

BioWire

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BioWire Outline

- Overview
- Types of propagation
- Weiss Circuit
- Our Circuit and Timing
- Modeling
- BioBricks and Other Materials
- Experiments
- Debugging and Backup Plans
- Photolithography

BioWire Overview

Our Mission:

To engineer bacteria capable of propagating signals on a macroscopic level.

And to make it look wicked cool.

Our Plan:

Use photolithography techniques to create a “wire” of bacteria.

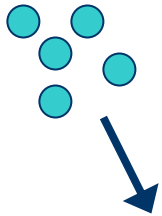
Use cell-to-cell signaling and transcriptional regulation to create a chemical “pulse” that travels down the length of the wire.

Two Types of Propagation

- “Pulse”
- “Line”
- Both transmitted via cell-cell signaling
- We are planning on building a pulse BioWire; the line BioWire is our backup.

BioWire: “Pulse” Propagation

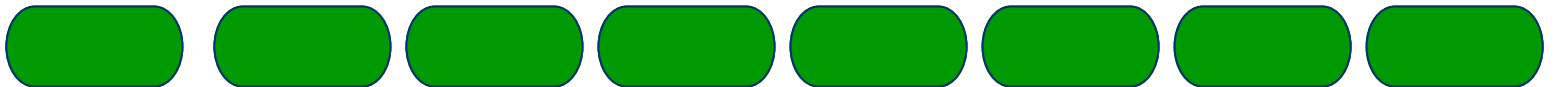
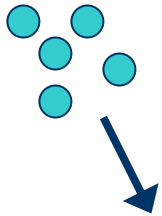
Initial Signal



Cells have refractory period

BioWire: “Line” Propagation

Initial Signal



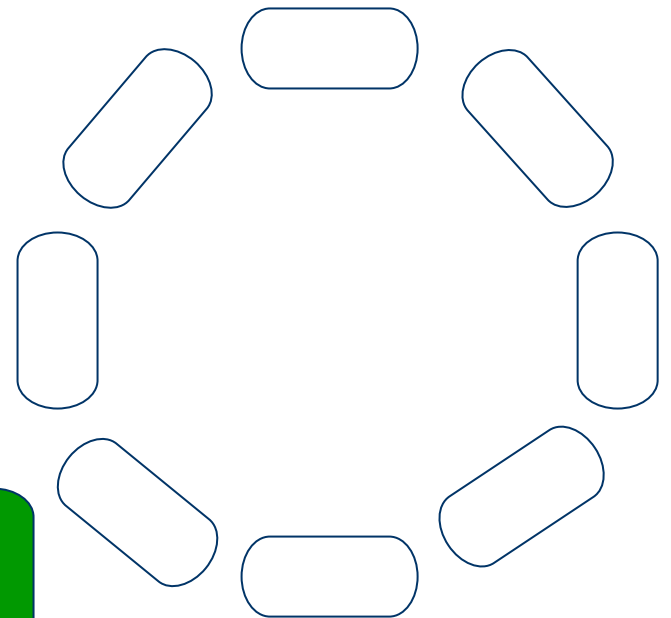
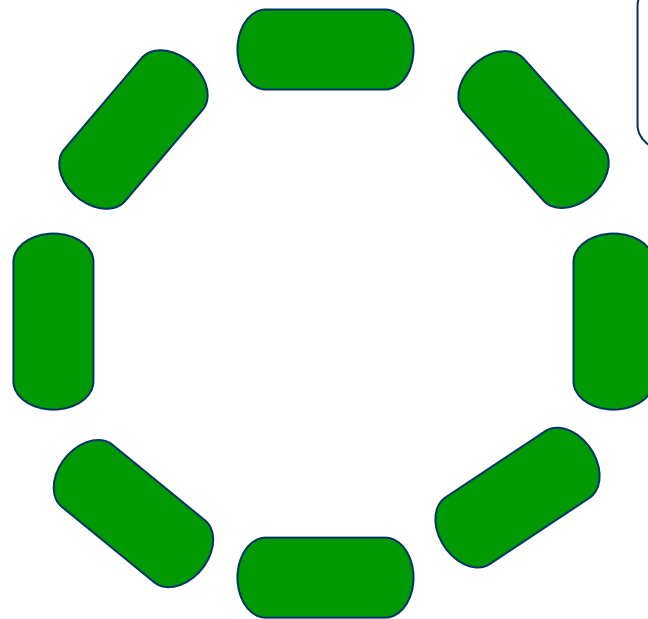
Cells do not have refractory period

