Chapter 6: Biological Networks

6.4 Engineering Synthetic Networks

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Overview

- Constructing regulatory gates
- A genetic toggle switch; repressilator; biosensors…
- Circuit design notes; directed evolution…
- Synthetic life…

  iGEM; http://parts2.mit.edu/wiki/index.php/Main_Page

GREETINGS FROM BACTERIA glow in this device entered in the 2004 iGEM competition by a team from the University of Texas at Austin. The group incorporated multiple light-receiving and color-producing genetic parts into *Escherichia coli* to turn a biofilm into biological film that displays images inscribed by light on its surface. In a nod to computer programming tradition, the machine's first message was "Hello World."

Baker D, Church G, Collins J, Endy D, Jacobson J, Keasling J, Modrich P, Smolke C, Weiss R. Engineering life: building a FAB for biology. Scientific American 294: 44-51 (2006).

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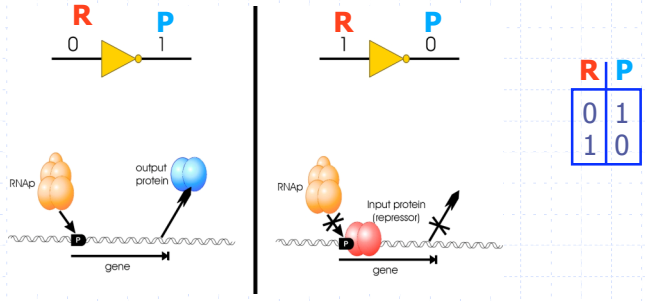
Regulatory Gates

Gene Circuits: Basic Inverter (NOT Gate)

- Signal = TF synthesis rate
- Computation = TF production & decay
- Gates = Boolean functions of TFs



R	P
0	1
1	0

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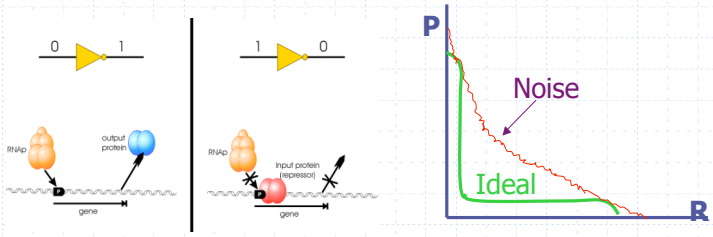
Gene Circuits May be Noisy

- Noise = fluctuations in expression rates
- Gate may amplify/damp input noise
- Noise can be an important tool for regulatory control



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Negative Feedback Improves Signal/Noise

- Auto-repression can reduce noise
  - Why? --feedback stabilizes expression rate and damps noise

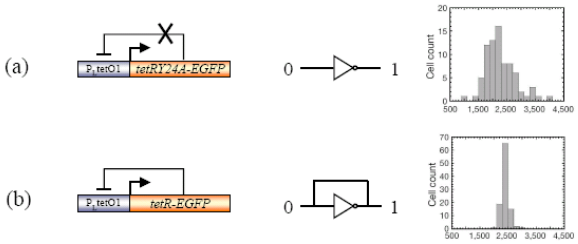

Figure 7. The auto-repressor circuit consists of a $P_L$tetO-1 promoter that regulates a tetR/EGFP fusion protein. In case (a) with a non-functional mutated version of tetR, the circuit behaves as a simple inverter because the tetR mutant protein (tetRY24A) cannot bind the tetO operator site on the promoter. The circuit exhibits wide fluctuations in gene expression. However, when the wild-type tetR is used in case (b), the negative feedback causes gene expression to be more uniform among the cell population.

(Ron Weiss thesis 2001; http://www.princeton.edu/~rweiss/)

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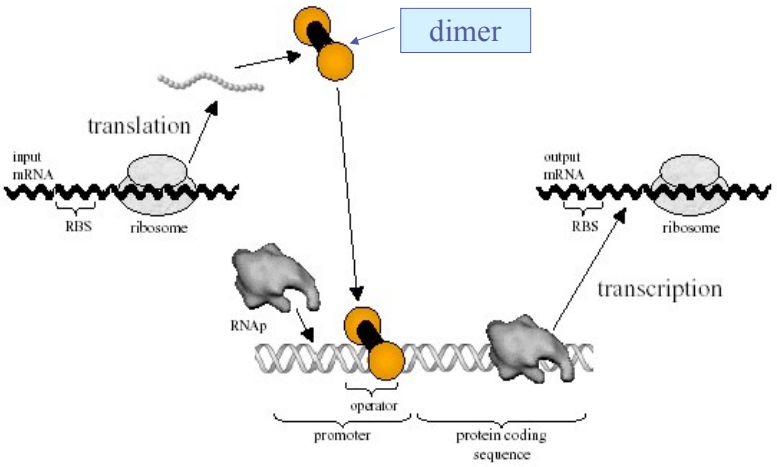
Noise Reduction Through Binding



