Cell-Cell Communications

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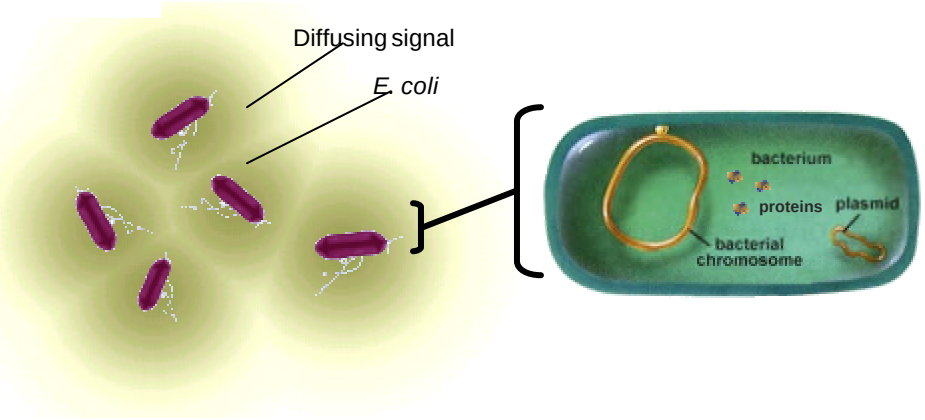
Computing Beyond Silicon Summer School, Caltech, Summer 2002

Programming Cell Communities

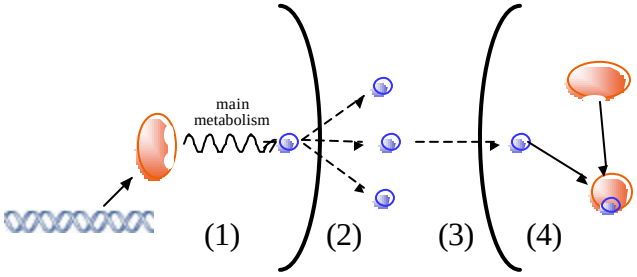


Program cells to perform various tasks using:

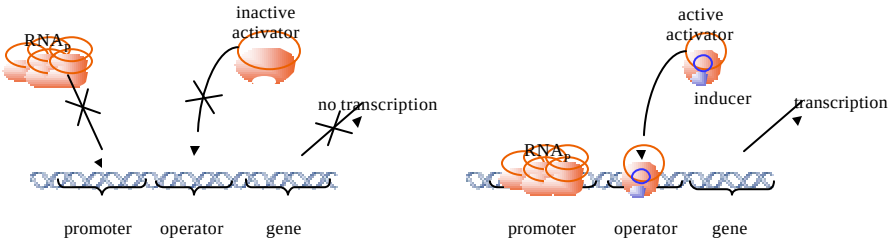
- Intra-cellular circuits
  - Digital & analog components
- Inter-cellular communication
  - Control outgoing signals, process incoming signals

Intercellular Communications

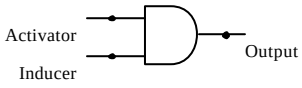
- Certain inducers useful for communications:
  1. A cell produces inducer
  2. Inducer diffuses outside the cell
  3. Inducer enters another cell
  4. Inducer interacts with repressor/activator → change signal



The Intercellular AND Gate



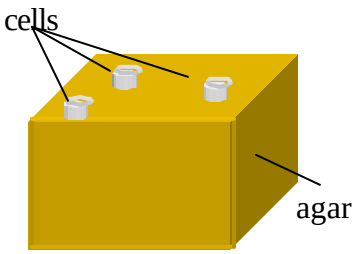
- Inducers can activate activators:
  - VAI (3-N-oxohexanoyl-L-Homoserine lacton) → luxR
- Use as a logical AND gate:

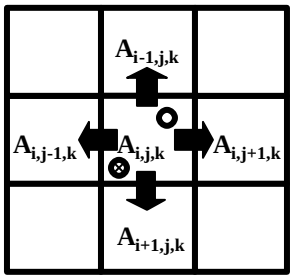


Activator	Inducer	Output
0	0	0
0	1	0
1	0	0
1	1	1

Communications Simulator

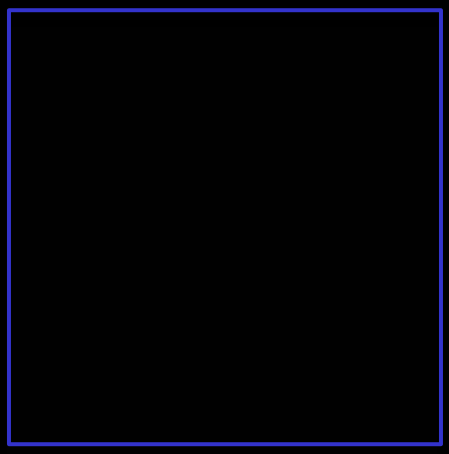
- Cells sit on 3D agar grid
- Model genetic networks in cells (ODE, stochastic)
- ODE diffusion model with reflective boundaries



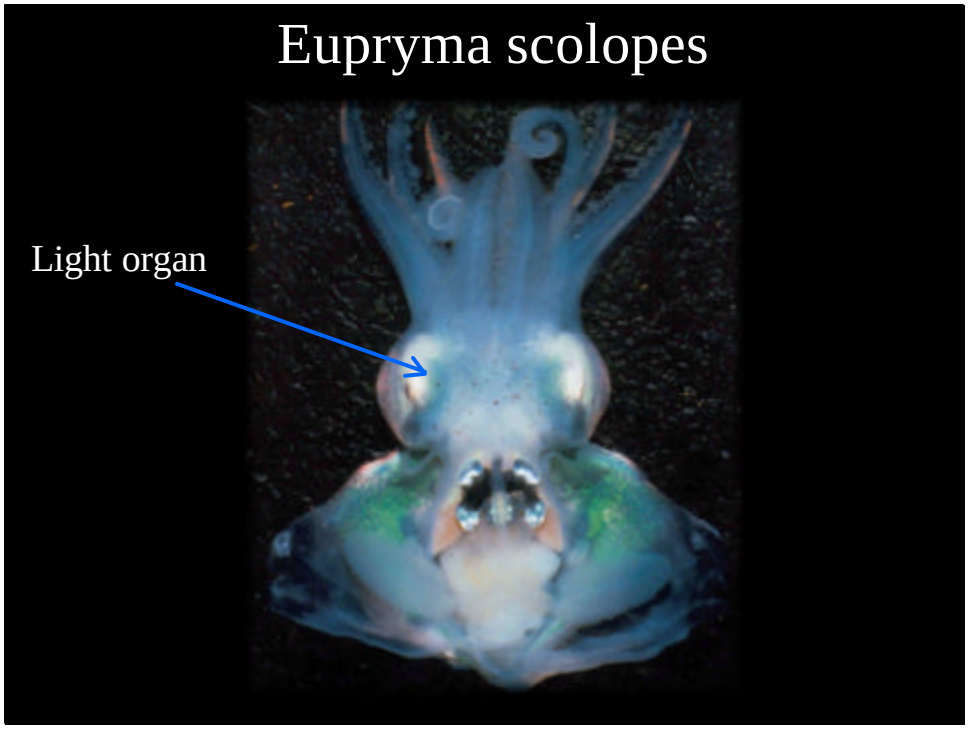


3D diffusion: $dA_{i,j,k}/dt = k_{diff}(A_{i,j-1,k} + A_{i,j+1,k} + A_{i-1,j,k} + A_{i+1,j,k} + A_{i,j,k+1} + A_{i,j,k-1} - 6A_{i,j-1,k})$

