

Synthetic Biology: A New Application Area for Design Automation Research

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Synthetic Biology

- Increasing number of labs are designing more ambitious and mission critical *synthetic biology* projects.
- These projects construct synthetic *genetic circuits* from DNA.
- These synthetic *genetic circuits* can potentially result in:
 - More efficient pathways for the production of antimalarial drugs (Dae et al.).
 - Bacteria that can metabolize toxic chemicals (Brazil et al.).
 - Bacteria that can hunt and kill tumors (Anderson et al.).

Genetic Engineering vs. Synthetic Biology

- *Genetic engineering* (last 30 years):
 - *Recombinant DNA* - constructing artificial DNA through combinations.
 - *Polymerase Chain Reaction (PCR)* - making many copies of this new DNA.
 - *Automated sequencing* - checking the resulting DNA sequence.
- Synthetic biology adds:
 - *Automated construction* - separate design from construction.
 - *Standards* - create repositories of parts that can be easily composed.
 - *Abstraction* - high-level models to facilitate design.

Genetic Design Automation (GDA)

- *Electronic Design Automation* (EDA) tools have facilitated the design of ever more complex integrated circuits each year.
- Crucial to the success of synthetic biology is an improvement in methods and tools for *Genetic Design Automation* (GDA).
- Existing GDA tools require biologists to design at the molecular level.
- Roughly equivalent to designing electronic circuits at the layout level.
- Analysis of genetic circuits is also performed at this very low level.
- A GDA tool that supports higher levels of abstraction is essential.

Adventures in Synthetic Biology

OK, PAY ATTENTION!
AN INVERTER IS A
COMBINATION OF BASIC
DNA PARTS THAT-

-WORKING
TOGETHER, TURN
SOMETHING UPSIDE
DOWN.

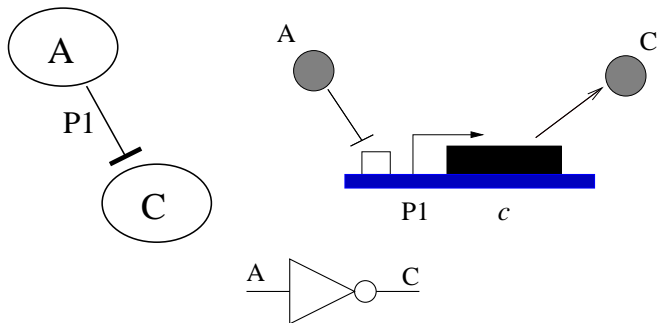
ON BECOMES OFF,
LOW BECOMES HIGH,
AND SO ON.

Parts of an Inverter

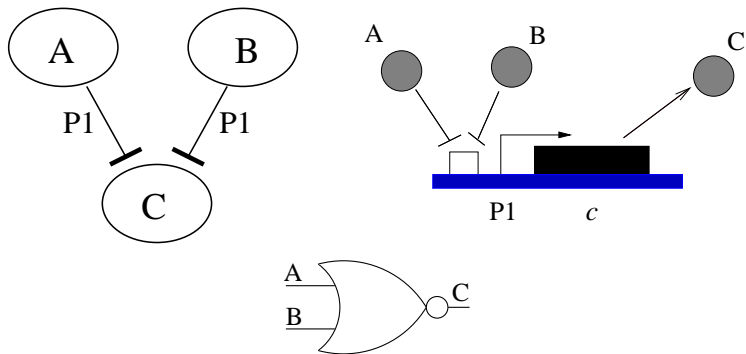
- 1. Ribosome Binding Site (RBS)** - Basic elements that start the process of protein synthesis.
- 2. Repressor** - A gene that encodes a particular type of protein that will bind DNA sites in a specific Operator part and cause changes in the rate of gene expression.
- 3. Terminator** - Special elements that decrease the flow of RNA polymerase along DNA, sometimes to zero!
- 4. Operator** - Stretches of DNA that contain Repressor protein binding sites and RNA polymerase binding and initiation sites. With a Repressor protein, the Operator part will be turned OFF. Without a Repressor protein, the Operator part will be turned ON, allowing RNA polymerase to bind and initiate a HIGH output signal.

(From "Adventures in Synthetic Biology" - Endy et al.)