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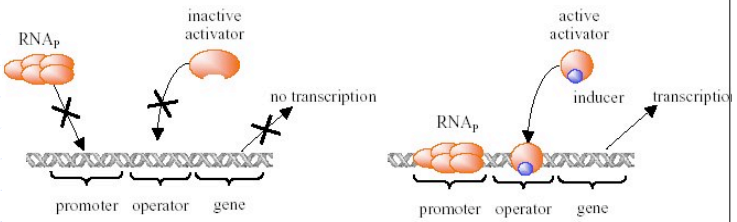
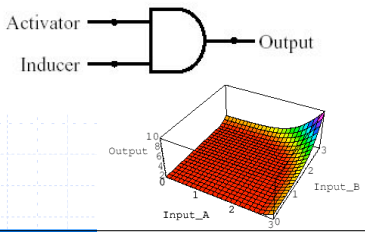
AND Gate Using Cooperative Binding

- AND (activator, inducer)
- Cooperative binding helps noise resiliency





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

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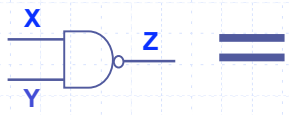
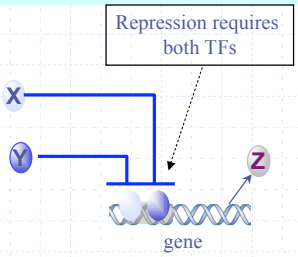
NAND Gate:

X	Y	Z
0	0	1
1	0	1
0	1	1
1	1	0



NAND(X,Y)

=



NAND(x,y)=NOT(AND(x,y))

- Example:
  - X=repressor, Y=inducer activating X
- Noise considerations:
  - The "NOT" (repressor) gating can control noise through feedback
  - The "AND" gating filters noise through cooperative binding

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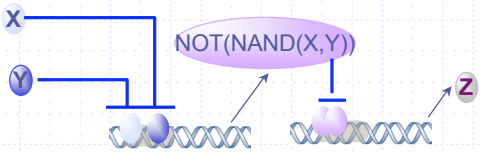
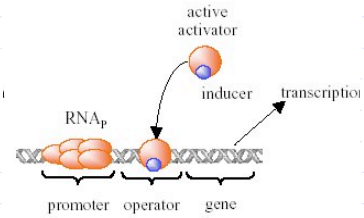
{NAND, NOT} Are Universal Primitives

- Can construct any Boolean function
- E.g consider "AND" gate via AND(x,y)=NOT(NAND(x,y))
  - Note: this gate uses repressors only





- Contrast the two circuits:
  - Which one handles noise better?
  - Which one results in higher delays?

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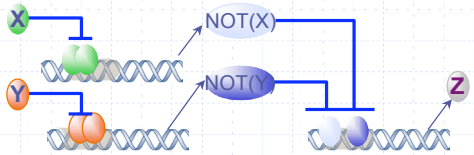
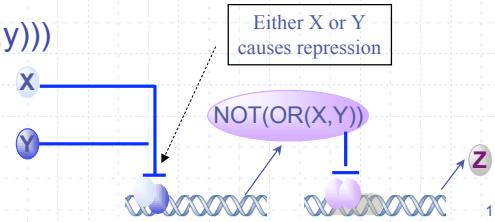
Constructing an "OR" Gate

- Direct implementation: OR(activator1, activator2)
  - Noise sensitive
- Using NAND: OR(x,y)=NAND(NOT(x),NOT(y))
  - Note: circuit uses repressors only



X	Y	Z
0	0	0
1	0	1
0	1	1
1	1	1

- But: is this a "good" circuit?
- OR(x,y)=NOT(NOT(OR(x,y)))



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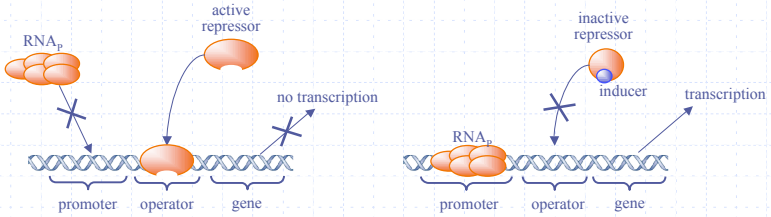
IMPLY Gate Using Cooperative Binding

- Cooperative binding improves signal shape



- Consider inducers that inactivate a repressor:
  - IPTG (Isopropylthio-ß-galactoside) → Lac repressor
  - aTc (Anhydrotetracycline) → Tet repressor
- Use as a logical *Implies* gate:

(NOT R) OR I

Repressor	Inducer	Output
0	0	1
0	1	1
1	0	0
1	1	1

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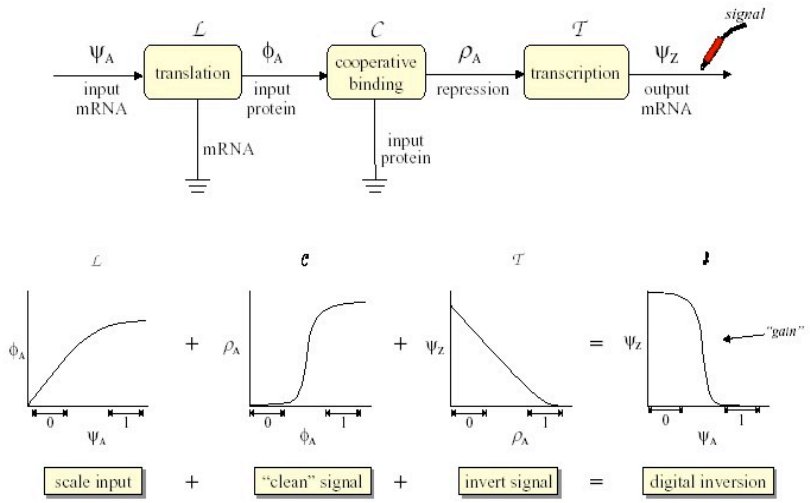
Signal Shaping (R. Weiss)



