The Device Physics of Cellular Logic Gates

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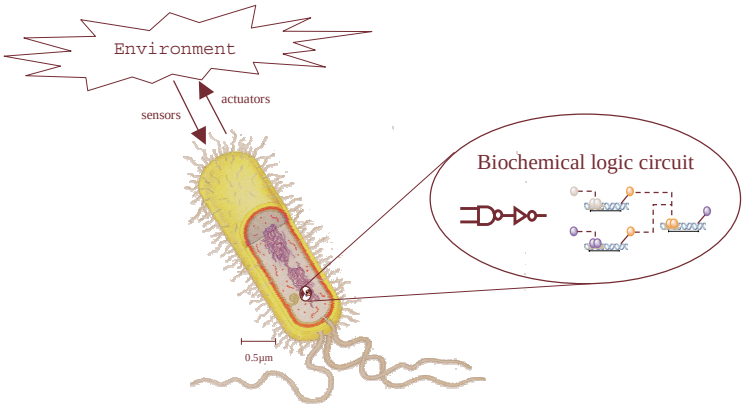
NSC-1

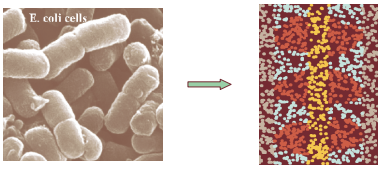
Programming Cells



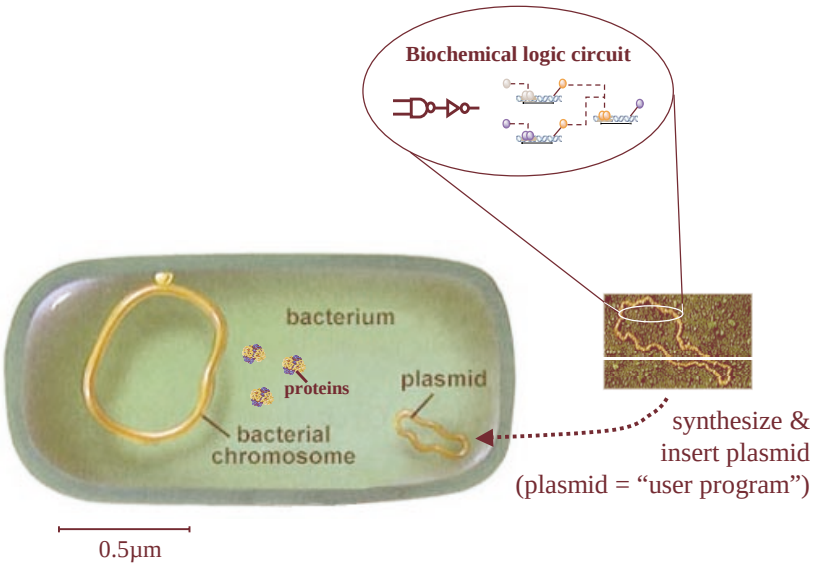
- Understand and engineer:
  - Genetic regulatory networks
  - Cell-cell communications

Programmed Cell Applications

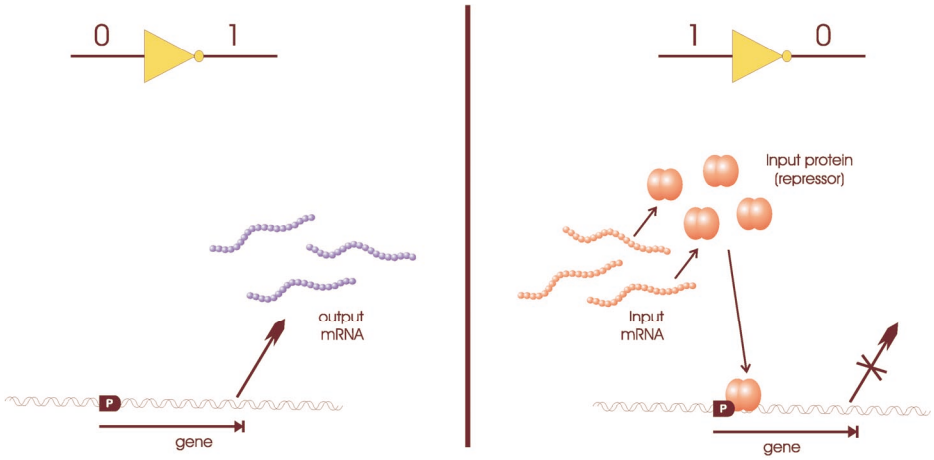
- "Real time" cellular debugger
  - detect conditions that satisfy logic statements
  - maintain history of cellular events
- Environmental
  - sense & respond to complex environmental conditions
- Biomedical
  - combinatorial gene regulation with few inputs
- Molecular-scale fabrication
  - cellular robots that manufacture complex scaffolds



Programming Cells



A Biochemical Inverter



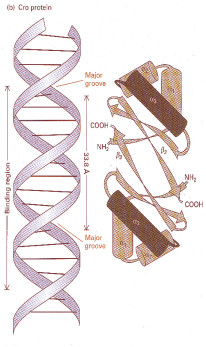
signal = concentration of specific molecules (mRNA)
computation = regulated mRNA and protein synthesis + decay

Outline

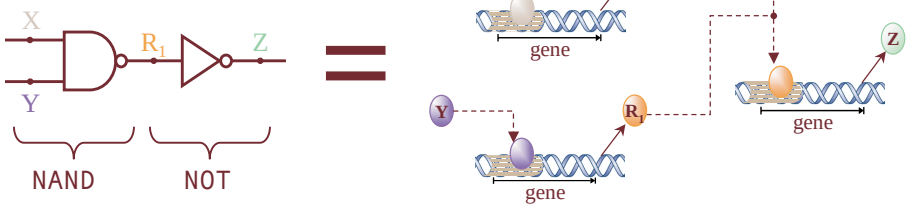
- *In-vivo* digital circuits
- Cellular gates: *Inverter, Implies*
- BioSPICE circuit simulations & design
- Measuring & modifying "device physics"
- Cell-cell Signaling

Why Digital?

