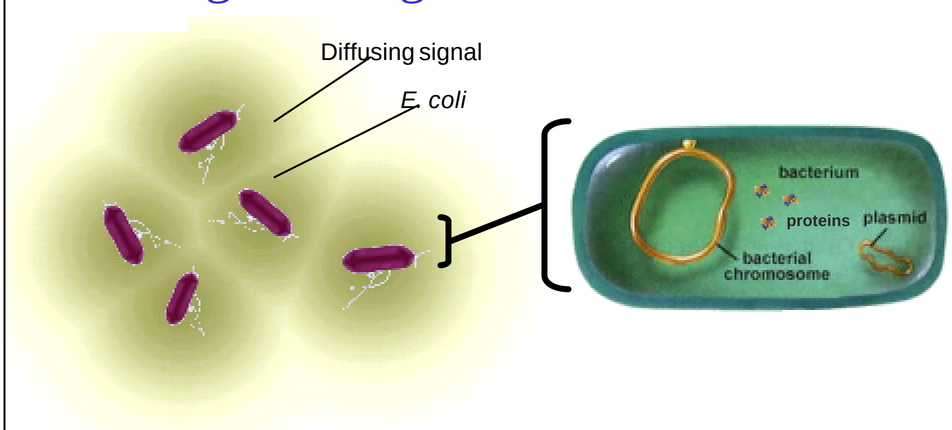
Design of Digital Logic by Genetic Regulatory Networks

Ron Weiss
Department of Electrical Engineering
Princeton University

Computing Beyond Silicon Summer School, Caltech, Summer 2002

Programming Cell Communities

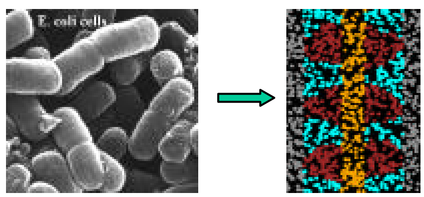


Program cells to perform various tasks using:

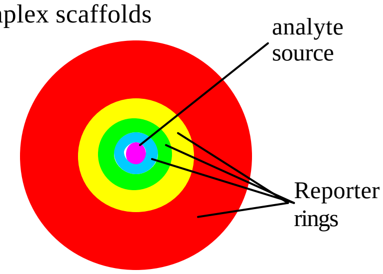
- Intra-cellular circuits
  - Digital & analog components
- Inter-cellular communication
  - Control outgoing signals, process incoming signals

Programmed Cell Applications

- Biomedical
  - combinatorial gene regulation with few inputs; tissue engineering
- Environmental sensing and effecting
  - recognize and respond to complex environmental conditions
- Engineered crops
  - toggle switches control expression of growth hormones, pesticides
- Cellular-scale fabrication
  - cellular robots that manufacture complex scaffolds



Pattern formation

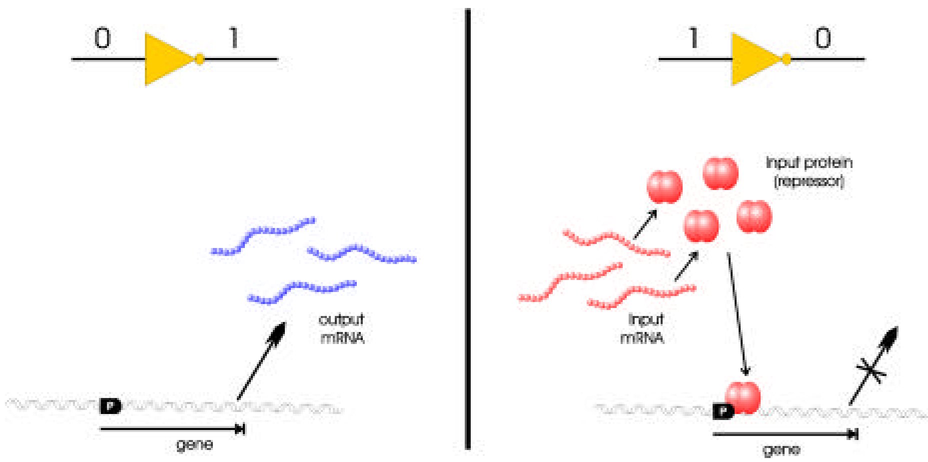


Analyte source detection

Outline

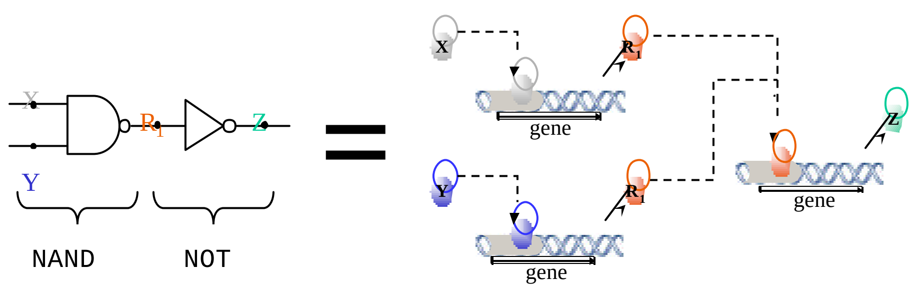
- *In-vivo* logic circuits
- Intercellular communications
- Signal processing and analog circuits
- Programming cell aggregates

A Genetic Circuit Building Block



- Digital Inverter
- Amplifier
- Threshold Detector
- Delay

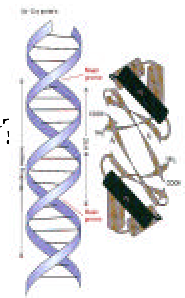
Logic Circuits based on Inverters



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

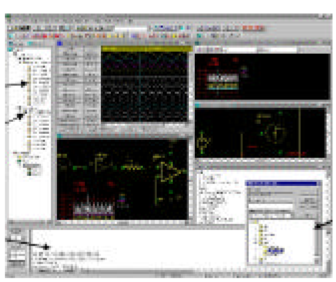
Why Digital?