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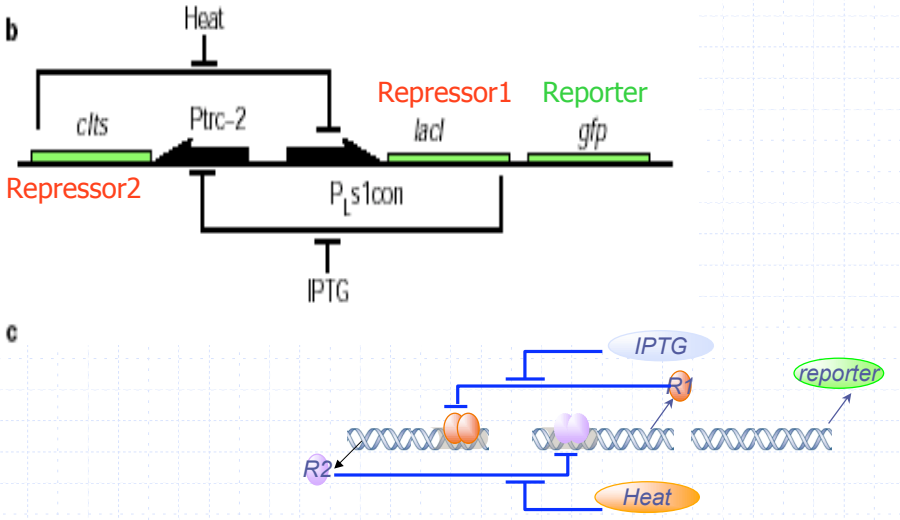
A Toggle Switch

Gardner, T.S., Cantor, C. R. and Collins, J.J.
Construction of a genetic toggle switch in *Escherichia coli* . Nature: 2000, 403

Toggle switch



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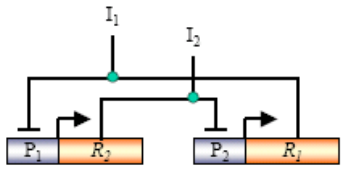
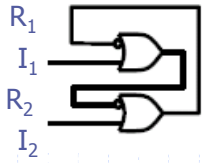
Circuit Diagram





Two IMPLY circuits

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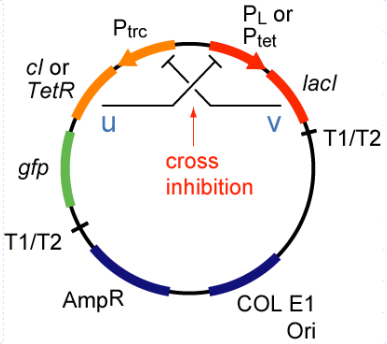
Toggle Switch Implementation

- 4 different switches made.
- One repressor was temperature sensitive, so switch was induced by thermal pulse.



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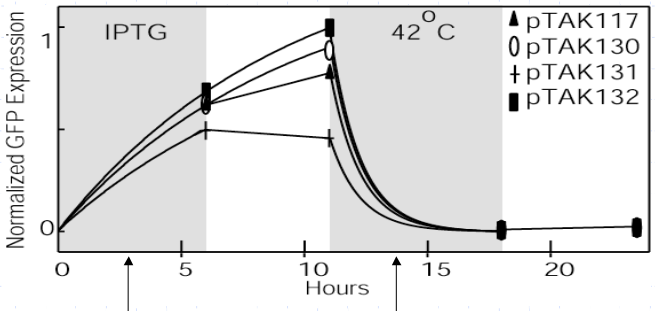
Bistable Switching



IPTG induces "high" state

Heat induces "low" state

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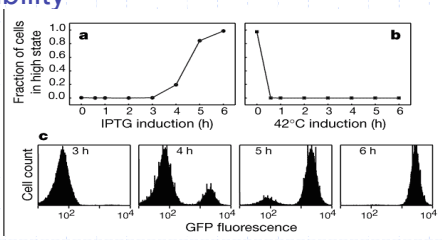
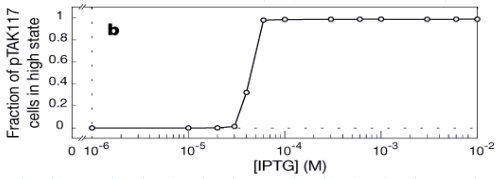
Toggle Switch Results

- Robust bi-stability and reversibility



- Switch demonstrates threshold behavior:



- Switch delay range from 35 min (thermal) to 6 hours (IPTG inducer) after pulse.