- We know how to program with it
  - Signal restoration + modularity = robust complex circuits
- Cells do it
  - Phage λ cI repressor: Lysis or Lysogeny? [Ptashne, A Genetic Switch, 1992]
  - Circuit simulation of phage λ [McAdams & Shapiro, Science, 1995]
- Also working on combining analog & digital circuitry

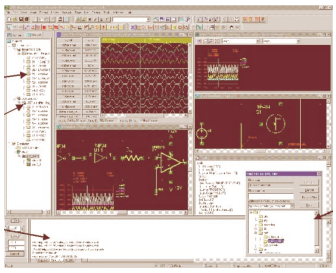


Logic Circuits based on Inverters

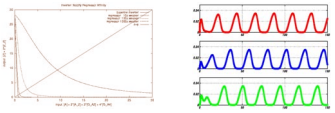


- Proteins are the wires/signals
- Promoter + decay implement the gates
- NAND gate is a universal logic element:
  - any (finite) digital circuit can be built!

BioCircuit Computer-Aided Design



SPICE



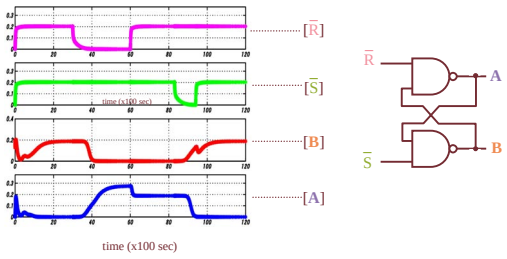
BioSPICE

- BioSPICE: a prototype biocircuit CAD tool
  - simulates protein and chemical concentrations
  - intracellular circuits, intercellular communication
  - single cells, small cell aggregates

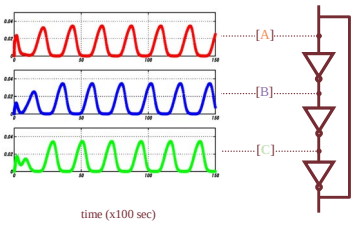
"Proof of Concept" Circuits

- Work in BioSPICE simulations [Weiss, Homsy, Nagpal, 1998]

RS-Latch ("flip-flop")



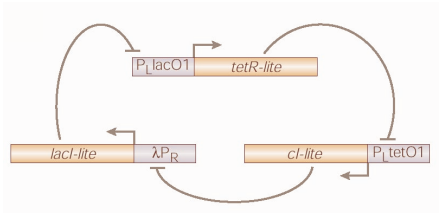
Ring oscillator

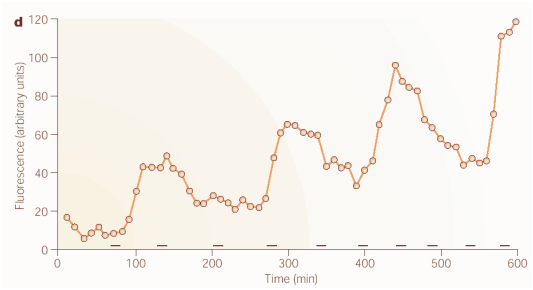


- They work in vivo
  - Flip-flop [Gardner & Collins, 2000], Ring oscillator [Elowitz & Leibler, 2000]
- Models poorly predict their behavior

Actual Behavior of Ring Oscillator

[Elowitz & Leibler, 2000]

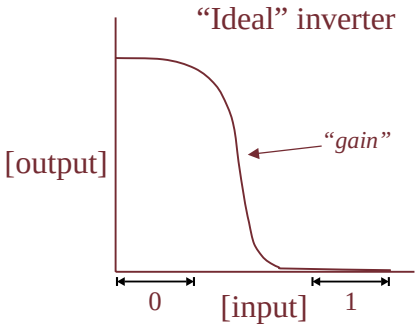




The Cellular Gate Library

- Assembled and characterized a library of gates
  - Constructed and measured gates using 4 genetic elements
    - *lac, tet, cI, lux*
- Genetic process engineering
  - Different elements have widely varying characteristics
    - Modify "device physics" of gates until they match
  - Created 16 variations of *cI* in order to match with *lac*:
    - modified repressor/operator affinity
    - modified RBS efficiency
- Established component evaluation criteria
  - Initially, focused on steady state behavior

Device Physics in Steady State



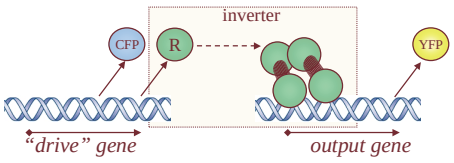
Transfer curve:

- gain (flat,steep,flat)
- adequate noise margins

- Curve can be achieved with certain dna-binding proteins
- Inverters with these properties can be used to build complex circuits