Patterns

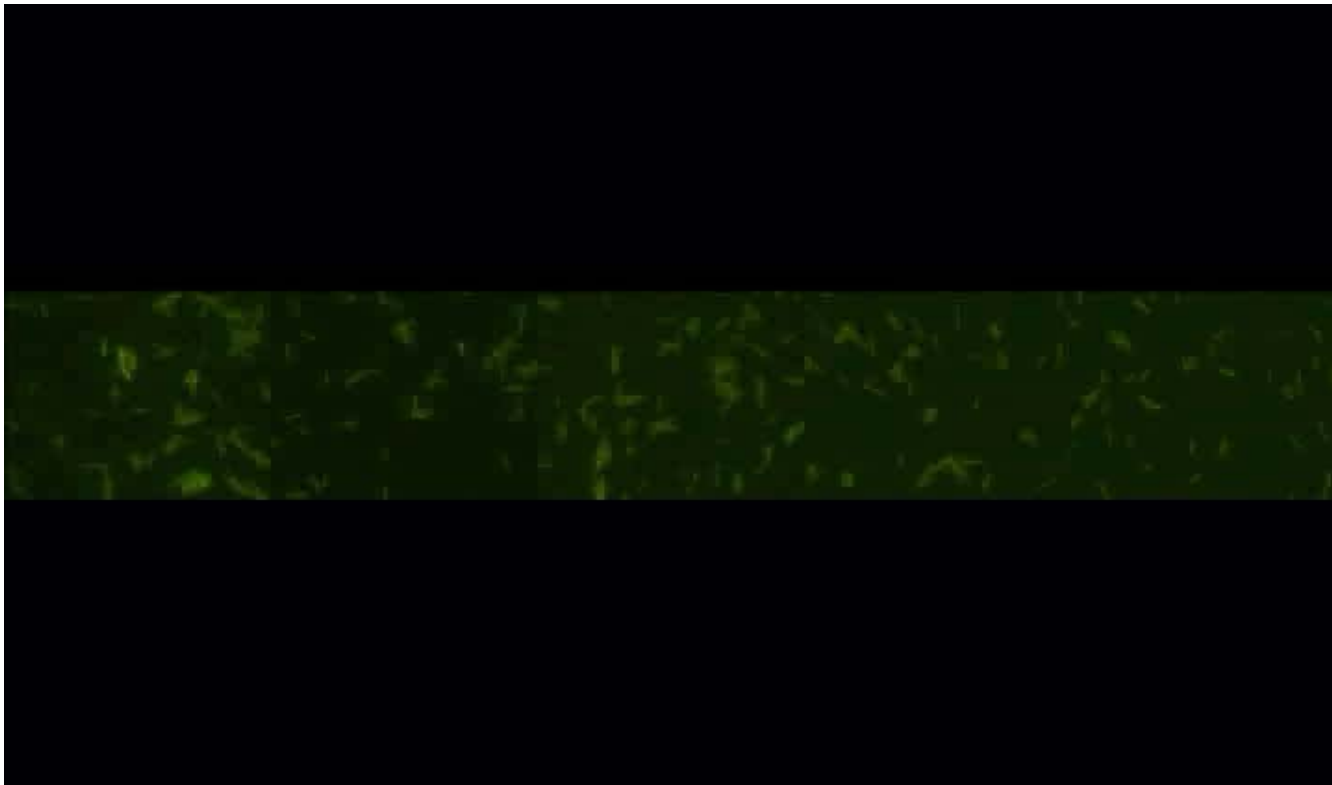
- Initially start with line
 - Bacteria can be arranged in a line either through streaking or by photolithography
- Alternatively, different temporal patterns can be produced by different spatial arrangement of bacteria.