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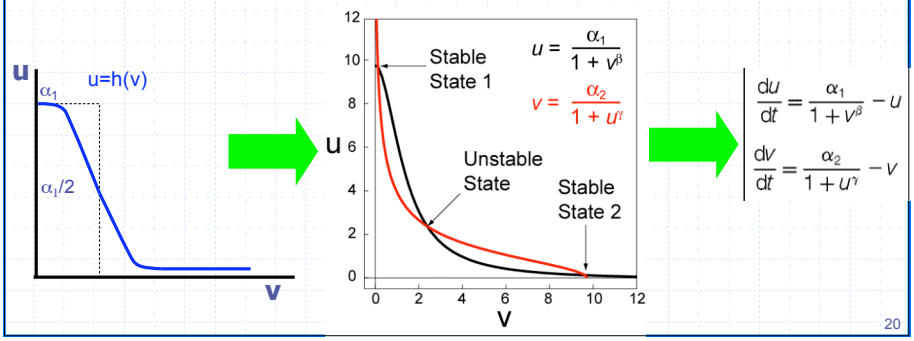
Modeling

- Model:
  - u,v are concentrations of the two repressors
  - α_i is the rate of synthesis of repressor i
  - β,γ are cooperativity factors
- Hill function models repressor
  - $u=h(v)=\alpha/(1+v^{\beta})$
  - Hill function approximates a step function when $\beta \rightarrow \infty$
  - Steady state: intersection of h(u) and h(v)



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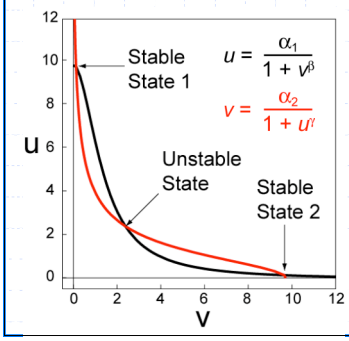
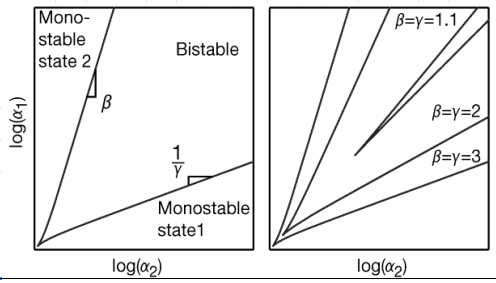
Analysis

- Start with Hill function dynamics
- Consider steady state behaviors
  - du/dt=dv/dt=0
  - β,γ tune the bistable/monostable operating regions

$$\frac{du}{dt} = \frac{\alpha_1}{1+v^{\beta}} - u$$

$$\frac{dv}{dt} = \frac{\alpha_2}{1+u^{\gamma}} - v$$



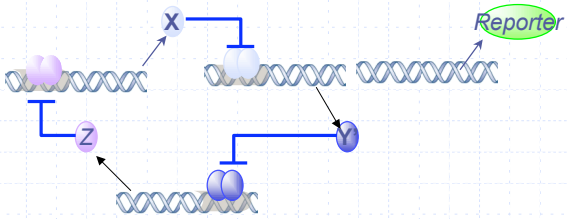


Repressilator: A Genetic Oscillator

Elowitz and Leibler, 2000.
A synthetic oscillatory network of transcriptional regulators. Nature:403

Repressilator Architecture

- Basic Design: Daisy chain repressors



- One promoter linked to “reporter” gene for gfp (green fluorescent protein)

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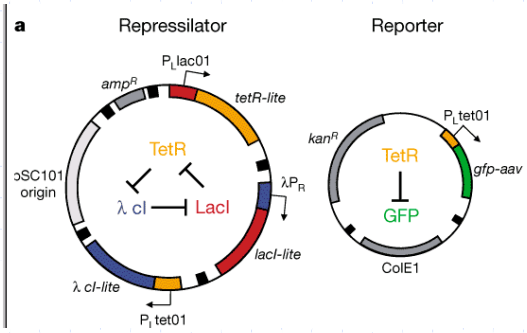
Implementation

- Genes used: LacI from e-coli, tetR from Tetracycline resistance transposon, λ cI from λ-phage.



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Repressilator Oscillations

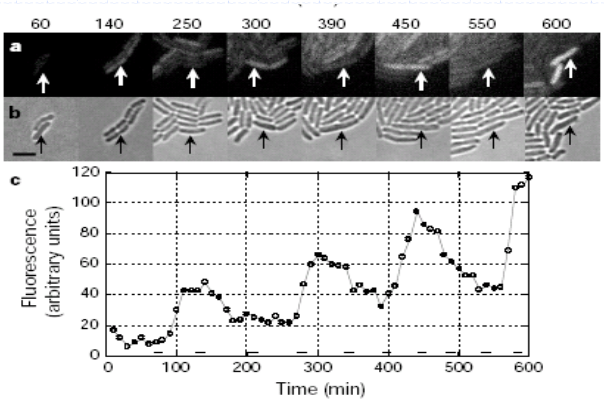

Figure 2 Repressilation in living bacteria. **a**, **b**, The growth and timecourse of GFP expression for a single cell of *E. coli* host strain MC4100 containing the repressilator plasmids (Fig. 1a). Snapshots of a growing microcolony were taken periodically both in fluorescence (**a**) and bright-field (**b**). **c**, The pictures in **a** and **b** correspond to peaks and troughs in the timecourse of GFP fluorescence density of the selected cell. Scale bar, 4 μm. Bars at the bottom of **c** indicate the timing of septation events, as estimated from bright-field images.