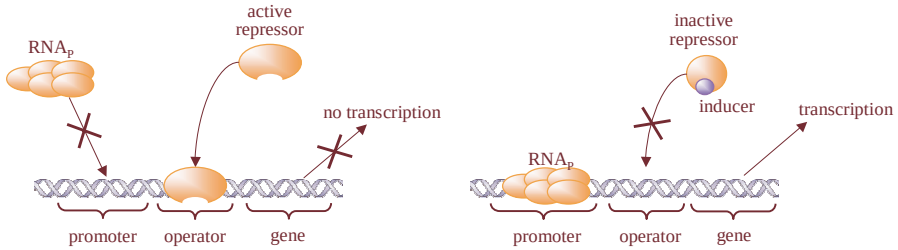
Measuring a Transfer Curve

- Construct a circuit that allows:
  - Control and observation of input protein levels
  - Simultaneous observation of resulting output levels



- Also, need to normalize CFP vs YFP

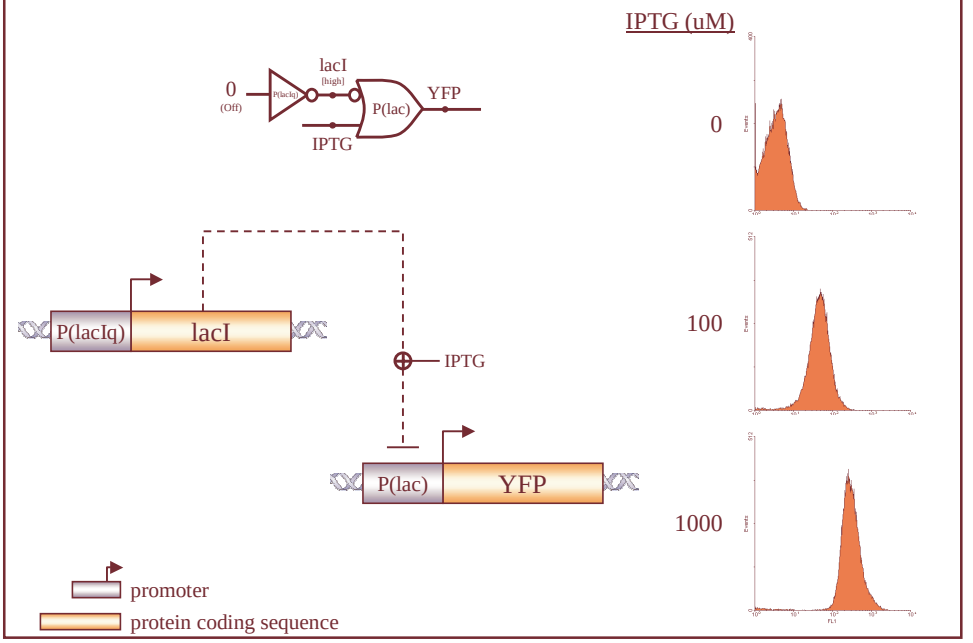
The IMPLIES Gate



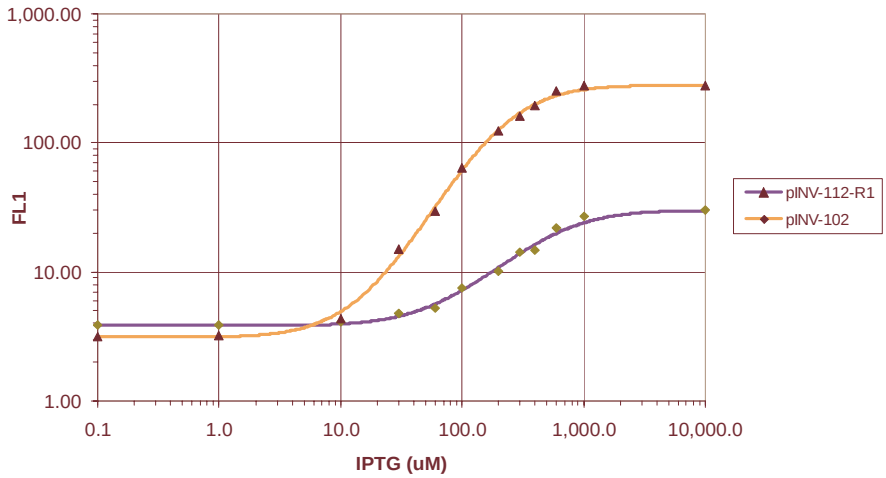
- Inducers that inactivate repressors:
  - IPTG (Isopropylthio-ß-galactoside) → Lac repressor
  - aTc (Anhydrotetracycline) → Tet repressor
- Use as a logical *Implies* gate: `(NOT R) OR I`

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

Drive Input Levels by Varying Inducer



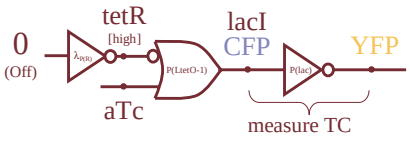
Controlling Input Levels

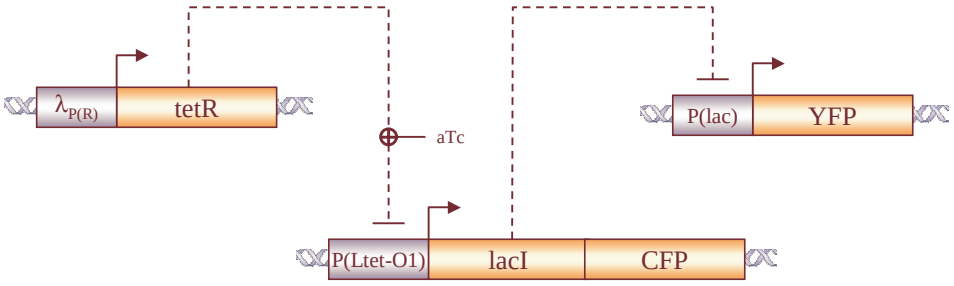


Also use for CFP/YFP calibration

Measuring a Transfer Curve

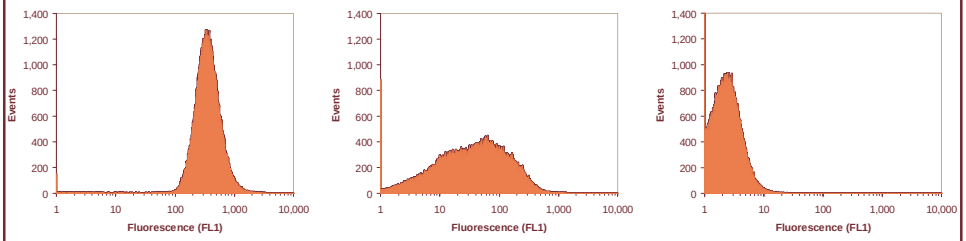
for lacI/p(lac)