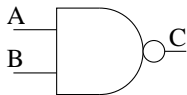
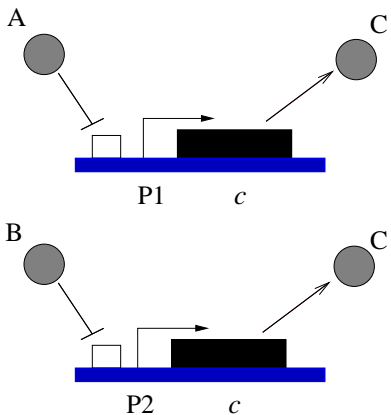
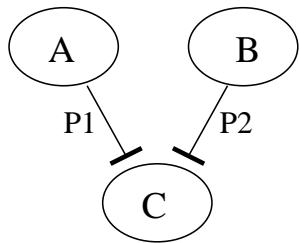
A Genetic Not Gate



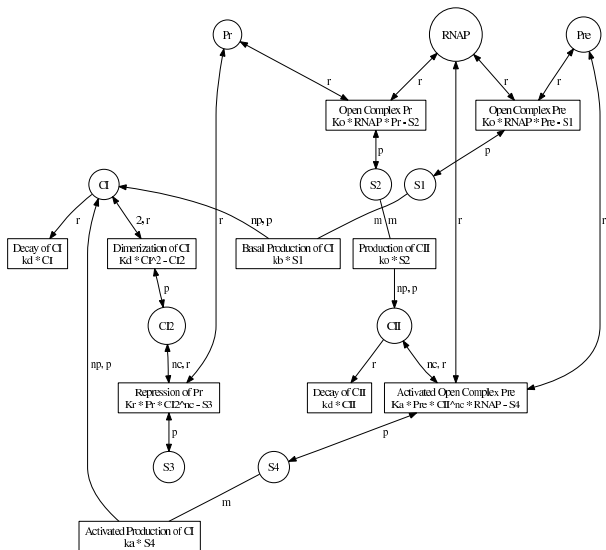
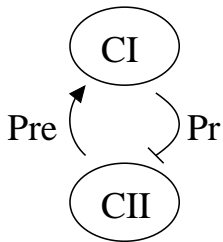
A Genetic Nor Gate



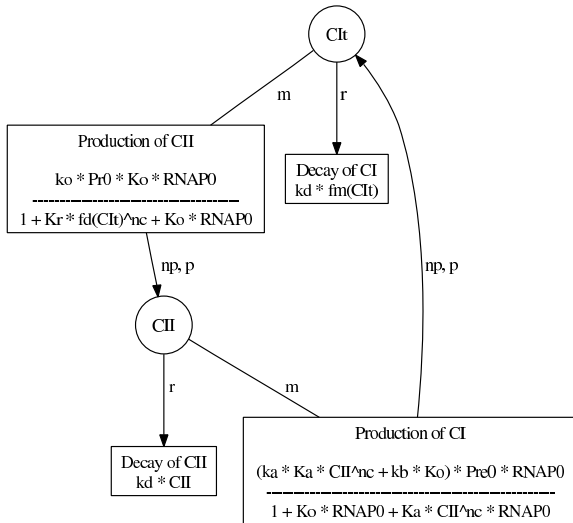
A Genetic Nand Gate



Genetic Circuit versus Molecular Representation



Final Molecular Model After Abstraction



10 species and 10 reactions reduced to 2 species and 4 reactions

Classical Chemical Kinetics

- Uses *ordinary differential equations* (ODE) to represent the system to be analyzed, and it assumes:
 - Molecule counts are high, so concentrations can be continuous variables.
 - Reactions occur continuously and deterministically.
- Genetic circuits have:
 - Small molecule counts which must be considered as discrete variables.
 - Gene expression reactions that occur sporadically.
- ODEs do not capture non-deterministic behavior.

NYTimes: Expressing Our Individuality, the Way E. Coli Do

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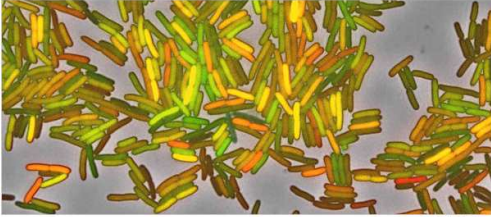
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Expressing Our Individuality, the Way E. Coli Do



Dr. Michael Elowitz

A colony of genetically identical E. coli is actually a mob of individuals. Under identical conditions, they behave in different ways.