Two Cell Simulation



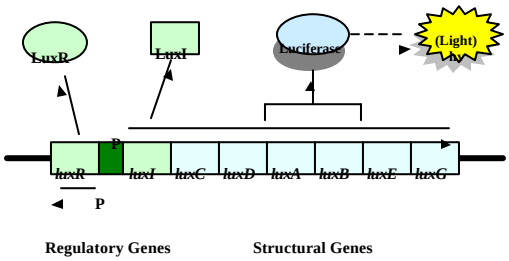
Eupryma scolopes



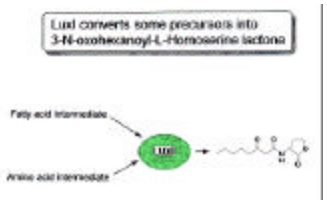
Quorum Sensing

- Cell density dependent gene expression

Example: *Vibrio fischeri* [density dependent bioluminscence]

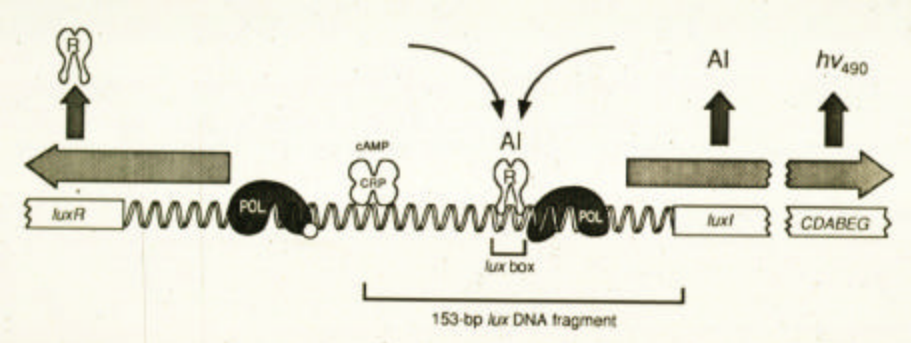


The lux Operon

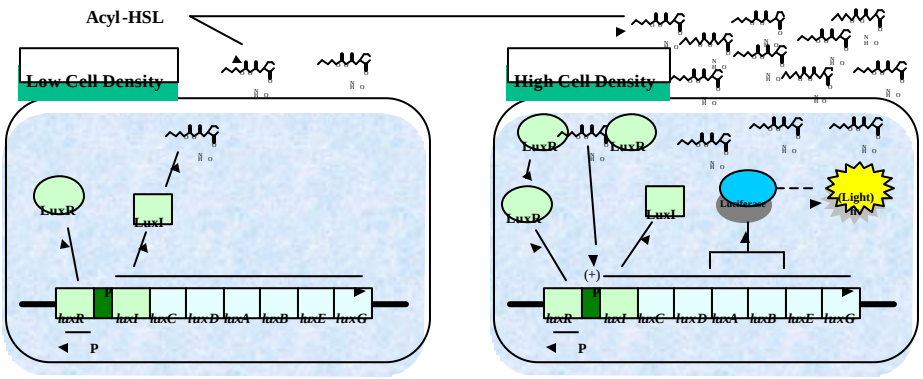


LuxI metabolism
→ *autoinducer* (VAI)

The lux box



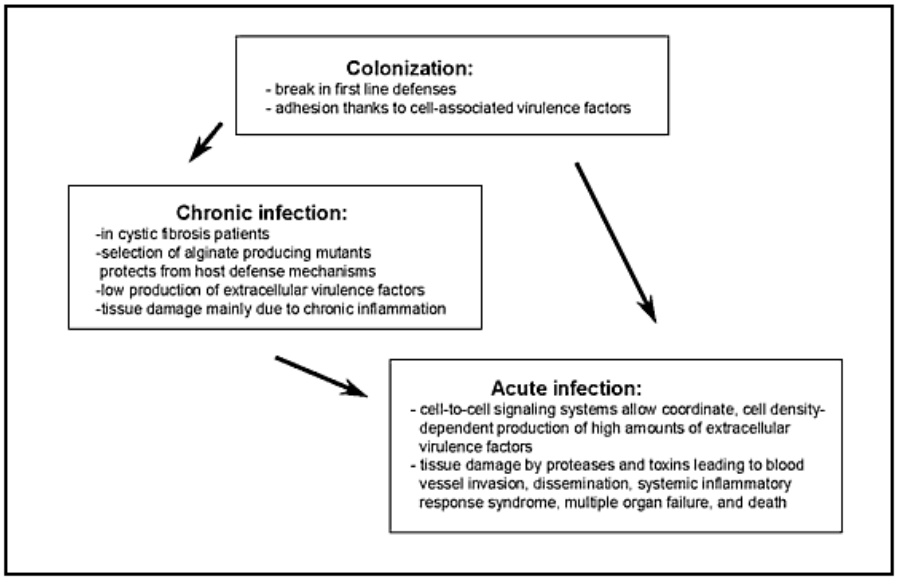
Low and High Cell Densities



free living, 10 cells/liter
<0.8 photons/second/cell

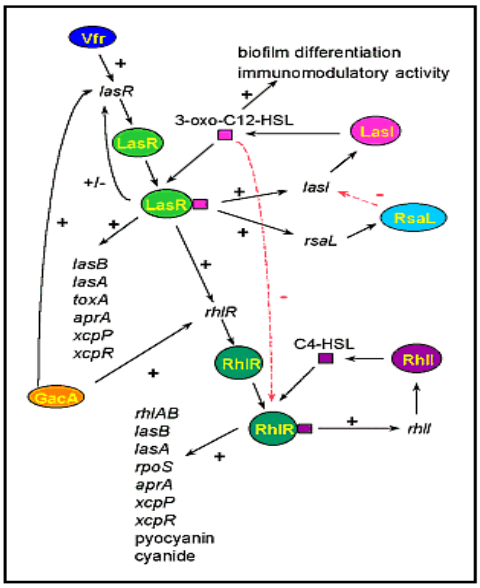
symbiotic, 10^{10} cells/liter
800 photons/second/cell

P. Aeruginosa



P. Aeruginosa

- Two autoinducer systems regulate virulence/biofilm formation
- Secrete virulence factors when population high enough to overcome host defenses