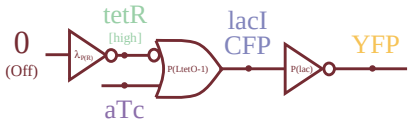
Transfer Curve Data Points

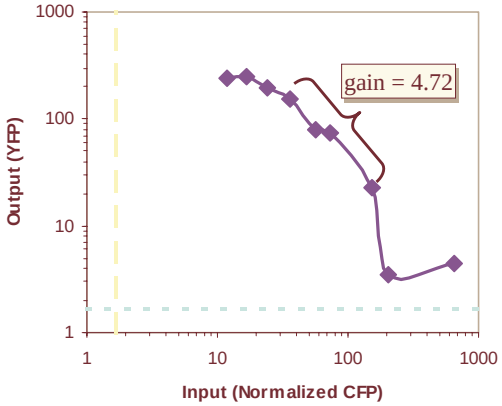
0➲1 | undefined | 1➲0



1 ng/ml aTc | 10 ng/ml aTc | 100 ng/ml aTc

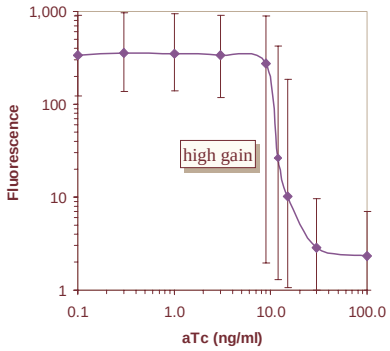
lacI/p(lac) Transfer Curve



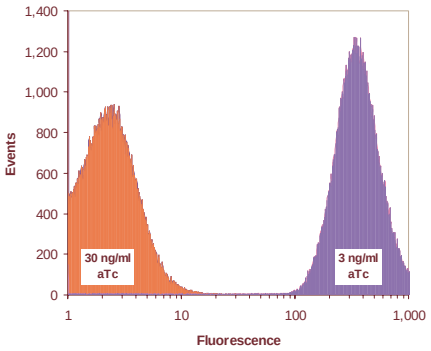


Evaluating the Transfer Curve

- Gain / Signal restoration:



- Noise margins:

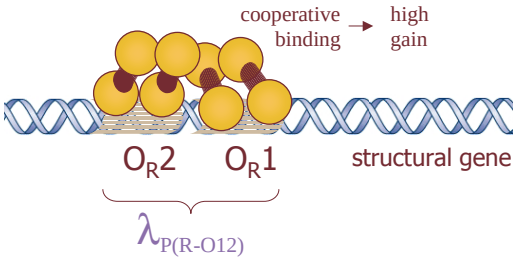


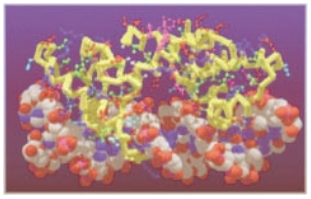
* note: graphing vs. aTc
(i.e. transfer curve of 2 gates)

