Synthetic Biology:

Rational Engineering of Biological Systems

James Brown

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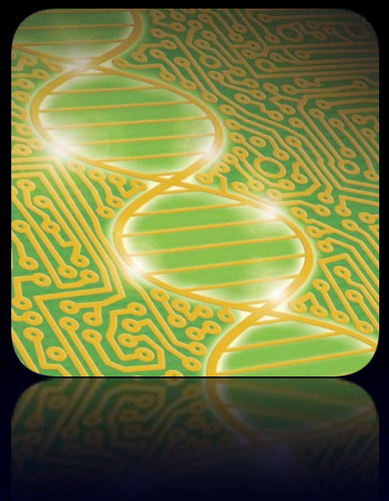
Dept. of Plant Sciences
University of Cambridge

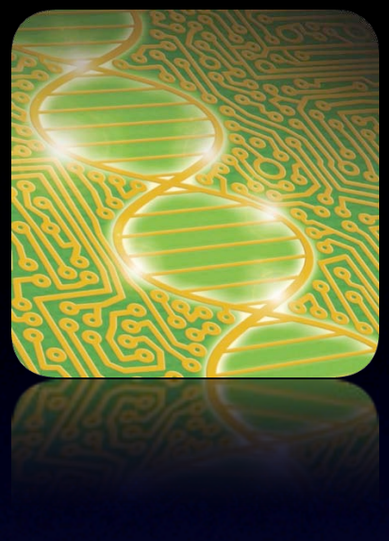
"Scientists discover the world that exists;
Engineers create the world that never was."

Theodore von Karman



Synthetic Biology

- Foundational Principles
- Fundamental Research Review



Synthetic Biology

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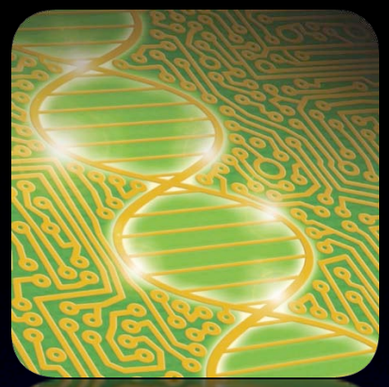
The Registry of Biological Parts

- Overview
- BioBricks & Standard Assembly
- Rational Design



Synthetic Biology

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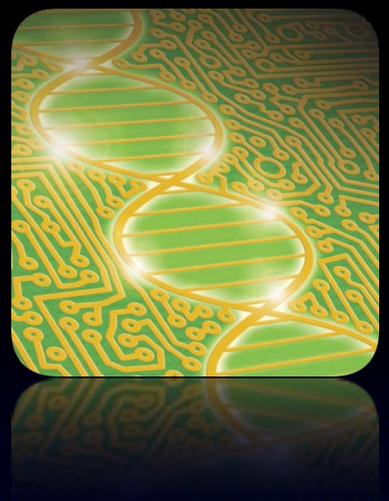
The iGEM Competition

- History of Competition
- Project examples



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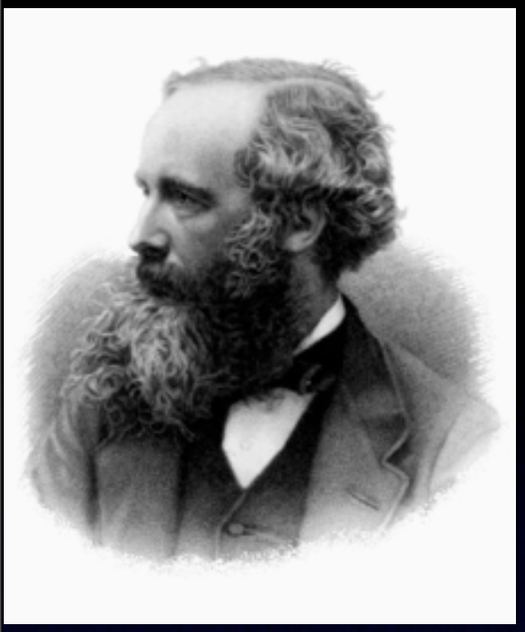

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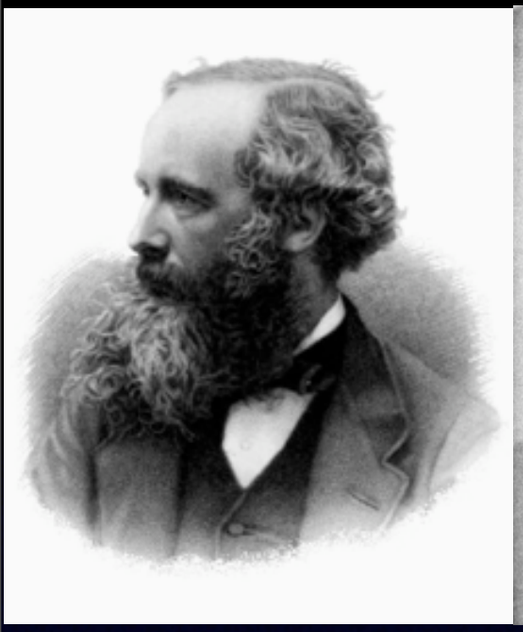

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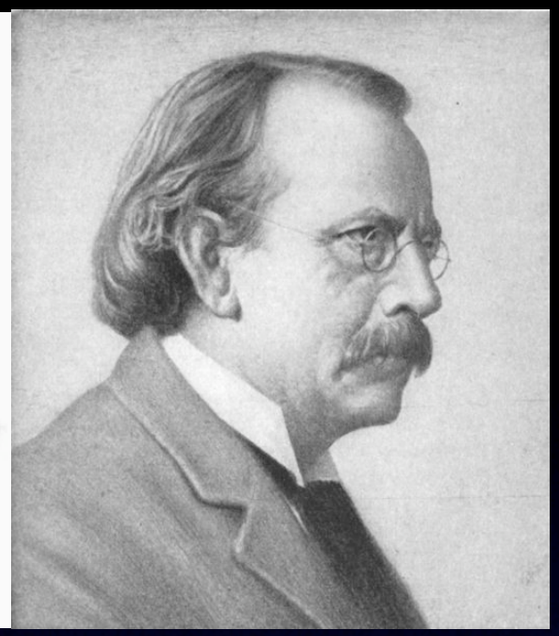

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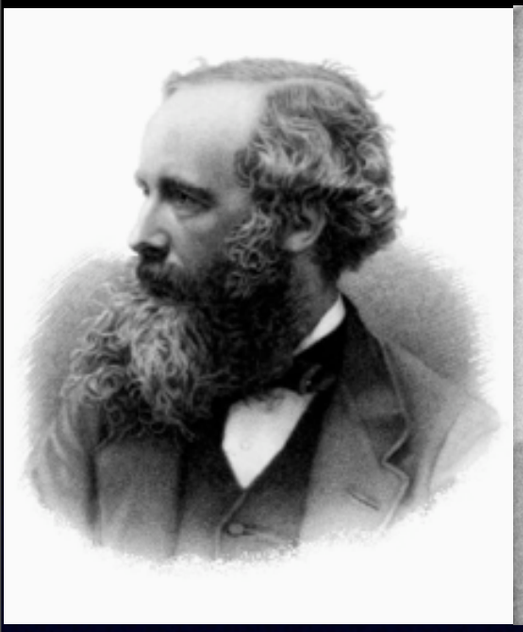

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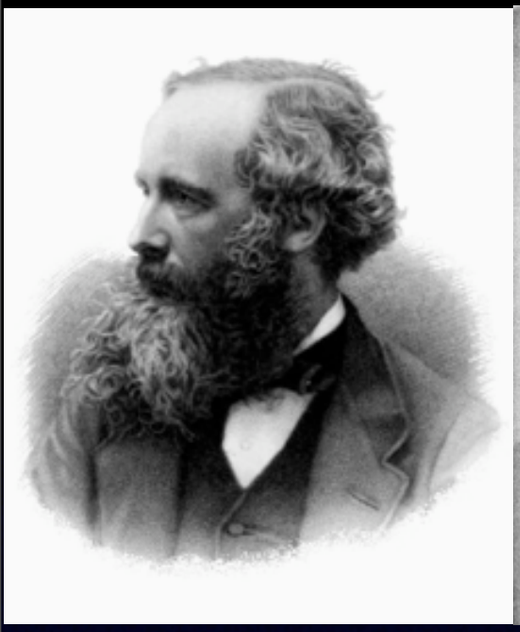

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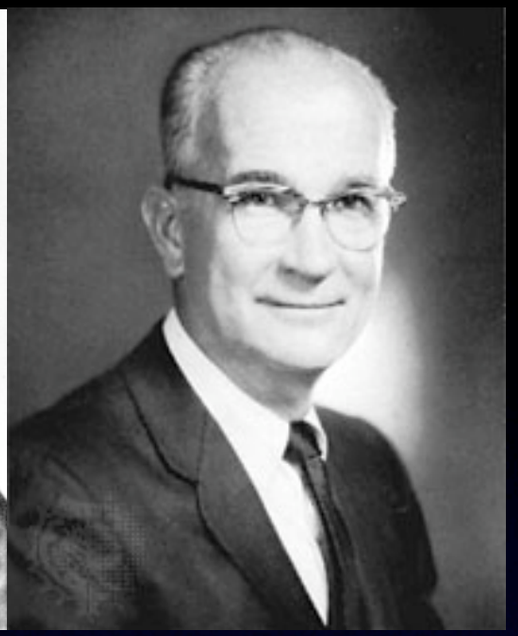

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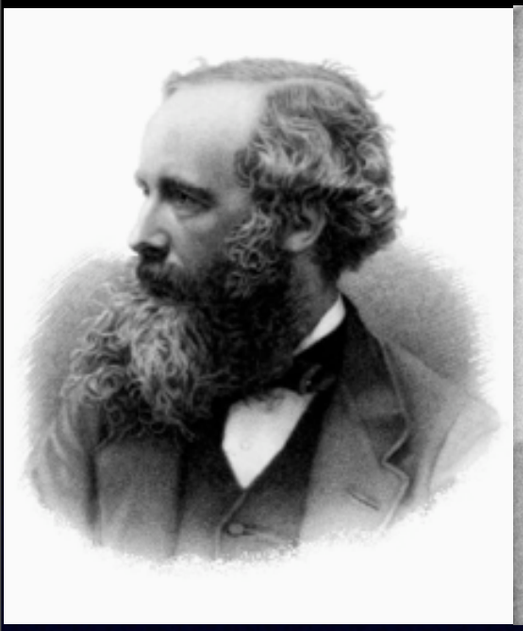

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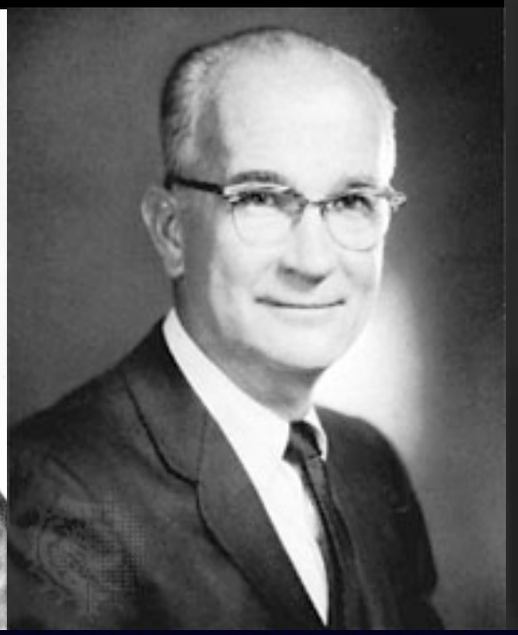

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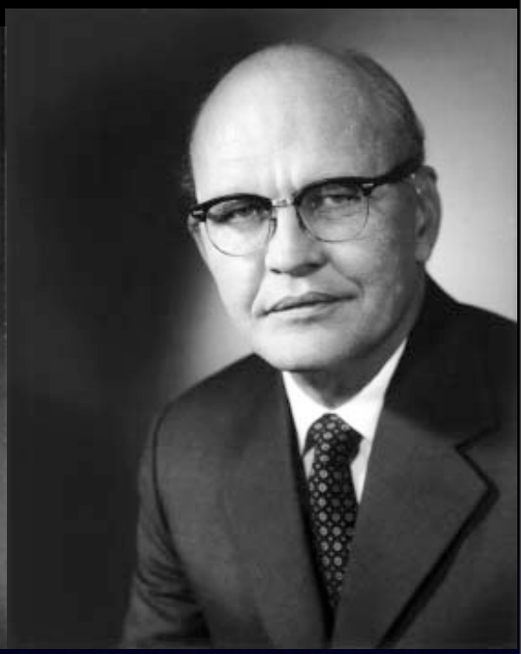

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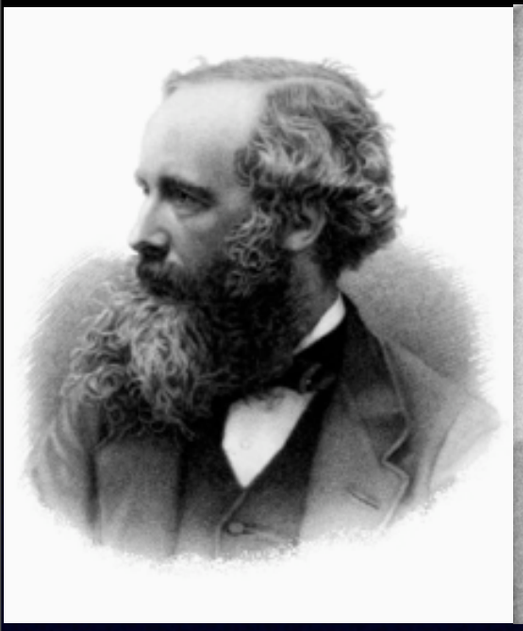

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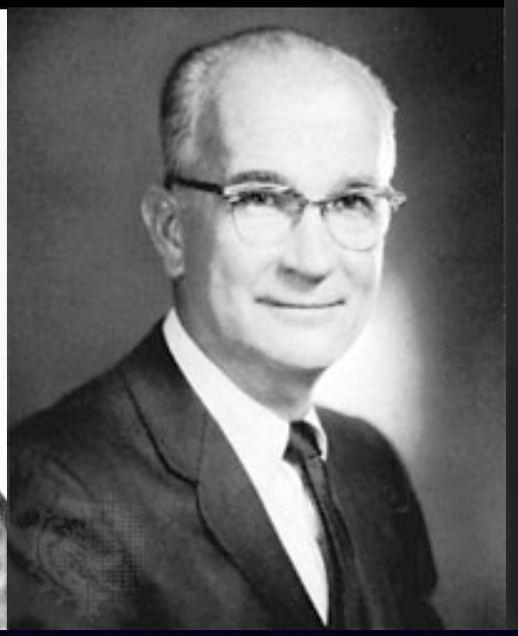

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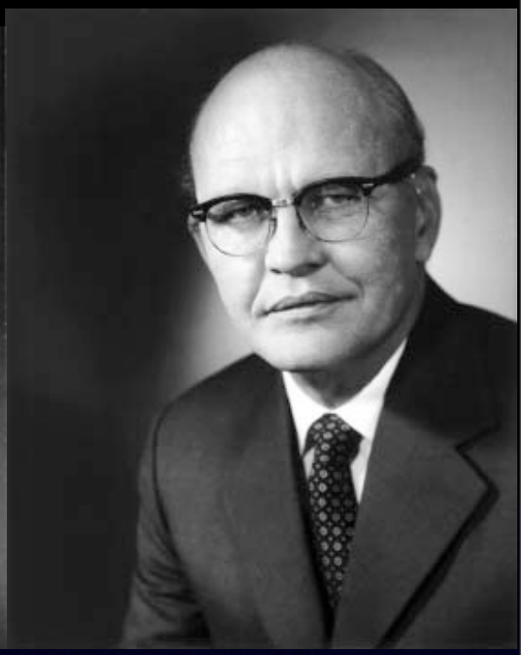

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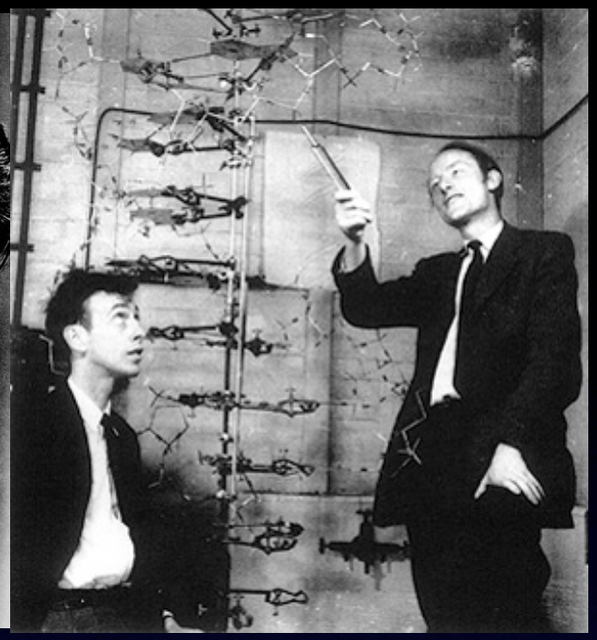

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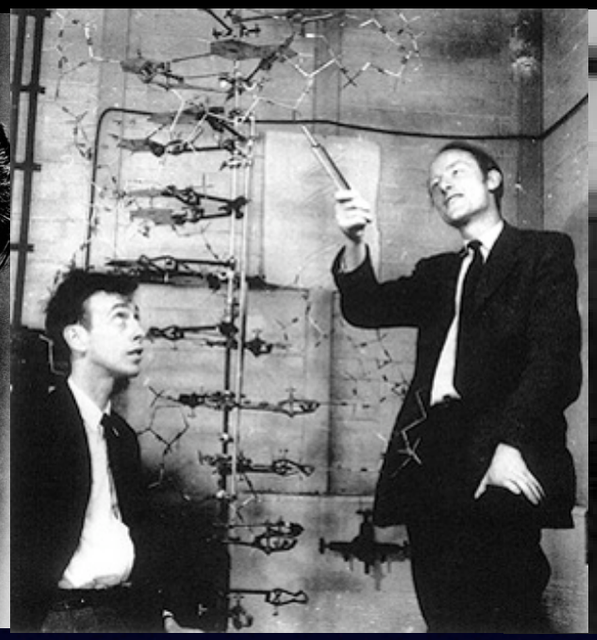

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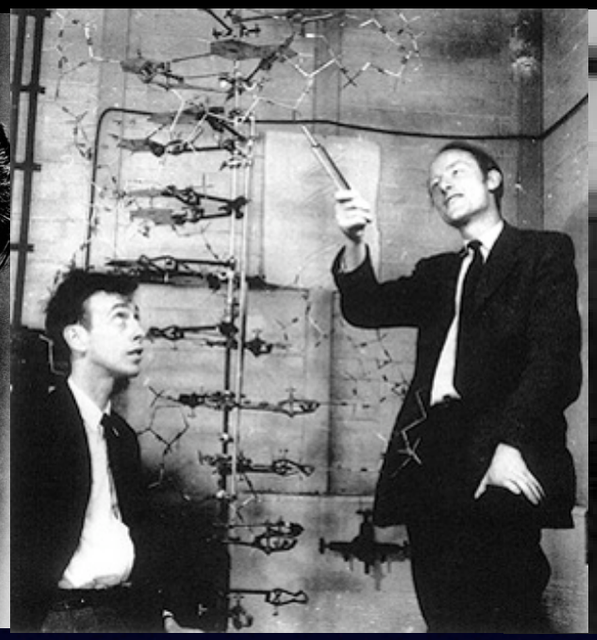

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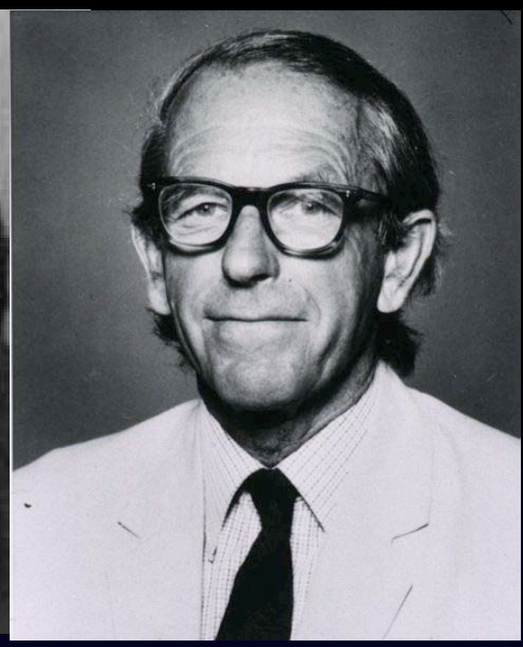

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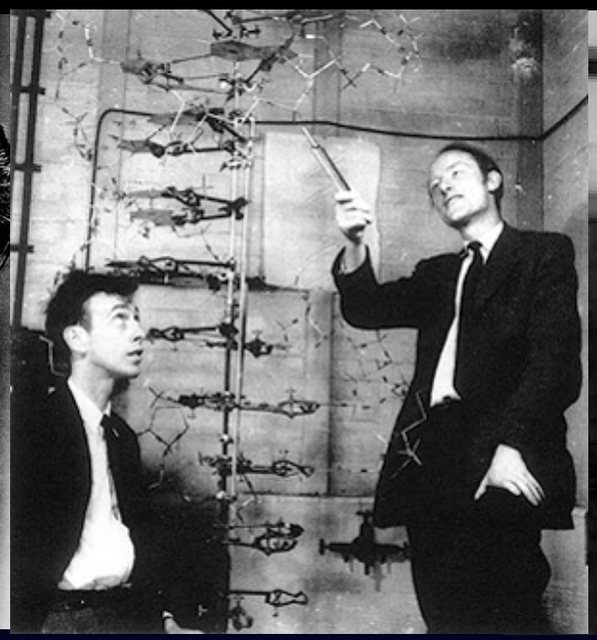

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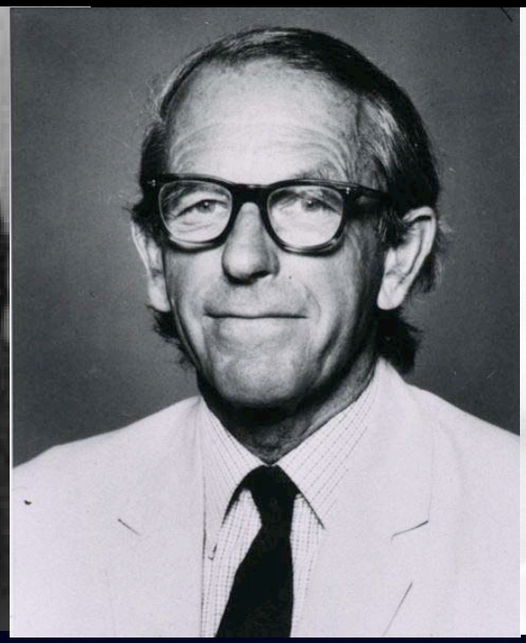

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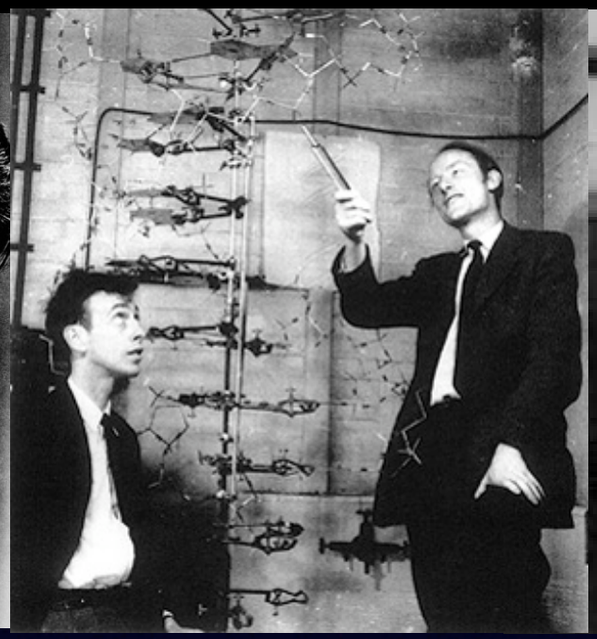