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Oscillator simulation

- Deterministic -- 6 coupled ODEs: 3 repressor and 3 mRNA transcript concentrations.

$$\frac{dm_i}{dt} = -m_i + \frac{\alpha}{1 + p_j^n} + \alpha_0$$

$$\frac{dp_i}{dt} = \beta(p_i - m_i)$$

-i,j = "successive" genes in the chain.
-$m_i(t)$ = [mRNA]
-$p_i(t)$ = [repressor]
-α = promoter rate (- α_0)without repressor
-α_0 = leakiness term in saturating repressor
-β = ratio of protein decay rate to mRNA decay rate
-n = Hill coefficient of the repressor

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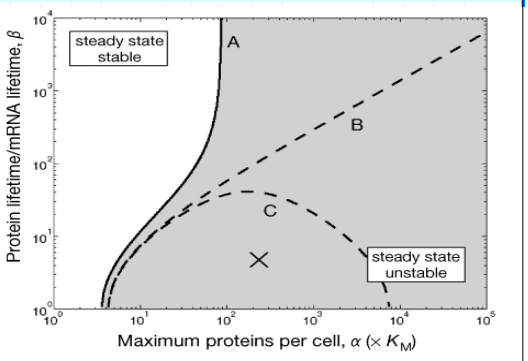
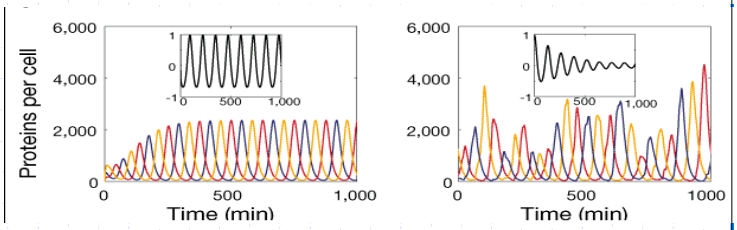
Simulation results

- Stability Diagram:



- Oscillations:



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Repressilator -- Results

- 40% of cells show oscillation (consistent with results of stochastic simulation)
- Period of oscillations range from 120 - 200 min (cell division time 50-70 minutes)
- State is transmitted across generations; becomes decorrelated in 95+-10 minutes

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Synthetic Signaling

Applications To Cell Signaling

Weiss & Knight 2000

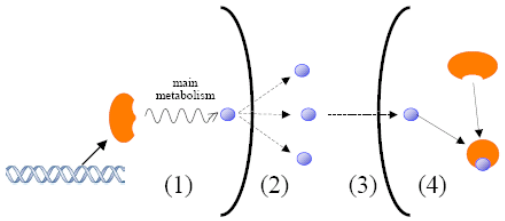

Figure 17. Cell-cell communication schematics: (1) The sender cell produces small signal molecules using certain metabolic pathways. (2) The small molecules diffuse outside the membrane and into the environment. (3) The signals then diffuse into neighboring cells (4) and interact with proteins in the receiver cells, and thereby change signal values.

Cells use signaling to sense quorum, food, mates…

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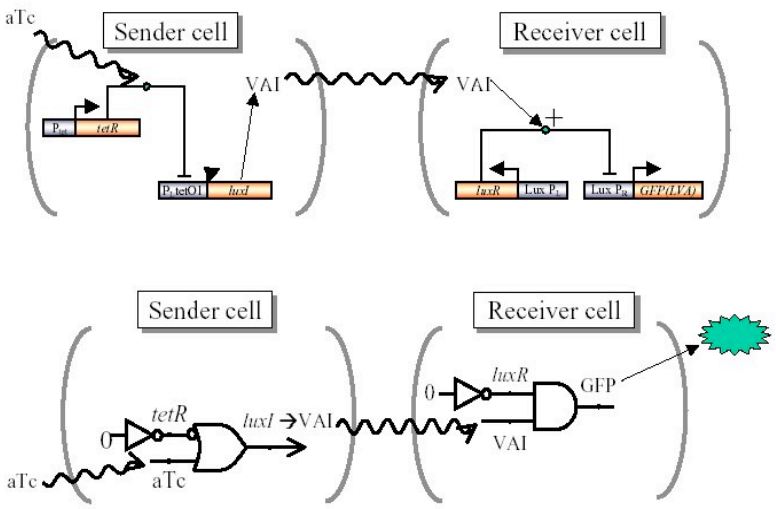
Cell to cell communication



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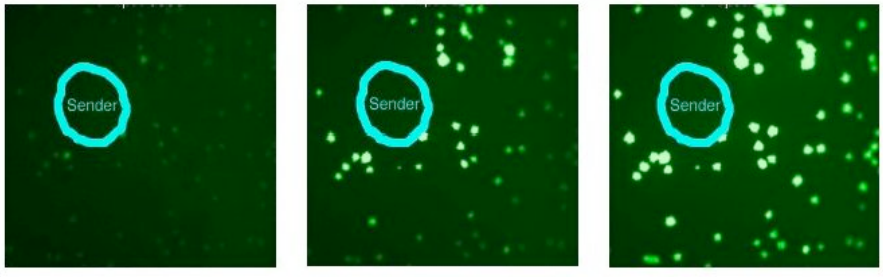
Bacteria Colonies Communicating