The Cellular Gate Library

Add the cI/$\lambda_{P(R)}$ Inverter

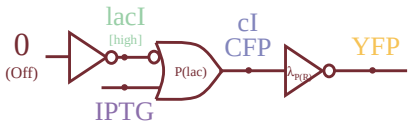
- *cI* is a highly efficient repressor



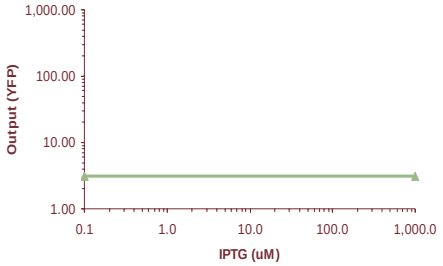


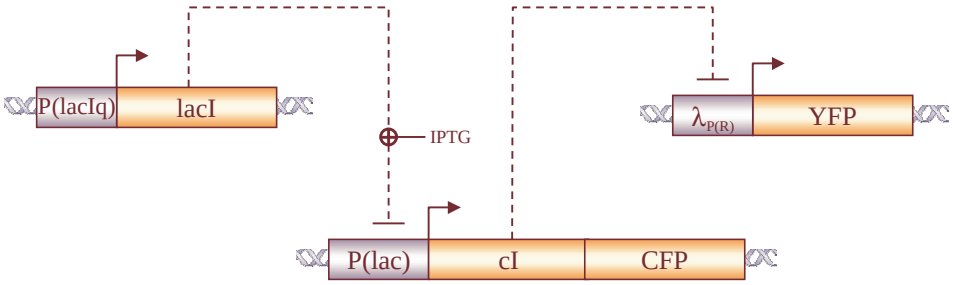
cI bound to DNA

- Use lacI/p(lac) as driver

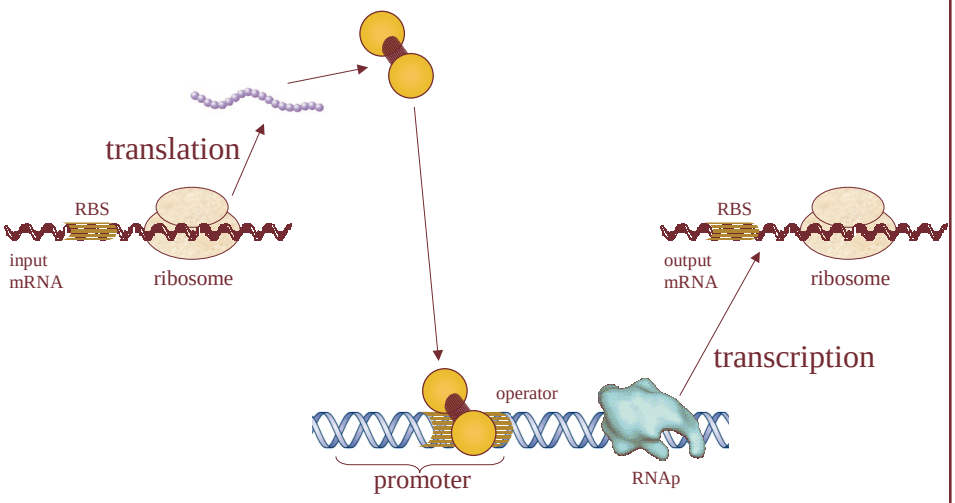


Initial Transfer Curve for cI/$\lambda_{P(R)}$

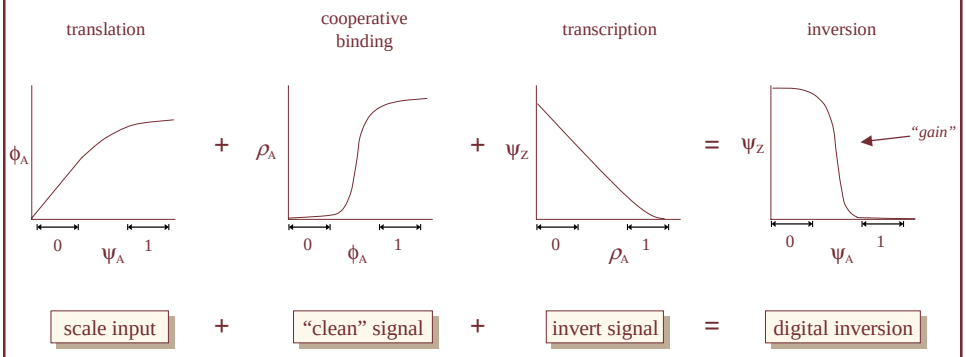




Inverter Components

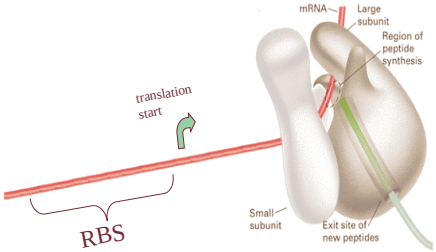


Functional Composition of an Inverter

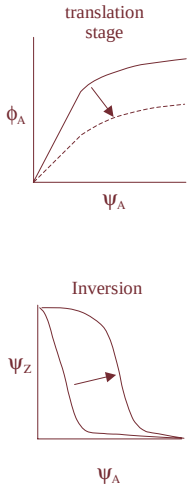


ψ_A = input mRNA
ϕ_A = input protein
ρ_A = bound operators
ψ_Z = output mRNA

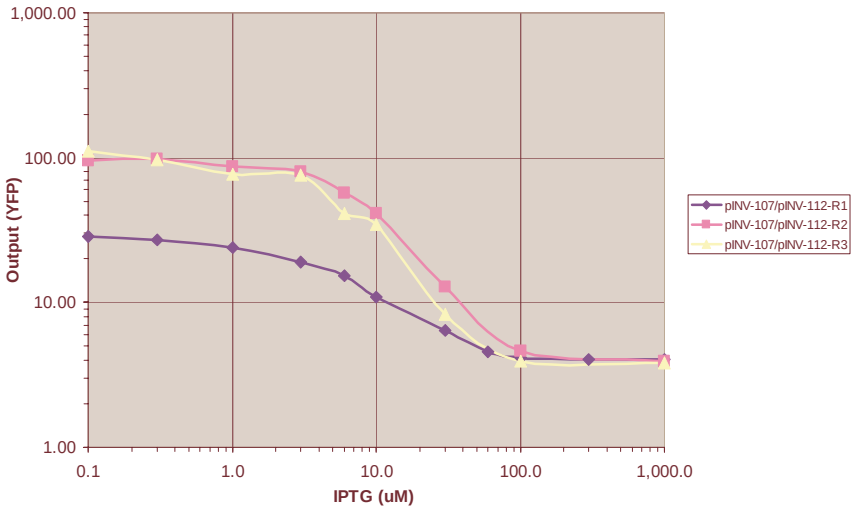
Genetic Process Engineering I:
Reducing Ribosome Binding Site Efficiency




```
Orig:  ATTAAAGAGGAGAAATTAAGCATG   strong
RBS-1:  TCACACAGGAAACCGGTTCGATG     |
RBS-2:   TCACACAGGAAAGGCCTCGATG     |
RBS-3:     TCACACAGGACGGCCGGATG     weak
```