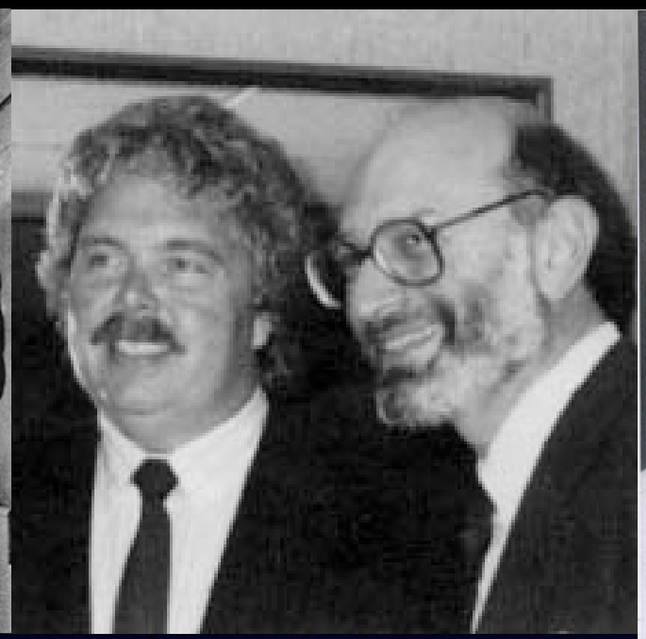

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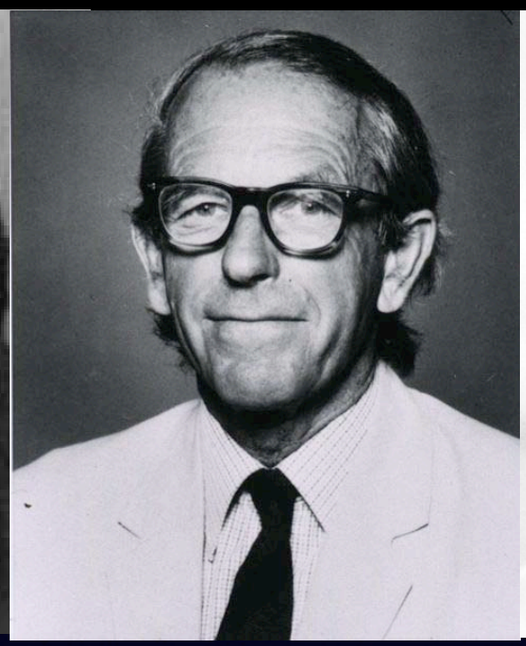

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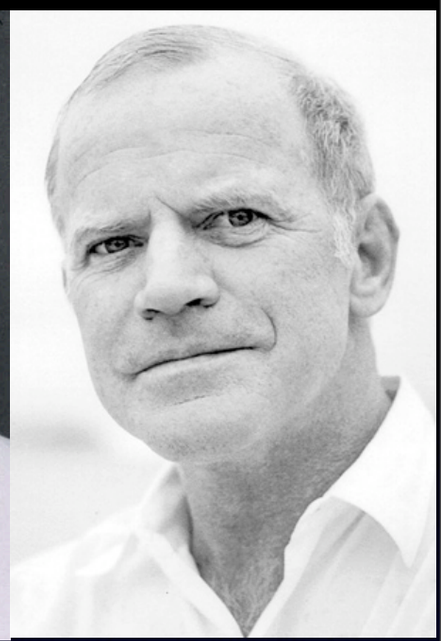

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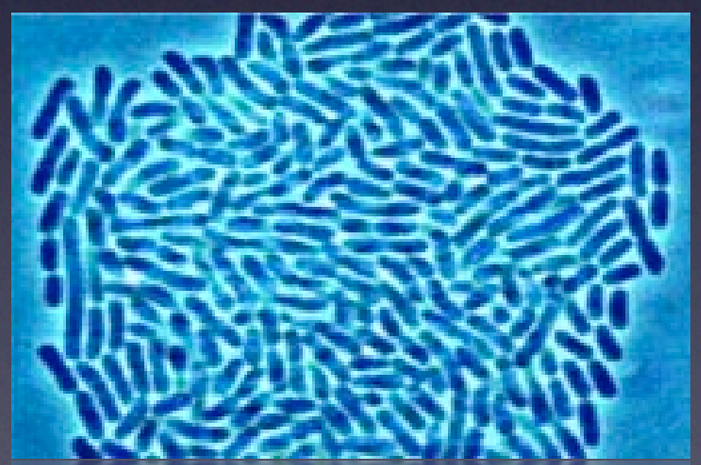
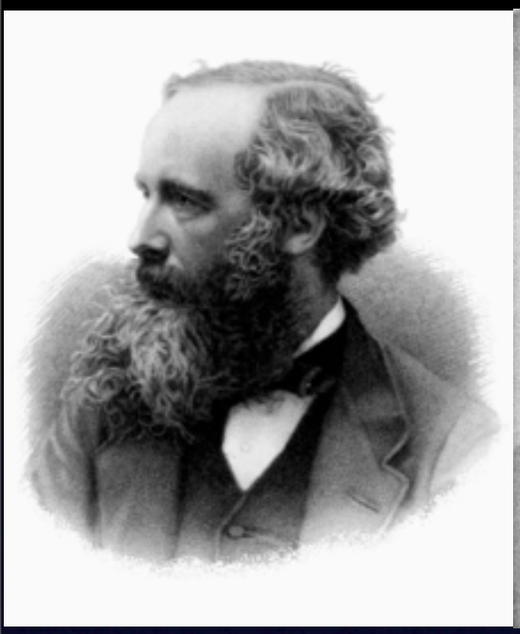


Physical Sciences



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Electrical/Electronic Engineering

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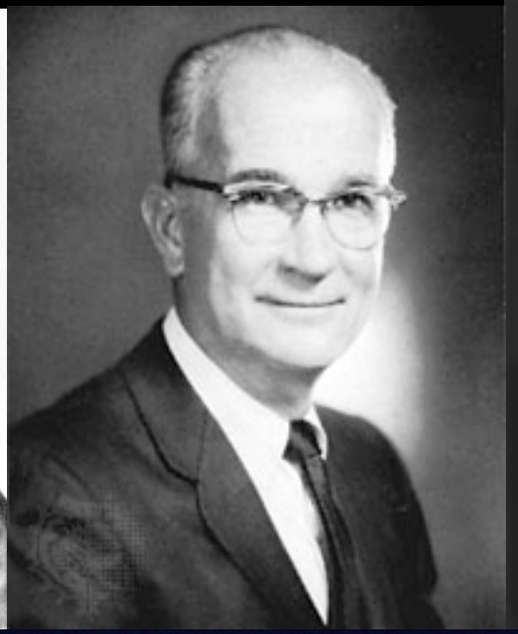

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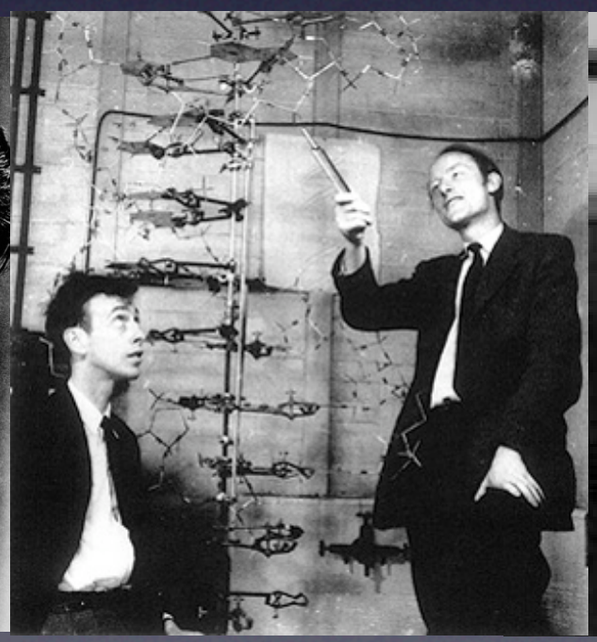

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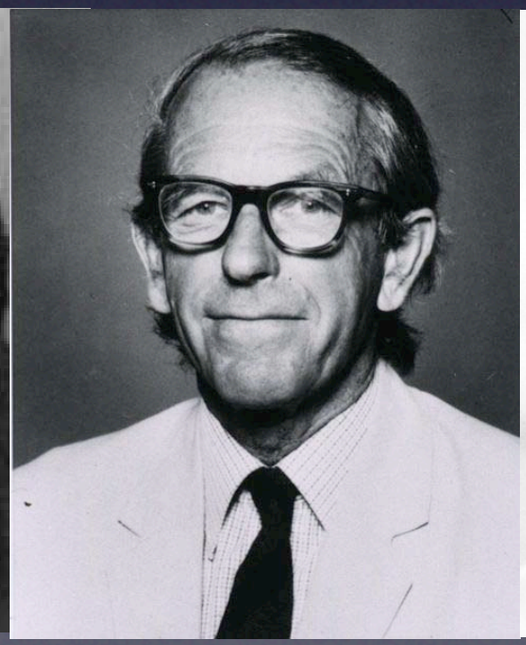
Biological Engineering?



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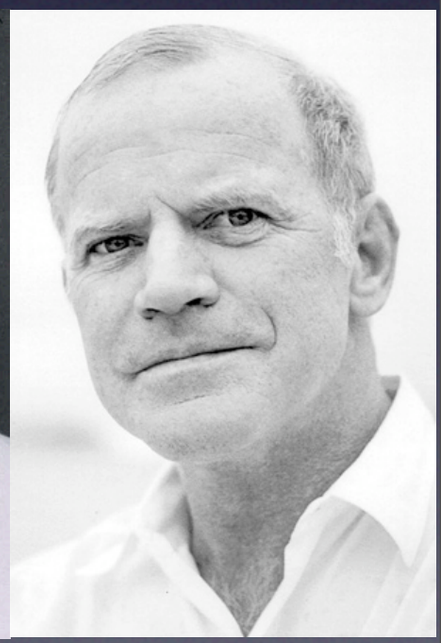

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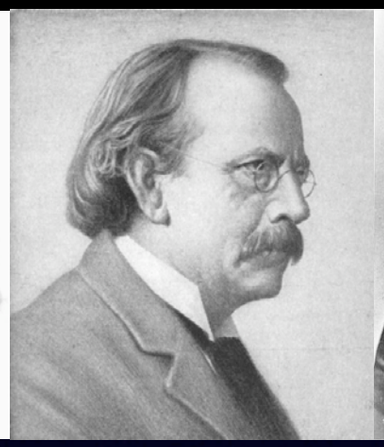

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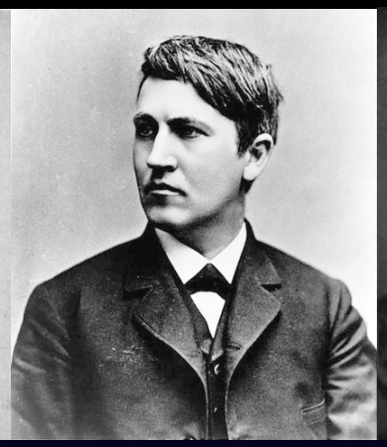

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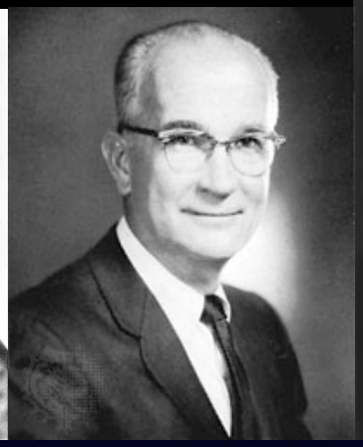

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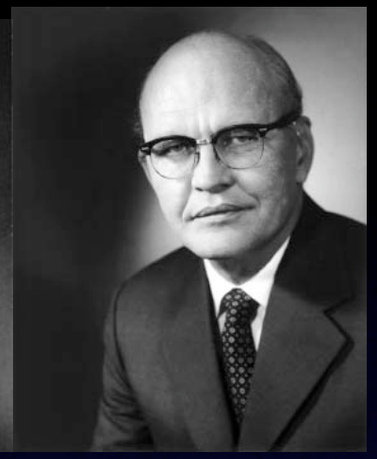

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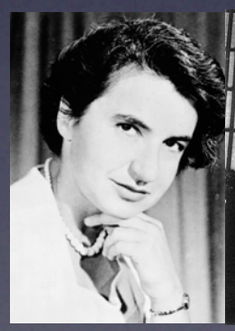

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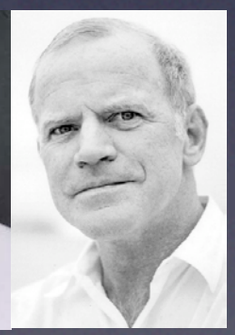

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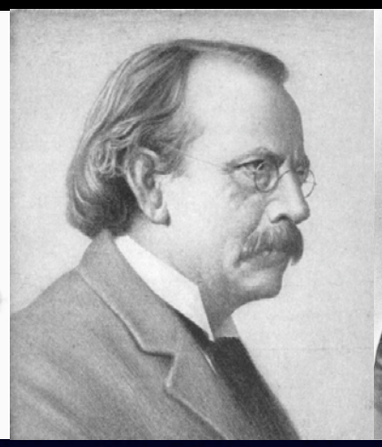

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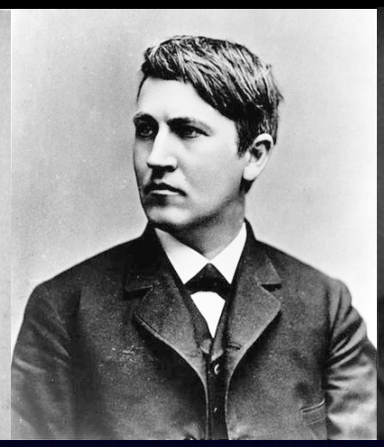

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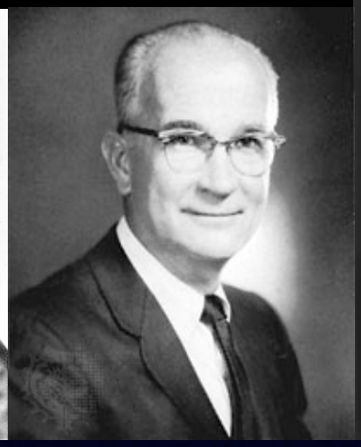

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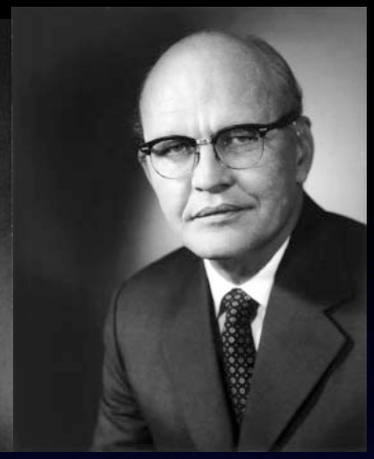

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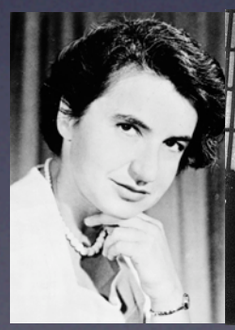

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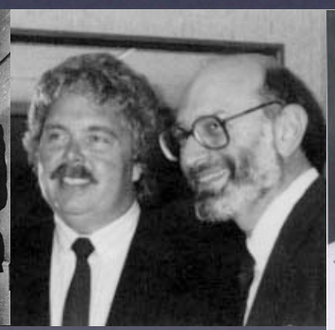

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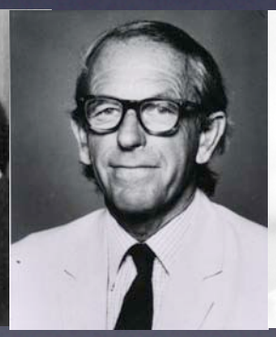

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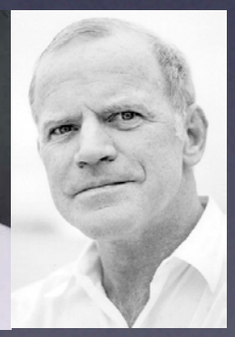

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Biological Engineering

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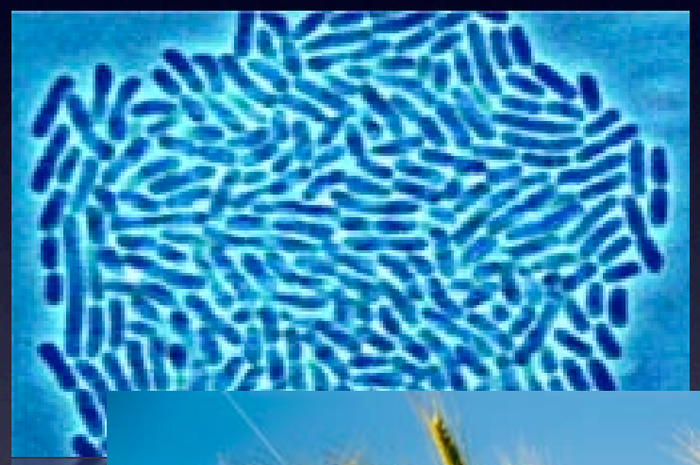
Existing Technologies

Biological research focuses on:

- study & analysis of naturally evolved systems
- ad-hoc construction of 'genetically-engineered' solutions



eg. insulin-production in bacteria
pesticide-resistance in crops

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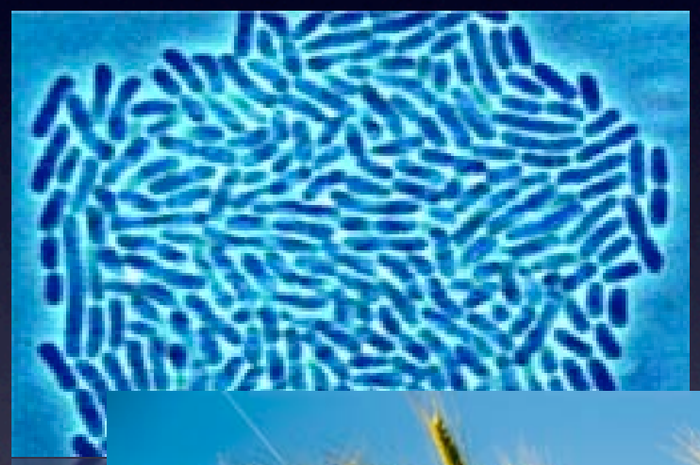

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1st Generation Biotech.

Enabling Technologies:

Polymerase Chain Reaction

Recombinant DNA

Emerging Technologies

2nd Generation Biotech.

Enabling Technologies:

Polymerase Chain Reaction

Recombinant DNA

Emerging Technologies

2nd Generation Biotech.

Enabling Technologies:

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Recombinant DNA

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DNA Synthesis

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Computational Modelling & Design

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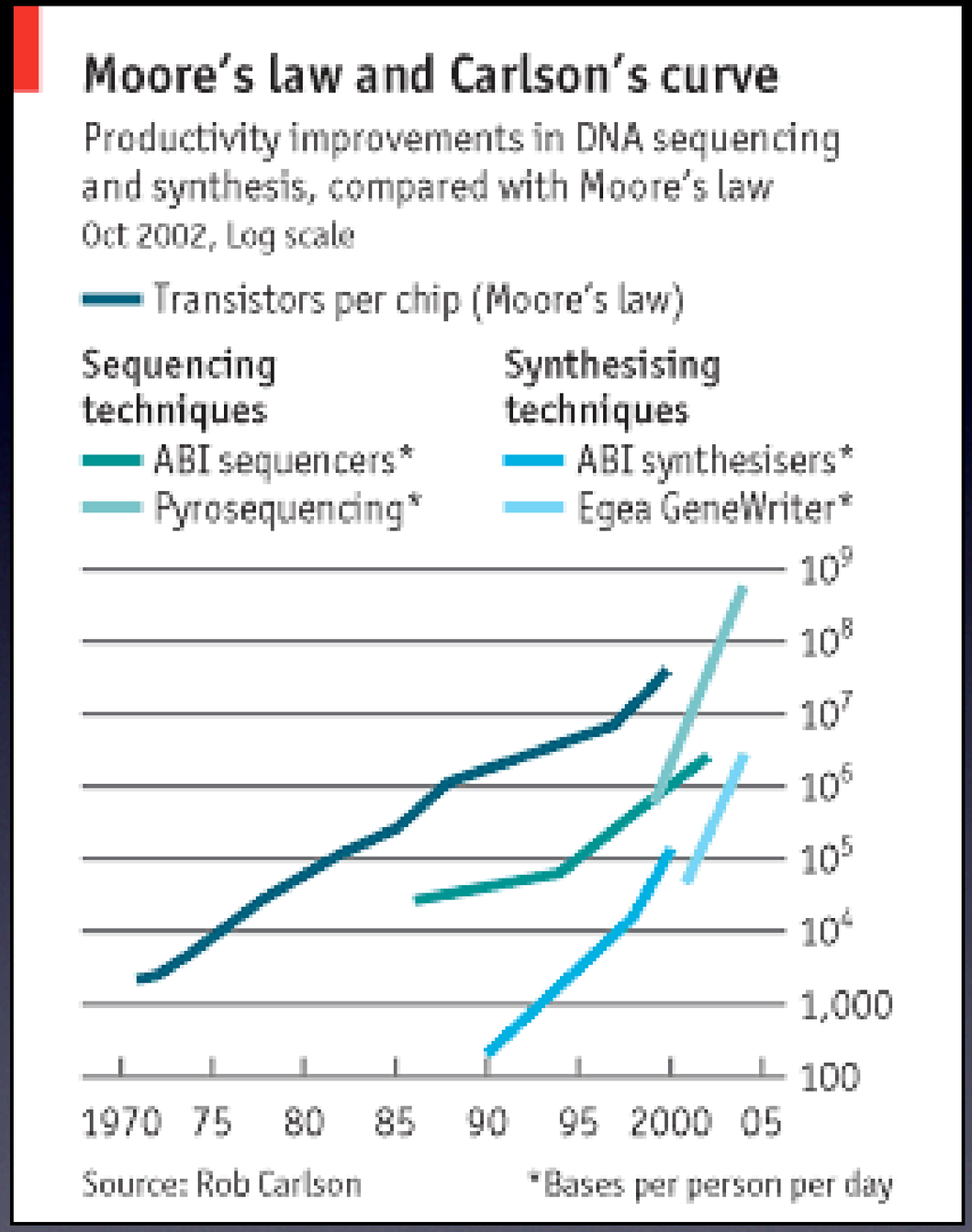
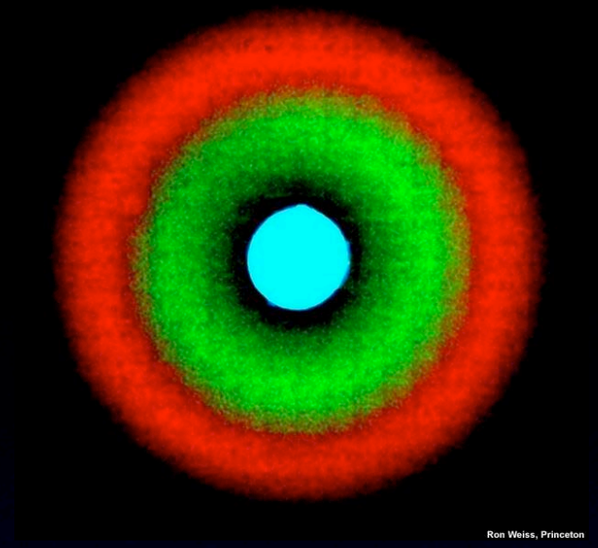


'Synthetic Biology - Life 2.0'
The Economist, August 31st 2006

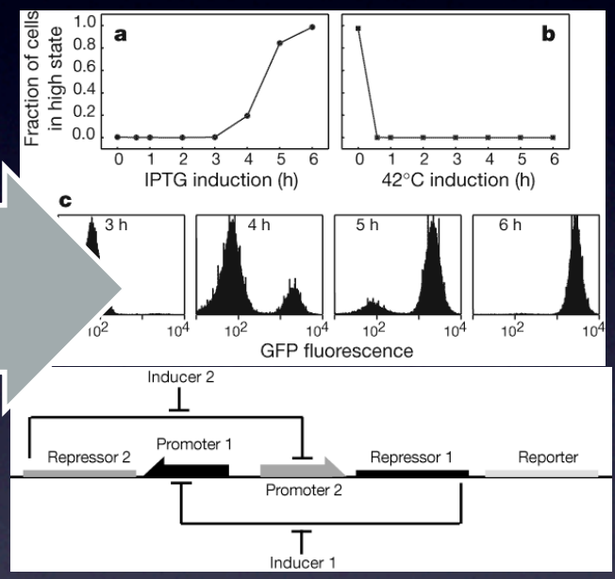
Existing Biotechnology

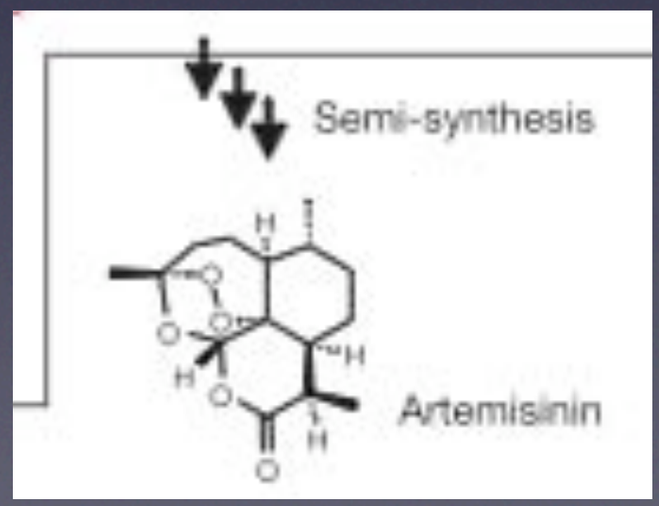


Design & Construction

Devices???