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

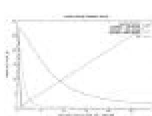


- Also working on combining analog & digital circuitry

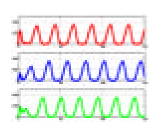
BioCircuit Computer-Aided Design



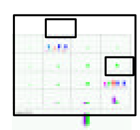
SPICE



steady state



dynamics

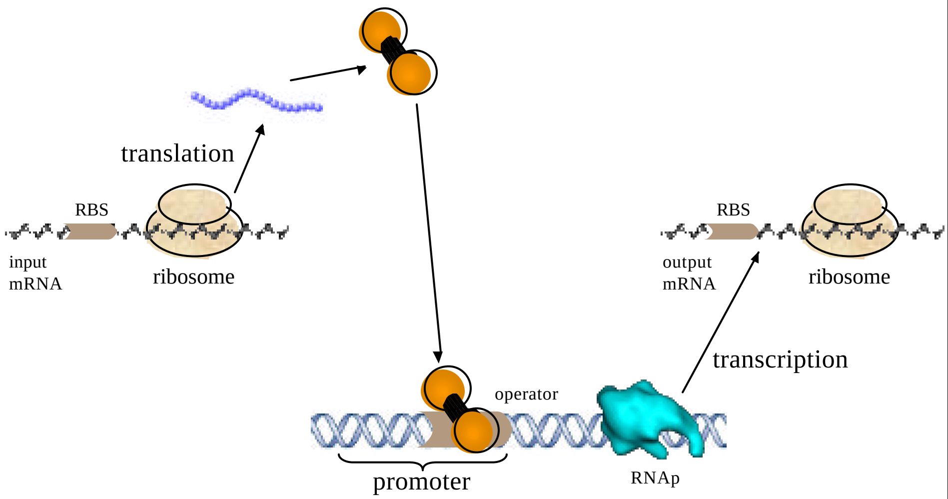


intercellular

BioSPICE

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

Genetic Circuit Elements



Modeling a Biochemical Inverter

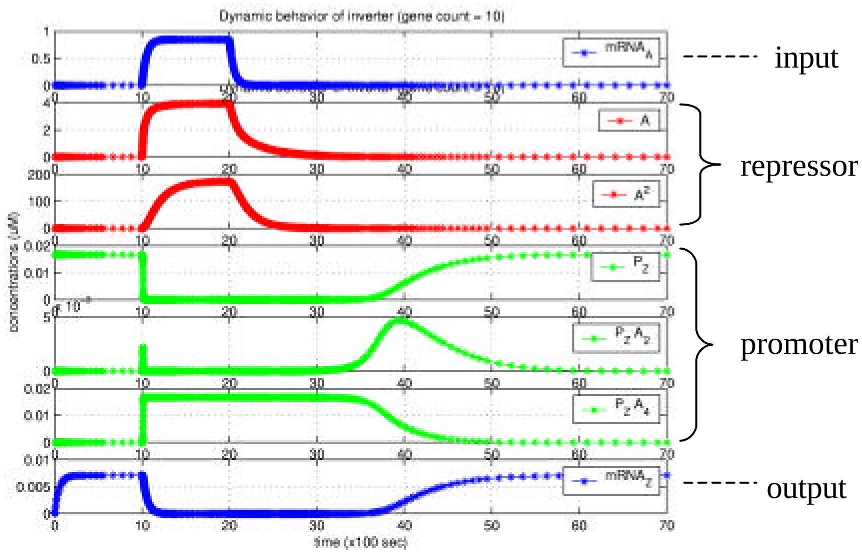
$$\left.\begin{aligned}
&mRNA_A + rRNA \xrightarrow[\text{translate}]{k_{\mathrm{xlate}}} mRNA_A + rRNA + A && (1)\\
&mRNA_A \xrightarrow[\text{decay}]{k_{\mathrm{dec(mrna)}}} && (2)
\end{aligned}\right\}\ \text{input}$$

$$\left.\begin{aligned}
&A + A \xrightarrow[\text{dimerization}]{k_{\mathrm{dim(a)}}} A_2 \quad (3) && A \xrightarrow[\text{decay}]{k_{\mathrm{dec(a)}}} \quad (5)\\
&A_2 \xrightarrow[\text{single}]{k_{\mathrm{sngl(a)}}} A + A \quad (4) && A_2 \xrightarrow[\text{decay}]{k_{\mathrm{dec(a2)}}} \quad (6)
\end{aligned}\right\}\ \text{repressor}$$

$$\left.\begin{aligned}
&P_Z + A_2 \xrightarrow[\text{repress 1}]{k_{\mathrm{rprs(a2)}}} P_Z A_2 \quad (7) && P_Z A_4 \xrightarrow[\text{decay}]{k_{\mathrm{dec(ga4)}}} P_Z A_2 \quad (10)\\
&P_Z A_2 \xrightarrow[\text{dissociation}]{k_{\mathrm{dis(a2)}}} P_Z + A_2 \quad (8) && P_Z A_2 + A_2 \xrightarrow[\text{repress 2}]{k_{\mathrm{rprs(a4)}}} P_Z A_4 \quad (11)\\
&P_Z A_2 \xrightarrow[\text{decay}]{k_{\mathrm{dec(ga2)}}} P_Z \quad (9) && P_Z A_4 \xrightarrow[\text{dissociation}]{k_{\mathrm{dis(a4)}}} P_Z A_2 + A_2 \quad (12)
\end{aligned}\right\}\ \text{promoter}$$

$$\left.\begin{aligned}
&P_Z + RNA_p \xrightarrow[\text{transcribe}]{k_{\mathrm{xscribe}}} P_Z + RNA_p + mRNA_Z && (13)\\
&mRNA_Z \xrightarrow[\text{decay}]{k_{\mathrm{dec(mrna)}}} && (14)
\end{aligned}\right\}\ \text{output}$$

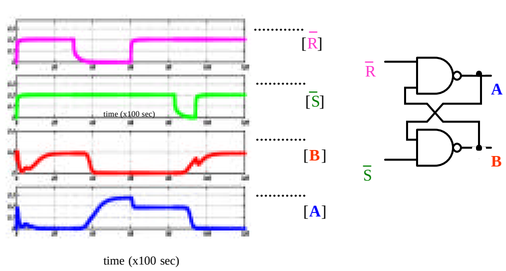
A BioSPICE Inverter Simulation



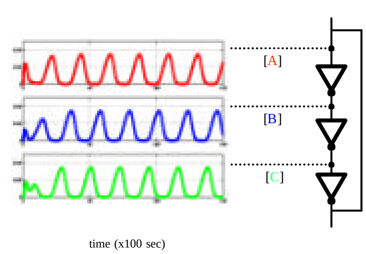
"Proof of Concept" Circuits

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

RS-Latch ("flip-flop")