Patterns

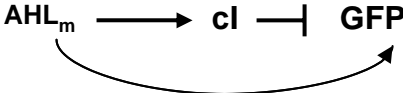
- Uniform (ring wave)
- Circle (oscillator?)
- Others?



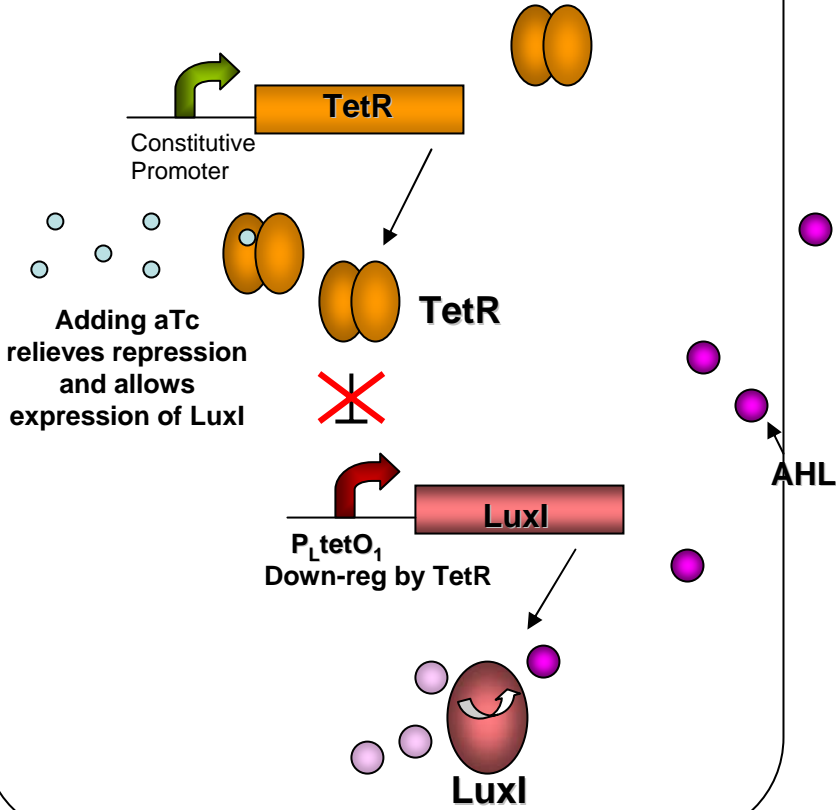
Ron Weiss Movie