Systems???





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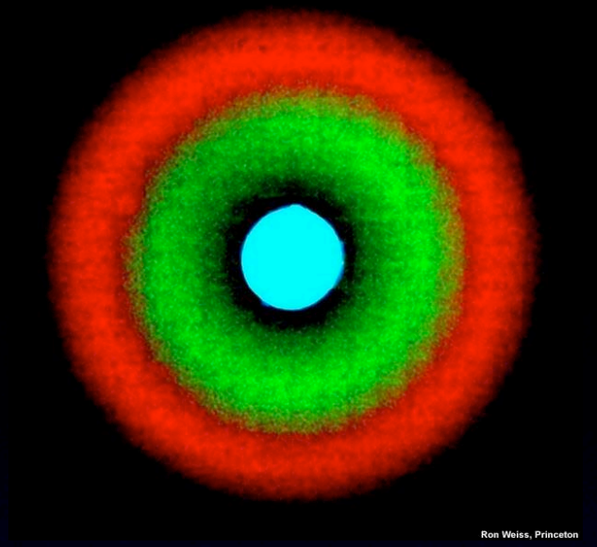
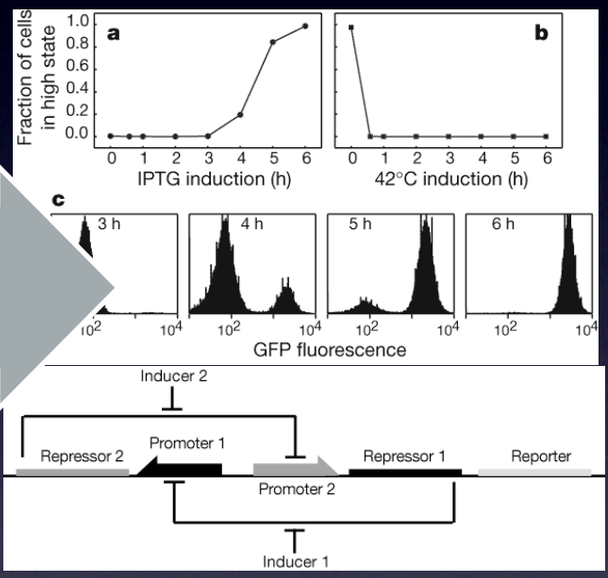
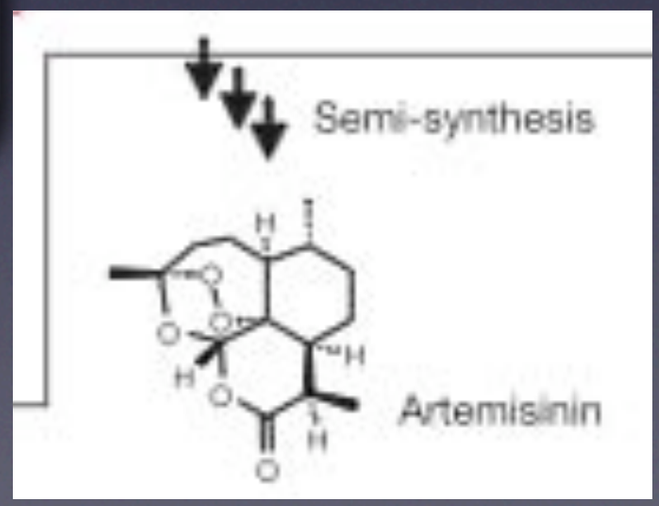


Design

Parts & Devices

Construction





Application of Engineering Principles to Biological Systems

Essential to apply engineering principles :

Modularity
Standardisation
Abstraction
Decoupling

Application of Engineering Principles to Biological Systems

Essential to apply engineering principles :

Modularity
Standardisation
Abstraction
Decoupling

Consider rational engineering of novel synthetic devices and systems

Engineering Principles for Biology

Decoupling

-Rules insulating design process from details of fabrication

-Enable parts, device, and system designers to work together

-VLSI electronics, 1970's

Engineering Principles for Biology

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Standardisation

-Predictable performance of parts, devices & systems
-Off-the-shelf standardised components
-Mech. Eng; 1800's

Engineering Principles for Biology

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Standardisation

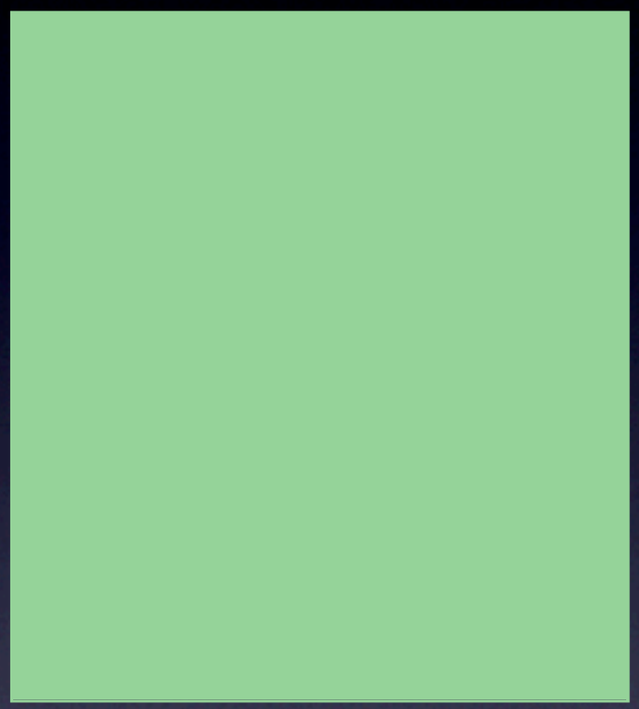
-Predictable performance of parts, devices & systems
-Off-the-shelf standardised components
-Mech. Eng; 1800's

Abstraction

-Insulate relevant characteristics from overwhelming detail
-Simple artifacts that can be used in combination
-From Physics to Elec. Eng; 1800's

Abstraction

Insulate relevant characteristics from excessive details



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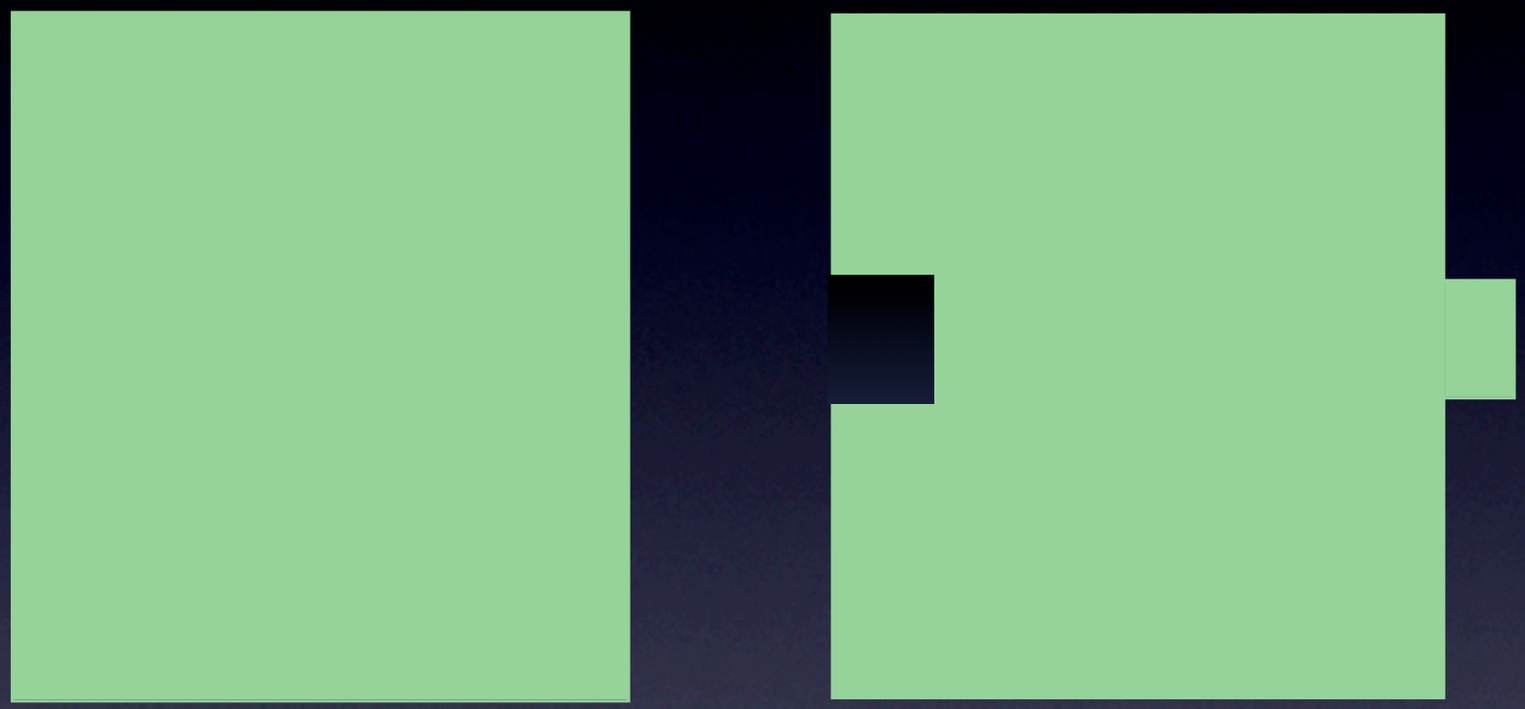
Standardisation

Construction from "off the shelf" parts with known characteristics



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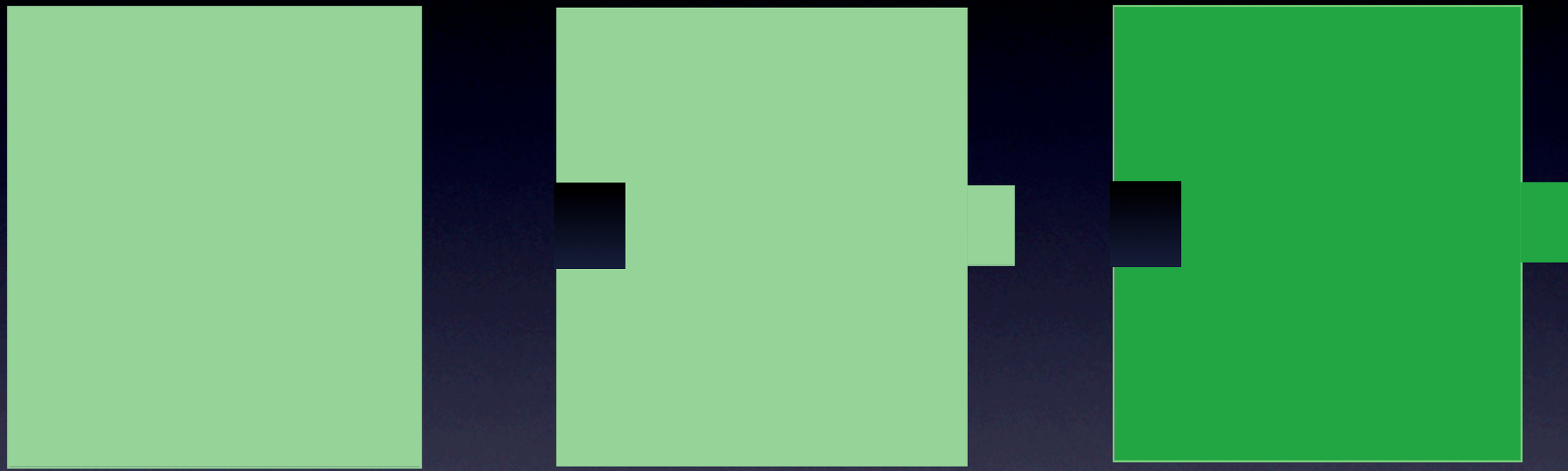
Decoupling

Insulate design process from fabrication details



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cgctagattcgtacgtgtgac

An Abstraction Hierarchy

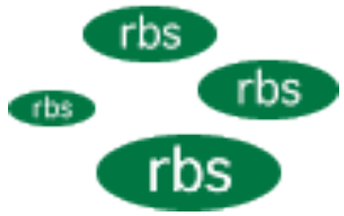
DNA

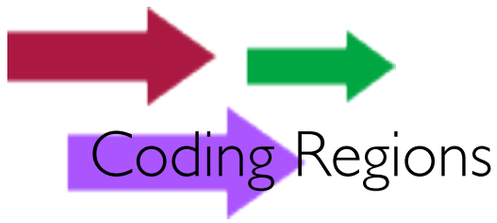
ATGCTTACCGGTACGTTTACGACTACGTAGCTAGCAT
GCTTACCGGTACGTTTACGACTACGTAGCTAGCATG
CTTACCGGTACGTTTACGACTACGTAGCTAGCATGCT
TACT…

An Abstraction Hierarchy

Parts

Promoters





Coding Regions

Terminators

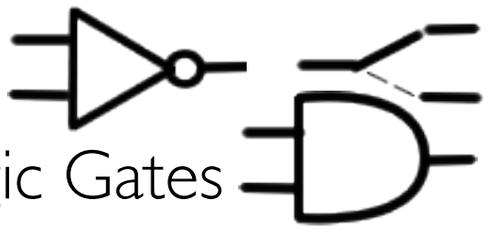
DNA

ATGCTTACCGGTACGTTTACGACTACGTAGCTAGCAT
GCTTACCGGTACGTTTACGACTACGTAGCTAGCATG
CTTACCGGTACGTTTACGACTACGTAGCTAGCATGCT
TACT...

An Abstraction Hierarchy

Devices

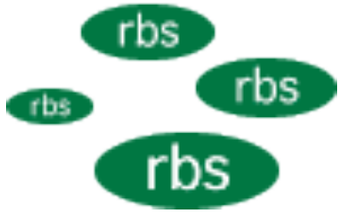
Inputs 

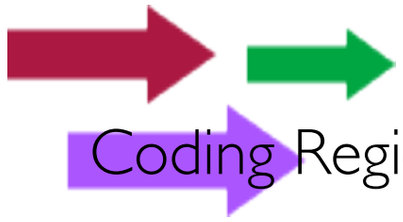
Logic Gates 

Outputs 

Parts

Promoters 





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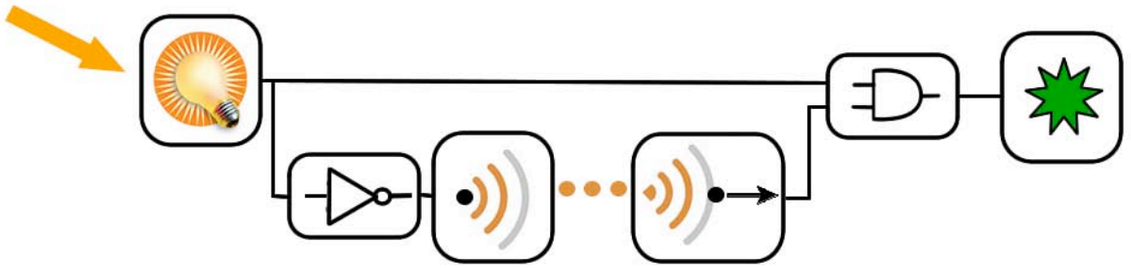
An Abstraction Hierarchy

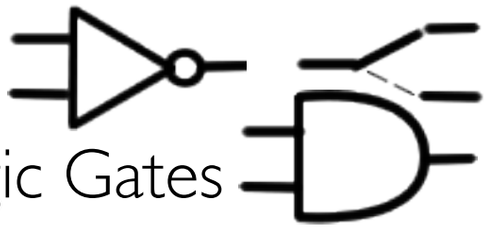
Systems



IF dark
    signal-out
ELSEIF (signal-in AND light-in)
    MAKE Pigment

Devices

Inputs   

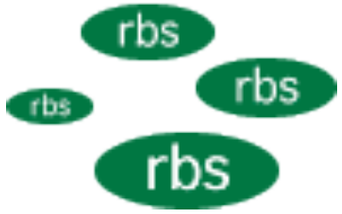


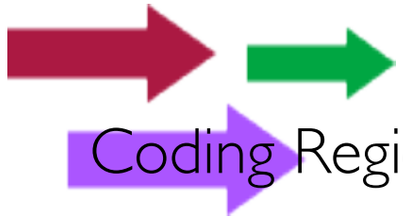
Logic Gates

Outputs 

Parts

Promoters





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GCTTACCGGTACGTTTACGACTACGTAGCTAGCATG
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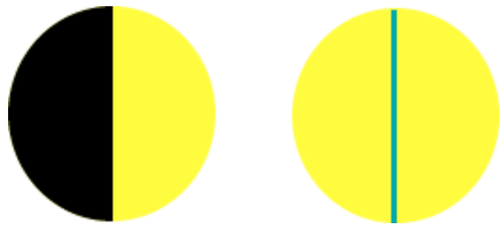
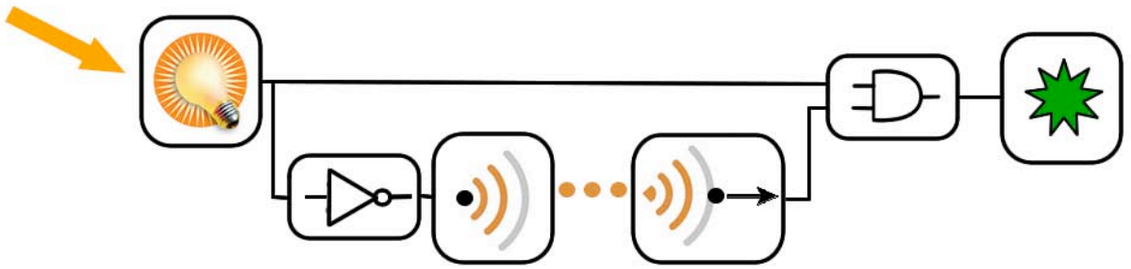
Applications



Systems



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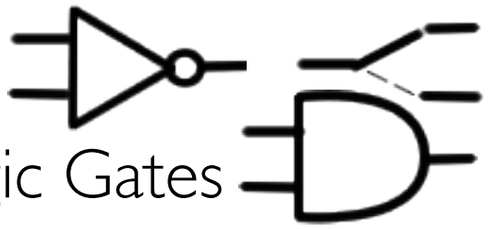
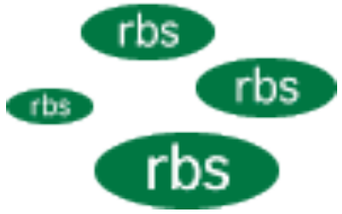
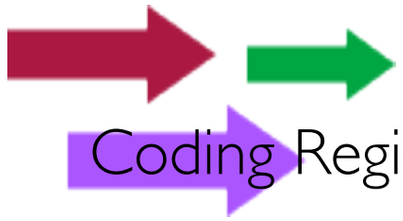
Logic Gates 