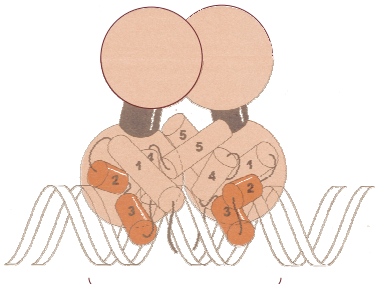


Experimental Results for cI/$\lambda_{P(R)}$ Inverter with Modified RBS



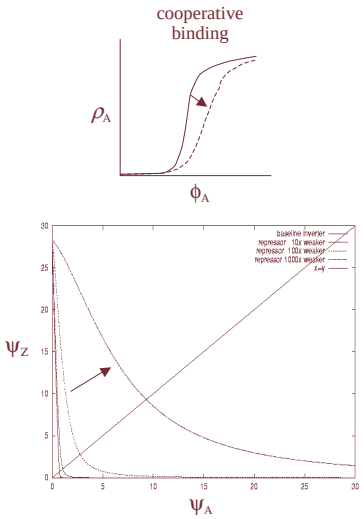
Genetic Process Engineering II:

Mutating the $\lambda_{P(R)}$ operator



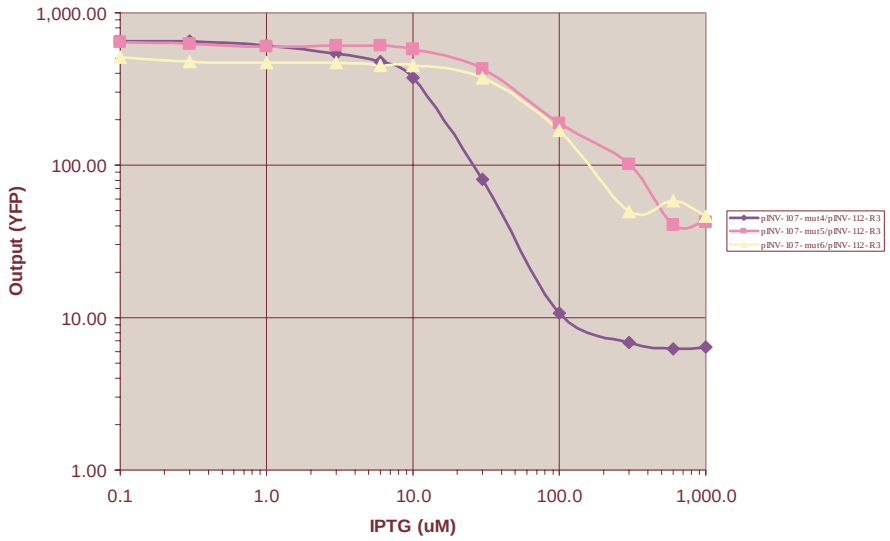
orig: TACCTCTGGCGGTGATA
mut4: TACATCTGGCGGTGATA
mut5: TACATATGGCGGTGATA
mut6 TACAGATGGCGGTGATA

O_R1

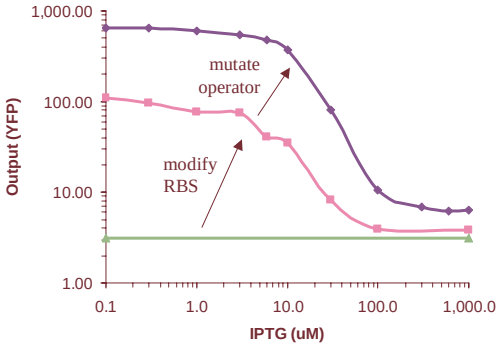


BioSPICE Simulation

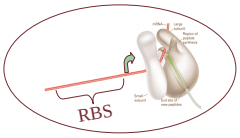
Experimental Results for Mutating $\lambda_{P(R)}$



Genetic Process Engineering



#2: mutate operator

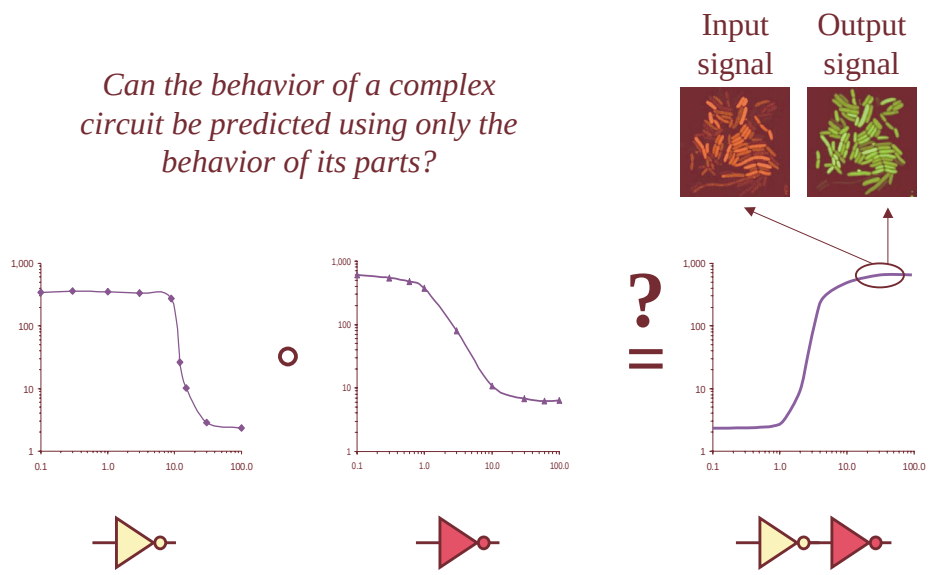


#1: modify RBS

- Genetic modifications required to make circuit work
- Need to understand “device physics” of gates
  - enables construction of complex circuits

Prediction of Circuit Behavior

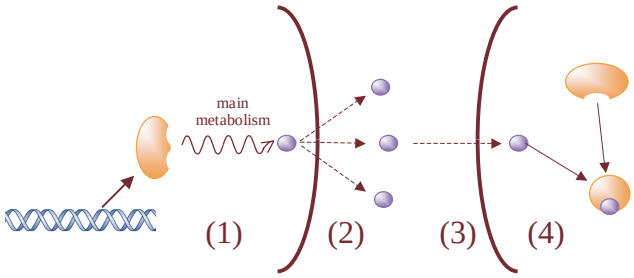
Can the behavior of a complex circuit be predicted using only the behavior of its parts?