40 minutes 5:00 hours 7:30 hours

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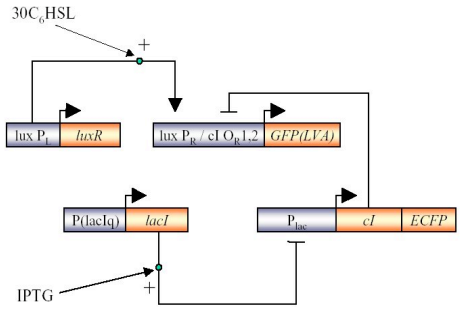
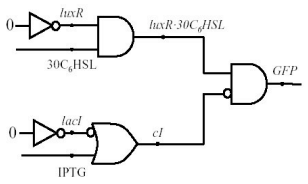
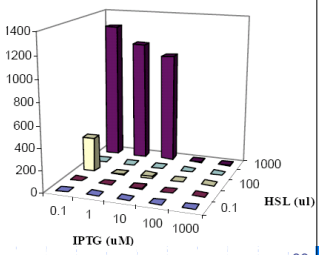
Decoding 2 incoming signals





HSL	IPTG	GFP
0	0	0
0	1	0
1	0	1
1	1	0



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Concentration band detector

- The challenge: search the source of certain molecules
  - E.g., detecting the source of pollutants

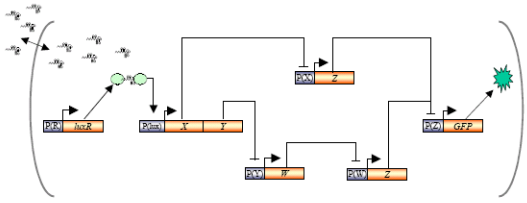

Figure 25. Gene Network for a chemical concentration band detector.

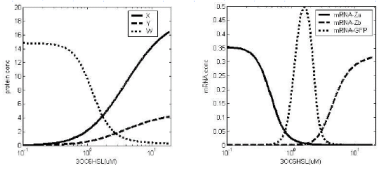

Figure 26. Simulation of the steady state behavior of the band detect circuit components.

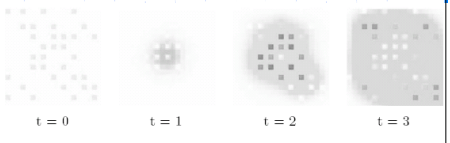

Figure 27. Simulation of the band detect circuit with a sender cell in the center, 35 receiver cells, and diffusion of $3OC_6HSL$.

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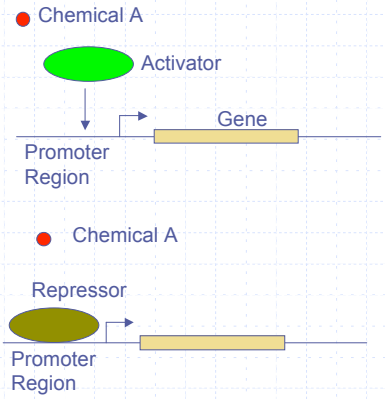
Biosensors

Can Cells Be Programmed?

- Sense and respond to environmental conditions
  - Presence of chemicals, heat, light…



Cellular Pseudocode

```
{
read in input; //light, chemical, etc

if (input is equal to "Chemical A")
        "Turn on Gene 1";

else
        "Gene 1 remains off";

}
```

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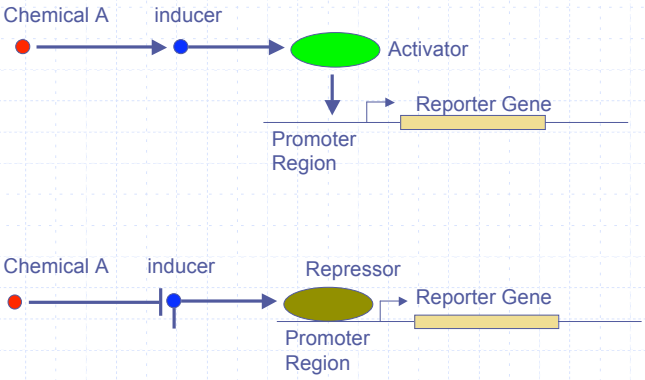
Can Cells Be Programmed?

- Connect sensing/reporting circuits



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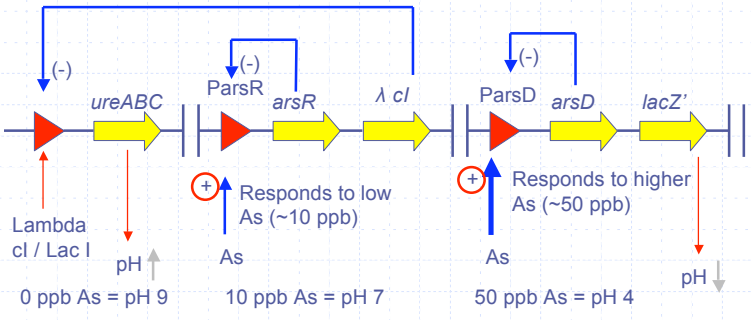
An Arsenic Biosensor Project

http://parts2.mit.edu/wiki/index.php/Arsenic_Biosensor

- The challenge: arsenic poison in water
  - Estimated to harm 100M people
  - Need simple detection process
- Key idea: use PH changes to signal arsenic presence
  - arsR and arsD are genes that respond to the presence of arsenate/arsenite
  - These genes are linked to control lacZ and λ-cI, which controls urease
  - lacZ metabolism or urease expressions result in PH changes
  - PH changes may be easily measured