Outputs 

Parts

Promoters 



Coding Regions 

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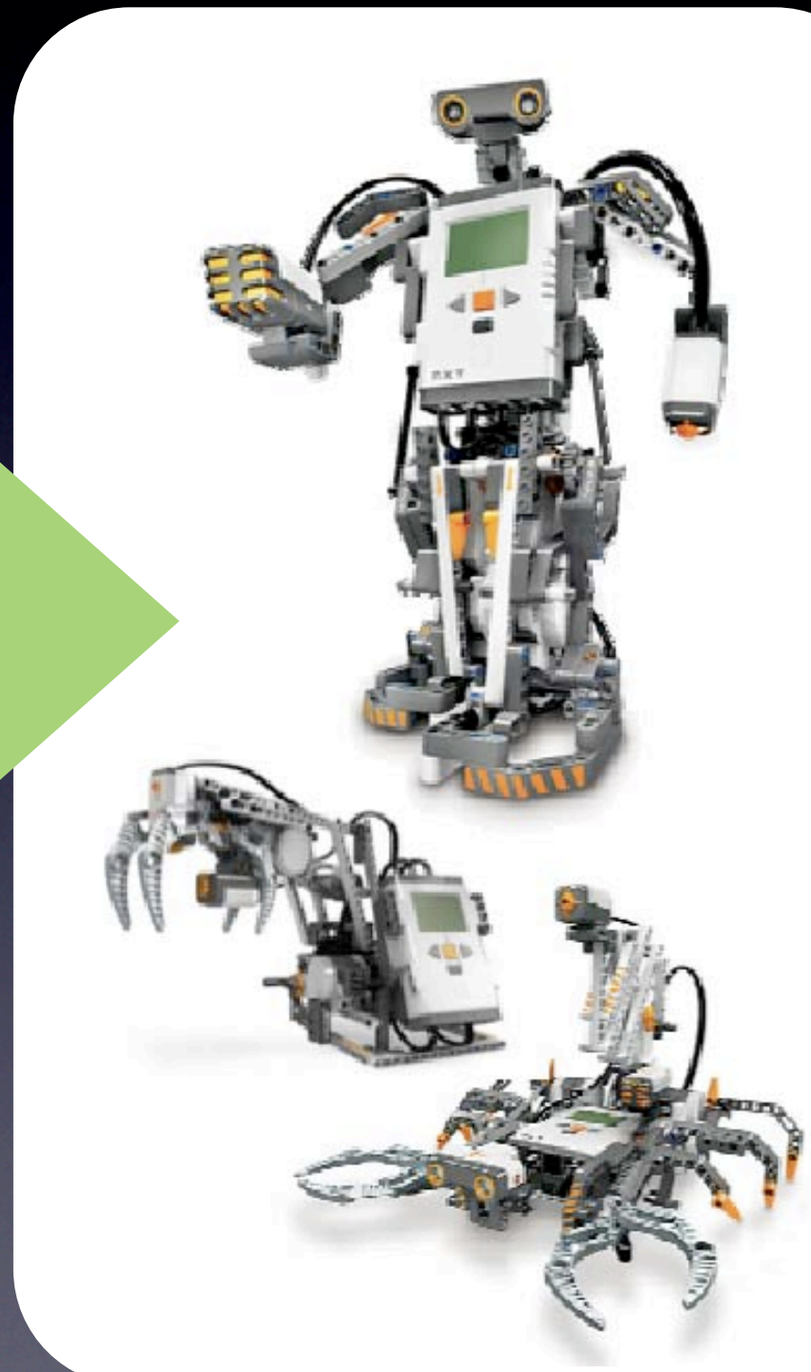
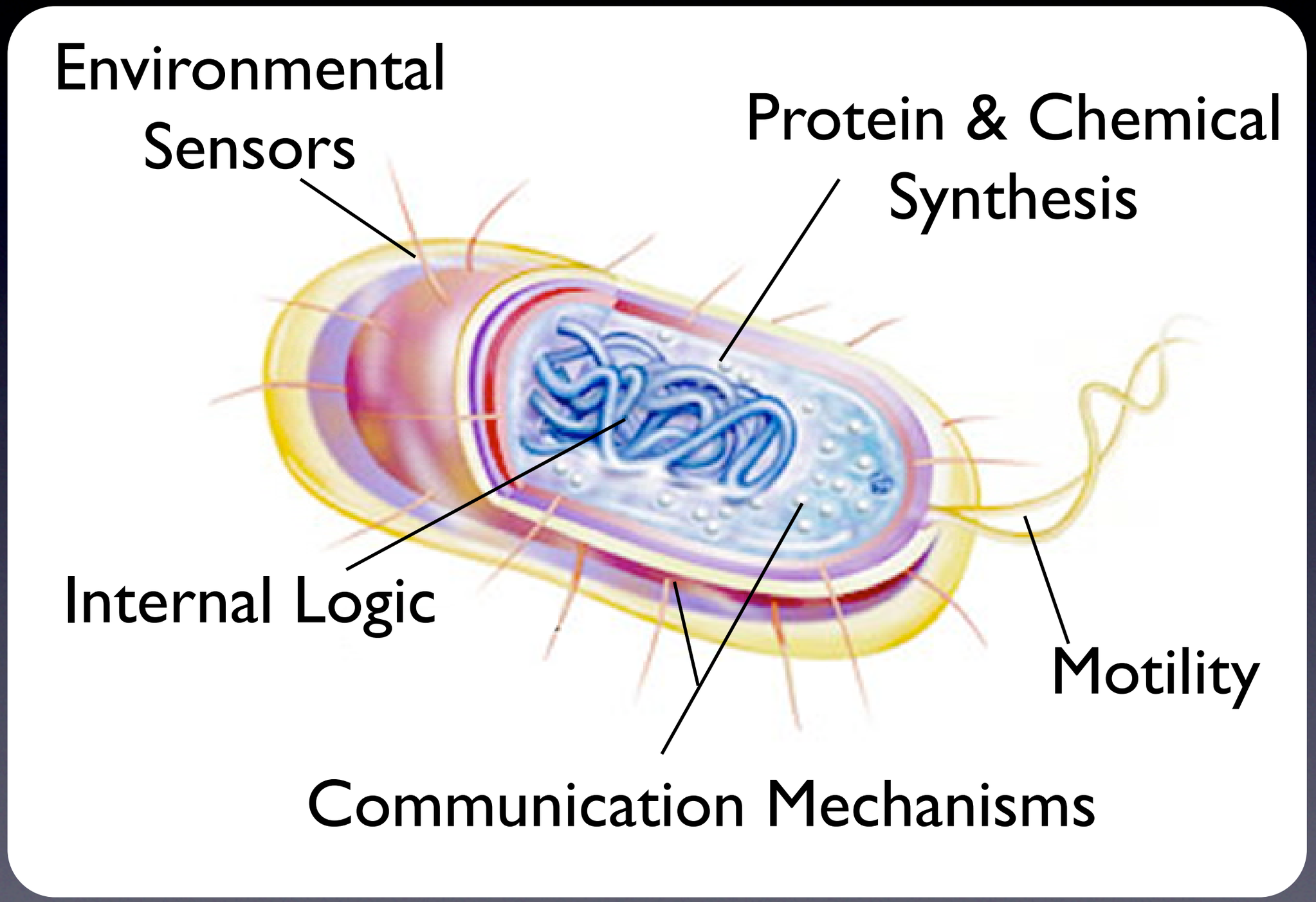
DNA

ATGCTTACCGGTACGTTTACGACTACGTAGCTAGCAT
GCTTACCGGTACGTTTACGACTACGTAGCTAGCATG
CTTACCGGTACGTTTACGACTACGTAGCTAGCATGCT
TACT…

Standard Interchangeable Parts



Micro-Organisms as Genetic Machines



Construction of a genetic toggle switch in *Escherichia coli*

Timothy S. Gardner*†, Charles R. Cantor* & James J. Collins*†

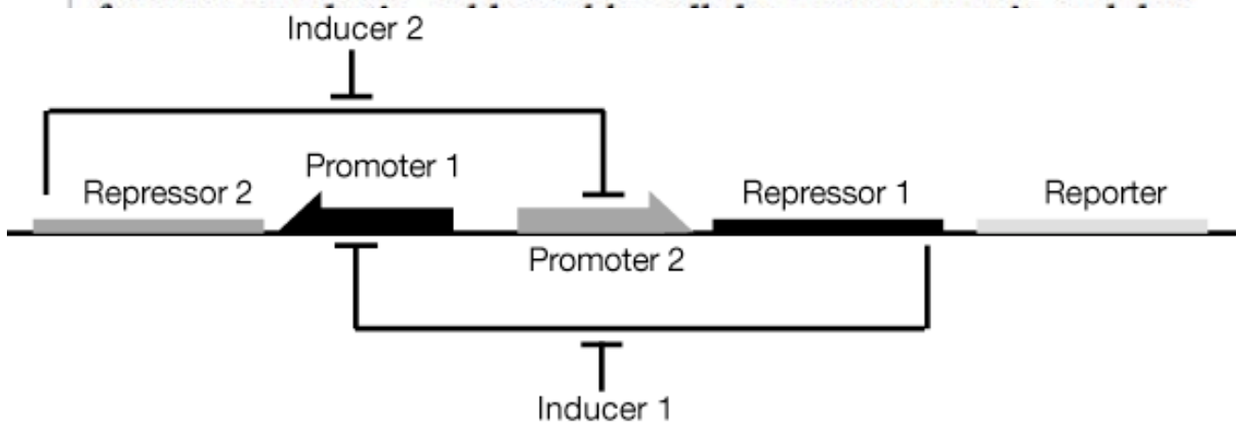
* *Department of Biomedical Engineering, † Center for BioDynamics and ‡ Center for Advanced Biotechnology, Boston University, 44 Cummington Street, Boston, Massachusetts 02215, USA*

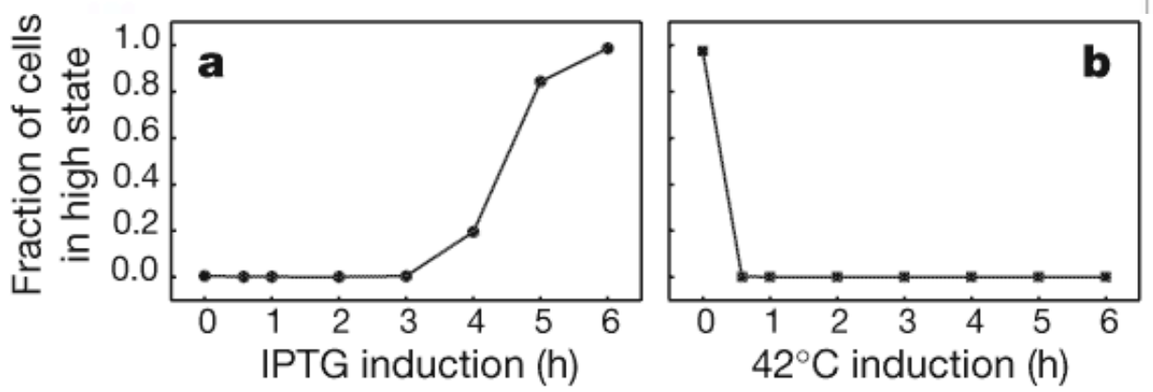
It has been proposed[1] that gene-regulatory circuits with virtually any desired property can be constructed from networks of simple regulatory elements. These properties, which include multistability and oscillations, have been found in specialized gene circuits such as the bacteriophage λ switch[2] and the Cyanobacteria circadian oscillator[3]. However, these behaviours have not been demonstrated in networks of non-specialized regulatory components. Here we present the construction of a genetic toggle switch—a synthetic, bistable gene-regulatory network—in *Escherichia coli* and provide a simple theory that predicts the conditions necessary for bistability. The toggle is constructed from any two repressible promoters arranged in a mutually inhibitory network. It is flipped between stable states using transient chemical or thermal induction and exhibits a nearly ideal switching threshold. As a practical device, the toggle switch

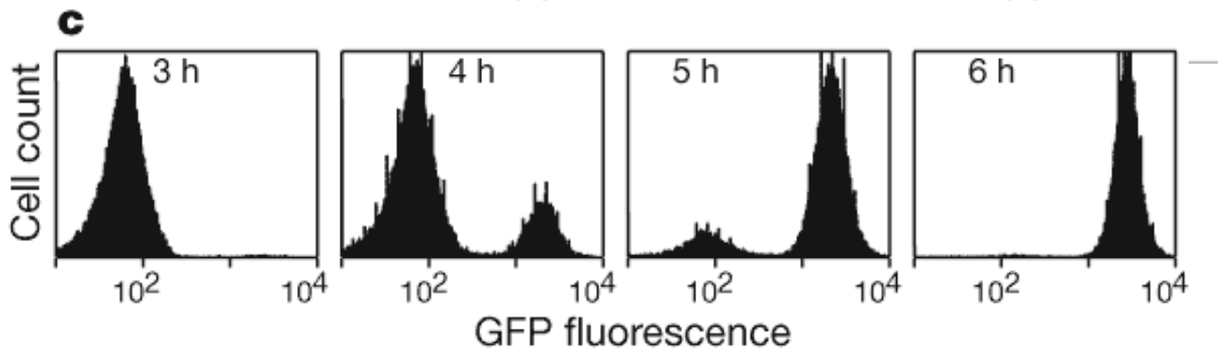
robust and more difficult to tune experimentally. In addition, the chosen toggle design does not require any specialized promoters, such as the P_R/P_{RM} promoter of bacteriophage λ. Bistability is possible with any set of promoters and repressors as long as they fulfil the minimum set of conditions described in Box 1 and Fig. 2.

The bistability of the toggle arises from the mutually inhibitory arrangement of the repressor genes. In the absence of inducers, two stable states are possible: one in which promoter 1 transcribes repressor 2, and one in which promoter 2 transcribes repressor 1. Switching is accomplished by transiently introducing an inducer of the currently active repressor. The inducer permits the opposing repressor to be maximally transcribed until it stably represses the originally active promoter.

All toggle switches are implemented on *E. coli* plasmids conferring ampicillin resistance and containing the pBR322 ColE1 replication origin. The toggle switch genes are arranged as a type IV plasmid, as shown in Fig. 3. Although all genes and promoters are







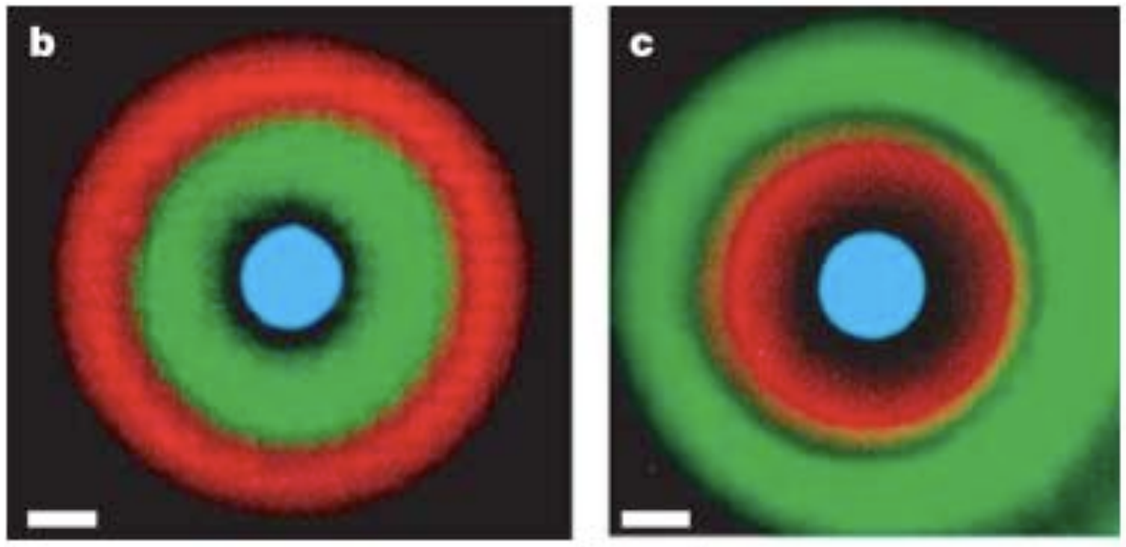
A synthetic multicellular system for programmed pattern formation

Subhayu Basu[1], Yoram Gerchman[1], Cynthia H. Collins[3], Frances H. Arnold[3] & Ron Weiss[1,2]

[1]*Department of Electrical Engineering and* [2]*Department of Molecular Biology, Princeton University, Princeton, New Jersey 08544, USA*
[3]*Division of Chemistry and Chemical Engineering, California Institute of Technology 210-41, Pasadena, California 91125, USA*

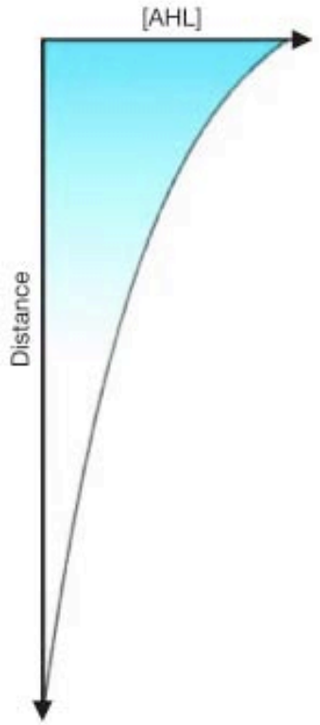
Pattern formation is a hallmark of coordinated cell behaviour in both single and multicellular organisms[1–3]. It typically involves cell–cell communication and intracellular signal processing. Here we show a synthetic multicellular system in which genetically engineered 'receiver' cells are programmed to form ring-like patterns of differentiation based on chemical gradients of an acyl-homoserine lactone (AHL) signal that is synthesized by 'sender' cells. In receiver cells, 'band-detect' gene networks respond to user-defined ranges of AHL concentrations. By fusing different fluorescent proteins as outputs of network variants, an initially undifferentiated 'lawn' of receivers is engineered to form a bullseye pattern around a sender colony. Other patterns, such as ellipses and clovers, are achieved by placing senders in different configurations. Experimental and theoretical analyses reveal which kinetic parameters most significantly affect ring development over time. Construction and study of such synthetic multicellular systems can improve our quantitative understanding of naturally occurring developmental processes and may foster applications in tissue engineering, biomaterial fabrication and biosensing.

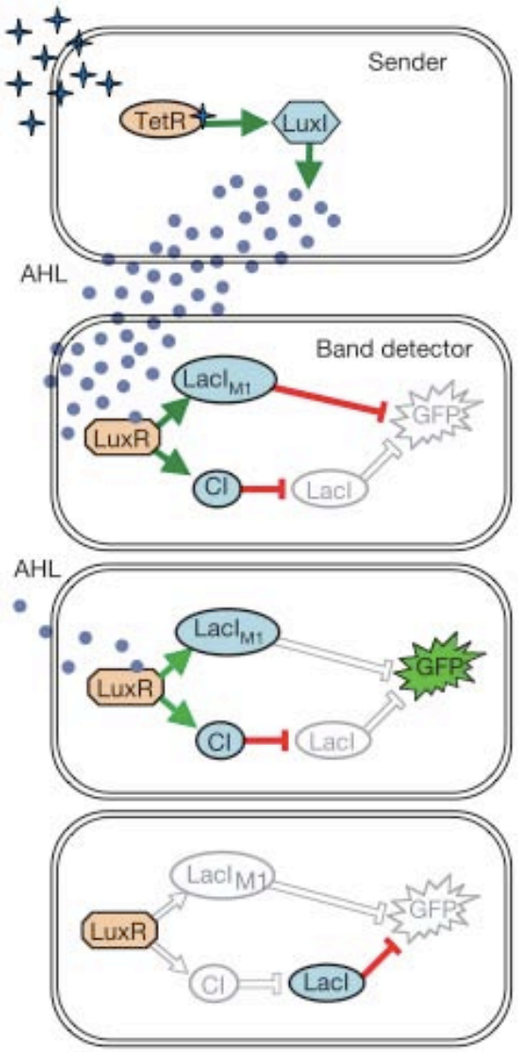
Figure 1a depicts the design of the synthetic bacterial multicellular system, showing how only receivers at intermediate distances from senders express the output protein. Cell–cell communication from the senders is initiated by expression of the



a

AHL	CI	$LacI_{M1}$	LacI	GFP
++	++	++	–	–
+	+	+	–	+
–	–	–	++	–





Environmentally Controlled Invasion of Cancer Cells by Engineered Bacteria

J. Christopher Anderson[1,3], Elizabeth J. Clarke[3], Adam P. Arkin[1,2*] and Christopher A. Voigt[2,3]

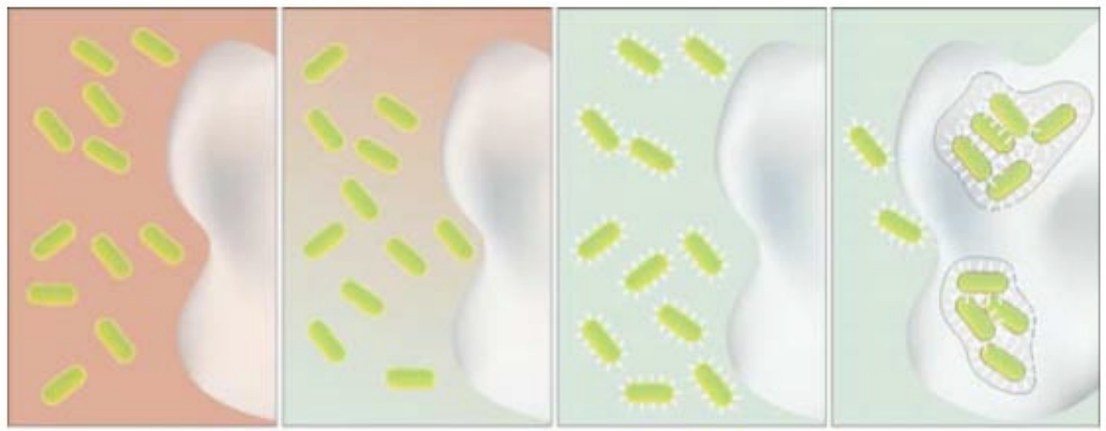
[1]*Howard Hughes Medical Institute, California Institute of Quantitative Biology Department of Bioengineering University of California, 717 Potter Street, Room 257 Berkeley, CA 94720, USA*

[2]*Physical Biosciences Division E.O. Lawrence Berkeley National Laboratory, 1 Cyclotron Road, MS 977-257 Berkeley, CA 94710, USA*

[3]*Biophysics Program Department of Pharmaceutical Chemistry, California Institute of Quantitative Biology The University of California San Francisco, 600 16th St. San Francisco, CA 94107 USA*

**Corresponding author*

Bacteria can sense their environment, distinguish between cell types, and deliver proteins to eukaryotic cells. Here, we engineer the interaction between bacteria and cancer cells to depend on heterologous environmental signals. We have characterized invasin from *Yersinia pseudotuburculosis* as an output module that enables *Escherichia coli* to invade cancer-derived cells, including HeLa, HepG2, and U2OS lines. To environmentally restrict invasion, we placed this module under the control of heterologous sensors. With the *Vibrio fischeri lux* quorum sensing circuit, the hypoxia-responsive *fdhF* promoter, or the arabinose-inducible *araBAD* promoter, the bacteria invade cells at densities greater than 10^8 bacteria/ml, after growth in an anaerobic growth chamber or in the presence of 0.02% arabinose, respectively. In the process, we developed a technique to tune the linkage between a sensor and output gene using ribosome binding site libraries and genetic selection. This approach could be used to engineer bacteria to sense the microenvironment of a tumor and respond by invading cancerous cells and releasing a cytotoxic agent.