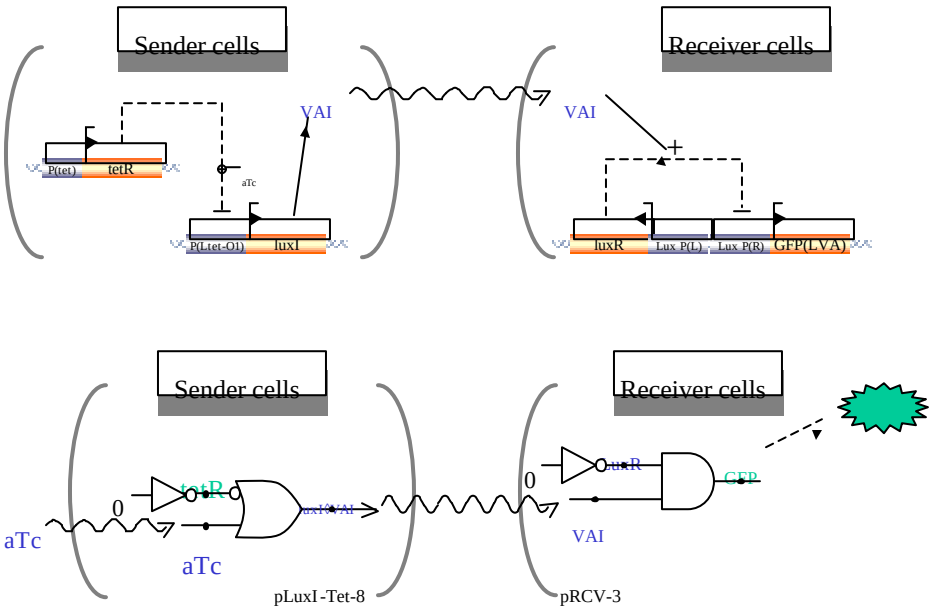


Sources for a Library of Signals

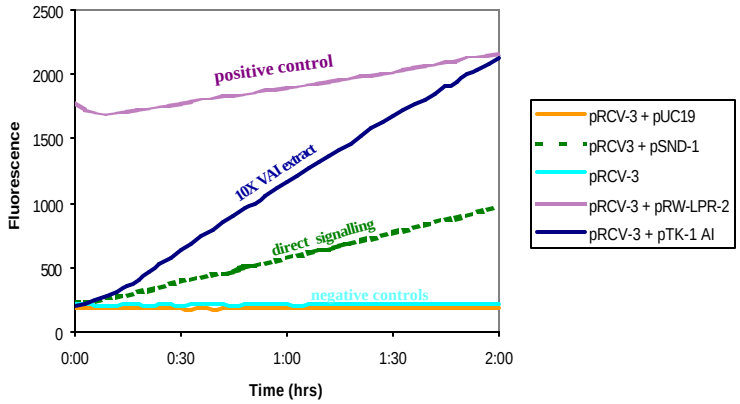
N-acyl-L-Homoserine Lactone Autoinducers in Bacteria

Species	Relation to Host	Regulate Production of	I Gene	R Gene
Vibrio fischeri	marine symbiont	Bioluminescence	*luxI*	*luxR*
Vibrio harveyi	marine symbiont	Bioluminescence	*luxL,M*	*luxN,P,Q*
Pseudomonas aeruginosa	Human pathogen	Virulence factors	*lasI*	*lasR*
		Rhamnolipids	*rhlI*	*rhlR*
Yersinia enterocolitica	Human pathogen	?	*yenI*	*yenR*
Chromobacterium violaceum	Human pathogen	Violaceumproduction Hemolysin Exoprotease	cviI	cviR
Enterobacter agglomerans	Human pathogen	?	*eagI*	?
Agrobacterium tumefaciens	Plant pathogen	Ti plasmid conjugation	*traI*	*traR*
Erwinia caratovora	Plant pathogen	Virulence factors Carbapenem production	*expI*	*expR*
Erwinia stewartii	Plant pathogen	Extracellular Capsule	*esaI*	*esaR*
Rhizobium leguminosarum	Plant symbiont	Rhizome interactions	*rhiI*	*rhiR*
Pseudomonas aureofaciens	Plant beneficial	Phenazine production	*phzI*	*phzR*

Cell-Cell Communication Circuits

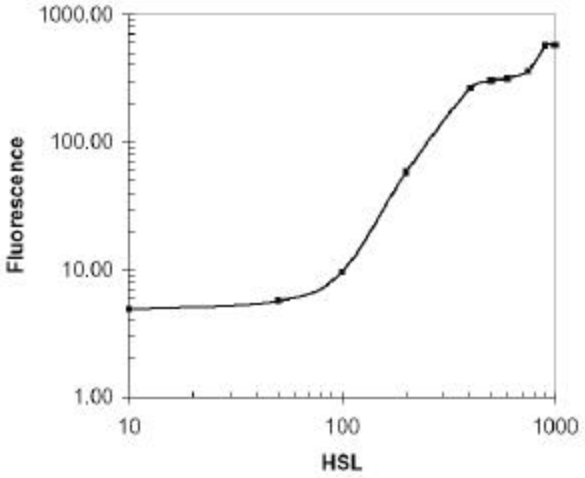


Time-Series Response to Signal



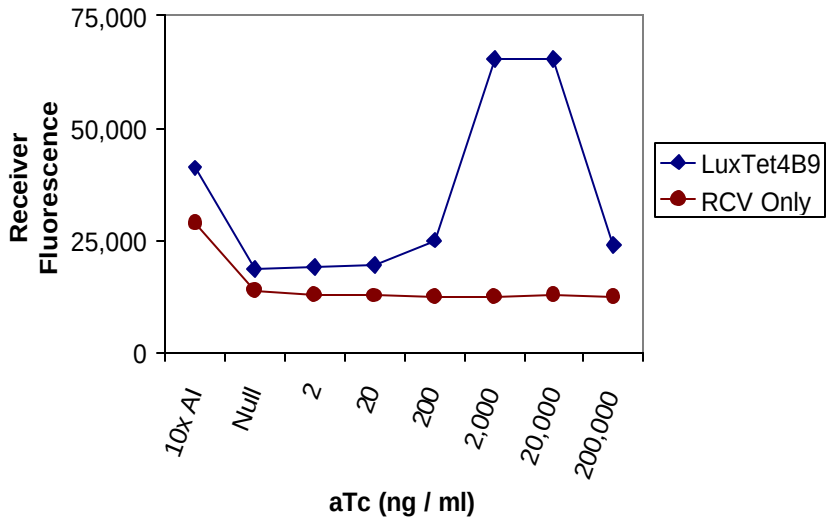
Fluorescence response of receiver (pRCV-3)