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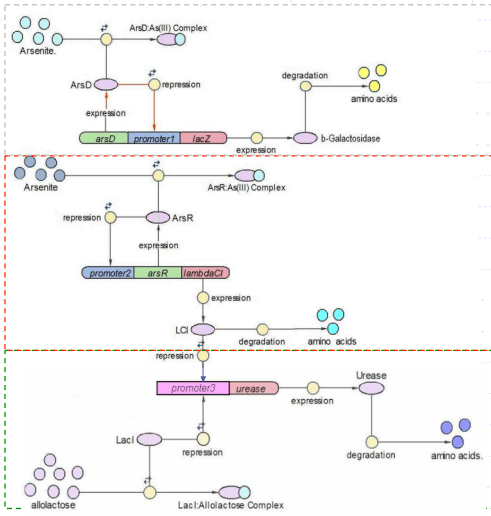
The System



- *LacZ* Operon
  - 6 Reactions
  - 10 Parameters
- λCI Operon
  - 6 Reactions
  - 10 Parameters
- Urease Operon
  - 5 Reactions
  - 9 Parameters

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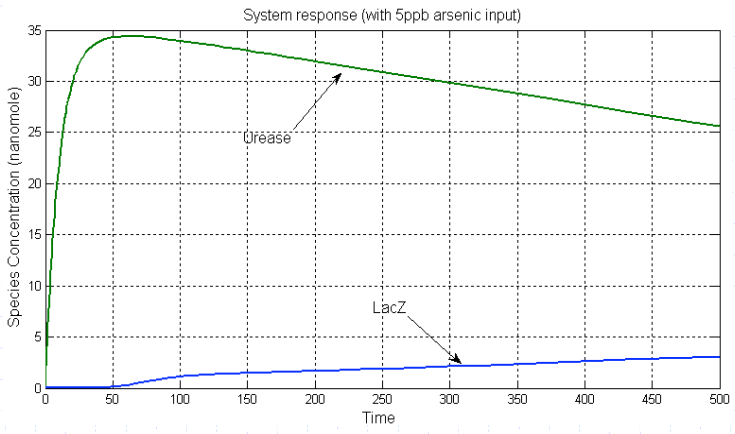
Modeling Result: Low Arsenic



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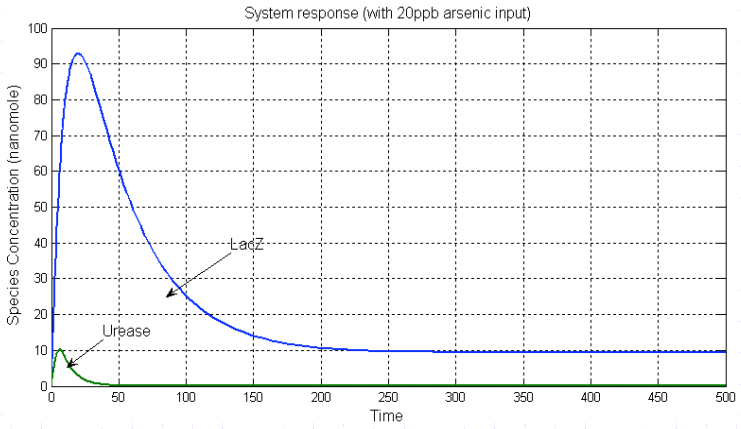
Modeling Result: High Arsenic



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Circuit Design

Challenges & Status

- Noise: difficult to control/predict signals (expression levels)
  - Signal is very sensitive
  - Interconnections can increase noise; limiting circuit sizes
- Timing is difficult to control/predict
- Circuit design is difficult
- Applications of small circuits

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Rational Design: Inverter Model (R. Weiss 2000)

Table I. Biochemical reactions that model an inverter. $mRNA_A$ is the input and $mRNA_Z$ the output.

$$mRNA_A + rRNA \xrightarrow{k_{\text{xlate}}} mRNA_A + rRNA + A \quad (1)$$

$$mRNA_A \xrightarrow{k_{\text{dec(mrna)}}} \quad (2)$$

$$A + A \xrightarrow{k_{\text{dim(a)}}} A_2 \quad (3)$$

$$A_2 \xrightarrow{k_{\text{sngl(a)}}} A + A \quad (4)$$

$$A \xrightarrow{k_{\text{dec(a)}}} \quad (5)$$

$$A_2 \xrightarrow{k_{\text{dec(a2)}}} \quad (6)$$

$$P_Z + A_2 \xrightarrow{k_{\text{rprs(a2)}}} P_Z A_2 \quad (7)$$

$$P_Z A_2 \xrightarrow{k_{\text{dis(a2)}}} P_Z + A_2 \quad (8)$$

$$P_Z A_2 \xrightarrow{k_{\text{dec(ga2)}}} P_Z \quad (9)$$

$$P_Z A_4 \xrightarrow{k_{\text{dec(ga4)}}} P_Z A_2 \quad (10)$$

$$P_Z A_2 + A_2 \xrightarrow{k_{\text{rprs(a4)}}} P_Z A_4 \quad (11)$$

$$P_Z A_4 \xrightarrow{k_{\text{dis(a4)}}} P_Z A_2 + A_2 \quad (12)$$

$$P_Z + RNA_p \xrightarrow{k_{\text{xscribe}}} P_Z + RNA_p + mRNA_Z \quad (13)$$

$$mRNA_Z \xrightarrow{k_{\text{dec(mrna)}}} \quad (14)$$

$$d(A) = 2 \cdot k_{sngl(a)} \cdot A_2 - k_{dec(a)} \cdot A + k_{xlate} \cdot rRNA \cdot mRNA_A - 2 \cdot k_{dim(a)} \cdot A^2$$