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LETTERS

Production of the antimalarial drug precursor artemisinic acid in engineered yeast

Dae-Kyun Ro[1]*, Eric M. Paradise[2]*, Mario Ouellet[1], Karl J. Fisher[6], Karyn L. Newman[1], John M. Ndu
Kimberly A. Ho[1], Rachel A. Eachus[1], Timothy S. Ham[4], James Kirby[2], Michelle C. Y. Chang[1], Sydno
Yoichiro Shiba[2], Richmond Sarpong[3] & Jay D. Keasling[1,2,4,5]

Malaria is a global health problem that threatens 300–500 million people and kills more than one million people annually[1]. Disease control is hampered by the occurrence of multi-drug-resistant strains of the malaria parasite *Plasmodium falciparum*[2,3]. Synthetic antimalarial drugs and malarial vaccines are currently being developed, but their efficacy against malaria awaits rigorous clinical testing[4,5]. Artemisinin, a sesquiterpene lactone endoperoxide extracted from *Artemisia annua* L. (family Asteraceae; commonly known as sweet wormwood), is highly effective against multi-drug-resistant *Plasmodium* spp., but is in short supply and unaffordable to most malaria sufferers[6]. Although total synthesis of artemisinin is difficult and costly[7], the semi-synthesis of artemisinin or any derivative from microbially sourced artemisinic acid, its immediate precursor, could be a cost-effective, environmentally friendly, high-quality and reliable source of

To increase FPP production in *S. cerevisiae*, t
several genes responsible for FPP synthesis was up
gene responsible for FPP conversion to sterols wa
All of these modifications to the host strain were
somal integration to ensure the genetic stability
Overexpression of a truncated, soluble form of 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (*tHMGR*)[12] improved amorphadiene production approximately fivefold (Fig. 2, strain EYP208). Down-regulation of *ERG9*, which encodes squalene synthase (the first step after FPP in the sterol biosynthetic pathway), using a methionine-repressible promoter (P_{MET3})[13] increased amorphadiene production an additional twofold (Fig. 2, strain EPY225). Although *upc2-1*, a semi-dominant mutant allele that enhances the activity of *UPC2* (a global transcription factor regulating the biosynthesis of sterols in *S. cerevisiae*)[14], had only a modest effect on amorphadiene pro-

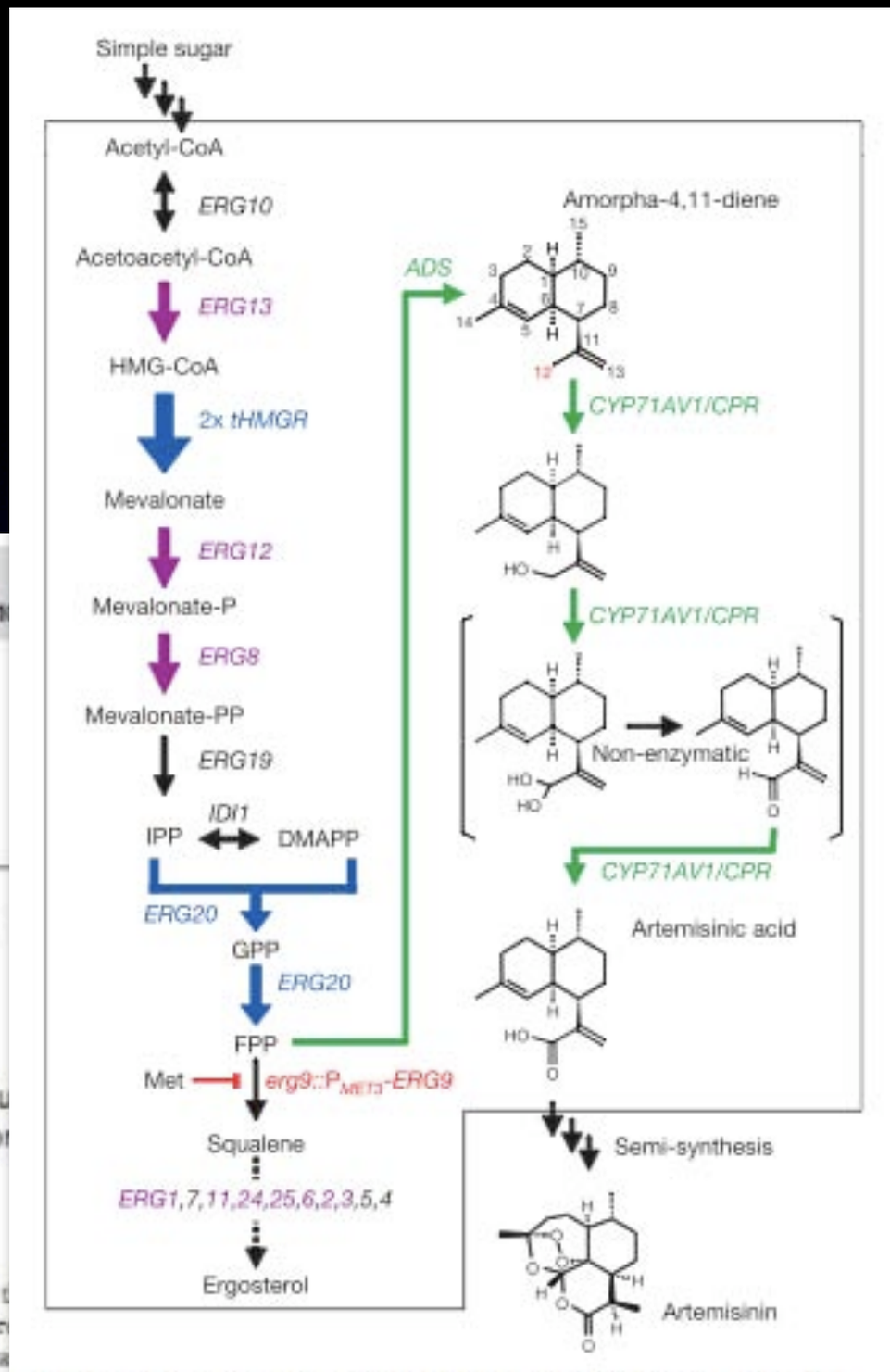
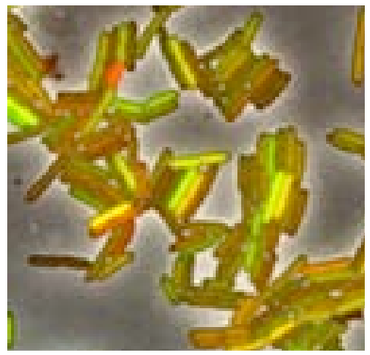


Figure 1 | Schematic representation of the engineered artemisinic acid biosynthetic pathway in *S. cerevisiae* strain EPY224 expressing *CYP71AV1*

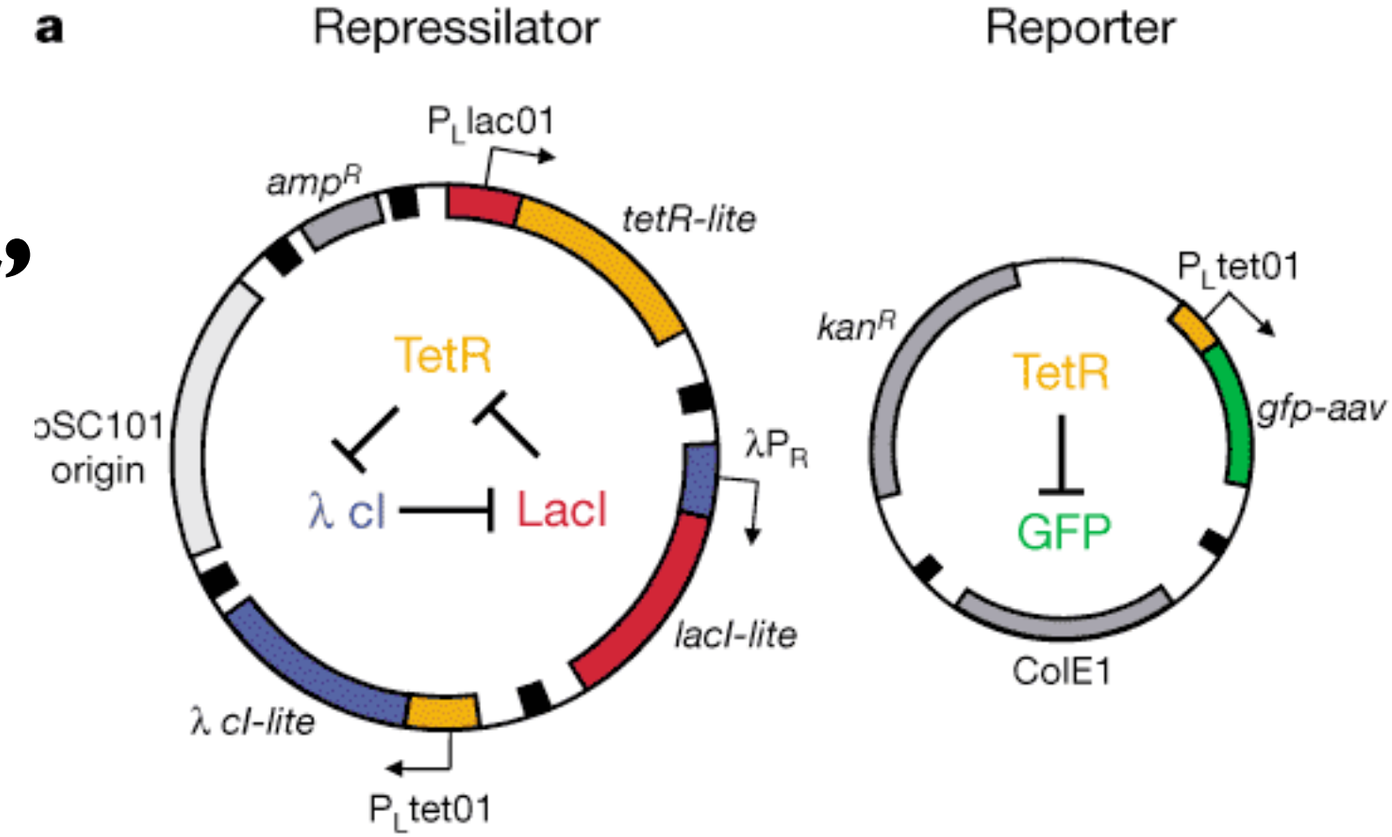


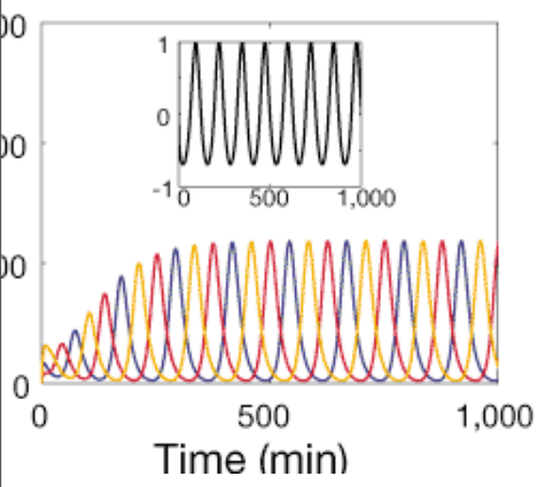
The ‘Repressilator’

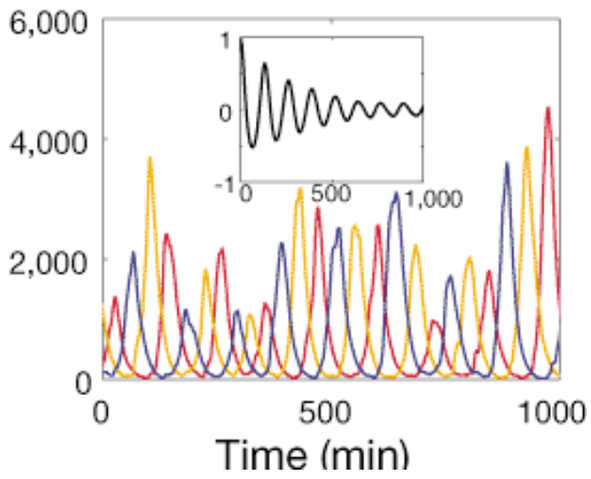
‘A synthetic oscillatory network of transcriptional regulators’

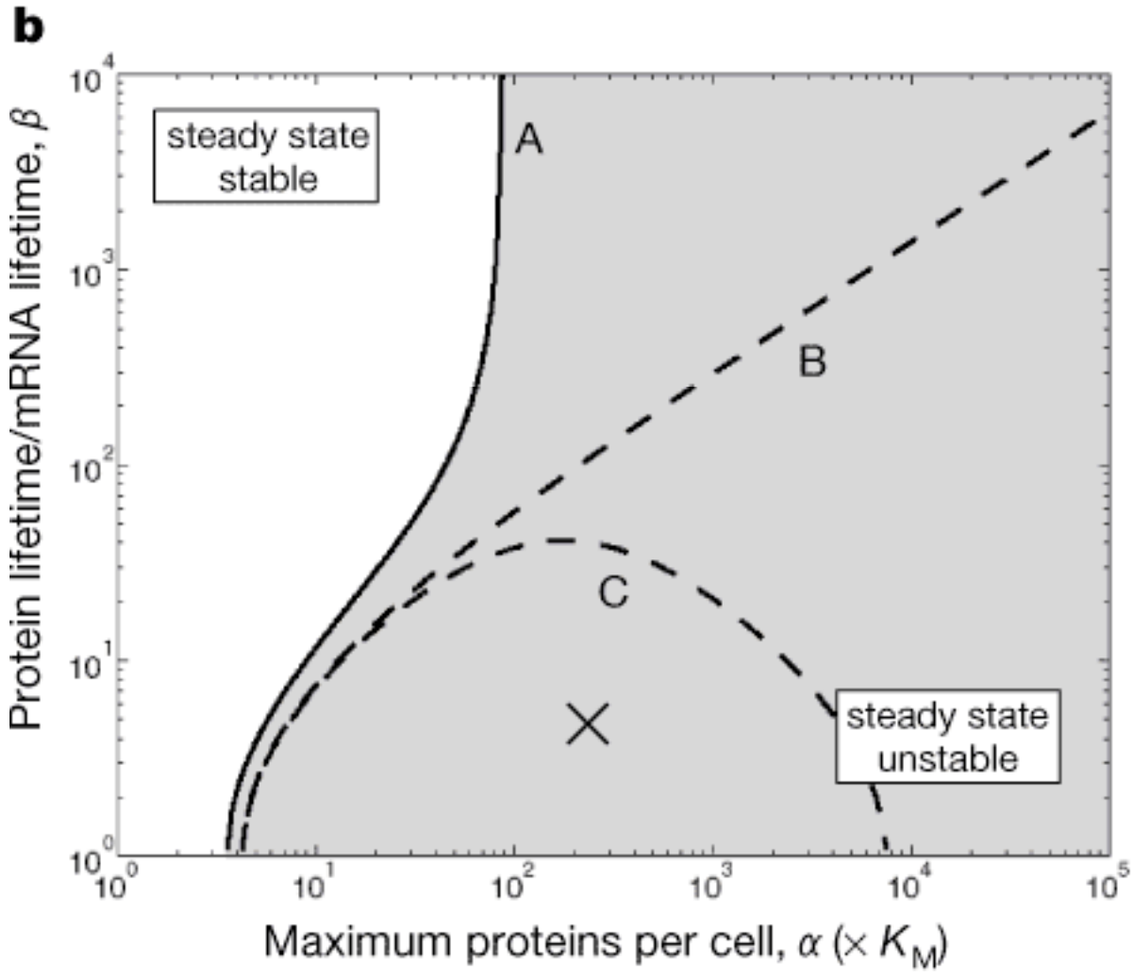
Elowitz & Liebler

Nature (2000)