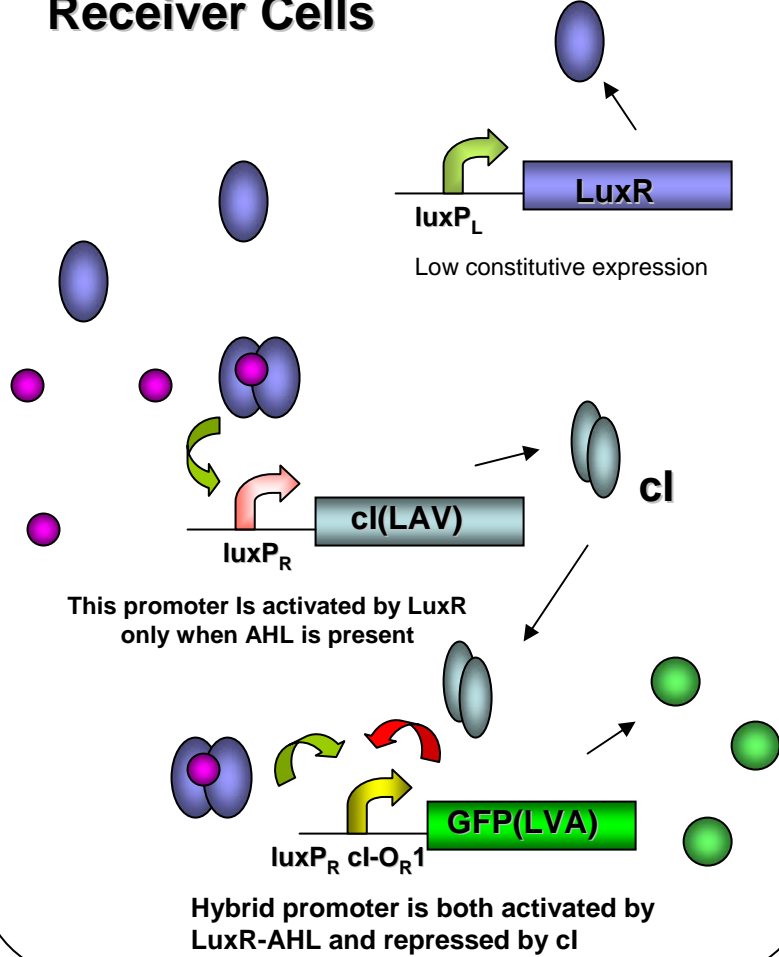
Weiss's Pulse-Generator



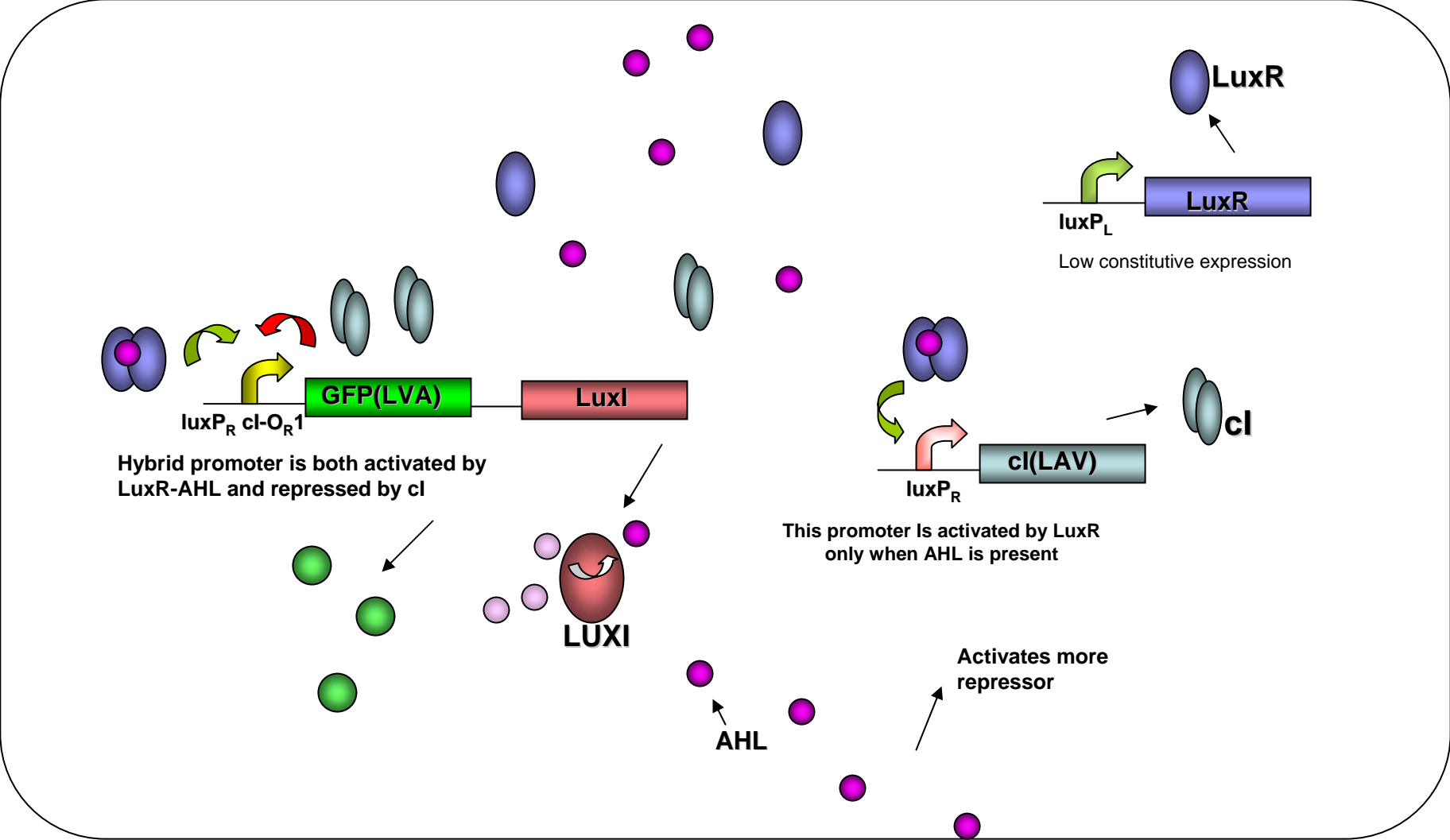
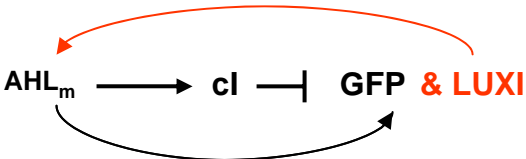
Sender Cells