Characterizing the Receiver

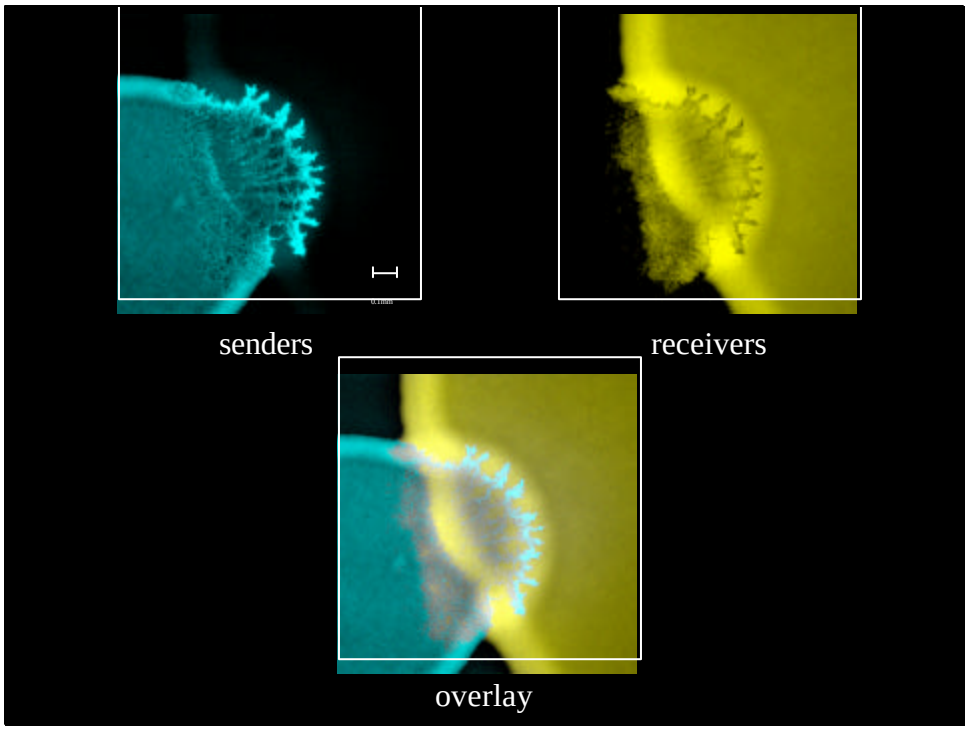


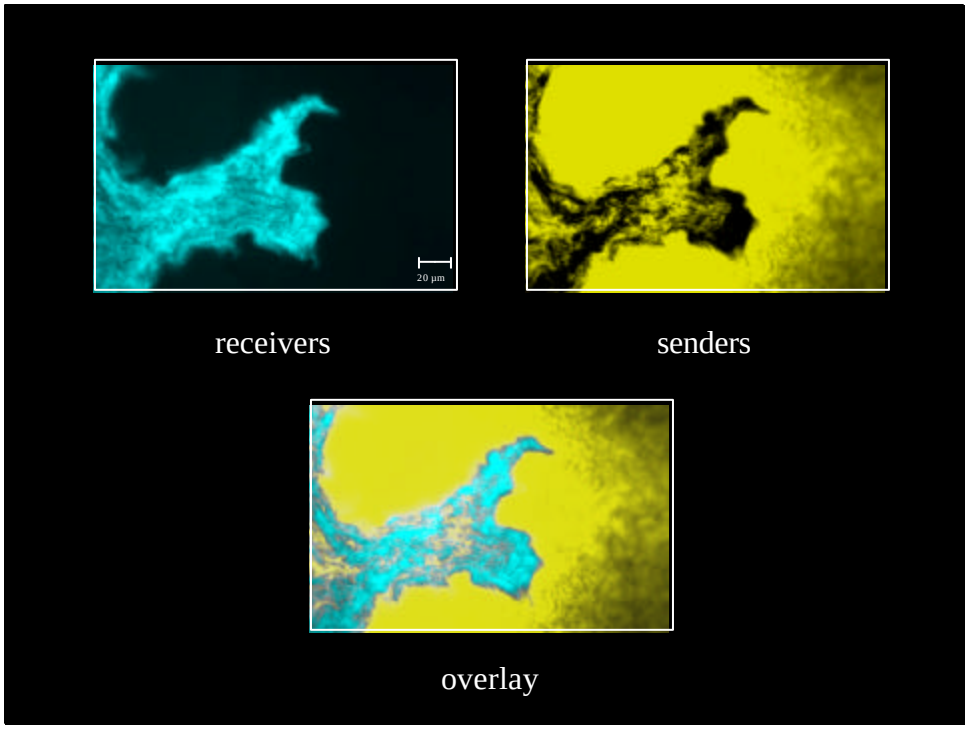
Response of receiver to different levels of VAI extract

Controlling the Sender's Signal Strength



Dose response of receiver cells to aTc induction of senders





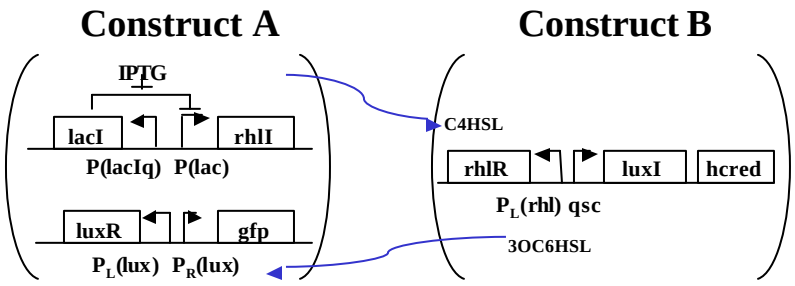
Engineered Microbial Communication

Ron Weiss
Tom Knight
Nick Papadakis

February, 2001

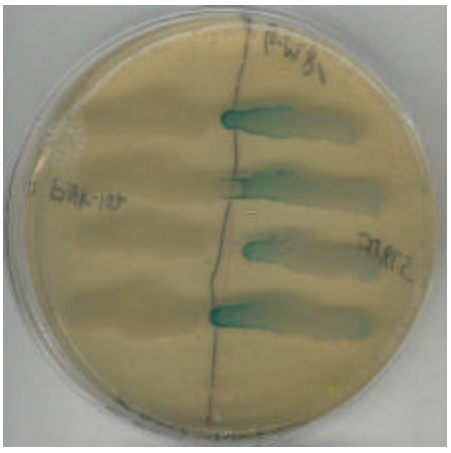
Bi-Directional Communication

[Karig, Weiss]



- Explore substrate properties
  - Crosstalk
  - Time scale/delay
  - Signal strength
- Create constructs useful in later systems

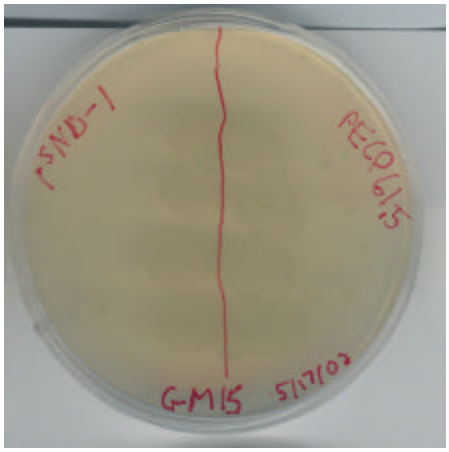
Demonstrating rhlI Communications



senders receivers

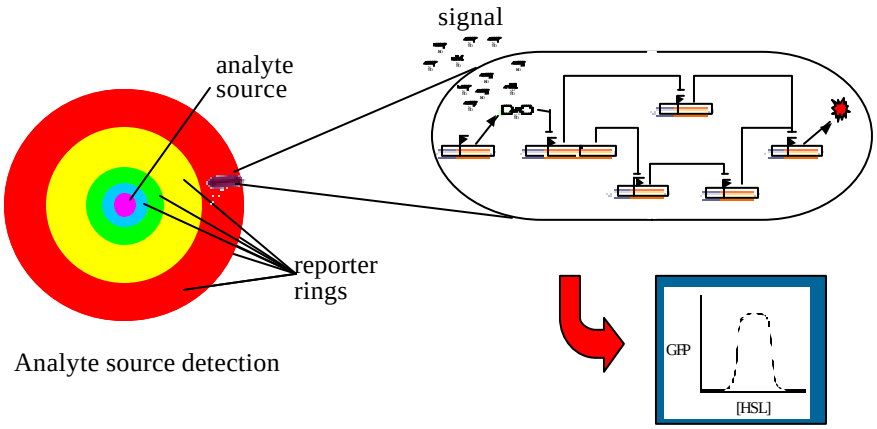
Testing Crosstalk

Does 3OC6HSL bind RhlR to activate transcription?