By CARL ZIMMER
Published: April 22, 2008

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Well

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Stochastic Chemical Kinetics

- To more accurately predict the temporal behavior of genetic circuits, *stochastic chemical kinetics* formalism can be used.
- Use Gillespie's *Stochastic Simulation Algorithm* which tracks the quantities of each molecular species and treats each reaction as a separate random event.
- Only practical for small systems with no major time-scale separations.
- Abstraction is essential for efficient analysis of any realistic system.

iBioSim: Genetic Circuit Editor

iBioSim File Edit View Tools Help

iBioSim

example
CICII.gcm

CICII.gcm

Main Elements Components

GCM Id: CICII SBML File: --none-- Biochemical abstraction: Dimerization abstraction:

List of Promoters:
PR
PRE

List of Species:
CI
CII

Add Promoter Remove Promoter Edit Promoter Add Species Remove Species Edit Species

List of Influences:
CI -| CII, Promoter PR
CII -> CI, Promoter PRE

List of Parameters:
Activated production rate (ka), Default, .25
Activation binding equilibrium (Ka), Default, .0033
Basal production rate (kb), Default, .0001
Biochemical equilibrium (Kb), Default, .05
Degradation rate (kd), Default, .0075
Degree of cooperativity (nc), Default, 2
Dimerization equilibrium (Kd), Default, .05
Initial RNAP count (nr), Default, 30
Initial promoter count (ng), Default, 2

Add Influence Remove Influence Edit Influence Edit Parameter

iBioSim: SBML Editor

iBioSim File Edit View Tools Help

iBioSim

example
CICII.gcm
CICII.xml

CICII.gcm x CICII.xml x

Main Elements Definitions/Types Initial Assignments/Rules/Constraints/Events

Model ID: CICII.xml Model Name: Created from CICII.xml

List of Compartments:
default 1.0

List of Species:
bound_PR_CII default 0.0
CII default 0.0
CII default 0.0
PR default 2.0
PRE default 2.0
RNAP default 30.0
RNAP_PR default 0.0
RNAP_PRE default 0.0
RNAP_PRE_CII default 0.0

Add Compartment Remove Compartment Edit Compartment Add Species Remove Species Edit Species

List of Reactions:
Degradation_CII
Degradation_CII
R_act_production_PRE_CII
R_basal_production_PRE
R_production_PR
R_repression_binding_PR_CII
R_RNAP_binding_PRE_CII
R_RNAP_PR
R_RNAP_PRE

List of Global Parameters:

Add Reaction Remove Reaction Edit Reaction Add Parameter Remove Parameter Edit Parameter

/Users/myers/nobackup/Projects/example/CICII.gcm

Saving GCM file as SBML file:
/Users/myers/nobackup/Projects/example/CICII.xml

Creating properties file:
/Users/myers/nobackup/Projects/example/sim/CICII.properties

iBioSim: ODE Analysis

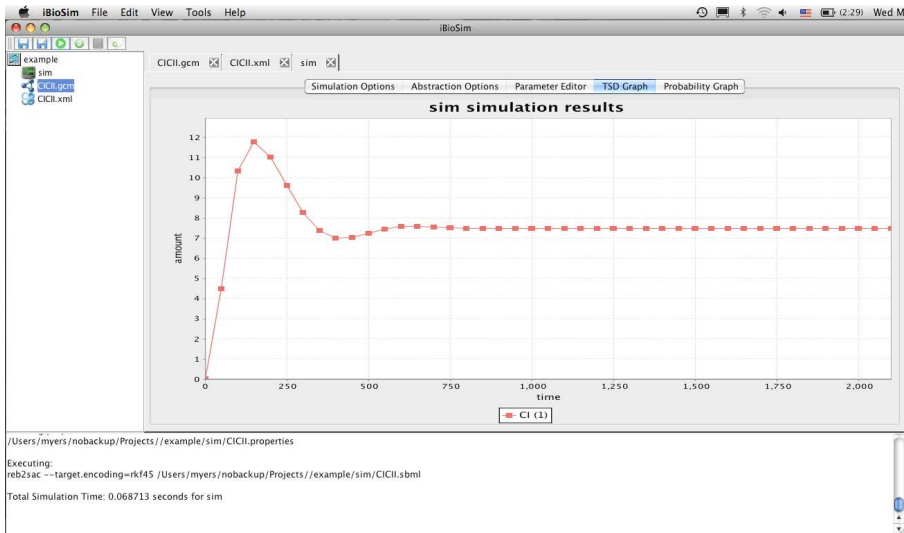
The screenshot shows the iBioSim application window. The title bar reads "iBioSim" and the menu bar includes "File", "Edit", "View", "Tools", and "Help". The window contains several tabs: "CICII.gcm", "CICII.xml", and "sim". The "Simulation Options" tab is active, showing the following settings:

- Model File: CICII.sbml
- Abstraction: Abstraction (Other options: None, Logical Abstraction)
- Simulation Type: ODE (Other options: Monte Carlo, Markov, SBML, Network, Browser)
- Choose One: Overwrite (Other option: Append)
- Possible Simulators/Analyzers: rkf45
- Description Of Selected Simulator: Embedded Runge-Kutta-Fehlberg (4, 5) method
- Time Limit: 2100.0
- Print Interval: 50.0
- Maximum Time Step: inf
- Absolute Error: 1.0E-9
- Random Seed: 314159
- Runs: 1
- Simulation ID: (empty field)

At the bottom of the window, a status bar displays the following information:

- Path: /Users/myers/nobackup/Projects//example//CICII.gcm
- Message: Saving GCM file as SBML file: /Users/myers/nobackup/Projects//example//CICII.xml
- Message: Creating properties file: /Users/myers/nobackup/Projects//example/sim/CICII.properties

iBioSim: ODE Simulation Results



iBioSim: Gillespie Analysis

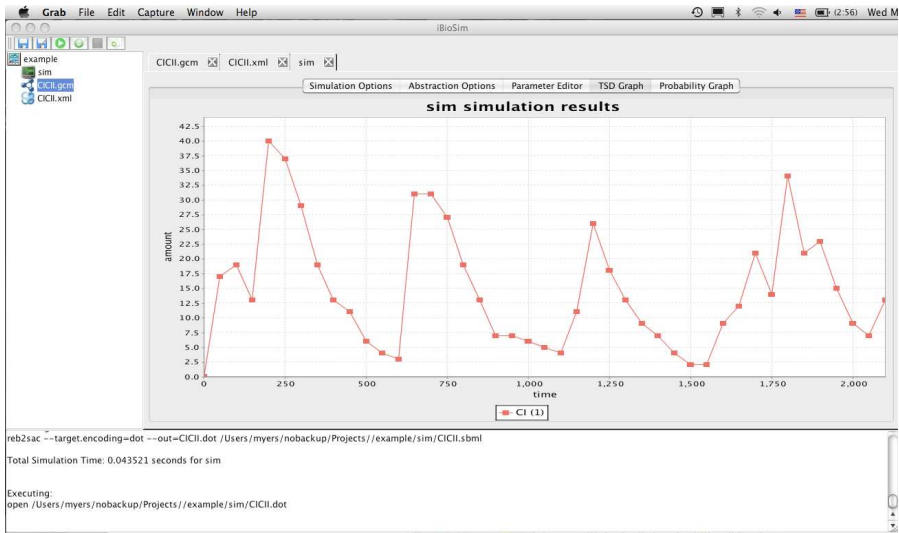
The screenshot shows the iBioSim application window. The title bar reads "iBioSim" and the menu bar includes "File", "Edit", "View", "Tools", and "Help". The window contains several tabs: "CICII.gcm", "CICII.xml", and "sim". The "Simulation Options" tab is active, showing the following settings:

- Model File: CICII.sbml
- Abstraction: Abstraction (Other options: None, Logical Abstraction)
- Simulation Type: Monte Carlo (Other options: ODE, Markov, SBML, Network, Browser)
- Choose One: Overwrite (Other option: Append)
- Possible Simulators/Analyzers: gillespie
- Description Of Selected Simulator: Gillespie's direct method
- Time Limit: 2100.0
- Print Interval: 50.0
- Maximum Time Step: inf
- Absolute Error: 1.0E-9
- Random Seed: 314159
- Runs: 10
- Simulation ID: (empty field)