$$d(P_Z A_2) = k_{rprs(a2)} \cdot P_Z \cdot A_2 - k_{dis(a2)} \cdot P_Z A_2 - k_{rprs(a4)} \cdot P_Z A_2 \cdot A_2 + k_{dec(ga4)} \cdot P_Z A_4 - k_{dec(ga2)} \cdot P_Z A_2 + k_{dis(a4)} \cdot P_Z A_4$$

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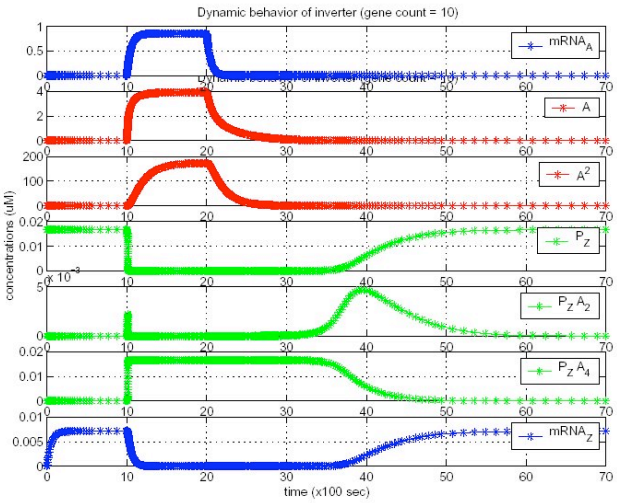
Inverter Analysis



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Languages & Tools For Circuit Design

- Process algebra
- Temporal logic
- Gene circuits design/simulation tools
- ….

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Can Evolution Optimize Synthetic Circuits?

Key idea: directed evolution (Guet, Elowitz et al, 2002)

- Design and optimization of circuits is difficult
- Instead, let evolution do the job
- Induce mutations and apply selection
- Results
  - Mutants tune network kinetics to produce the right behavior.
  - Produce expression levels that are difficult to implement artificially

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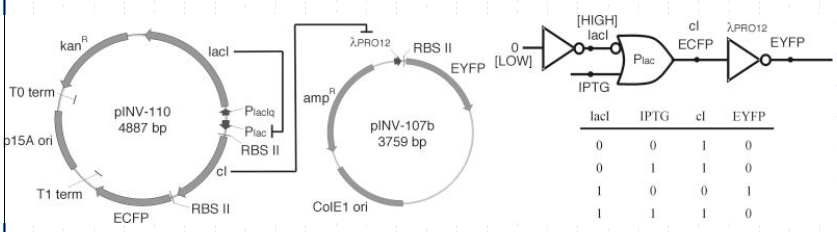
Circuit Design



lacI	IPTG	cI	EYFP
0	0	1	0
0	1	1	0
1	0	0	1
1	1	1	0

- Induce rich mutations of the CI repressor
- Select best performing mutants to evolve the circuit design

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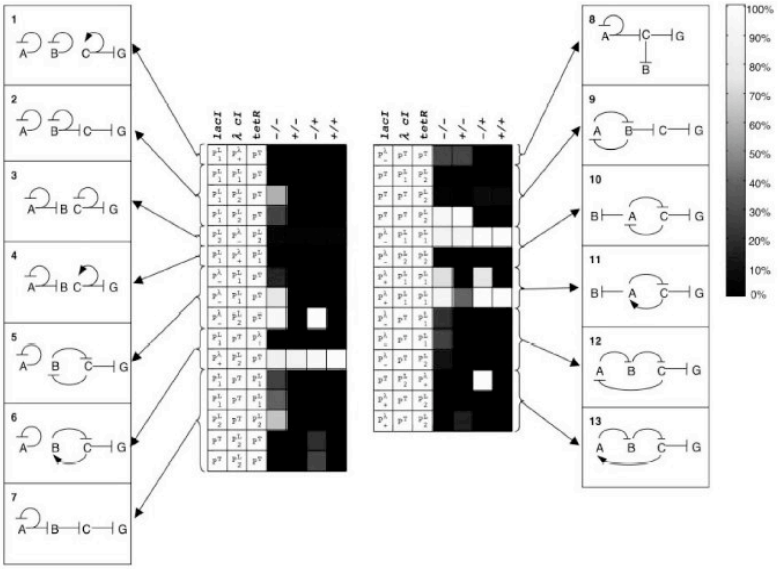
Directed evolution



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Conclusions

- Synthetic biology sheds new light on genomic networks
- Even small networks can perform “interesting” tasks
- Presents significant challenges
  - Improving circuit design and implementation technologies
  - Improving S/N ratio
  - Developing mechanisms for modular interconnection
  - Integrating regulatory, signaling and metabolic capabilities
  - Developing large-scale synthetic networks
  - Applying synthetic networks
  - IGEM: parts2.mit.edu/wiki/index.php/Main_Page

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