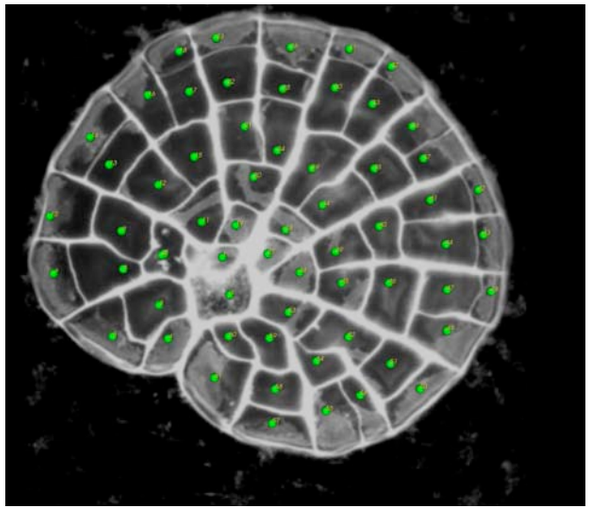


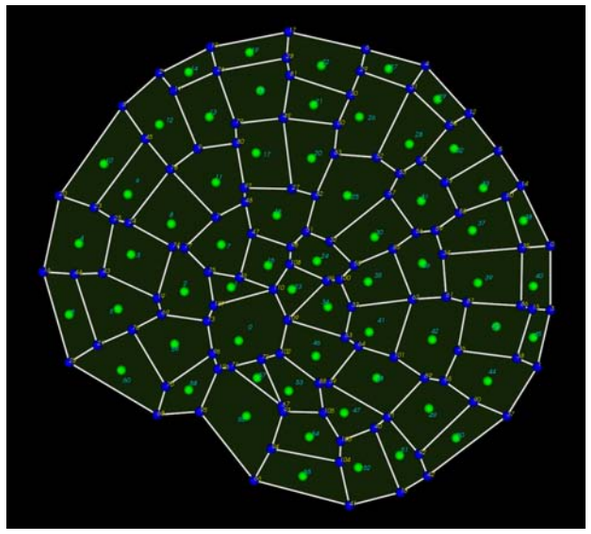


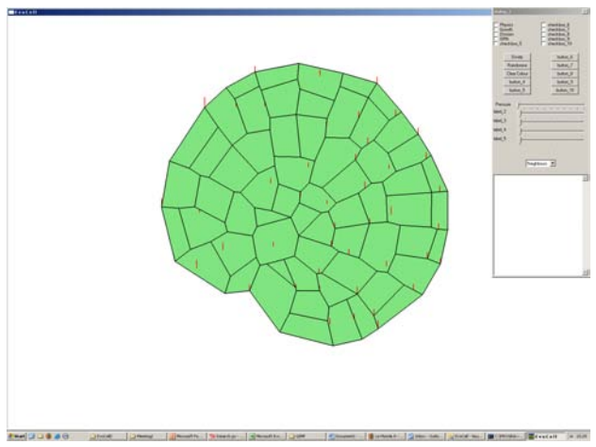


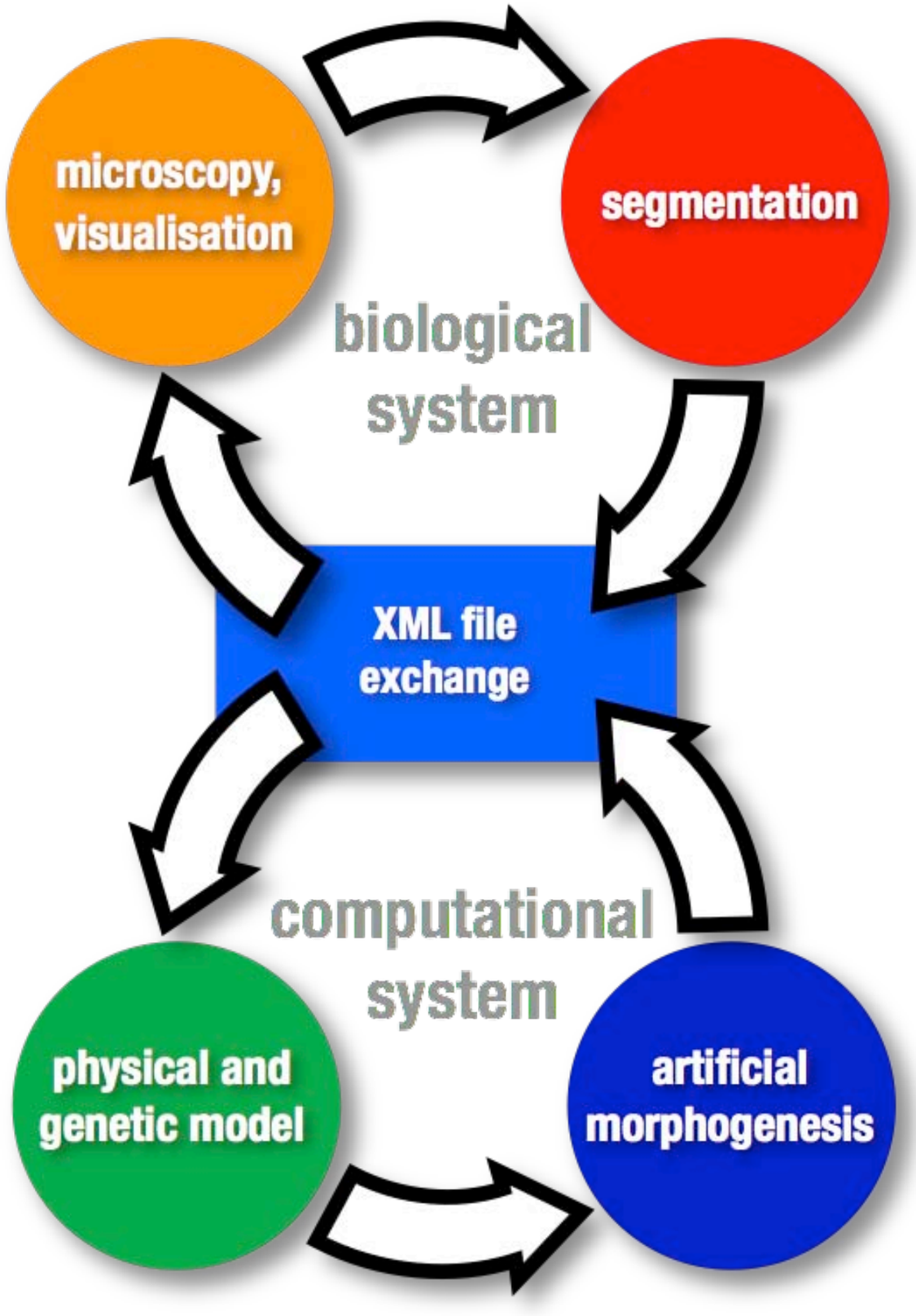


Linking *in vivo* and *in silico* experiments



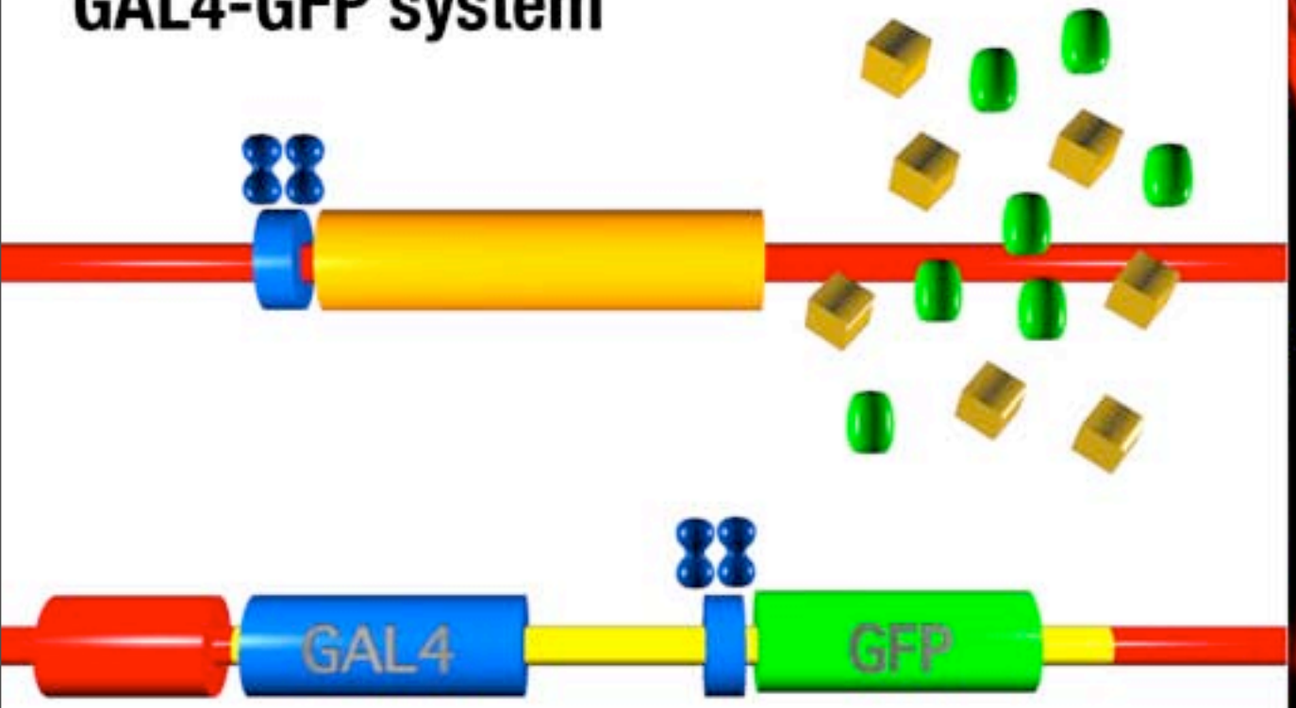




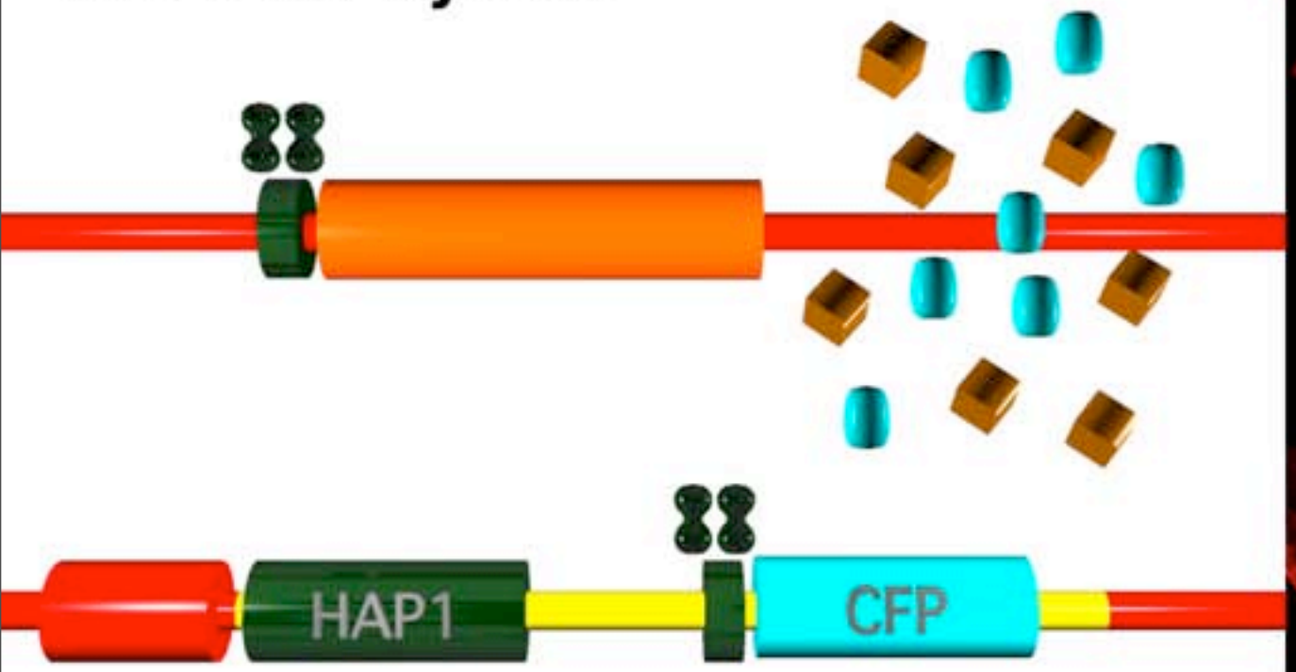


Lionel Dupuy & Jonathan Mackenzie

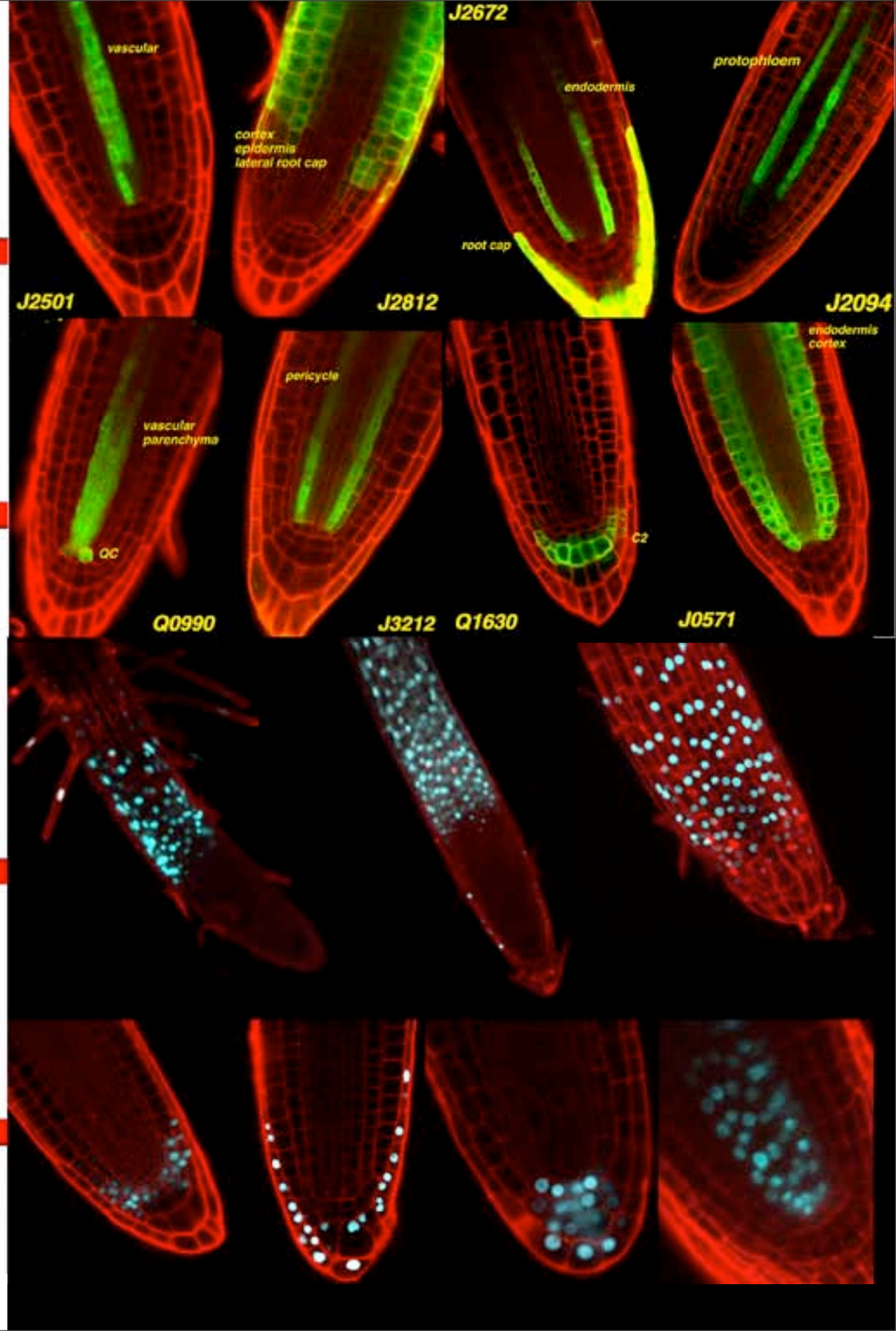
GAL4-GFP system



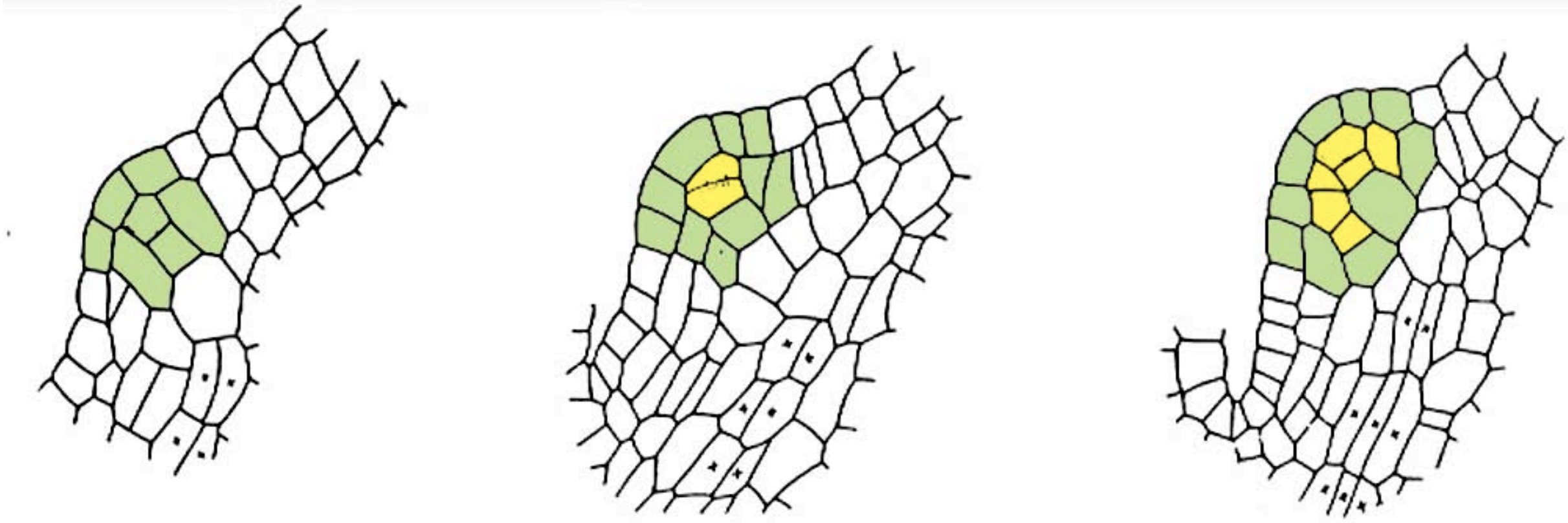
HAP1-CFP system



Triggers for gene expression



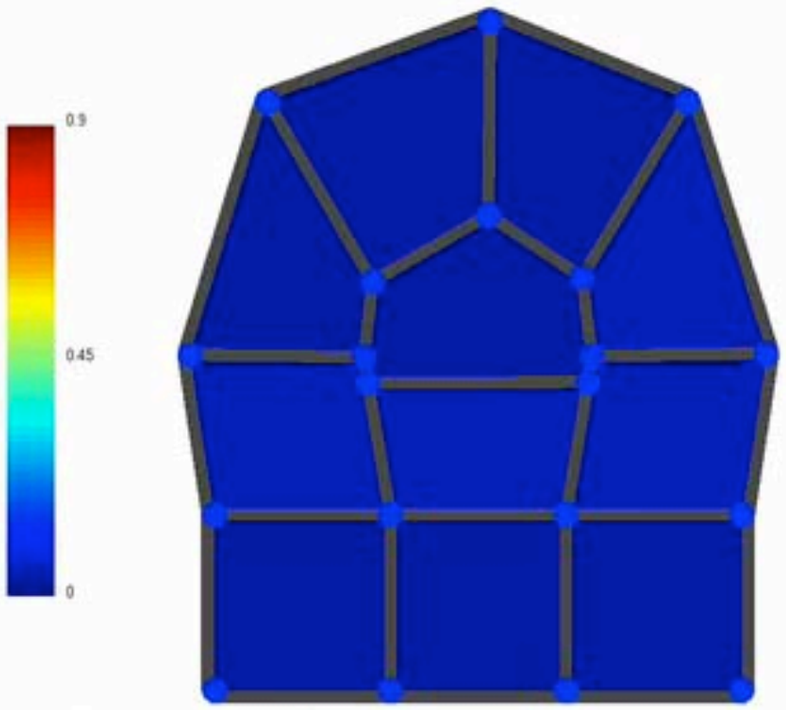
Neomorphogenesis



Trigger: initiate expression of a novel gene circuit during development

Patterning: define cohort of proliferating cells via intercellular signalling

Differentiation: confer new cell fates using



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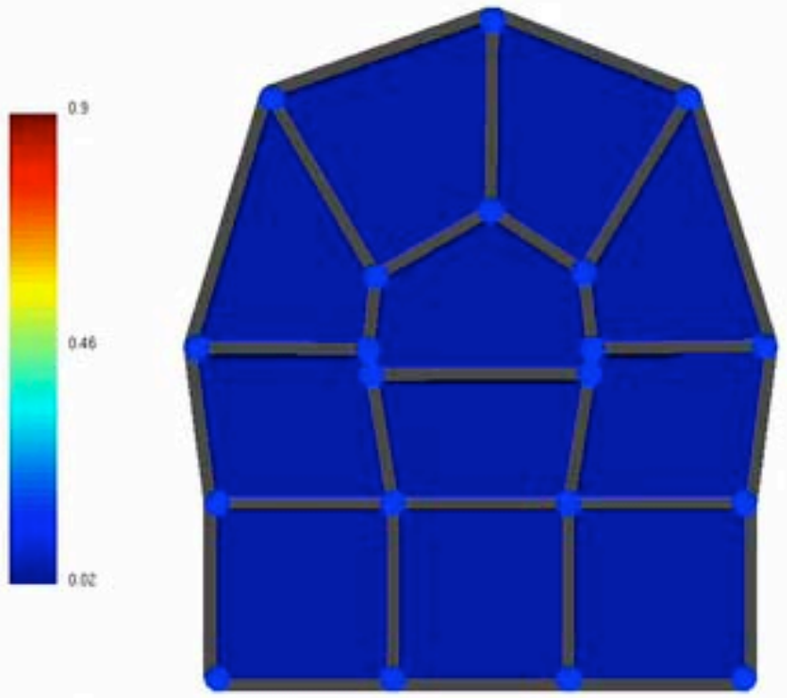
Time elapsed (h): 3
Scale of axes (10e-6 m): 93.7564



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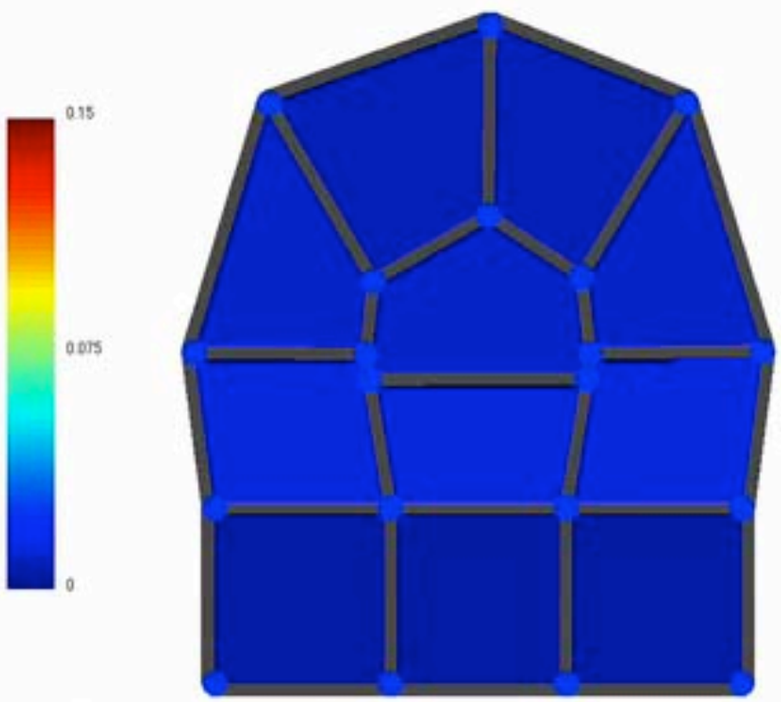
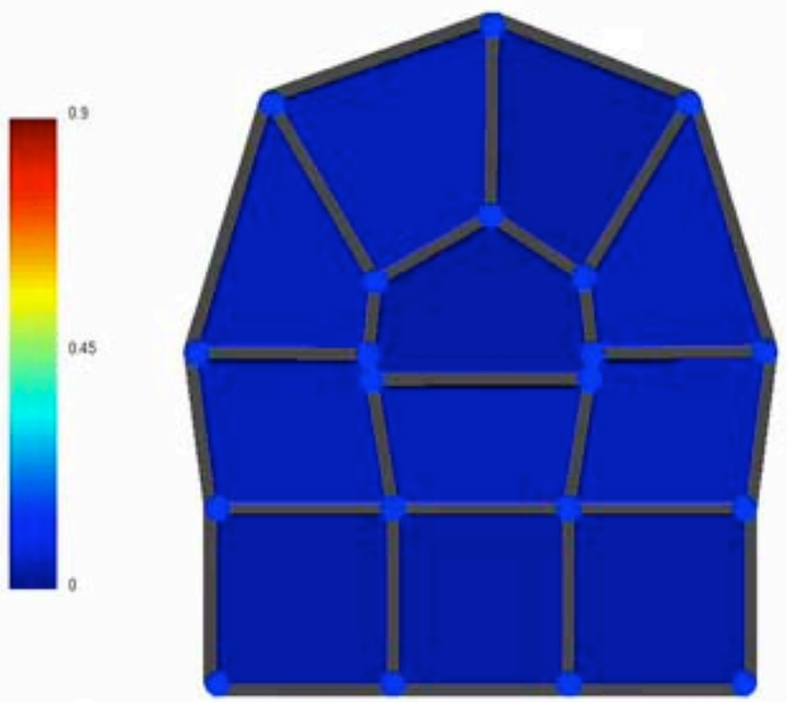
Time elapsed (h): 2.55
Scale of axes (10e-6 m): 93.7564



TITLE: CPC
Time elapsed (h): 2.7
Scale of axes (10e-6 m): 93.7564



TITLE: SIMULATION
Time elapsed (h): 2.4
Scale of axes (10e-6 m): 93.7564

Lionel Dupuy

Synthetic Biology

In this context, Synthetic Biology might be viewed as:

-The design and construction of new biological parts, devices and systems

-The re-design of existing, natural biological systems for useful purposes

Developing an Industry

An engineering discipline based on parts must develop catalogues and suppliers of those parts

The Registry of Standard Biological of Parts

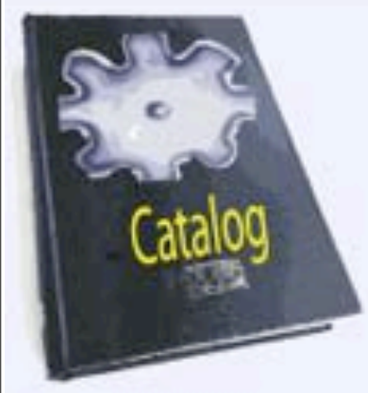
Engineering Biology @MIT

Randy Rettberg

Tom Knight

Drew Endy

http://parts.mit.edu



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Registry of Standard Biological Parts

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- For information about iGEM 2007, see www.igem2007.com
- We have a new tutorial for starting teams in the Help section
- iGEM 2007 team parts have new parts sandboxes and favorites available
- We are starting an editorial board for promoting well-defined and useful parts to BioBrick™ part status. To join this effort check the BioBrick™ Part Program
- Interested in a discussion of icons used in SB and the Registry? Check the Icon Discussion page - a new thread is below the horizontal line.

Part Types

Browse parts by projects:

iGEM 2007 | **iGEM 2006** | **iGEM 2005** | **Labs** | **Courses** | **Featured Parts**

Or browse parts by part categories:

Systems

- Measurement ?
- Measurement (Under Development) ?
- Projects(empty)

Devices

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- Inverters ?
- Signalling ?
- Protein Generator ?
- Composite Devices ?
- Measurement ?

Parts

- Ribosome Binding Sites ?
- Regulatory ?
- RNA ?
- DNA ?
- Protein Coding ?
- Terminators ?
- Conjugation ?

Chassis

- E.coli Strains ?
- Cell-Free Systems ?
- Mammalian

Vectors

- Plasmids ?

Other

- Yeast Parts ?
- A.B Construction Intermediate ?
- PCR Primer ?
- Tags ?
- Other
- Deleted
- Bacteriophage T7

Cell-Cell Signalling

Cell-cell signalling devices allow communication between an individual cell and its neighbors in culture or on a plate. This capability allows synchronized behavior across a cell population or the communication of information between cells hosting different systems. A cell can send a signal and it can receive an averaged signal from all its neighbors carrying the same signalling device. The two fundamental devices to perform cell-cell signalling are therefore a Sender device and a Receiver device. The current families of sender and receiver devices are all based on the Lux system of V. Fischeri or its analogs in other organisms (see references). These two families of devices are defined below.

Available signal senders

Show 5 more parts *Edit*

-?-	Name	Description	Family	Signalling Molecule	Control	Proteins	Molecules Cell Sec	Delay
A W	BBa_F1610	3OC$_6$HSL Sender Device		3OC$_6$HSL		LuxI		

Available signal receivers

Show 3 more parts *Edit*

-?-	Name	Description	Family	Signalling Molecule	Control	Proteins	Switch Point	Delay
A W	BBa_F2620	3OC$_6$HSL Receiver Device		3OC$_6$HSL	R0040	LuxR, TetR	2nM	Seconds
A W	BBa_F2621	3OC$_6$HSL Receiver Device		3OC$_6$HSL	R0063	LuxR	2nM	Seconds
A W	BBa_F2622	3OC$_6$HSL Receiver Device		3OC$_6$HSL	R0011	LuxR, LacI		

Available other signalling parts

Show 36 more parts *Edit*

-?-	Name	Description	Device Type
A W	BBa_I13261	Lux Receiver (I13263 with reversed part order)	
A W	BBa_I13263	Lux Receiver (HSL & R0063 driven)	
A W	BBa_I13272	YFP Producer Controlled by 3OC$_6$HSL Receiver Device	
A W	BBa_I13273	YFP Producer Controlled by 3OC$_6$HSL Receiver Device	
A W	BBa_T9002	GFP Producer Controlled by 3OC6HSL Receiver Device	
A	BBa_I0424	I0404.I6101	
A	BBa_I0426	I0406.I6107	
A	BBa_I0428	I0408.I6106	
A	BBa_I0466	RhlR Protein Generator	
A	BBa_I13018	LuxR Cassette under Ptet (Other)	
A	BBa_I13202	3OC$_6$HSL Sender Controlled by Lac Repressible Promoter	
A	BBa_I13207	HSL/aiiA test construct	
A	BBa_I13208	aiiA (LVA-) protein generator driven by plac	
A	BBa_I1466	RhlR protein generator (LVA-)	
A	BBa_J13040	pOmpR dependent 3OC6HSL sender device	

Cell-Cell Signalling

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Available signal receivers

Show 3 more parts *Edit*

-?-	Name	Description	Family	Signalling Molecule	Control	Proteins	Switch Point	Delay
A W	BBa_F2620	3OC$_6$HSL Receiver Device		3OC$_6$HSL	R0040	LuxR, TetR	2nM	Seconds
A W	BBa_F2621	3OC$_6$HSL Receiver Device		3OC$_6$HSL	R0063	LuxR	2nM	Seconds
A W	BBa_F2622	3OC$_6$HSL Receiver Device		3OC$_6$HSL	R0011	LuxR, LacI		

Available other signalling pa

-?-	Name	
A W	BBa_I13261	Lux Receiver
A W	BBa_I13263	Lux Receiver
A W	BBa_I13272	YFP Producer
A W	BBa_I13273	YFP Producer
A W	BBa_T9002	GFP Producer
A	BBa_I0424	I0404.I6101
A	BBa_I0426	I0406.I6107
A	BBa_I0428	I0408.I6106
A	BBa_I0466	RhlR Protein Generator
A	BBa_I13018	LuxR Cassette under Ptet (Other)
A	BBa_I13202	3OC$_6$HSL Sender Controlled by Lac Repressible Promoter
A	BBa_I13207	HSL/aiiA test construct
A	BBa_I13208	aiiA (LVA-) protein generator driven by plac
A	BBa_I1466	RhlR protein generator (LVA-)
A	BBa_J13040	pOmpR dependent 3OC6HSL sender device

Available signal receivers

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A W	BBa_F2620	3OC$_6$HSL Receiver Device		3OC$_6$HSL
A W	BBa_F2621	3OC$_6$HSL Receiver Device		3OC$_6$HSL
A W	BBa_F2622	3OC$_6$HSL Receiver Device		3OC$_6$HSL

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Part:BBa_F2620

DNA Available

Experience: Works

Designed by Barry Canton

Entered: 2004-08-09

3OC$_6$HSL Receiver Device

A transcription factor [LuxR] that is active in the presence of cell-cell signaling molecule [3OC6HSL] is controlled by an operator [TetR]. Device input is 3OC6HSL. Device output is PoPS produced at a LuxR-regulated operator.

Usage and Biology [edit]

Full PoPS output at high 3OC6HSL levels and high plasmid copy [e.g., pSB1A2] results in a reduced cell growth rate (see Load section). If used in a cell containing TetR then a second input signal [aTc] can be used to produce a logical AND function.

Sequence and Features

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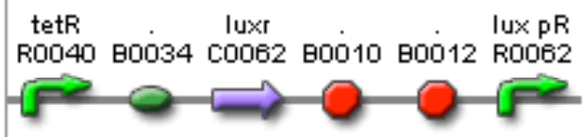
Get selected sequence



Device Characteristics [edit]

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Sequence and Features

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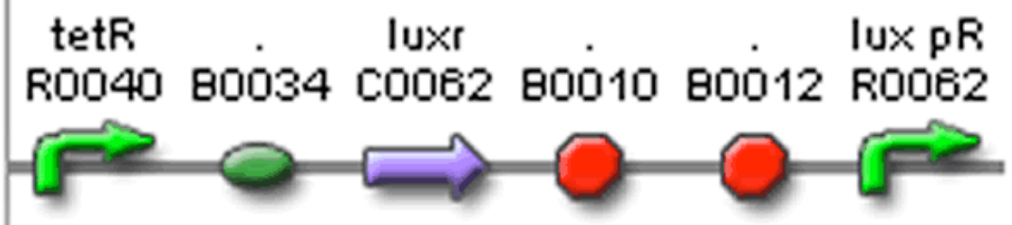

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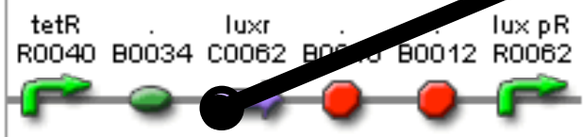
Get selected sequence



Device Characteristics [edit]

Get the device datasheet

Transfer Function [edit]

Latency [edit]

Specificity [edit]

Specificity preliminary data

Stability [edit]

Stability preliminary data

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DNA Available

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Designed by Barry Canton

Entered: 2004-08-09

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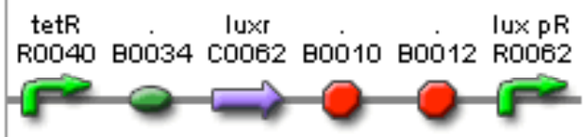
Sequence and Features

Format: Subparts | Ruler | SS | DS **Search:** **Length: 1061 bp** **Context: Part only**

Get selected sequence



Device Characteristics [edit]

Get the device datasheet

Transfer Function [edit]

Latency [edit]

Specificity [edit]

Specificity preliminary data

Stability [edit]

Stability preliminary data

F2620

Lux-receiver Device

BBa_F2620

$3OC_6HSL \rightarrow$ PoPS Receiver

Author(s): Barry Canton [bcanton@mit.edu]

Last Update: May 10, 2005

PDL Devices

Description

A transcription factor [LuxR] that is active in the presence of cell-cell signaling molecule [$3OC_6HSL$] is controlled by an operator [TetR]. *Device input* is $3OC_6HSL$. *Device output* is PoPS produced at a LuxR-regulated operator.