Receiver Cells



Our Proposed Design



Hybrid promoter is both activated by LuxR-AHL and repressed by ci

This promoter is activated by LuxR only when AHL is present

Activates more repressor

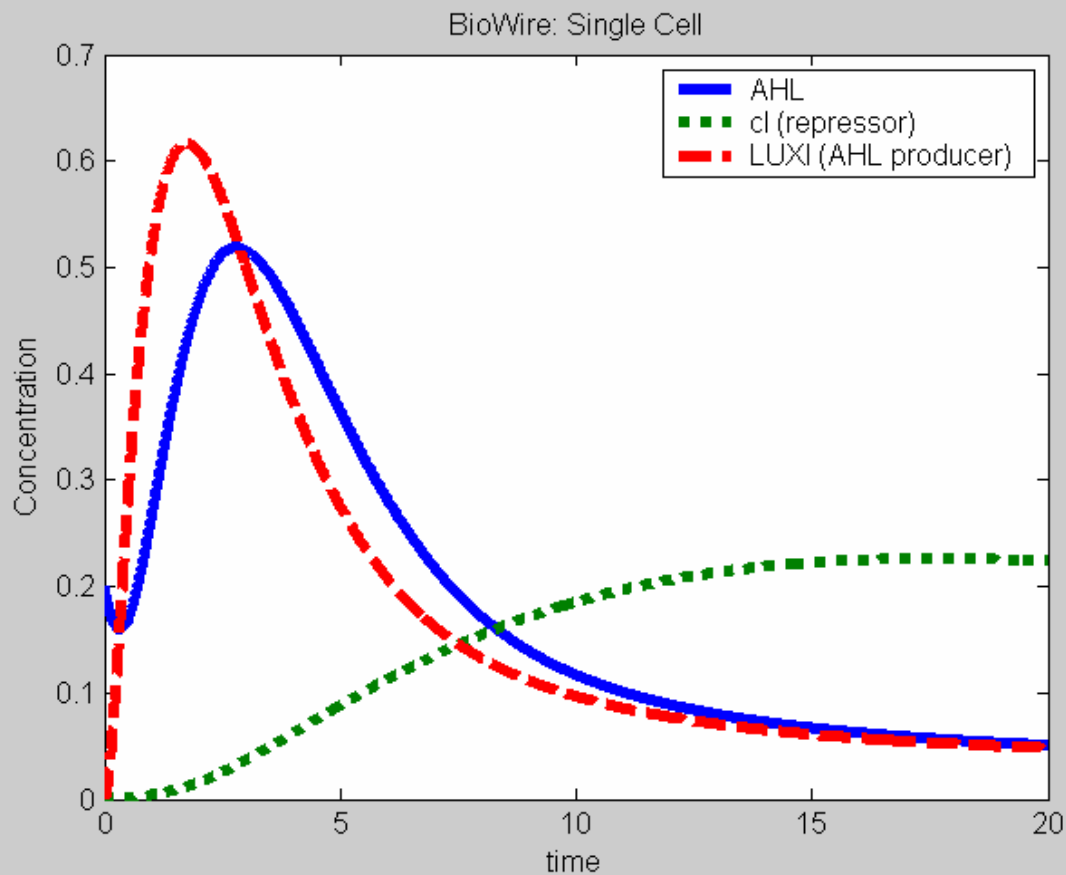
→ To neighboring cells

Timing

Steps:

- LuxR expressed constitutively in cell
- LUXR binds with AHLm
- LUXR-AHLm binds to
 - LuxPR
 - LuxBox
- CI & GFP (BioWire uses GFP-LuxI) produced
- CI binds to CI operator
- GFP (BioWire uses GFP-LuxI) repressed

BioWire Modeling: Single Cell



1. AHL binds to LUXR, forming LUXR-AHL complex
2. LUXR-AHL complex activates LuxI and cl
3. LUXI protein increases production of AHL in the short term; Cl eventually inhibits LuxI, shutting off AHL production

Modeling: Single Cell

- Modeling done in MATLAB using differential equations (continuum assumption, also used in Weiss)
- 5 species tracked: AHL, LUXR, LUXR-AHL, CI, LUXI.
- Still need to get good values for constants.
- Ultrasensitivity and noise—want our system to fire only if signal is above a certain threshold level.