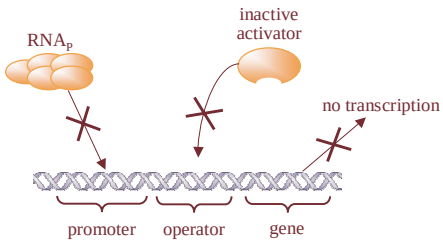
Cell-cell Signaling

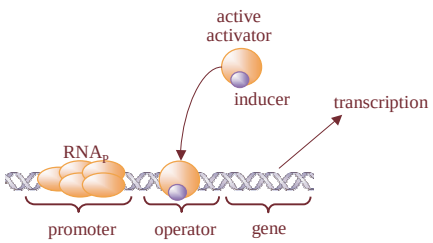
Intercellular Communications

- 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

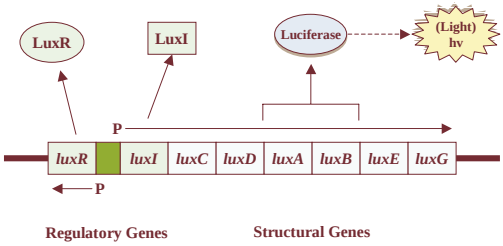
Eupryma scolopes

Light organ

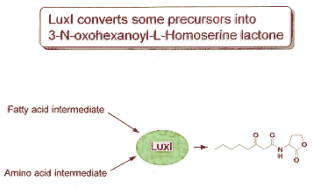
Quorum Sensing

- Cell density dependent gene expression

Example: *Vibrio fischeri* [density dependent bioluminscence]

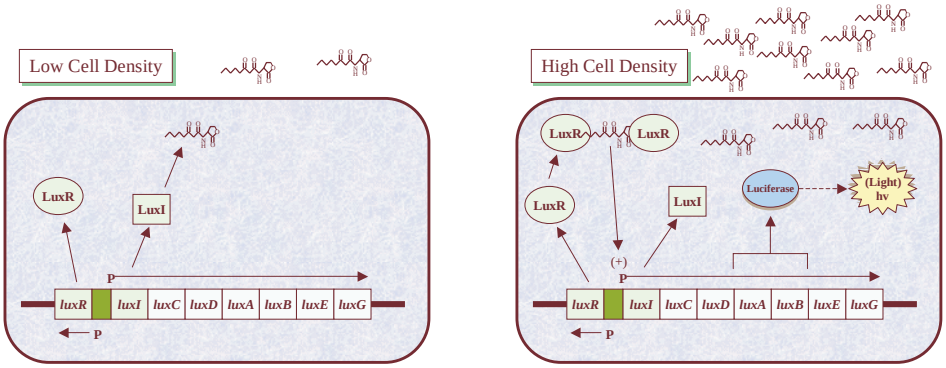


The lux Operon



LuxI metabolism
→ *autoinducer* (VAI)