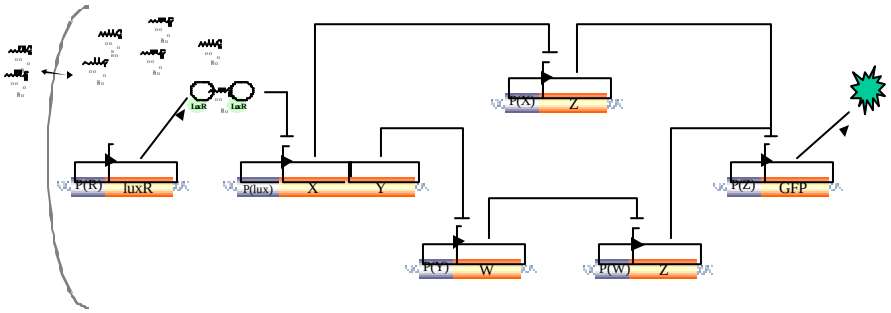
Signal Processing / Analog Circuits

Detecting Chemical Gradients



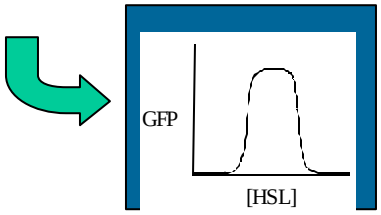
Analyte source detection

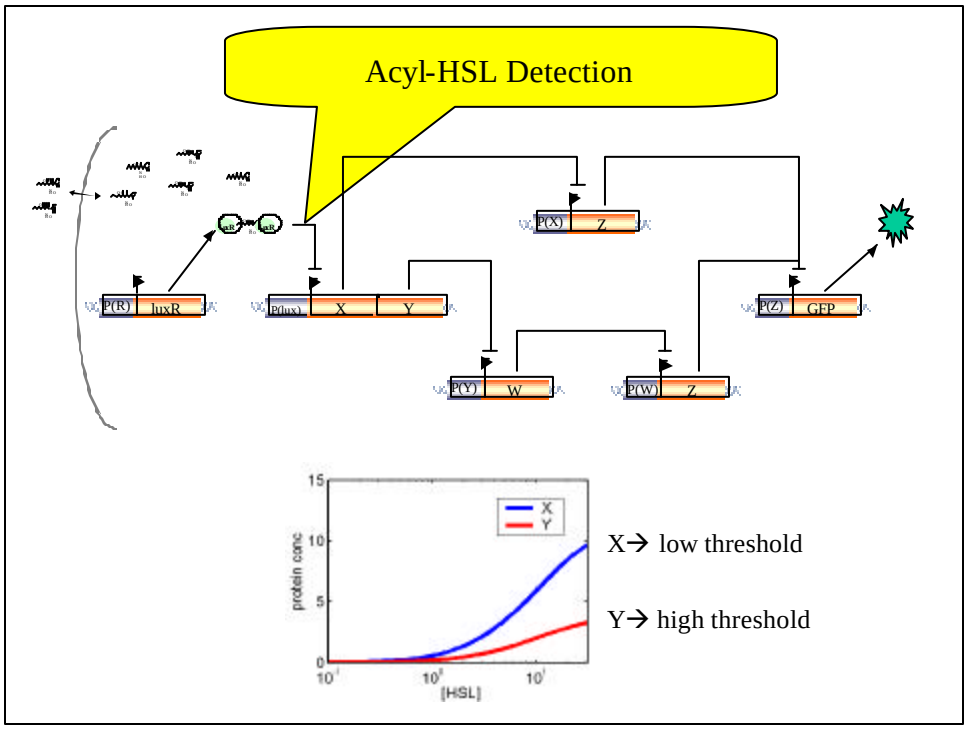
Circuit Components

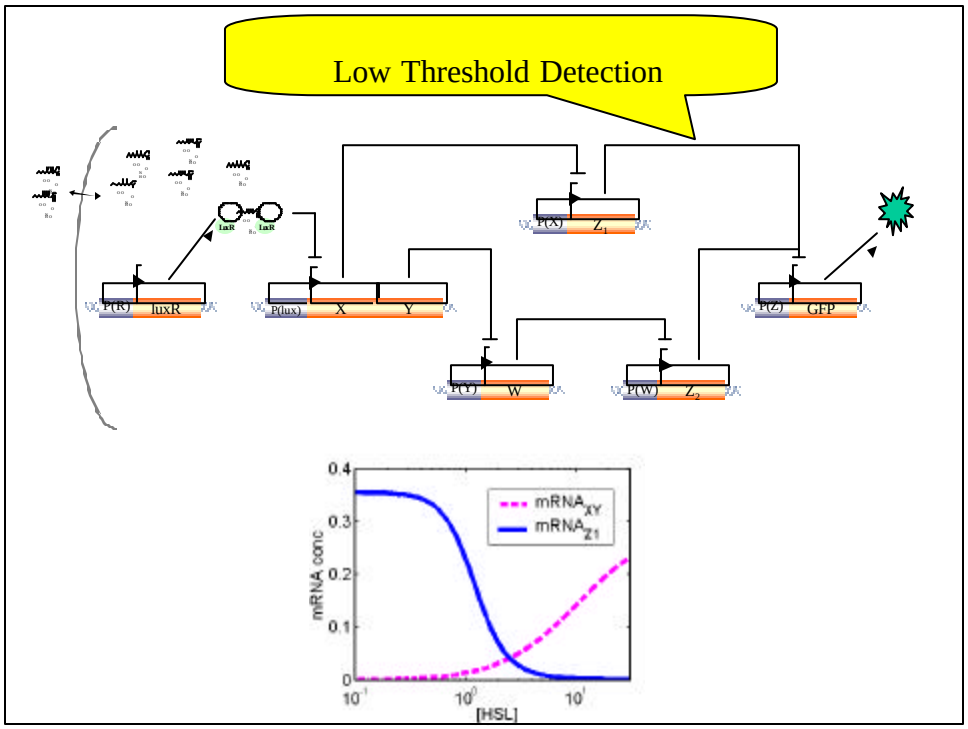


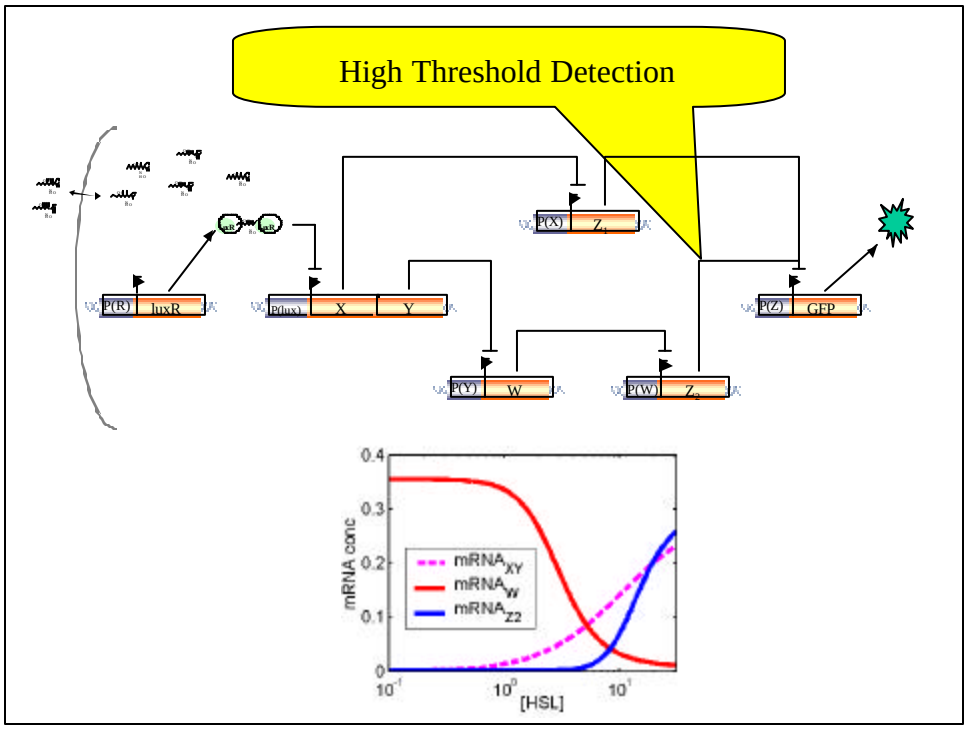
Components:

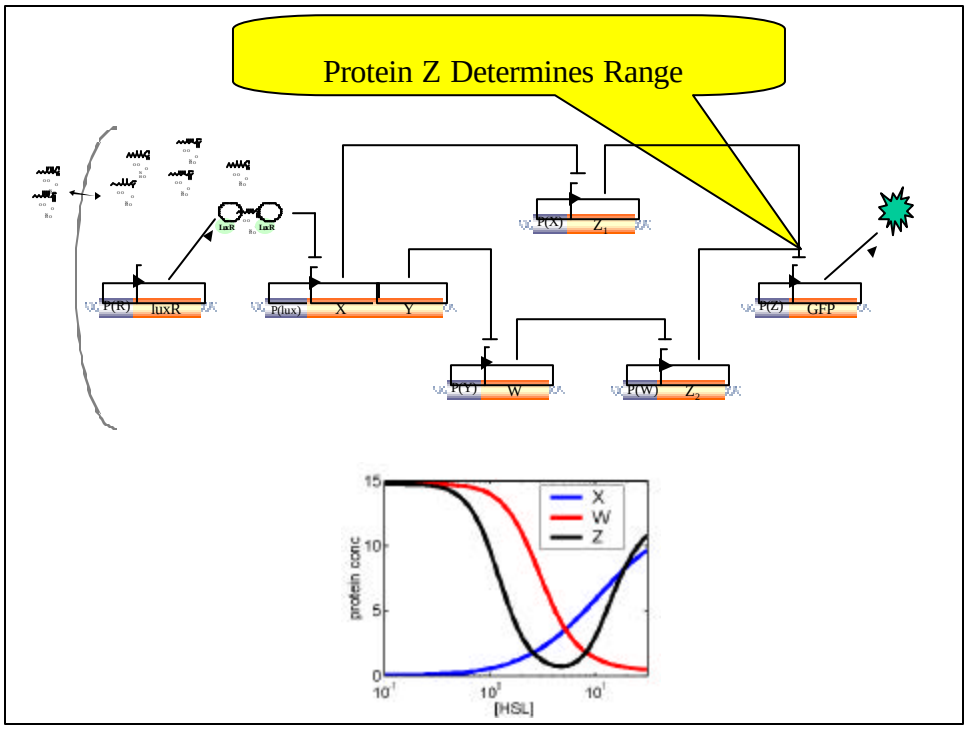
1. Acyl-HSL detect
2. Low threshold
3. High threshold
4. Negating combiner

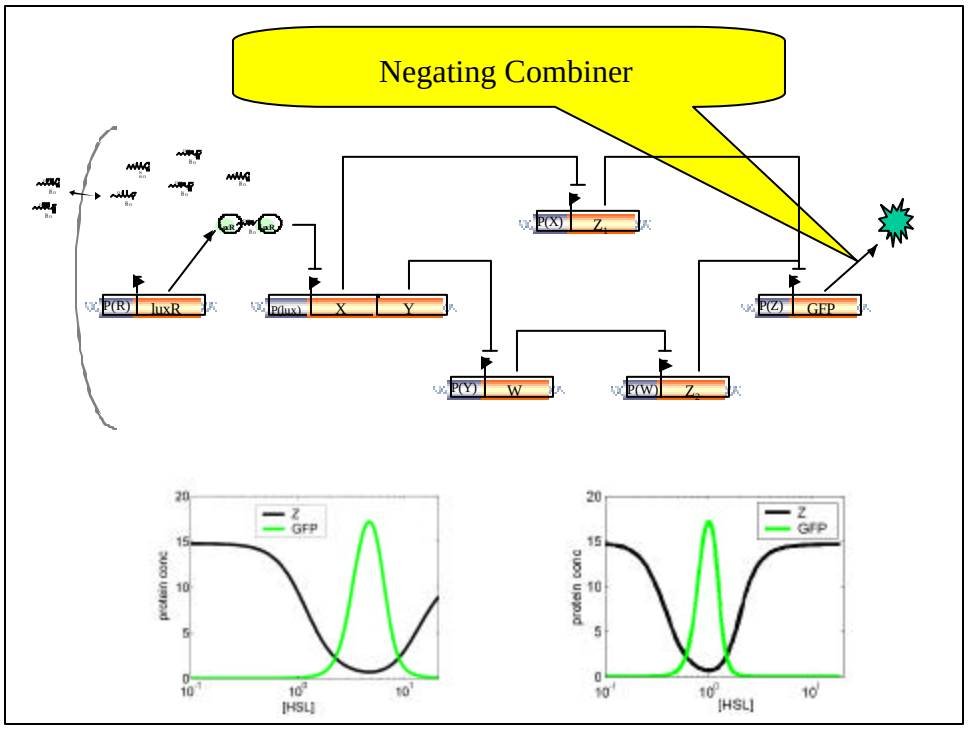



Acyl-HSL Detection
P(X)
Z
P(R)
luxR
P(lux)
X
Y
P(Z)
GFP
P(Y)
W
P(W)
Z
protein conc
X
Y
[HSL]
X→ low threshold
Y→ high threshold


Low Threshold Detection
P(X)
Z1
P(R)
luxR
P(lux)
X
Y
P(Z)
GFP
P(Y)
W
P(W)
Z2
mRNA conc
mRNA_XY
mRNA_Z1
[HSL]

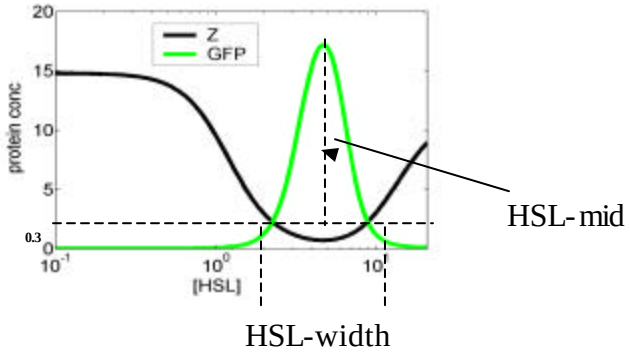

High Threshold Detection
P(R)
luxR
P(lux)
X
Y
P(X)
Z1
P(Y)
W
P(W)
Z2
P(Z)
GFP
mRNA conc
mRNA_XY
mRNA_W
mRNA_Z2
[HSL]


Protein Z Determines Range
P(R)
luxR
P(lux)
X
Y
P(X)
Z1
P(Y)
W
P(W)
Z2
P(Z)
GFP
protein conc
X
W
Z
[HSL]