Modeling: Cell-Cell



BioBricks Needed

- Weiss Sender:
 - BBa_F1610 -- 3OC6HSL Sender Device. This is the luxI; linked to a promoter. [ORDERED]
 - BBa_R0040 -- Promoter (tetR, negative). This is the PLtet0-1 promoter; linked to the HSL sender.
 - BBa_I13017 -- TetR under Plac control. This makes the constitutive promoter of tetR. ?Will this work?
- Weiss Receiver:
 - BBa_F2621 -- 3OC6HSL Receiver Device. Why is the promoter be regulated, instead of being constitutively present? [ORDERED]
 - BBa_C0051 -- Repressor, Lambda cl (RBS- LVA+). This is the CI(LVA)
 - BBa_B0015 -- Double terminator consisting of BBa_B0010 and BBa_B0012.
- Weiss Receiver:
 - BBa_R0065 -- Promoter (lambda cl and luxR regulated -- hybrid)
 - BBa_E0044 -- GFP-AAV. ?Does anyone have better ideas for a reporter?
- Ribosome Binding Sites:
 - BBa_B0030 (strong); BBa_B0031 (weak); BBa_B0032 (medium); BBa_B0033 (weaker)
- Backup Repressor
 - BBa_R0011 -- Promoter (lacI regulated, lambda pL hybrid)