Usage

Full PoPS output at high $3OC_6HSL$ levels and high plasmid copy [e.g., pSB1A2] results in a reduced cell growth rate (see *Load* section). If used in a cell containing TetR then a second input signal [aTc] can be used to produce a logical *AND* function.

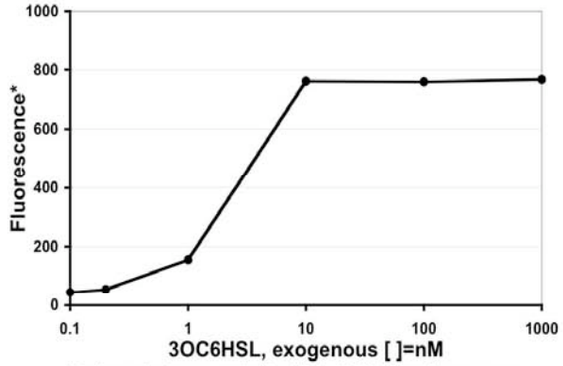
Characteristics

Input Swing: #-# nM $3OC_6HSL$, exogenous
Output Swing: #-# PoPS
Switch Point: 2 nM $3OC_6HSL$, exogenous
LH Latency: # seconds
HL Latency: # seconds

Key Components

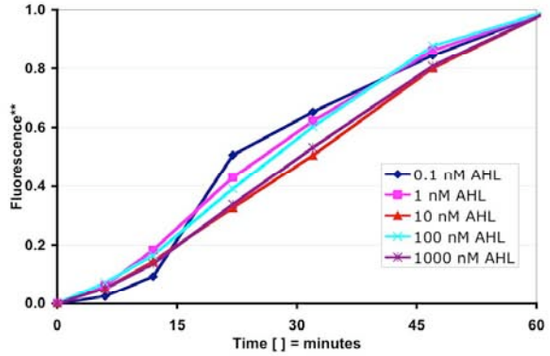
BBa_R0040: TetR-regulated operator
BBa_C0062: luxR ORF
BBa_R0062: LuxR-regulated operator

Transfer Function



*Device output measured indirectly via PoPS-driven fluorescence from *BBa_E0430*, [ ] = geometric mean, arbitrary units. Host cell *MC4100*, device carried on *pSB3K3*, 100ml batch flask, supplemented M9 media, FACScan cytometer [see *MIT SBWG FACS protocol*].

Latency



**Device output measured indirectly via PoPS-driven fluorescence from *BBa_E0430*, [ ] = geometric mean, arbitrary units. Host cell *MC4100*, device carried on *pSB3K3*, 200ul 96-well plate, VictorV plate reader [see *MIT SBWG plate reader protocol*].

Load

NTP/sec/copy: # NTP per second
AA/sec/copy: # AA per second

Stability

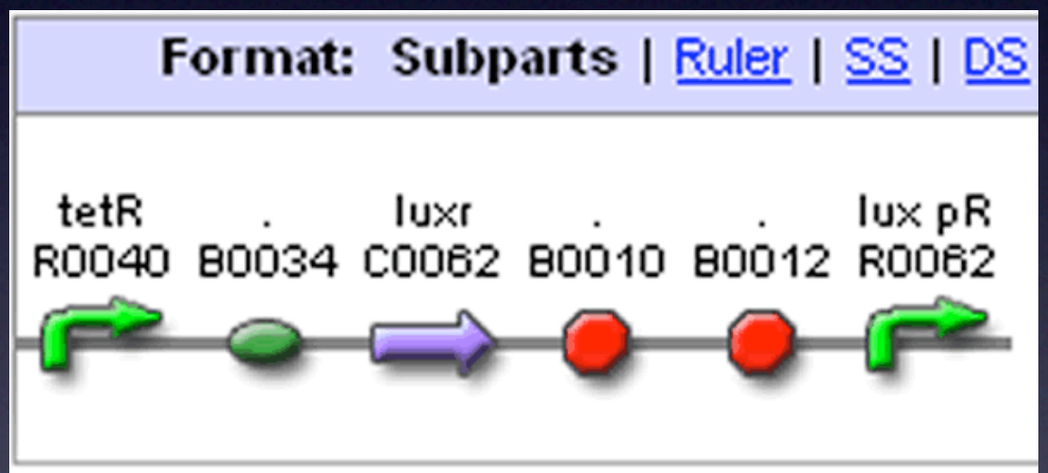
Genetic: > # replication events*
Operational: > # replication events*

Compatibility

Device has been shown to work in *MC4100*, *MG1655*, and *DH-5α*.
Device has been shown to with on *pSB3K3* and *pSB1A2*.
Device has been shown to work with *E0430* and *E0434*.
Crosstalk with input molecular similar to *$3OC_6HSL$*.
Crosstalk with systems containing *TetR*.

F2620

Lux-receiver Device



BBa_F2620

3OC_6HSL → PoPS Receiver

Author(s): Barry Canton [bcanton@mit.edu]

Last Update: May 10, 2005

PDL Devices

Description

A transcription factor [LuxR] that is active in the presence of cell-cell signaling molecule [3OC_6HSL] is controlled by an operator [TetR]. *Device input* is 3OC_6HSL. *Device output* is PoPS produced at a LuxR-regulated operator.

Usage

Full PoPS output at high 3OC_6HSL levels and high plasmid copy [e.g., pSB1A2] results in a reduced cell growth rate (see *Load* section). If used in a cell containing TetR then a second input signal [aTc] can be used to produce a logical *AND* function.

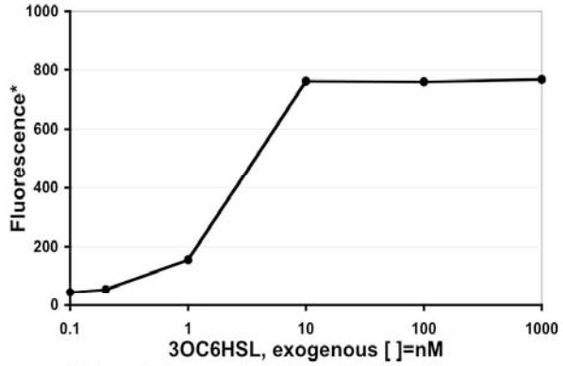
Characteristics

Input Swing: #-# nM 3OC_6HSL, exogenous
Output Swing: #-# PoPS
Switch Point: 2 nM 3OC_6HSL, exogenous
LH Latency: # seconds
HL Latency: # seconds

Key Components

BBa_R0040: TetR-regulated operator
BBa_C0062: luxR ORF
BBa_R0062: LuxR-regulated operator

Transfer Function



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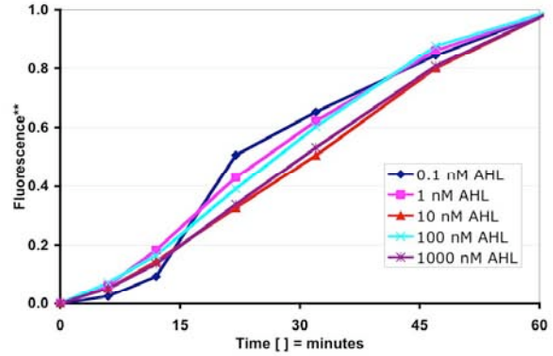
Latency



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F2620

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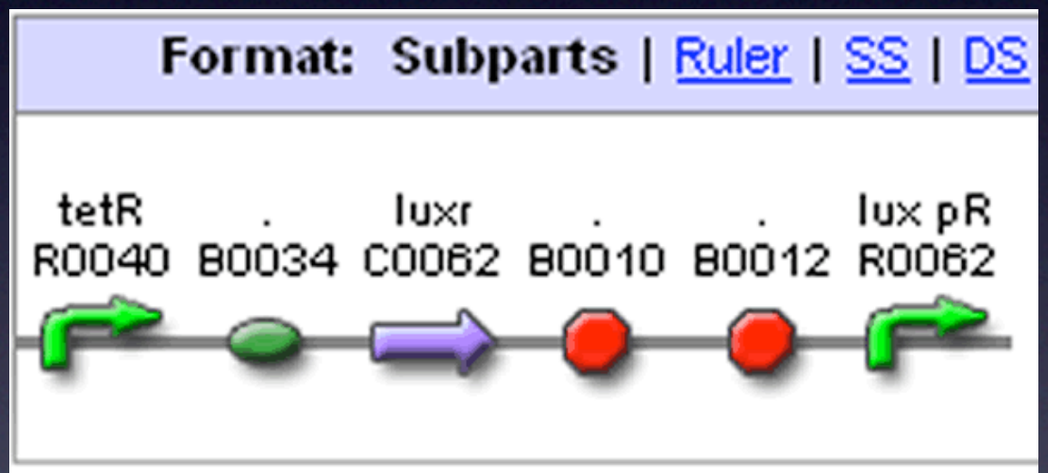

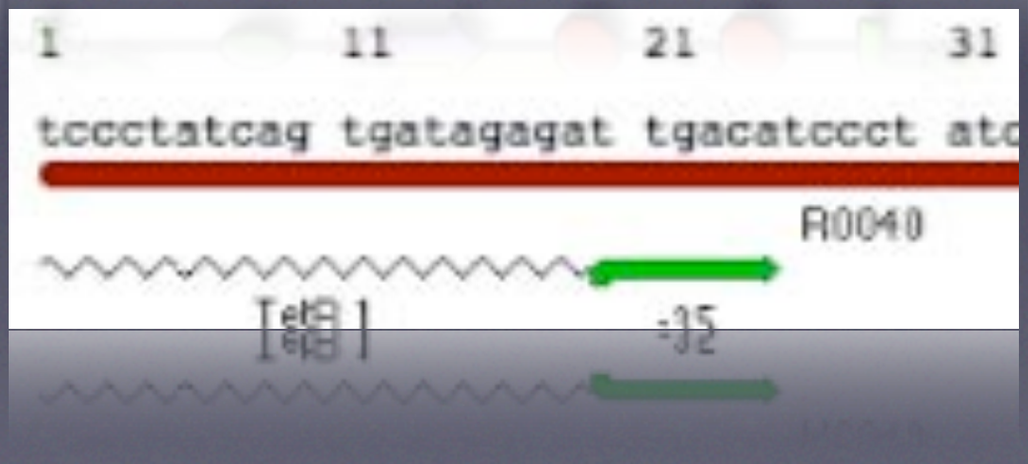

BBa_F2620

3OC$_6$HSL → PoPS Receiver

Author(s): Barry Canton [bcanton@mit.edu]

Last Update: May 10, 2005

PDL Devices

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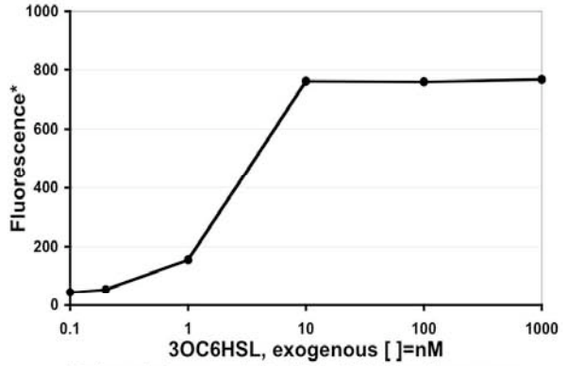

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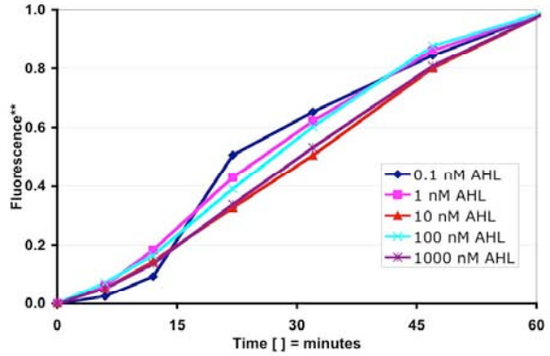

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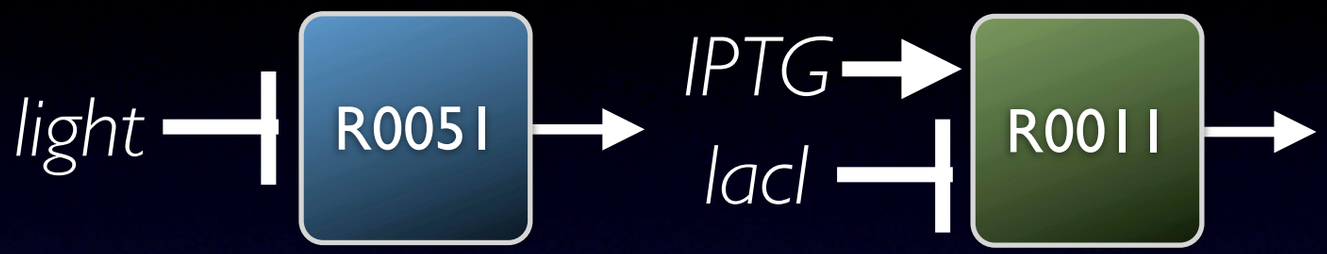
Wide range of parts & devices with modular functionality

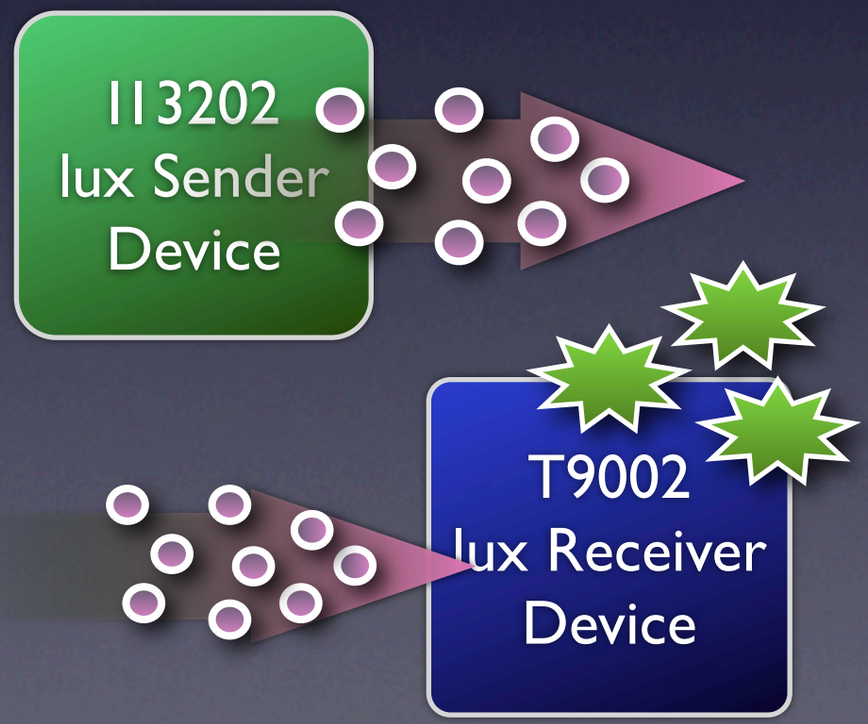
Switches



Generators

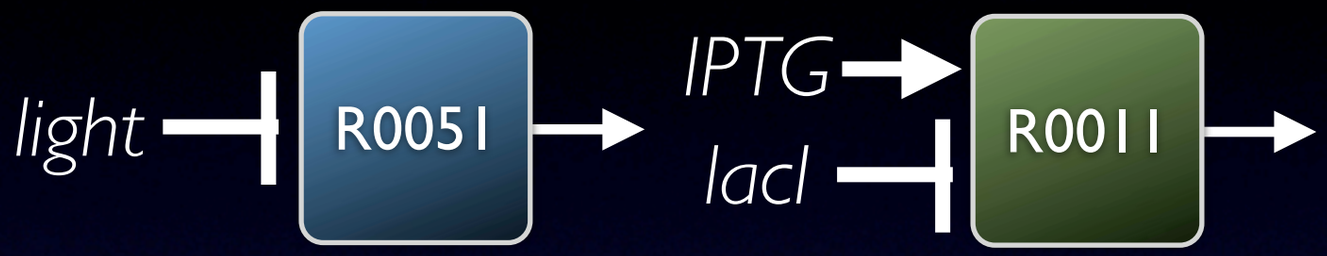
Reporters

Communication



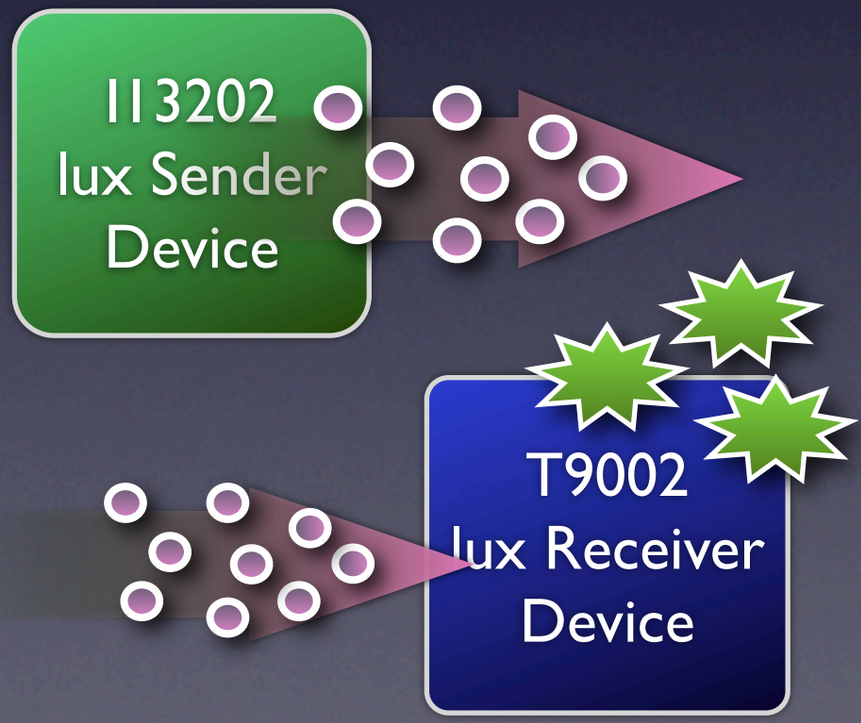
Wide range of parts & devices with modular functionality

Switches



Generators

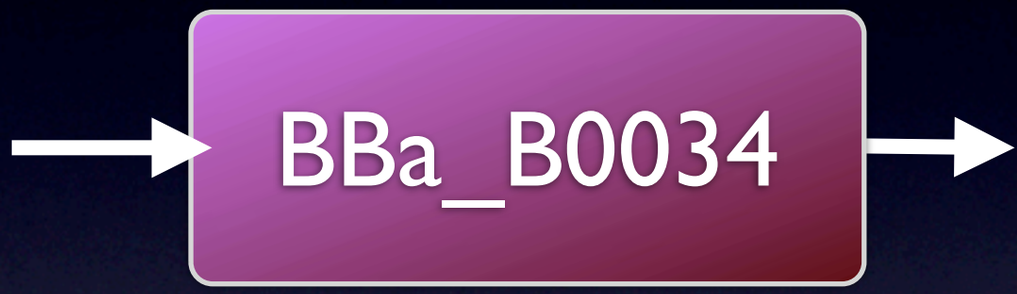
Communication



BioBricks

Standardised, interchangeable parts for Biology

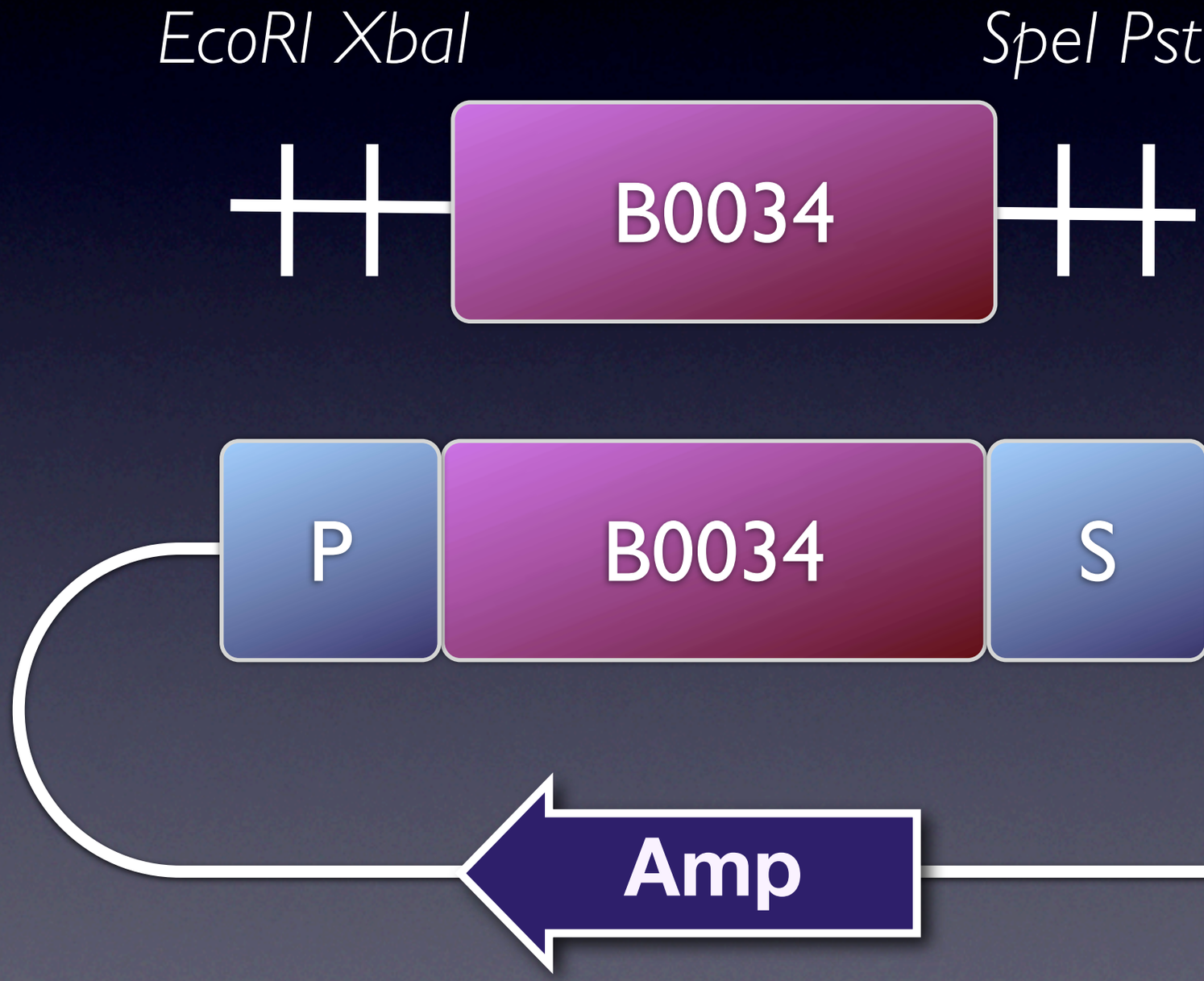
1. Physical Entity
- construction and test