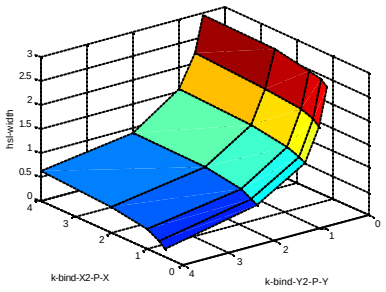


Engineering Circuit Characteristics

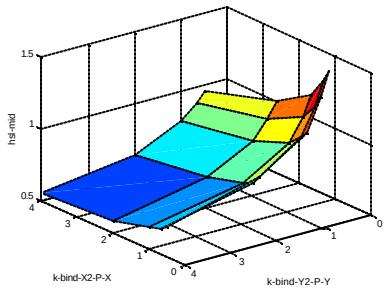
- HSL-mid: the midpoint where GFP has the highest concentration
- HSL-width: the range where GFP is above 0.3uM



Tuning the Range:
Repressor/Operator Affinities



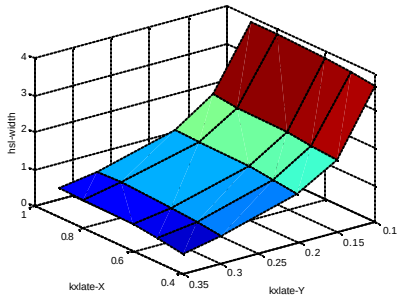
range width
versus
X & Y repressor efficiencies



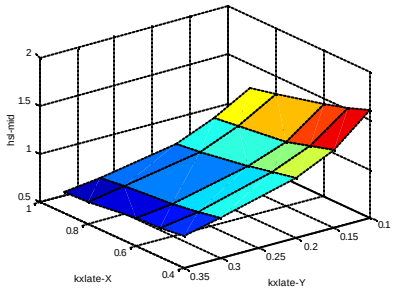
range mid-point
versus
X & Y repressor efficiencies

rep/op affinity increases → transfer-curve shifts left

Tuning the Range:
Ribosome Binding Sites



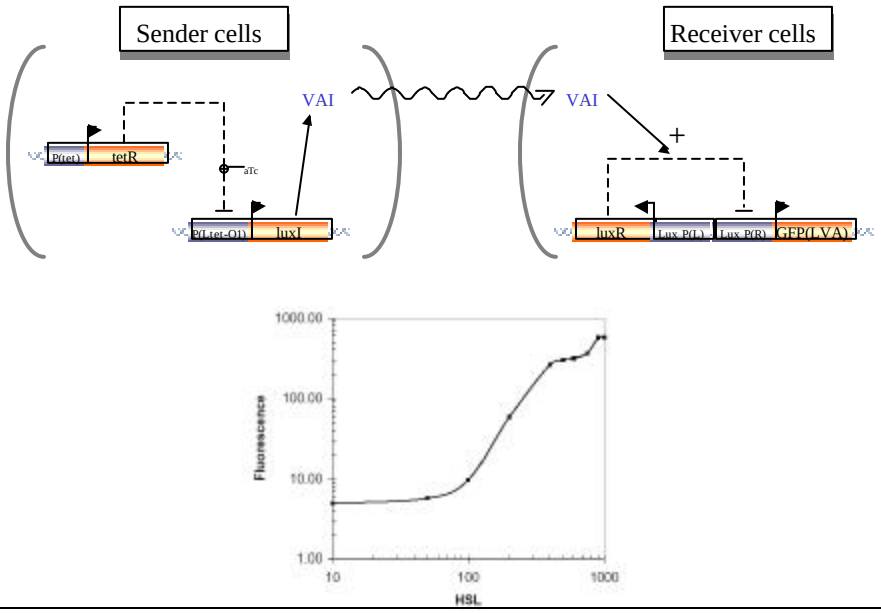
range width
versus
X & Y RBS efficiencies



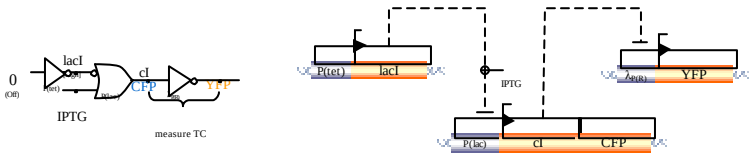
range mid-point
versus
X & Y RBS efficiencies

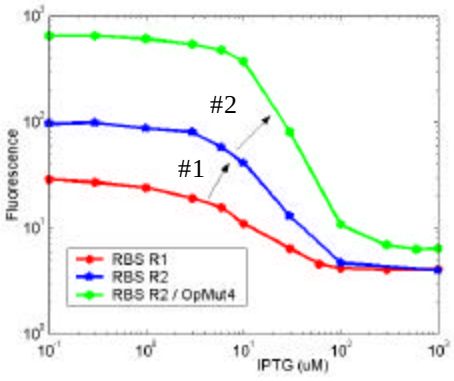
RBS efficiency increases → transfer-curve shifts left

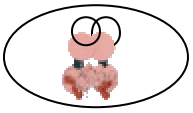
HSL Detection



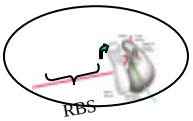
Low Threshold Component







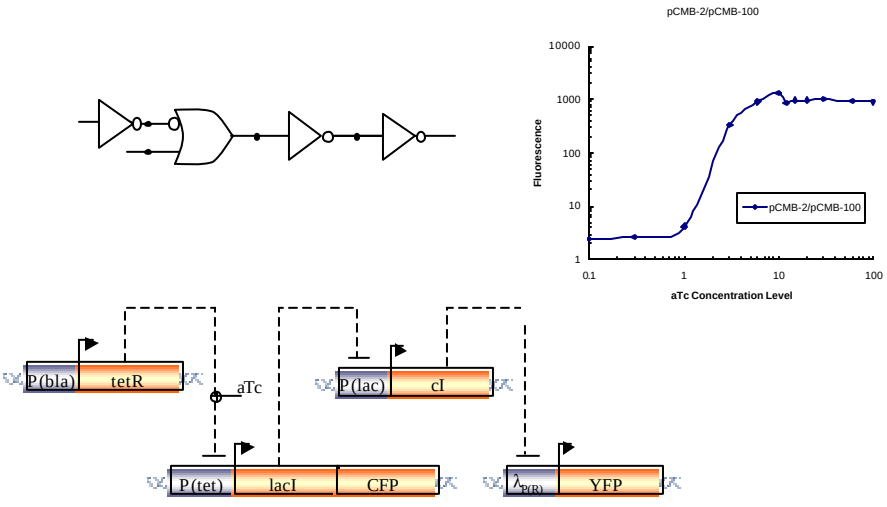
#2: mutate operator



#1: modify RBS

Weiss & Basu, NSC 2002

Genetic Circuit for High Threshold



Circuit Design Principles

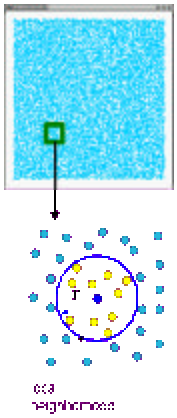
- Separation of low threshold and high threshold
  - RBS efficiency of X must be higher than that of Y
  - Binding affinity of X to its respective promoter has to be higher than that of Y
- Constants associated with Y have more impact on range-width and range-midpoint
  - Y passes through an additional gain stage
- Leakiness and sensitivity of lux promoter determines the lower bound of detection of acyl-HSL

Amorphous Computing

Programming Cell Aggregates

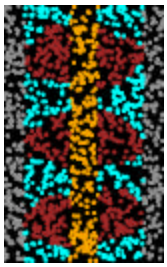
- Amorphous Computing:

  "How does one engineer prespecified, coherent behavior from the cooperation of vast numbers of unreliable parts that are interconnected in unknown, irregular, and time-varying ways."