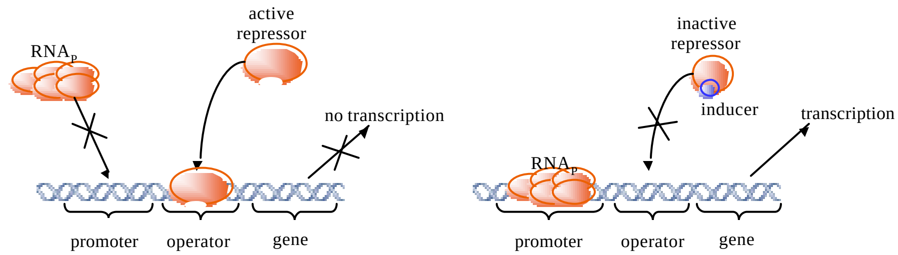


Ring oscillator



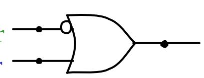
- They work in vivo
  - Flip-flop [Gardner & Collins, 2000]
  - Ring oscillator [Elowitz & Leibler, 2000]
- However, cells are very complex environments
  - Current modeling techniques poorly predict behavior

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

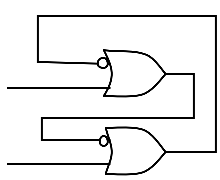


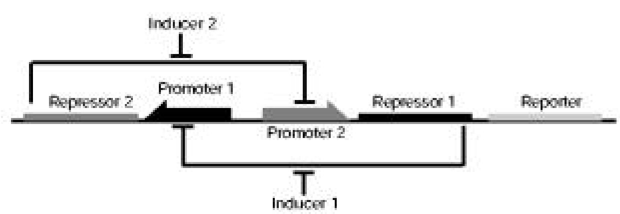
Output

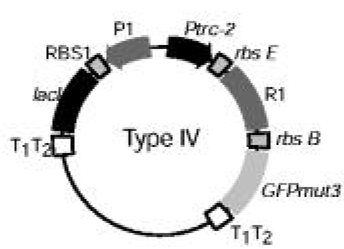
Repressor	Inducer	Output
0	0	1
0	1	1
1	0	0
1	1	1

The Toggle Switch

[Gardner & Collins, 2000]



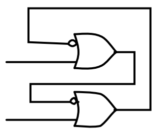


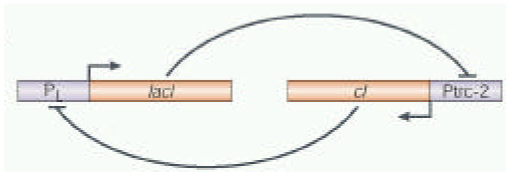


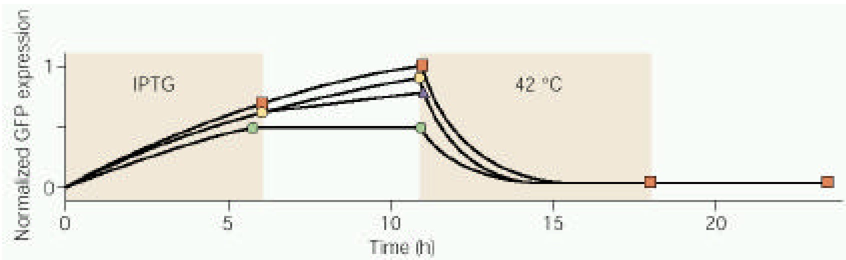
pIKE = *lac*/*tet*
pTAK = *lac*/*cIts*

Actual Behavior of Toggle Switch

[Gardner & Collins, 2000]





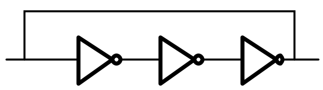


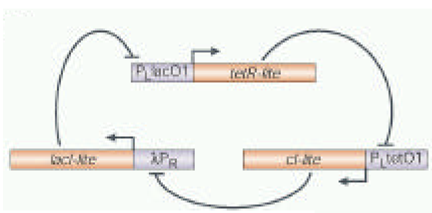
promoter

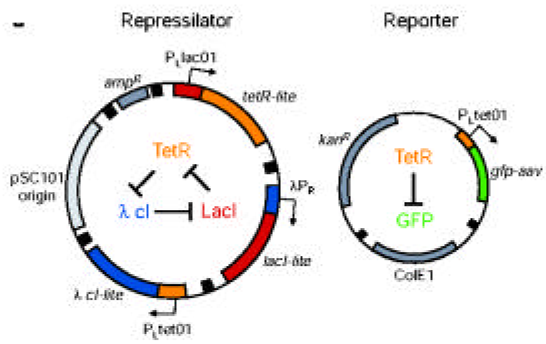
protein coding sequence

The Ring Oscillator

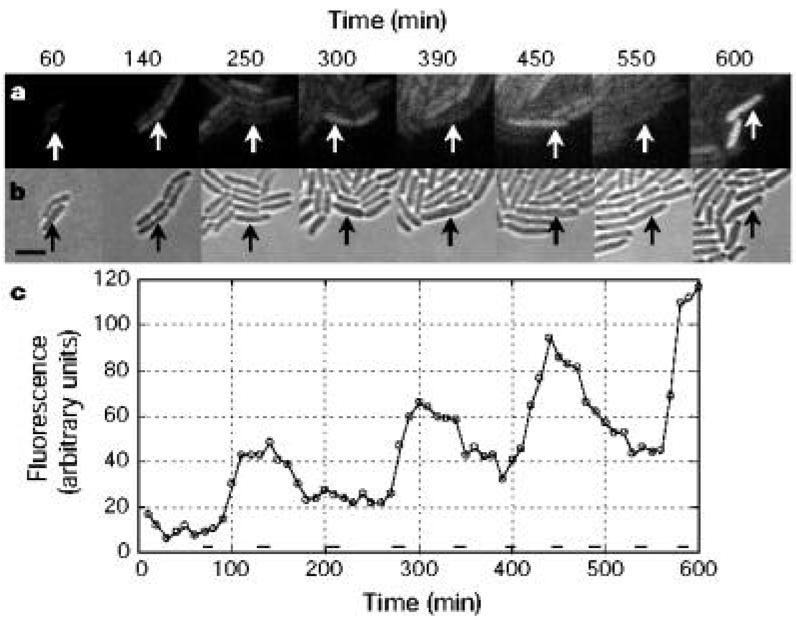
[Elowitz, Leibler 2000]



