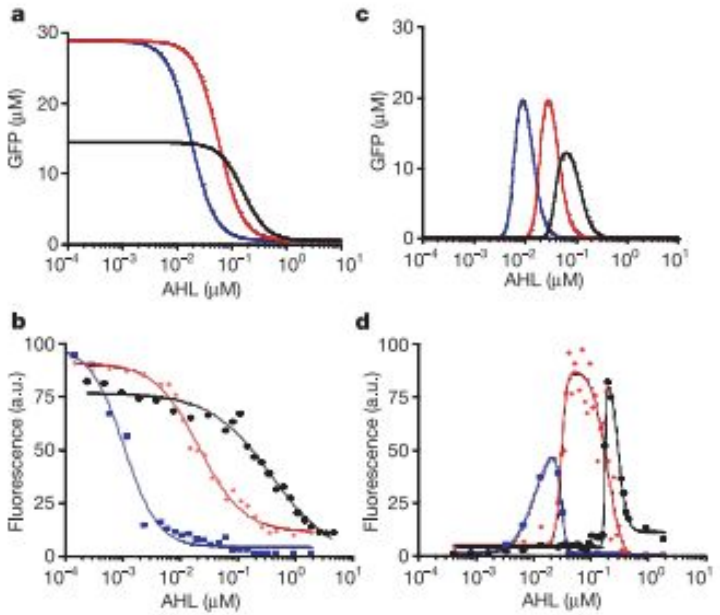


Figure 2: Model(a,b) and experimental (c,d) results of liquid culture with pHD{x} containing cells(a,c) and band detect plates with pHD{x} and pLD(c,d)

Experimental results

- Tested band-detect model
- Small differences occurred-growth rates and population densities

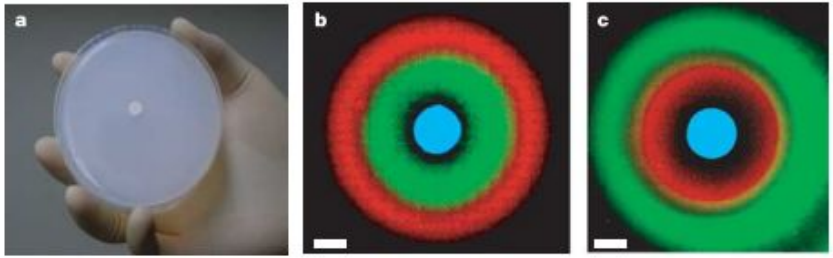


Figure 3. Experimental behavior. Scale bar, 5 mm
B. BD2-Red and BD3 cells
C. BD1 and BD2-Red cells

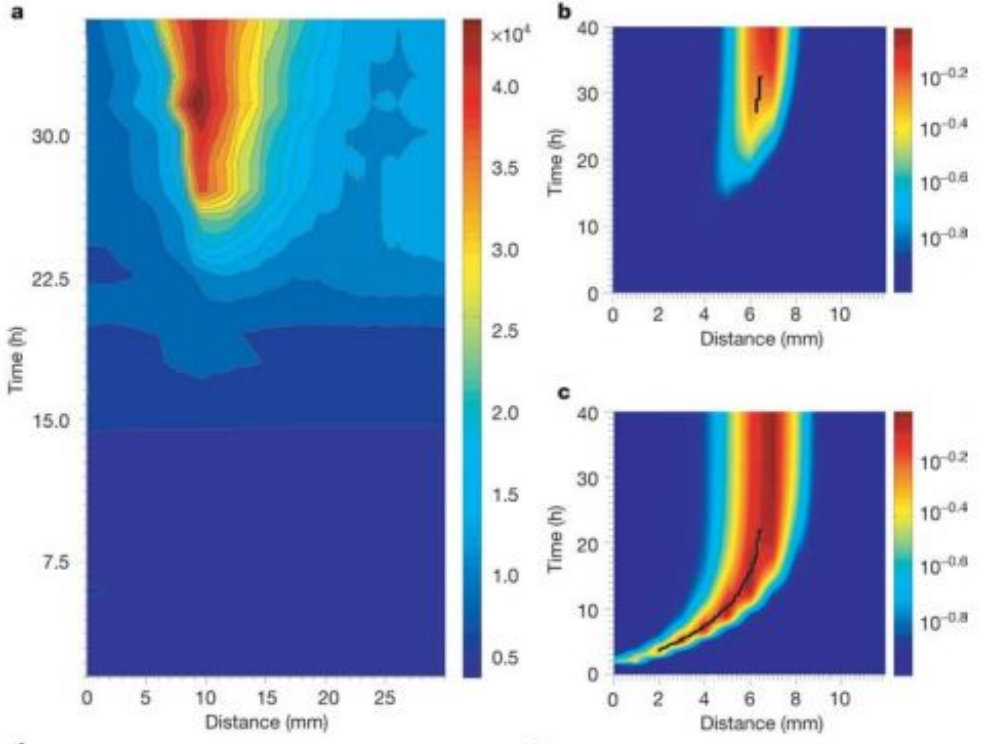


Figure 4. Ring formation dynamics. Red=max levels. Black lines=spatiotemporal shift of the ring

Variations on the basic model/experiment

- Patterns depends on distance from sender *and* placement of senders
- Rate of stability of LacI affects rate of fluorescence emergence
- Potential applications Ex. Tissue engineering

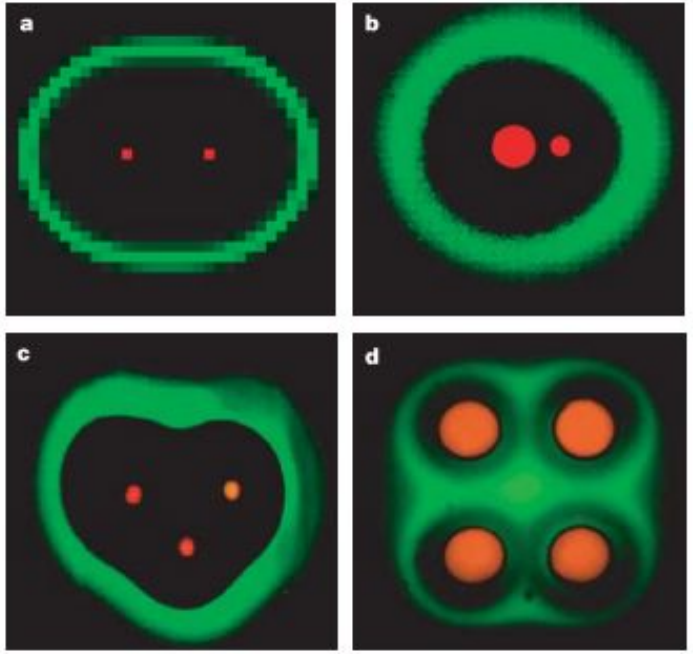


Figure 5. Formation of various patterns with different arrangements of senders (orange)

Summary and Approach Analysis

Motivation

- To effectively ID and demonstrate parts change to achieve a tunable circuit sensitive to a specified range of signal molecule concentration that allows for specific expression of desired genes

Strengths:

- Use of model to specify design options
- Mutations on characterized parts to further tunability
- Actually achieved a circuit with a tuneable activation range

Weaknesses:

- Activation range is the only tunable aspect
- The same approach for tunablity cannot be taken for genomic integrated parts
- Only designed for expression of one output of protein
    - more work needed for applications to tissue engineering and biomaterial fabrication