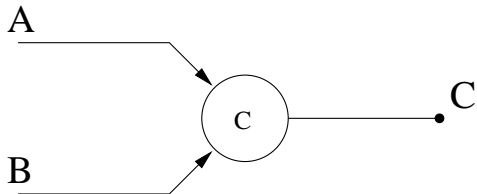
At the bottom of the window, the following text is displayed:

```
/Users/myers/nobackup/Projects//example/sim/CICII.properties  
Executing:  
reb2sac --target.encoding=rkf45 /Users/myers/nobackup/Projects//example/sim/CICII.sbml  
Total Simulation Time: 0.358468 seconds for sim
```

iBioSim: Stochastic Simulation Results

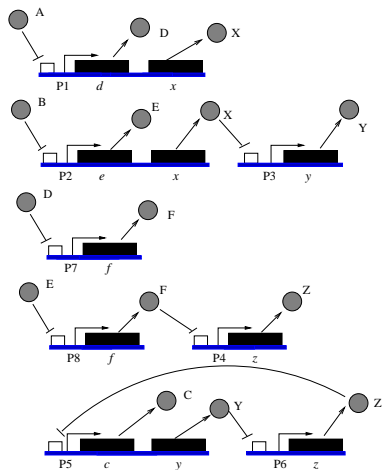
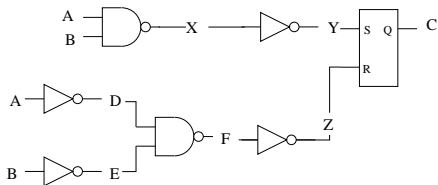


Genetic Muller C-Element

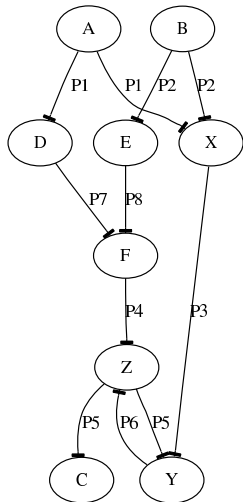
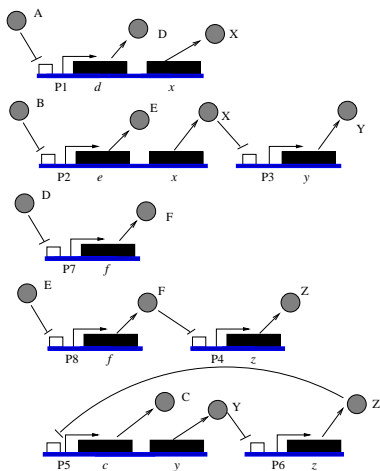


A	B	C'
0	0	0
0	1	C
1	0	C
1	1	1

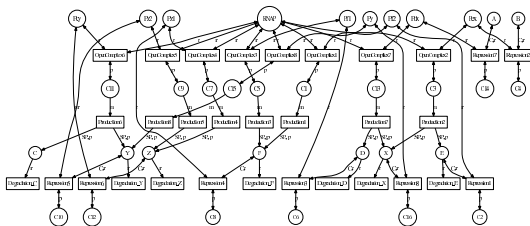
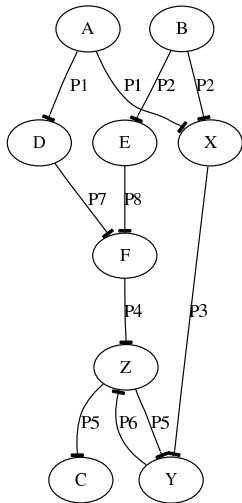
Toggle Switch C-Element (Genetic Circuit)



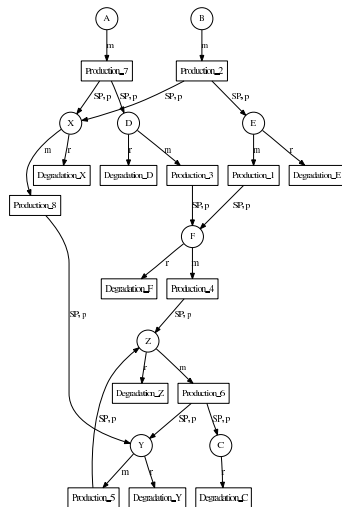
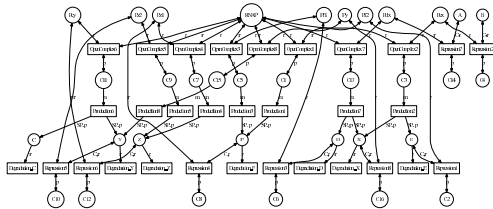
Toggle Switch C-Element (GCM)