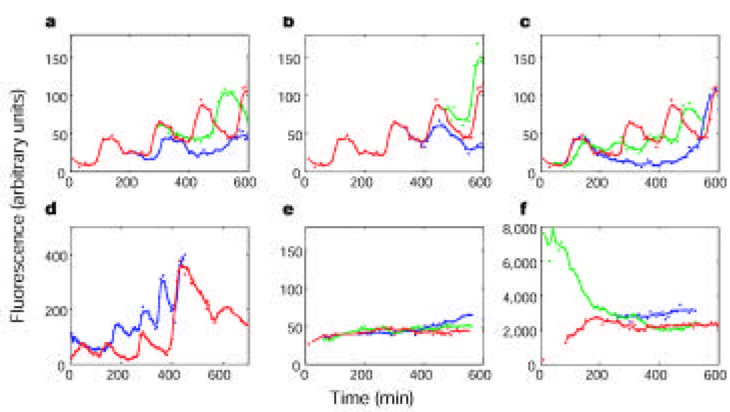




Example of Oscillation



Evaluation of the Ring Oscillator

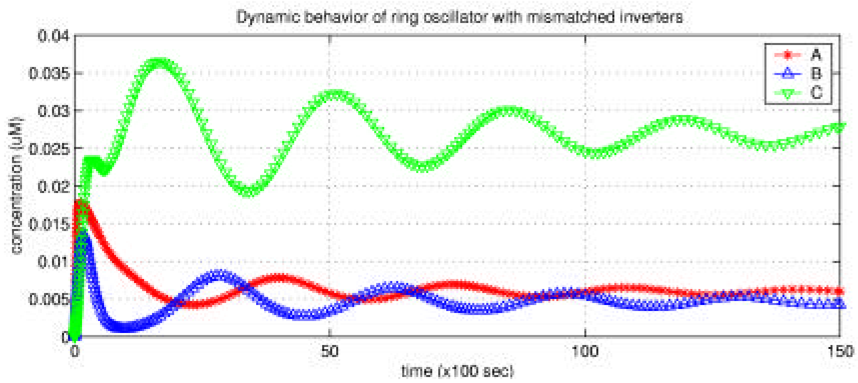


[Elowitz & Leibler, 2000]

Reliable long-term oscillation doesn't work yet:

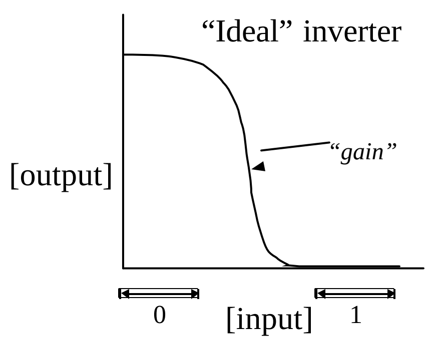
- Will matching gates help?
- Need to better understand noise
- Need better models for circuit design

A Ring Oscillator with Mismatched Inverters



A = original cI/?$_{P(R)}$
B = repressor binding 3X weaker
C = transcription 2X stronger

Device Physics in Steady State

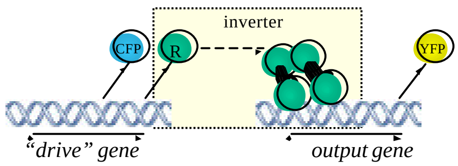


Transfer curve:
- gain (flat,steep,flat)
- adequate noise margins

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

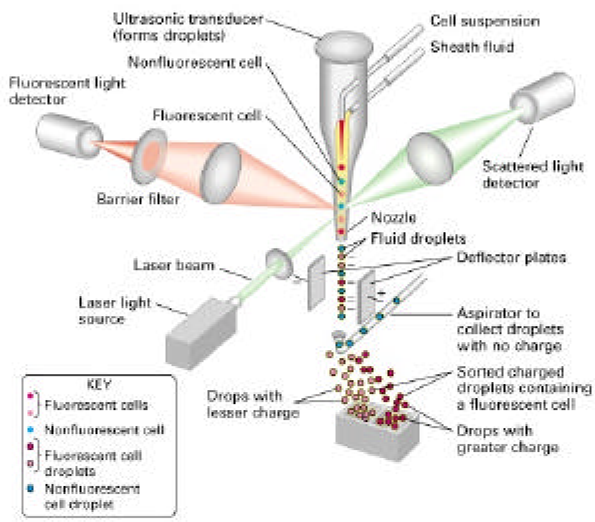
Measuring a Transfer Curve

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

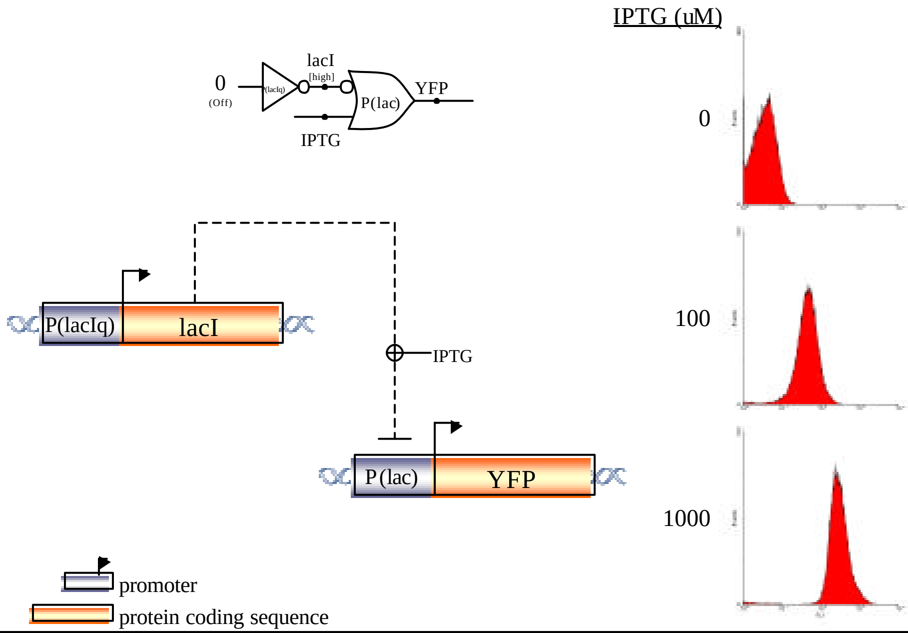


- Also, need to normalize CFP vs YFP

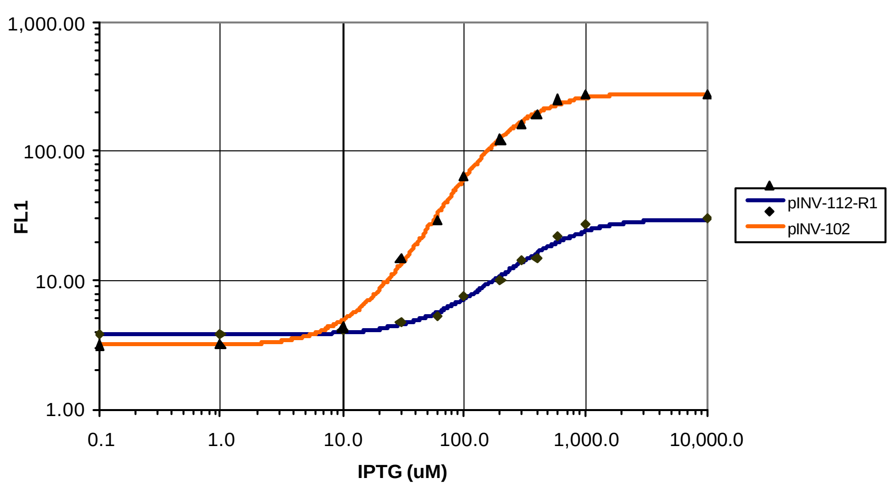
Flow Cytometry (FACS)