Density Dependent Bioluminescence

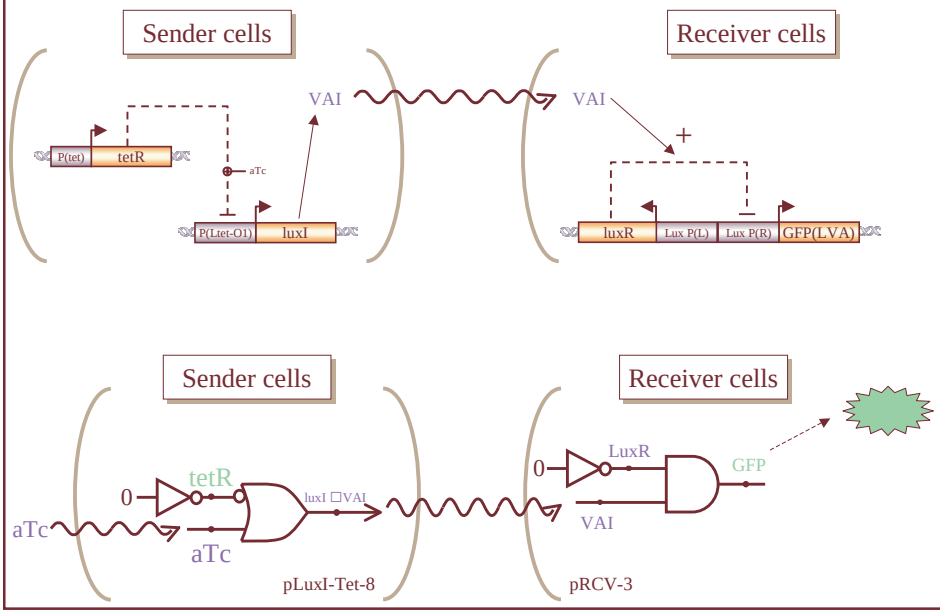


free living, 10 cells/liter
<0.8 photons/second/cell

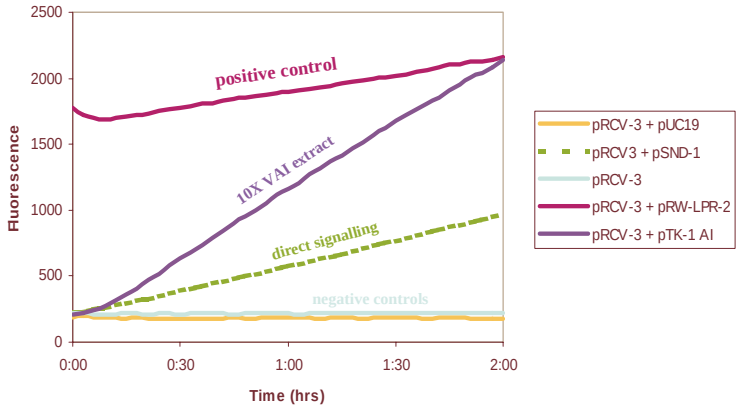
symbiotic, 10^{10} cells/liter
800 photons/second/cell

- A positive feedback circuit

Circuits for Controlled Sender & Receiver

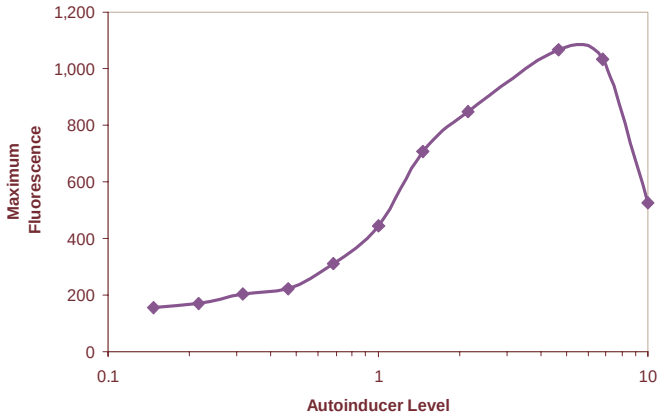


Time-Series Response to Signal



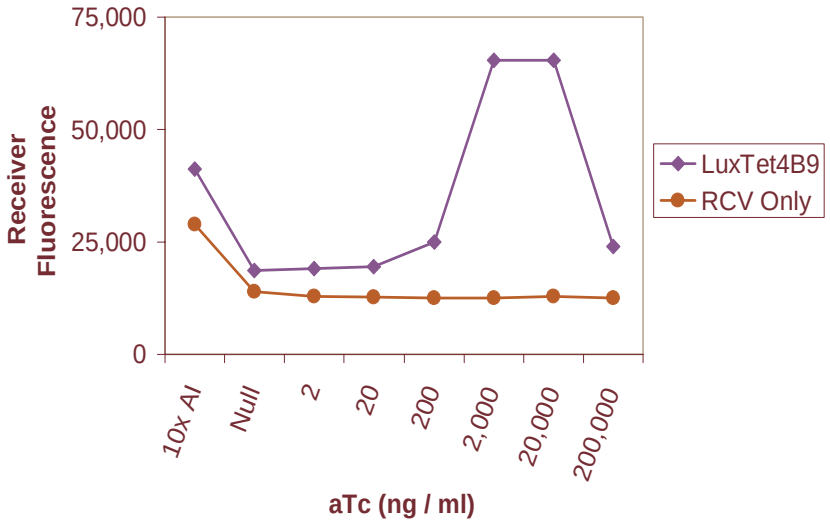
Fluorescence response of receiver (pRCV-3)

Characterizing the Receiver

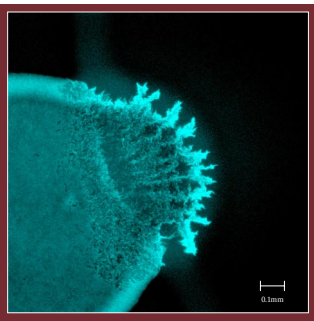


Response of receiver to different levels of VAI extract

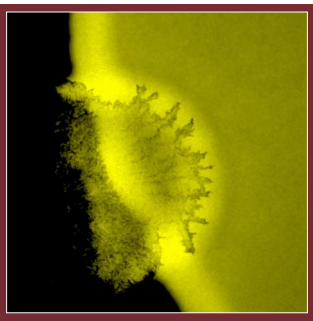
Controlling the Sender's Signal Strength



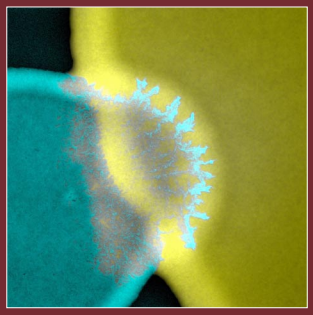
Dose response of receiver cells to aTc induction of senders



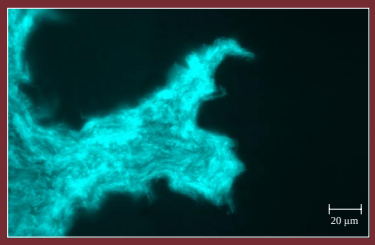
senders



receivers



overlay



receivers

senders

overlay

Engineered Microbial Communication

Ron Weiss
Tom Knight
Nick Papadakis

February, 2001

Summary

- Built and characterized an initial cellular gate library
- Genetic process engineering
  - mutated logic elements to have desired behavior
- Using parts that match, built and tested several small *in-vivo* digital circuits
  - Reliable circuits with predictable behavior from reliable components with known behavior
- BioSPICE for circuit design/verification
- Cell-cell signaling to control gene expression

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