Toggle Switch C-Element (SBML)

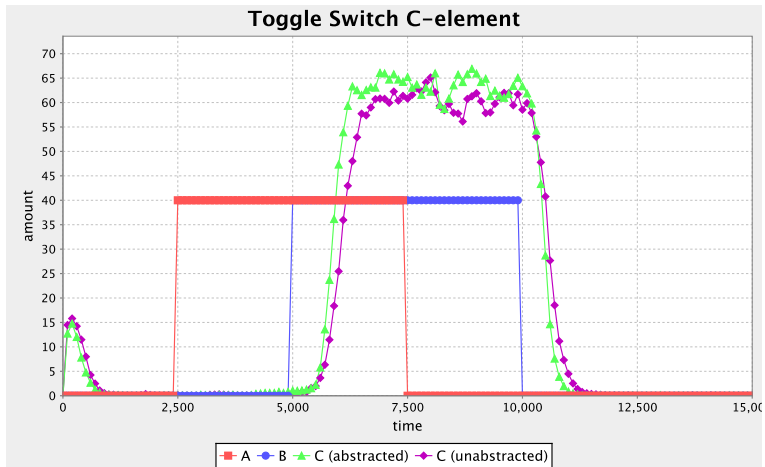


Toggle Switch C-Element (Abstracted)



Reduced from 34 species and 31 reactions to 9 species and 15 reactions.

Toggle Switch C-Element (Simulation)

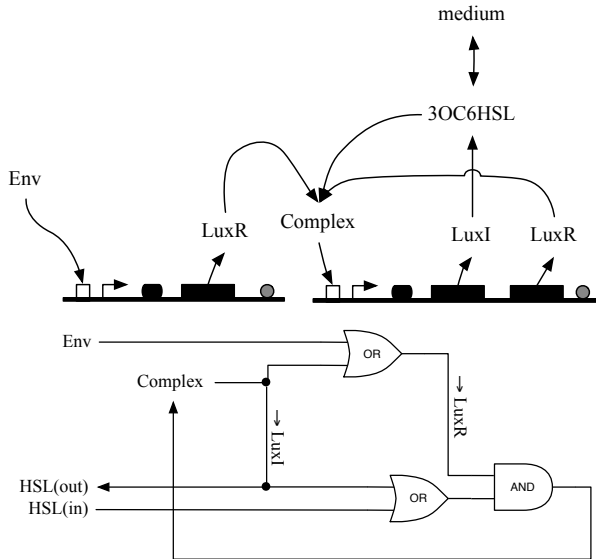


Simulation time improved from 312 seconds to 20 seconds.

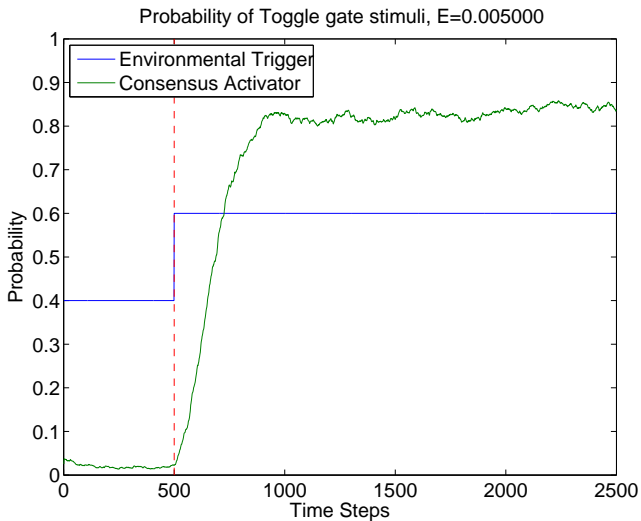
Application: Bacterial Consensus

- One interesting application is designing bacteria that can hunt and kill tumor cells (Anderson et al.).
- Care must be taken in determining when to attack potential tumor cells.
- Can use a genetic Muller C-element and a bacterial consensus mechanism known as *quorum sensing*.
- C-element combines a noisy environmental trigger signal and a density dependent quorum sensing signal.
- Activated bacteria signal their neighbors to reach consensus.
- C-elements behave unreliably (i.e., have probability of switching state).