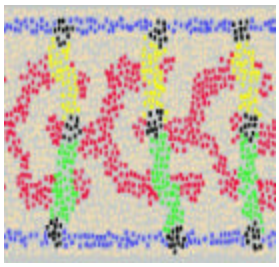


- An aggregate of cells is an example of an amorphous computing substrate

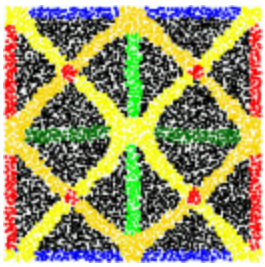
Engineering Coordinated Behavior



MCL
[Weiss, 1998]



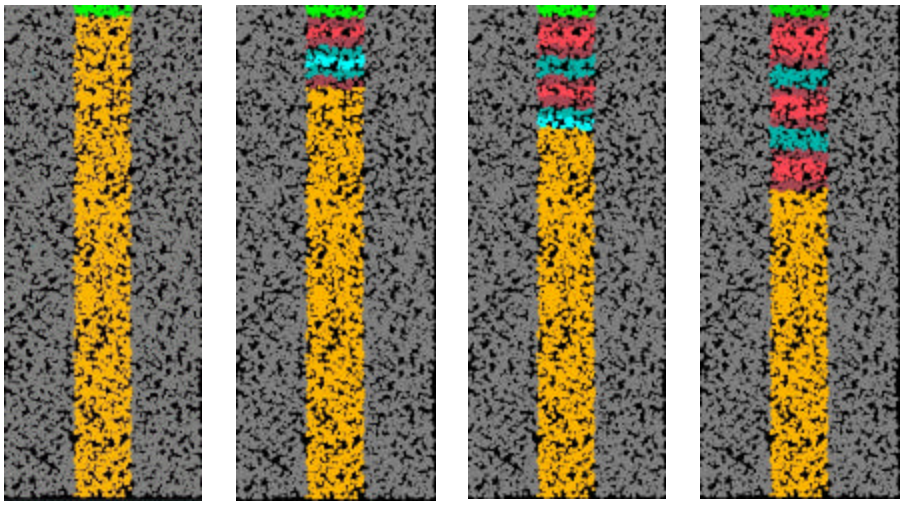
GPL
[Coore, 1997]



Origami
[Nagpal, 2001]

- High-level specifications for pattern formations
- Translate programs to genetic circuits

Another Example: Differentiation



Cells differentiate into bands of alternating C and D type segments.

Microbial Colony Language (MCL)

A program for creating segments:

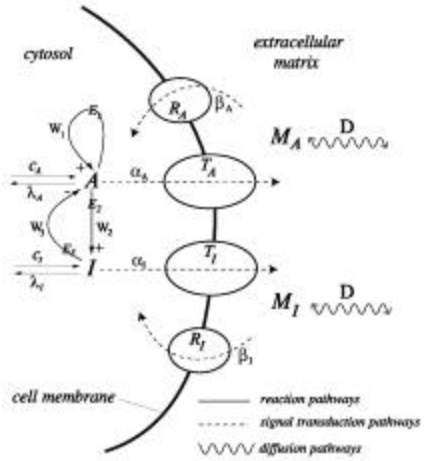
```
(start                                  (created                    }message
 Crest                                  (or C D)                    }condition
 ((send (make-seg C 1) 3)))             ((set Waiting 10)))         }actions

((make-seg seg-type seg-index)          (*
 (and Tube (not C) (not D))              (and Bottom C 1 (Waiting (= 0)))
 ((set seg-type)                         ((send (make-seg D 1) 3)))
  (set seg-index)
  (send created 3)))                    (*
                                         (and Bottom D 1 (Waiting (= 0)))
(((make-seg) (= 0))                      ((send (make-seg C 2) 3)))
 Tube
 ((set Bottom)))                        (*
                                         (and Bottom C 2 (Waiting (= 0)))
(((make-seg) (> 0))                      ((send (make-seg D 2) 3)))
 Tube
 ((unset Bottom)))                      (*
                                         (and Bottom D 2 (Waiting (= 0)))
                                         ((send (make-seg C 3) 3)))
```

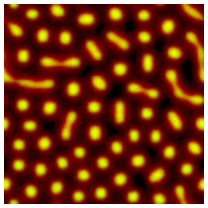
The Microbial Colony Language

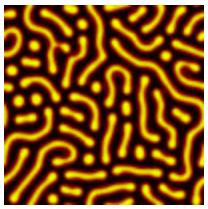
- Language primitives:
  - asynrchronous rules
  - boolean state variables
  - boolean logic
  - local communications with chemical diffusion
- These primitives can be mapped to engineered biochemical processes

Reaction/Diffusion Pattern Formation

[Millonas/Rauch]

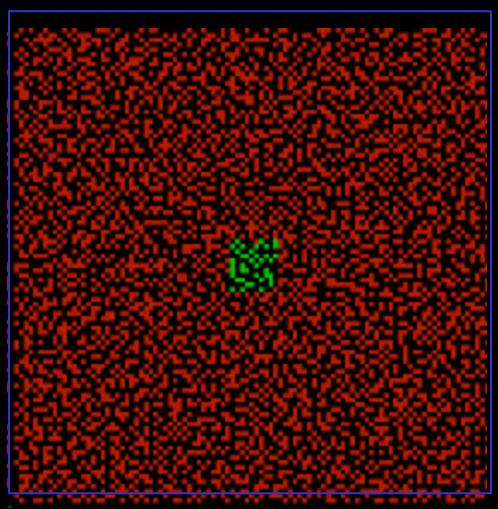




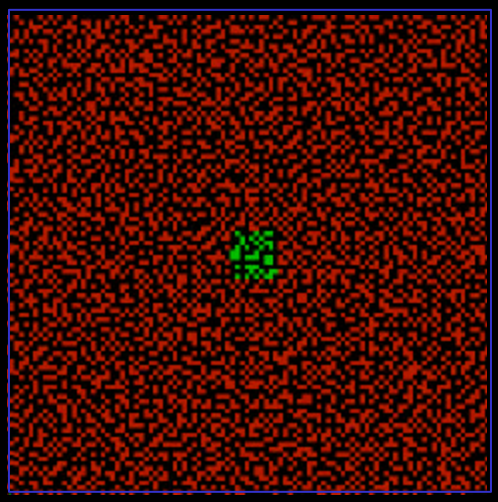


Kinetic rates determine emergence of patterns

Reaction/Diffusion Simulation



Reaction/Diffusion Simulation II



Future Work

- Quantitative prediction of engineered cell behavior
- Self-perfecting genetic circuits
- Intercellular communication architectures
- Signal processing circuits
- Additional CAD tools
- Bio-fab
  - Large scale circuit design, production, and testing
- Simpler & more complex organisms:
  - Eukaryotes
  - Mycoplasmas

vs.

- Biologically inspired logic gates
- Molecular scale fabrication