BioBricks + Materials Ordered

- Weiss Sender:
 - BBa_F1610 -- 3OC6HSL Sender Device. This is the luxI; linked to a promoter.
- Weiss Receiver:
 - BBa_F2621 -- 3OC6HSL Receiver Device. Why is the promoter regulated, instead of being constitutively present?
- Miscellaneous Parts
 - BBa_I13272 -- YFP producer controlled by 3OC6HSL Receiver Device
 - BBa_I12224 -- LacI protein generator (LVA-)
 - BBa_E0422 – Reporter ECFP (RBS+ LVA+ TERM) (B0034 E0022 B0015)
- Other Materials
 - AHL
 - aTc

Experiments: Plans and Expected Results (I)

This week

- **Test the AHL receiver**
 - [BioBrick and AHL should arrive by Tuesday]
 - Manually add AHL to the system in varying concentrations
 - Control: Add water instead of AHL
 - BioBrick comes with YFP as a reporter
 - *Expected Result.* YFP is expressed, but not in control plates.
 - Note: replicate experiment using GFP/mCherry as decided
- **Test the AHL sender**
 - [Components will be ordered Monday]
 - Replicate Weiss sender cell, adding GFP as a reporter
 - Manually add aTc to the system in varying concentrations
 - Control: Add water instead of aTc
 - *Expected Result.* GFP is expressed, but not in control plates.

Experiments: Plans and Expected Results (II)

Beginning Next Week

- **Test sender and receiver together**
 - Use a sender component without GFP
 - Manually add aTc to the system in varying concentrations
 - Control: Add water instead of aTc
 - *Expected Result:* Fluorescence is expressed, but not in control plates.
- **Test repressor by reconstructing Weiss circuit**
 - [Components will be ordered on Monday]
 - Manually add aTc to the system in varying concentrations
 - Control: Add water instead of aTc
 - *Expected Result:* Fluorescent pulse is expressed, but not in control plates.

Experiments: Plans and Expected Results (III)

By July 15th

- **Test cell signaling density**
 - Vary concentrations of sender and receiver cells in Weiss circuit (include concentration of 0 as control).
 - *Expected Result.* The number of cells needed is reasonable. (From Weiss' paper, it should only be 1 sender cell.)
- **Photolithography testing**
 - [Start ASAP, integrate into our experiments when ready]
 - Do the bacteria stick?
 - How long do they live?
 - Do we need to get them into stationary phase?
 - *Expected Result.* Bacteria stick, stay alive, and look cool

Experiments: Plans and Expected Results (IV)

By mid-August

- **Test our circuit**

- Us (Propagation):



- Control (Diffusion):



Visualization

- Use XY motor to move the slide between frames
- ~200 ms / exposure
- ~1 sec / motor movement
- 1 frame / 30 sec

Debugging the System

- Changing the strength of the RBS (weaker, weak, medium, strong)
- Altering repressor/operator affinity through mutation in the operator region
- Mutating promoter sequences
- Increasing AHL degradation rate through raising the pH of the medium
- Lowering plasmid copy number through mutations in replication origin (if repressor and AHL/GFP are on separate plasmids)
- Trying different repressors (lacI, araC etc.), requires inserting different operator regions

Backup Plans

- If repressor fails, we will get an extending line of GFP.
- We could send out a repressor wave slower than the AHL wave. The repressor would be produced independent of AHL/LuxR. We would see an increasing line segment.
- If lithography fails, we can streak cells in a narrow line.

Photolithography

- *E. Coli* is 1-1.5 microns long, 0.25-0.50 microns in diameter.
- Precisely pattern biological molecules that *E. Coli* can stick to. Need to choose a molecule to which our strain will adhere.
- Whitesides microcontact printing, pattern gold substrate surface with thiol-based SAMs to which mannose can bind. *E. Coli* at tips of pili
- Cornell- dry lift-off method, protein binds to parylene polymer on substrate

Photolithography

- Possible Methods

- 16-mercaptohexadecanoic acid patterned microarrays

Methods for Fabricating Microarrays of Motile Bacteria, Rozhok, Shen, Littler, et al.