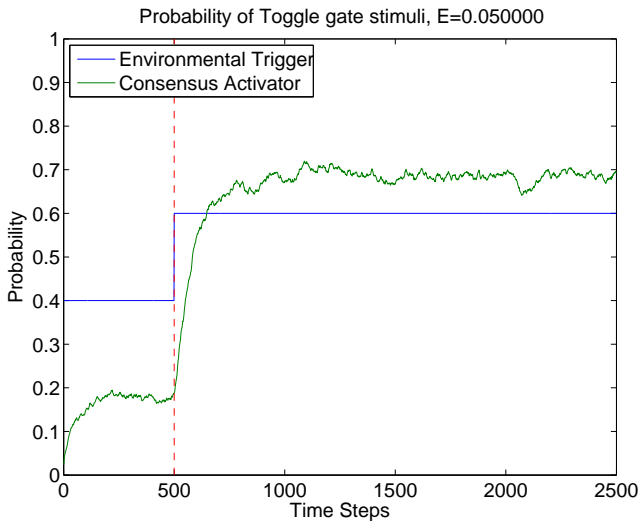
Quorum Trigger Circuit



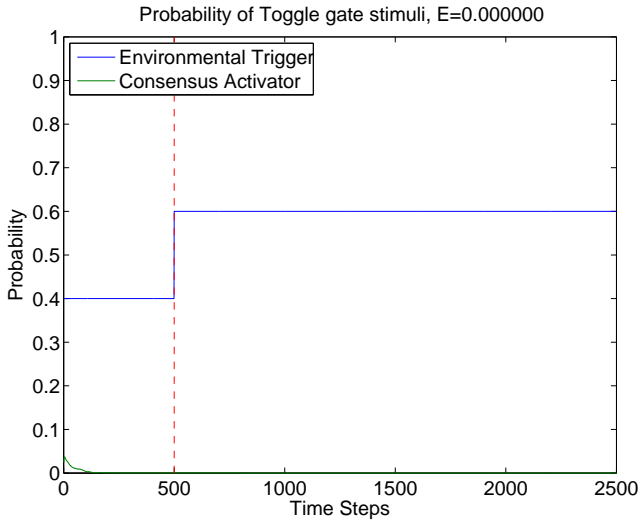
Application: Results



Application: Results



Application: Results



Future GDA Research Directions

- Genetic circuits have no signal isolation.
- Circuit products may interfere with each other and host cell.
- Gates in a genetic circuit library usually can only be used once.
- Behavior of circuits are non-deterministic in nature.
- No global clock, so timing is difficult to characterize.
- We plan to adapt asynchronous tools to genetic circuit technology.

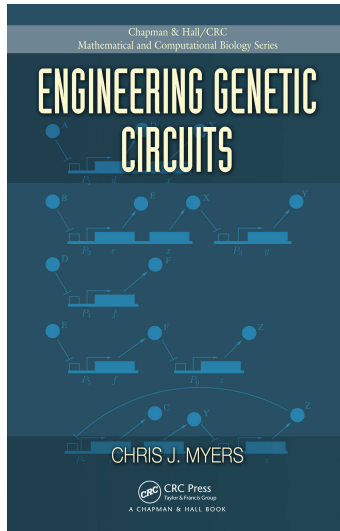
Biologically Inspired Circuit Design

- Human inner ear performs the equivalent of one billion floating point operations per second and consumes only $14 \mu\text{W}$ while a game console with similar performance burns about 50 W (Sarpeshkar, 2006).
- We believe this difference is due to over designing components in order to achieve an extremely low probability of failure in every device.
- Future silicon and nano-devices will be much less reliable.
- For Moore's law to continue, future design methods should support the design of reliable systems using unreliable components.
- Biological systems constructed from very noisy and unreliable devices.
- GDA tools may be useful for future integrated circuit technologies.

More Information

- 1st International Workshop on Bio-Design Automation
July 27th in San Francisco at DAC.
- Linux/Windows/Mac versions of iBioSim are freely available from:
<http://www.async.ece.utah.edu/iBioSim/>
- Publications:
<http://www.async.ece.utah.edu/publications/>
- Course materials:
<http://www.async.ece.utah.edu/~myers/ece6760/>
<http://www.async.ece.utah.edu/~myers/math6790/>

Engineering Genetic Circuits



Acknowledgments



Nathan Barker



Hiroyuki Kuwahara



Nam Nguyen



Curtis Madsen



Chris Winstead



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