Drive Input Levels by Varying Inducer

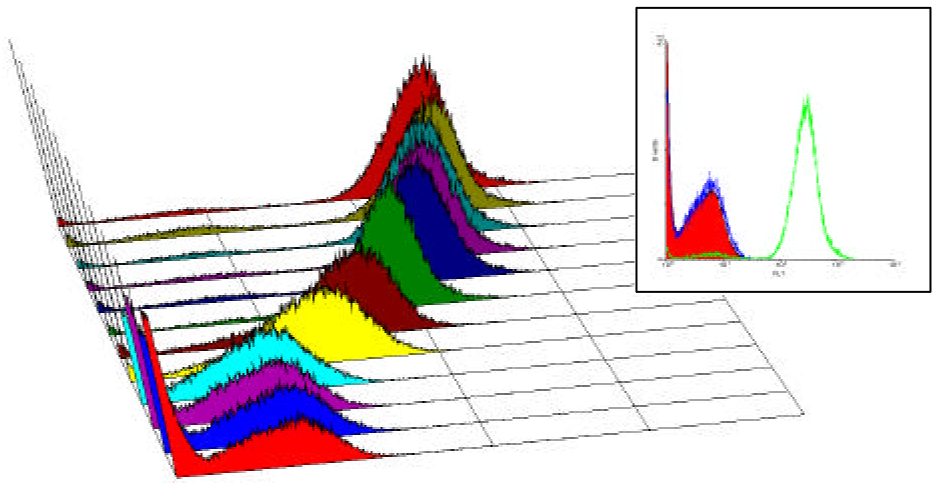


Controlling Input Levels



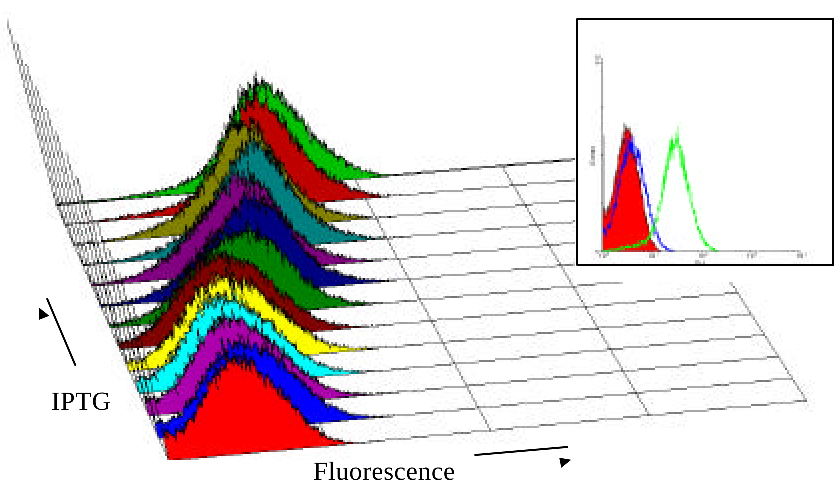
Also use for CFP/YFP calibration

Cell Population Behavior



Red = pPROLAR
Rest = pINV-102 with IPTG (0.1 to 1000 uM)

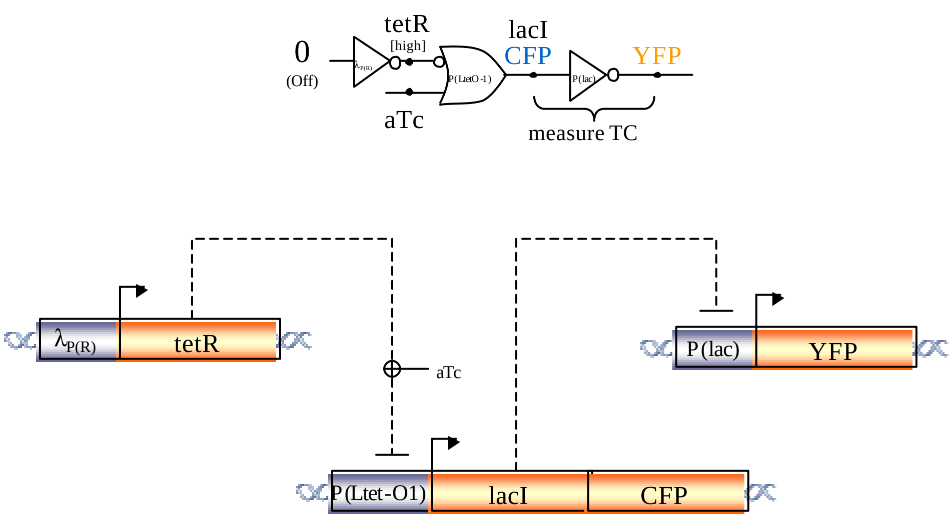
CFP: a Weak Fluorescent Protein



Induction of CFP expression

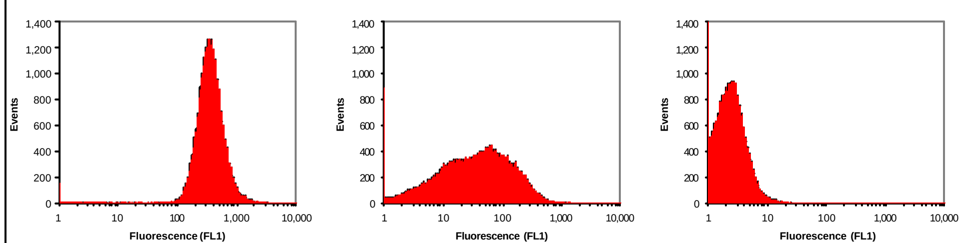
Measuring a Transfer Curve

for lacI/p(lac)