- patterned poly(ethylene glycol) (PEG) hydrogel microstructures

Control of Mammalian Cell and Bacteria Adhesion..., Koh, Revzin, Simonian, Reeves, Pishko

- microcontact printing (μ CP) to pattern... such as poly-L-lysine

Chemical and topographical patterning for directed cell attachment, Craighead, James, Turner

- Whitesides: r-D-mannopyranoside (adhesion of uropathogenic *E. Coli*)

Arrays of self-assembled monolayers for studying inhibition of bacterial adhesion, Qian, Metallo, et al.

- Concerns

- Will the *E. Coli* stick?
- How long will they live?
- Can we get them into stationary phase?

Photolithography

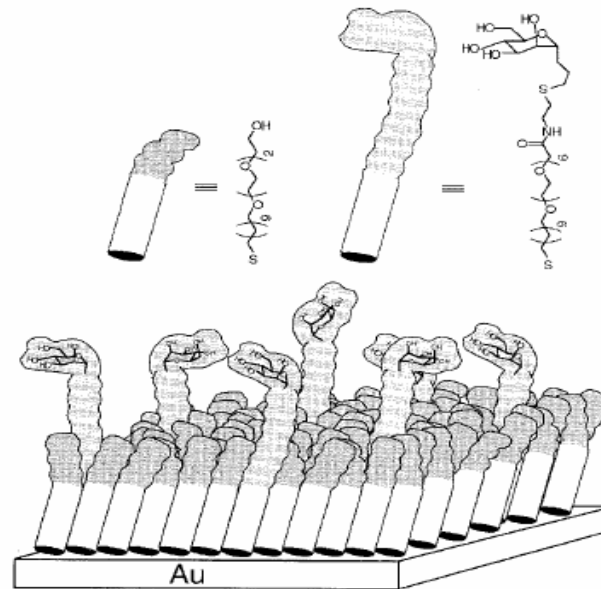


Fig. 1. Schematic representation of a mixed SAM in which an (EG)₆ group terminating in a mannose ligand is presented above a background of (EG)₃ groups. In this illustration, a SAM with $\chi_{\text{Man}} = 10^{-1}$ is shown. The triethylene glycol thiol extends 22 Å from the gold surface, whereas the extended mannose thiol, with three extra ethylene glycol units, extends 30 Å from the gold surface (18). The (EG)_n groups prevent nonspecific adhesion of proteins or bacteria to the surface of the SAM (18).

Whitesides et. al., *Measuring the forces involved in polyvalent adhesion of uropathogenic Escherichia coli to mannose-presenting surfaces*

Photolithography

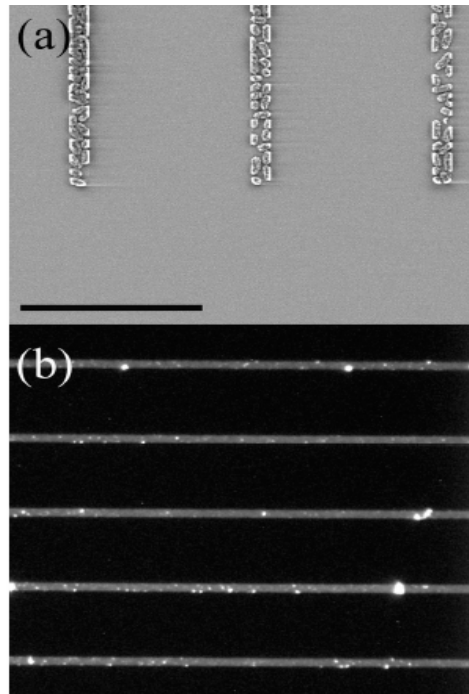


Fig. 3. (a) SEM micrograph of 2 μm wide lines of E. coli cells (scale bar = 20 μm). (b) Fluorescent micrograph of fluorescent spheres functionalized with aldehyde-sulfate bound to 5 μm wide APTS lines. From Ilic et al., 2000.