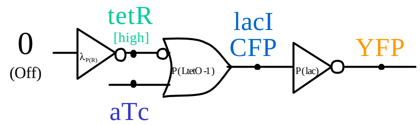
Transfer Curve Data Points

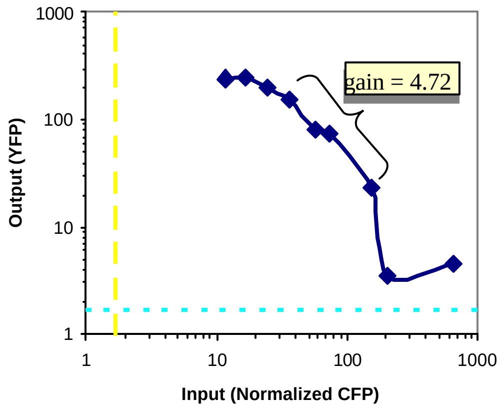
0➲1 | undefined | 1➲0



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

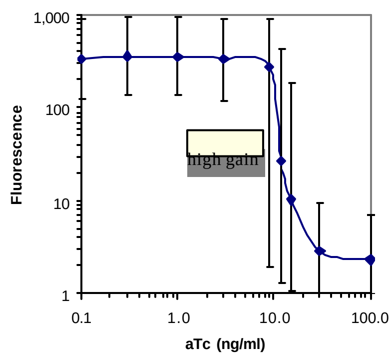
lacI/p(lac) Transfer Curve



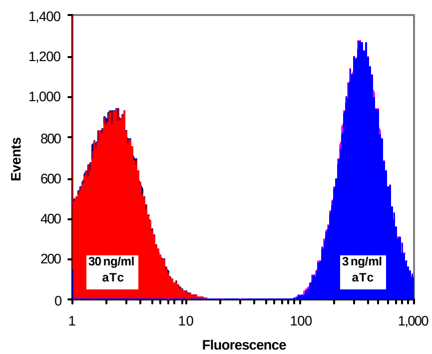


Evaluating the Transfer Curve

- Gain / Signal restoration:

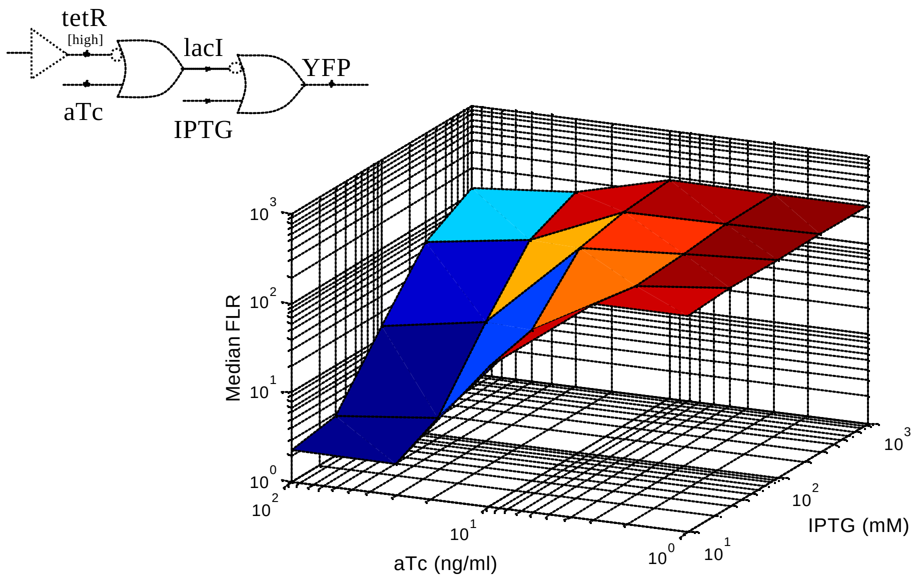


- Noise margins:



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

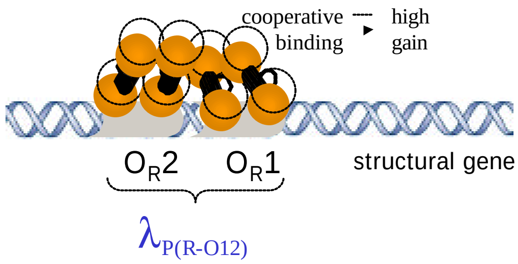
Transfer Curve of *Implies*

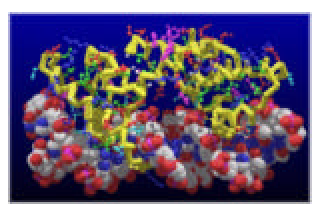


The Cellular Gate Library

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

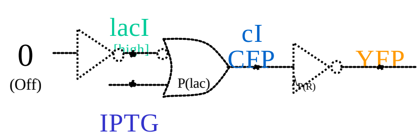
- *cI* is a highly efficient repressor



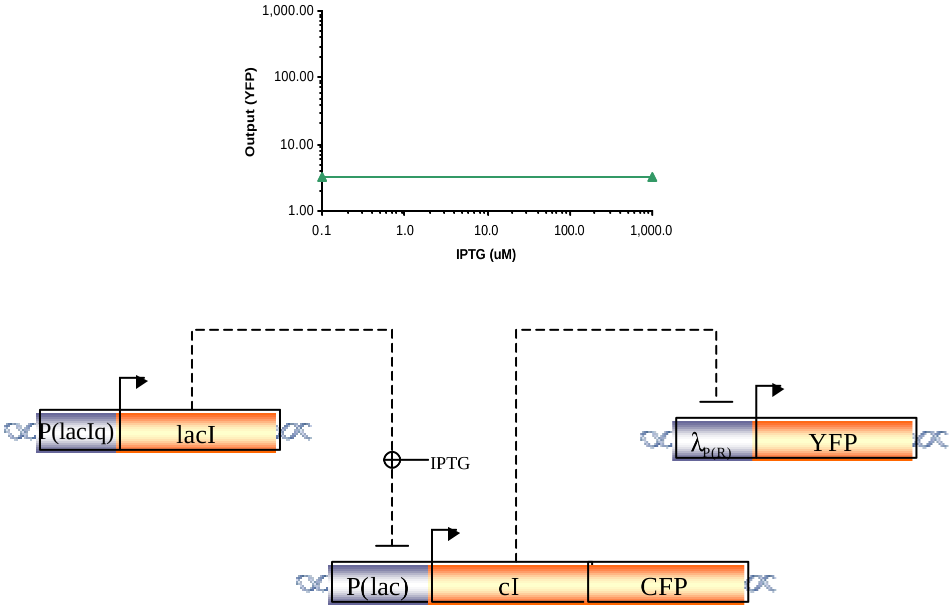


cI bound to DNA

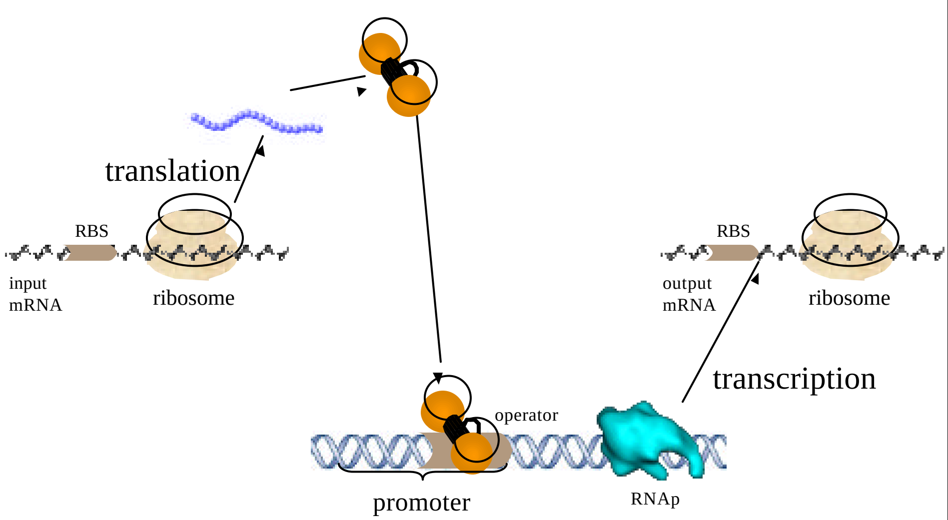
- Use lacI/p(lac) as driver



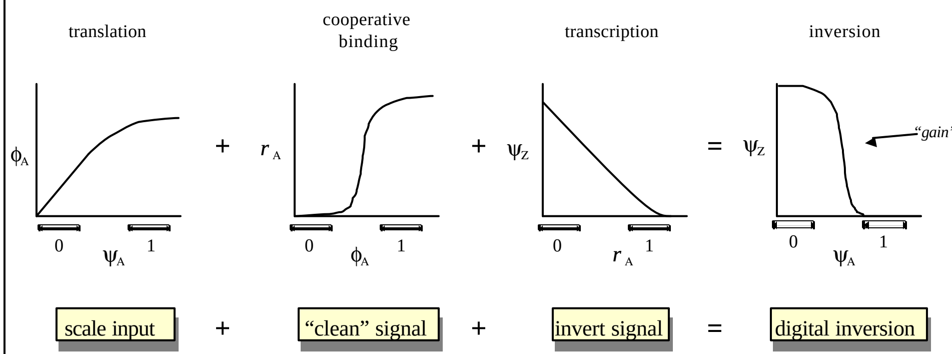
Initial Transfer Curve for cI/$\lambda_{P(R)}$



Recall Inverter Components

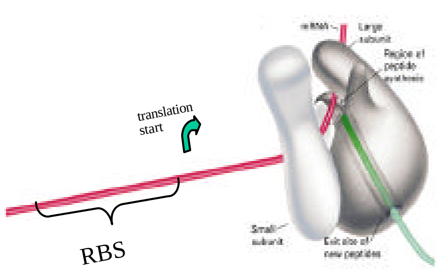


Functional Composition of an Inverter

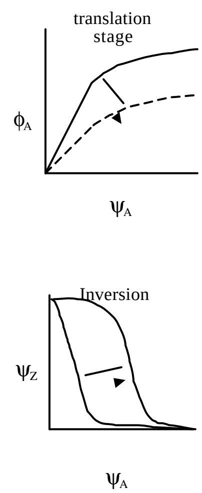


ψ_A = input mRNA r_A = bound operators
ϕ_A = input protein ψ_Z = output mRNA

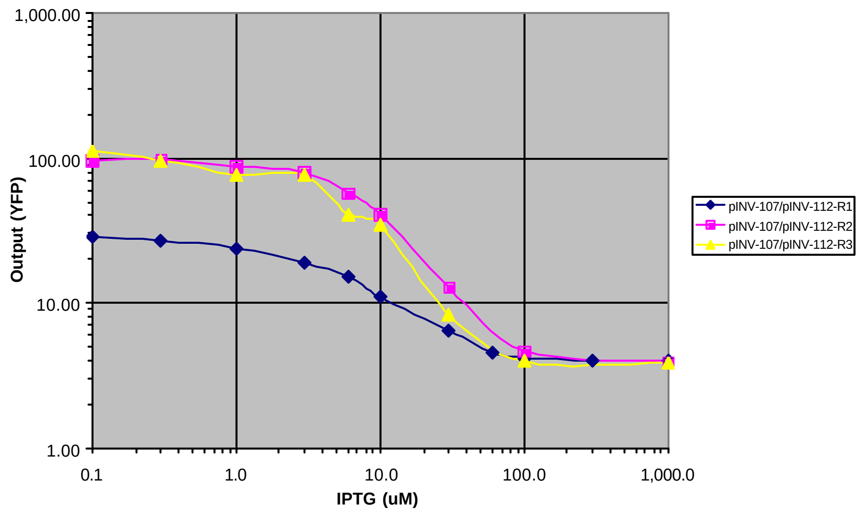
Genetic Process Engineering I:
Reducing Ribosome Binding Site Efficiency




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

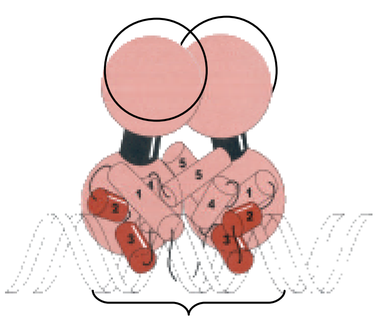


Experimental Results for $cI/\lambda_{P(R)}$ Inverter with Modified RBS



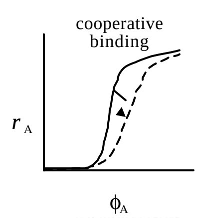
Genetic Process Engineering II:

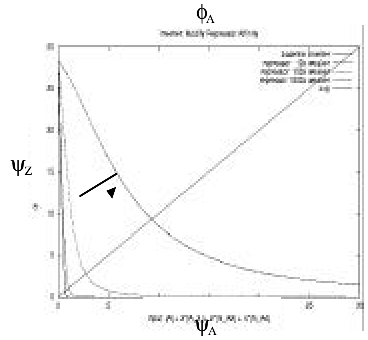
Mutating the $\lambda_{P(R)}$ operator



orig:	TACCTCTGGCGGTGATA
mut4:	TACATCTGGCGGTGATA
mut5:	TACATATGGCGGTGATA
mut6	TACAGATGGCGGTGATA

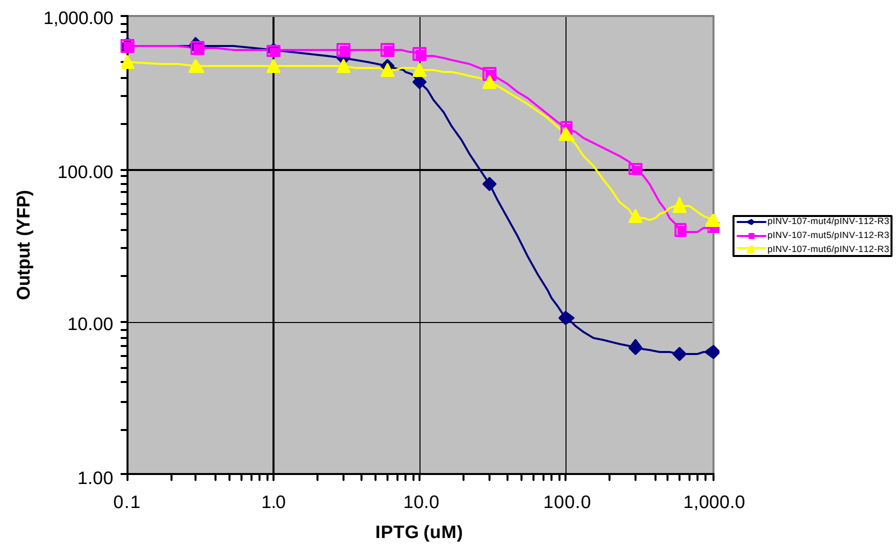
O_R1



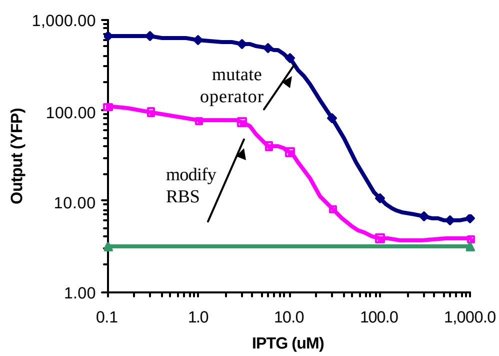


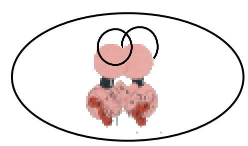
BioSPICE Simulation

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

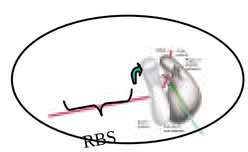


Genetic Process Engineering





#2: mutate operator



#1: modify RBS

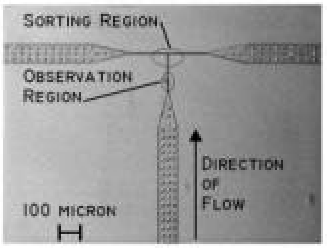
- Genetic modifications required to make circuit work
- Need to understand "device physics" of gates
  - enables construction of complex circuits

Self-perfecting Genetic Circuits

[Arnold, Yokobayashi, Weiss]

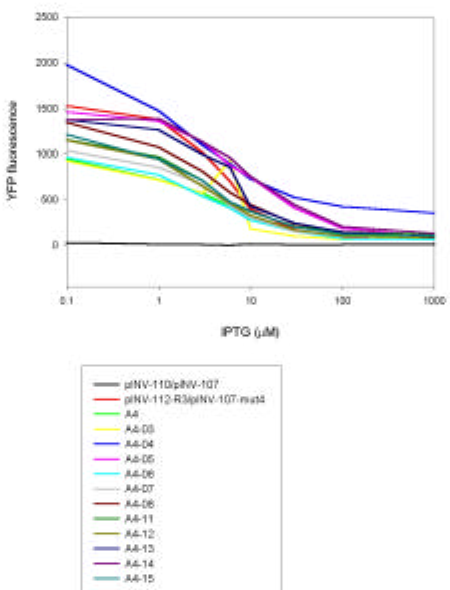
- Use directed evolution to optimize circuits
- Screening criteria based on transfer curve
- Initial results are promising

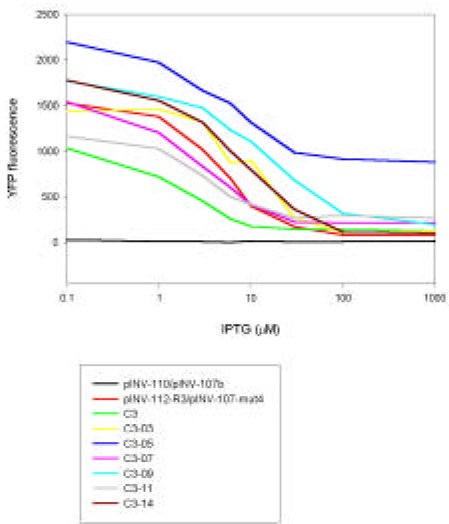
Lab-on-a-chip: µFACS [Quake]



optical micrograph of the µFACS device

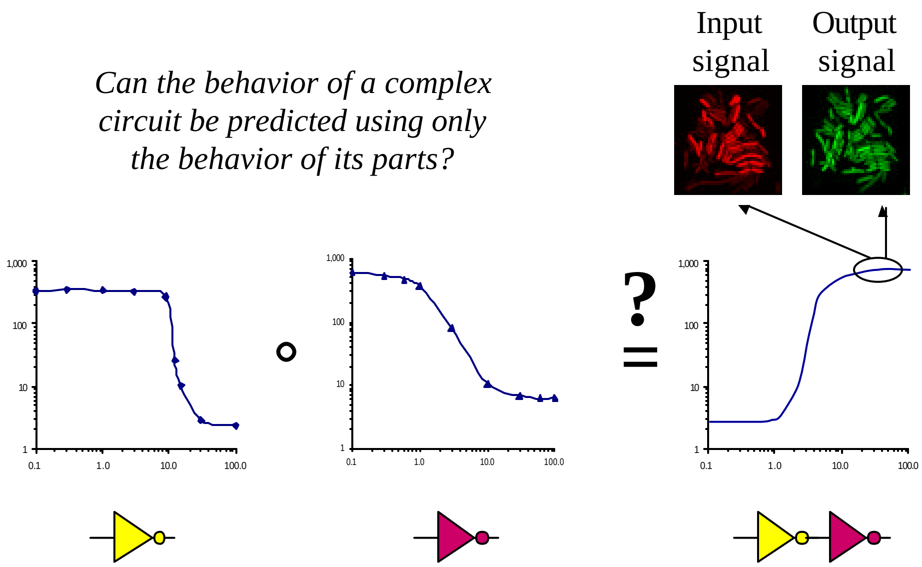
Molecular Evolution of the Circuit





Prediction of Circuit Behavior

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



Prediction of Circuit Behavior

preliminary results