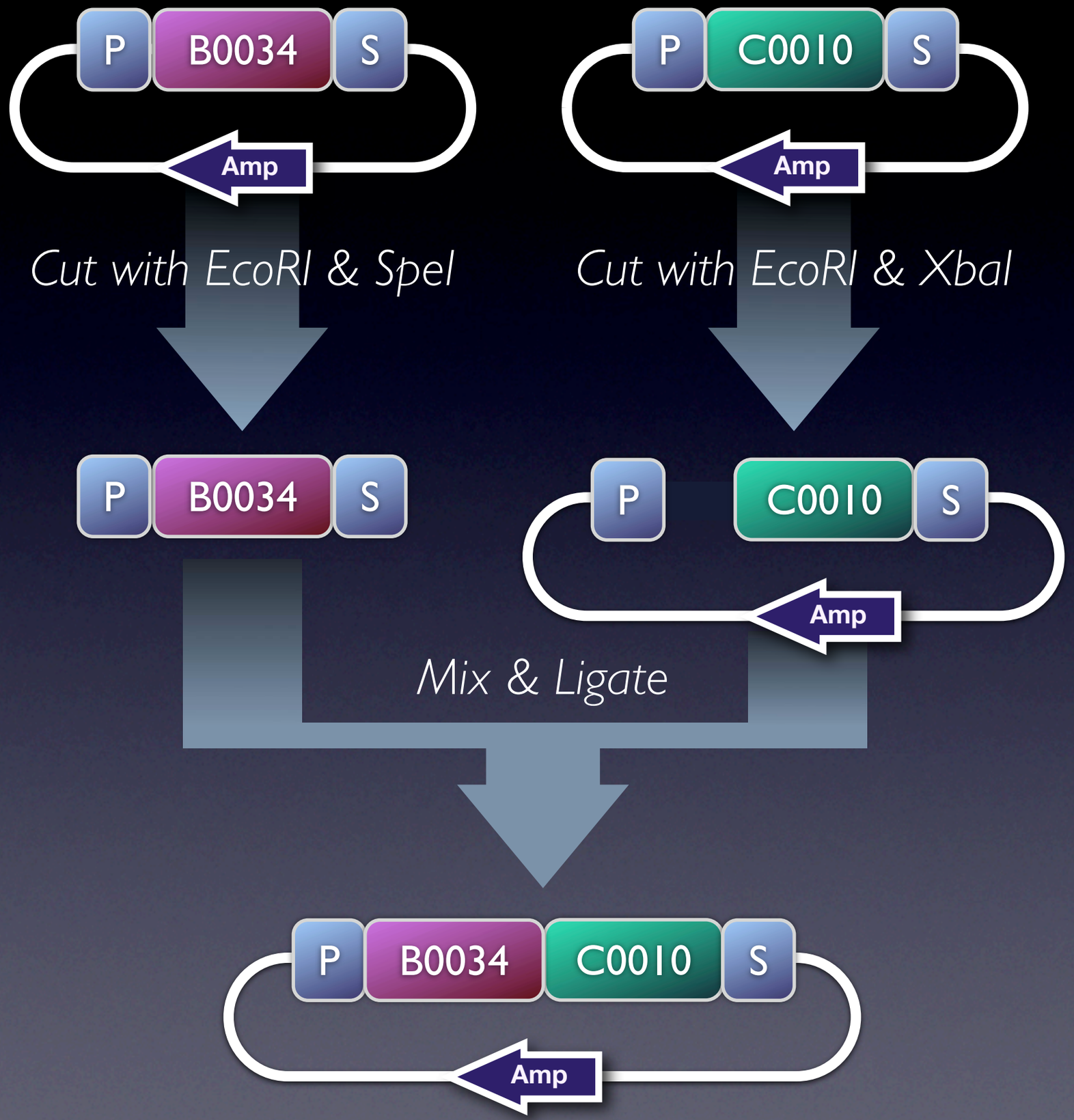
2. Information Modules
- encode function allowing design & modeling

BioBricks

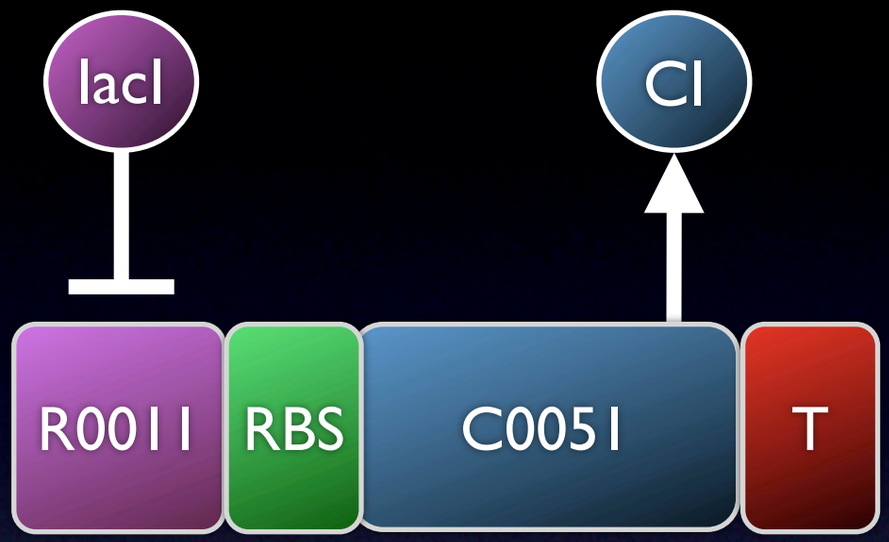
Standardised, interchangeable parts for Biology



1. Standard Assembly



2. Device Design: Making Biology Modular



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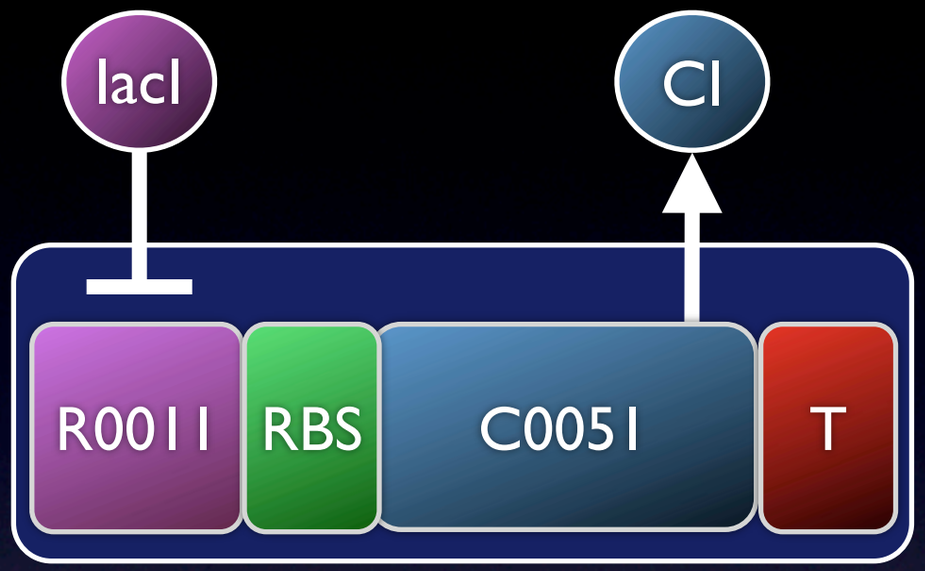

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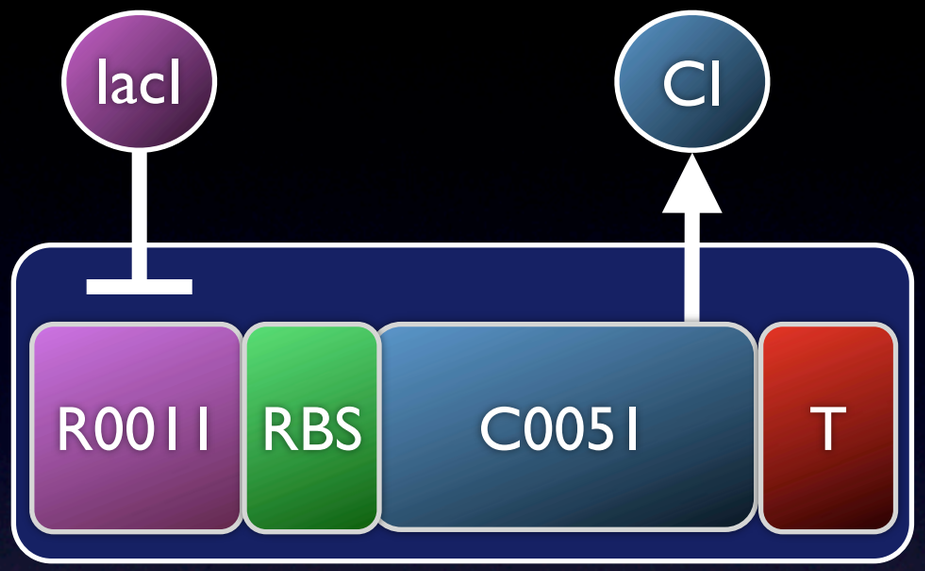

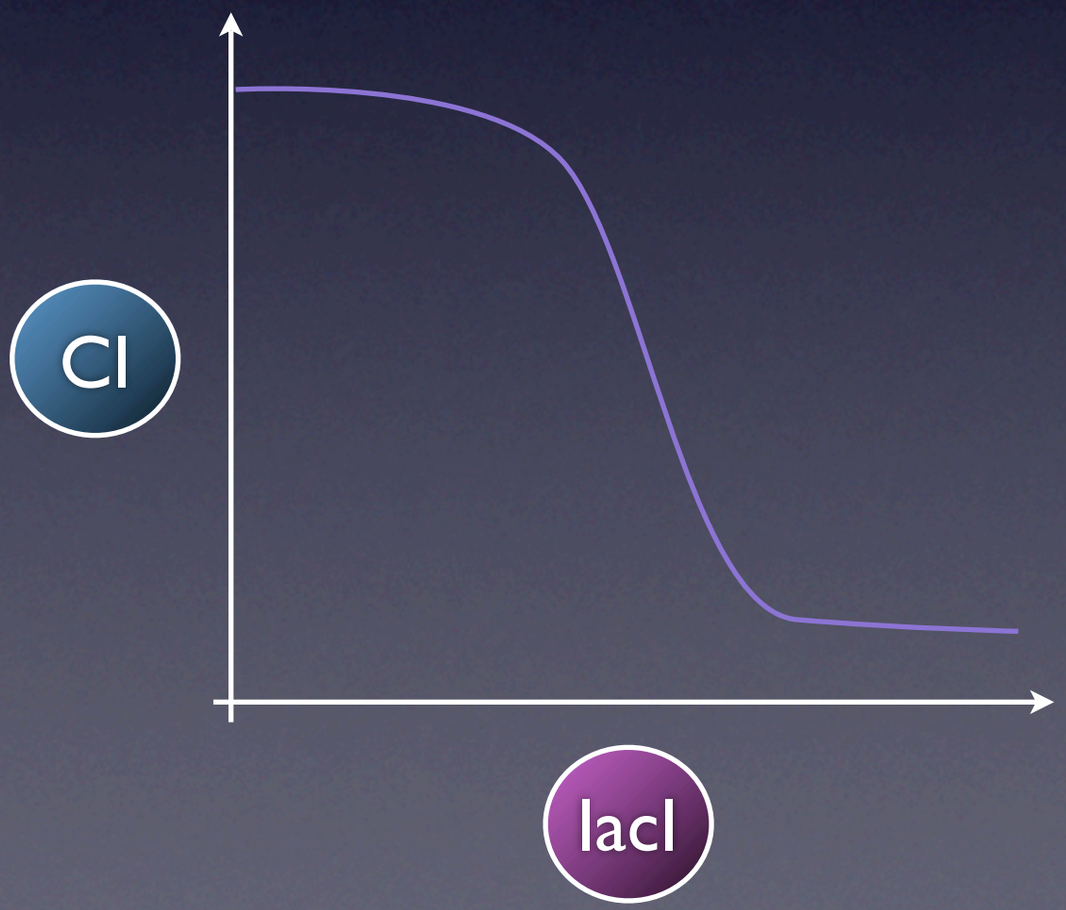

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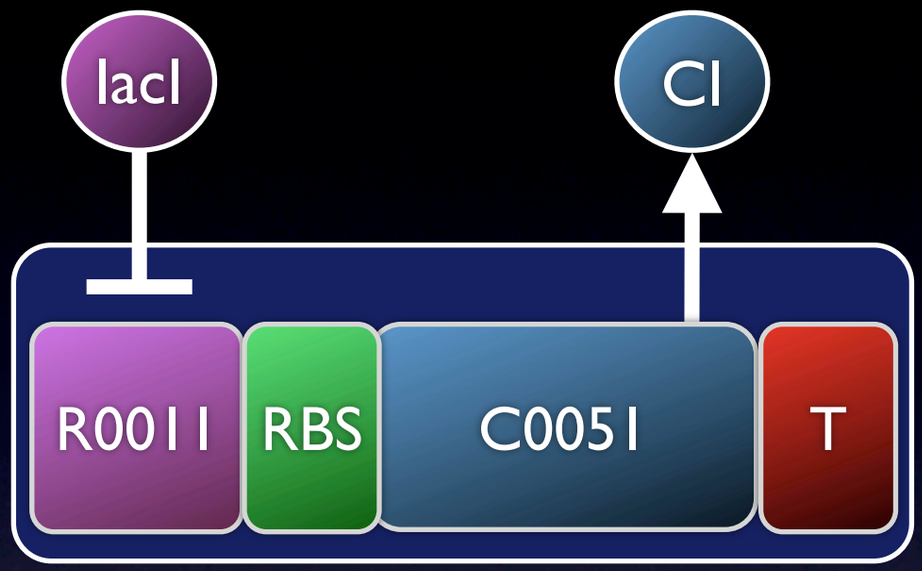

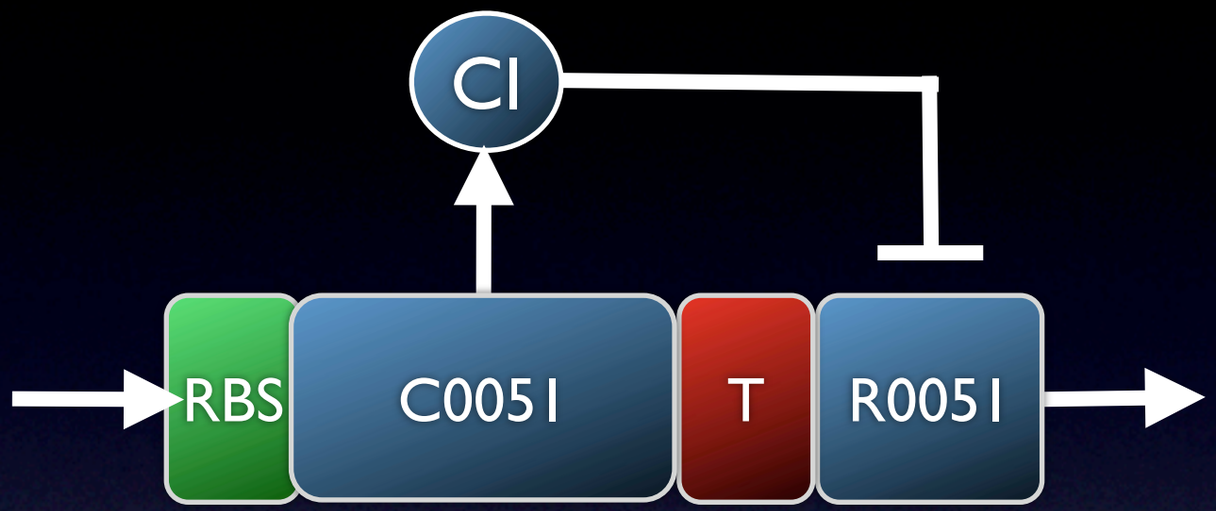

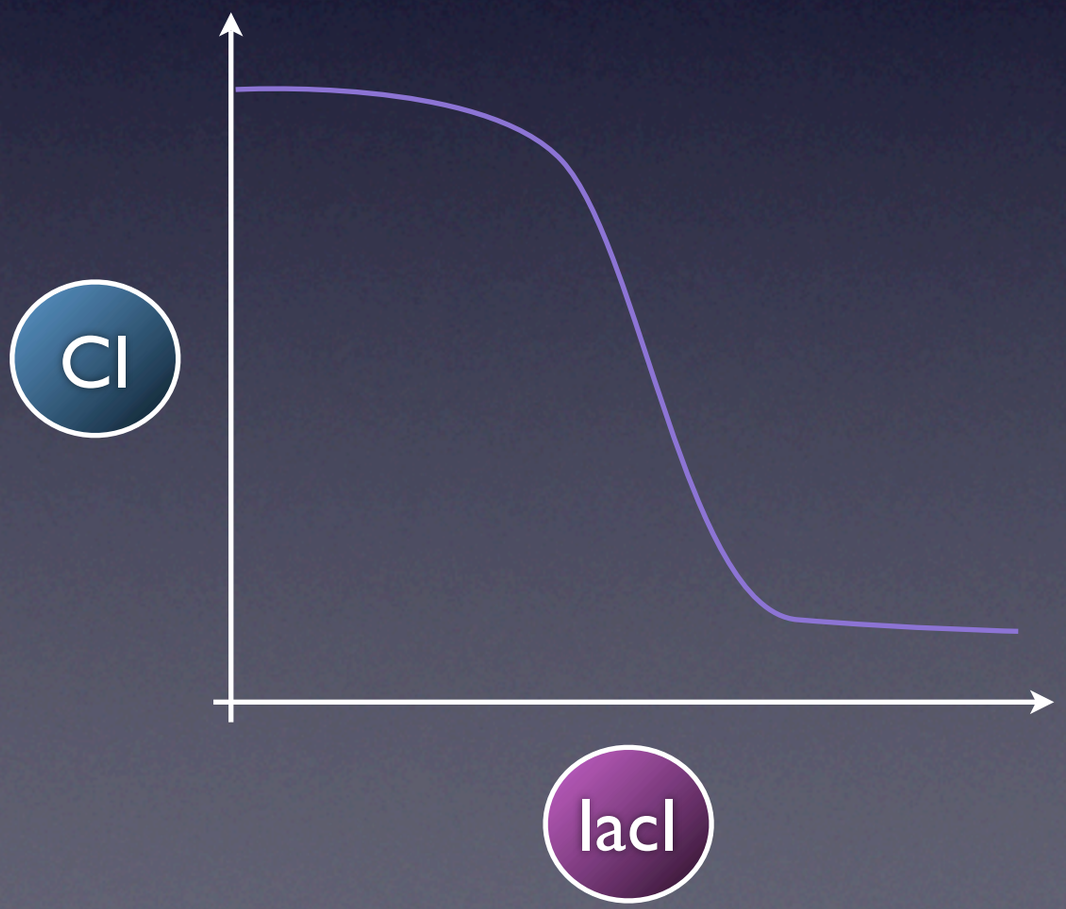

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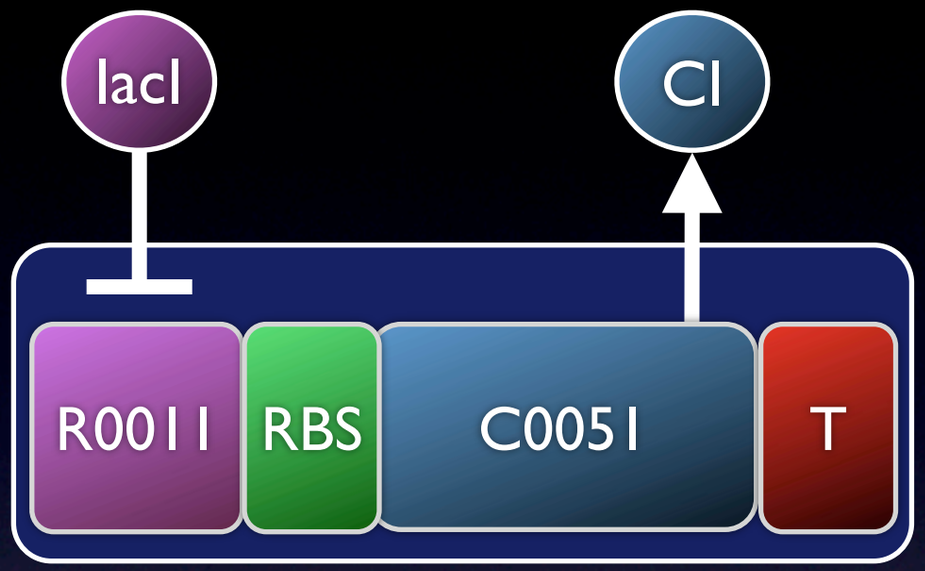

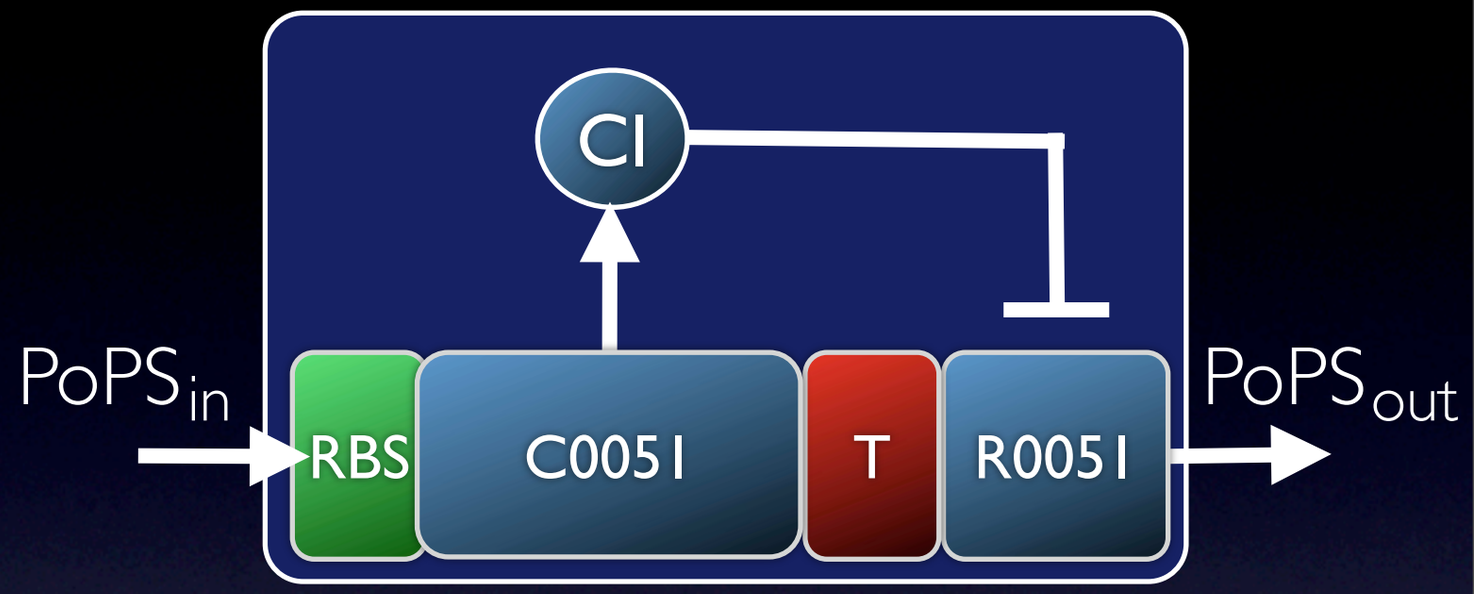

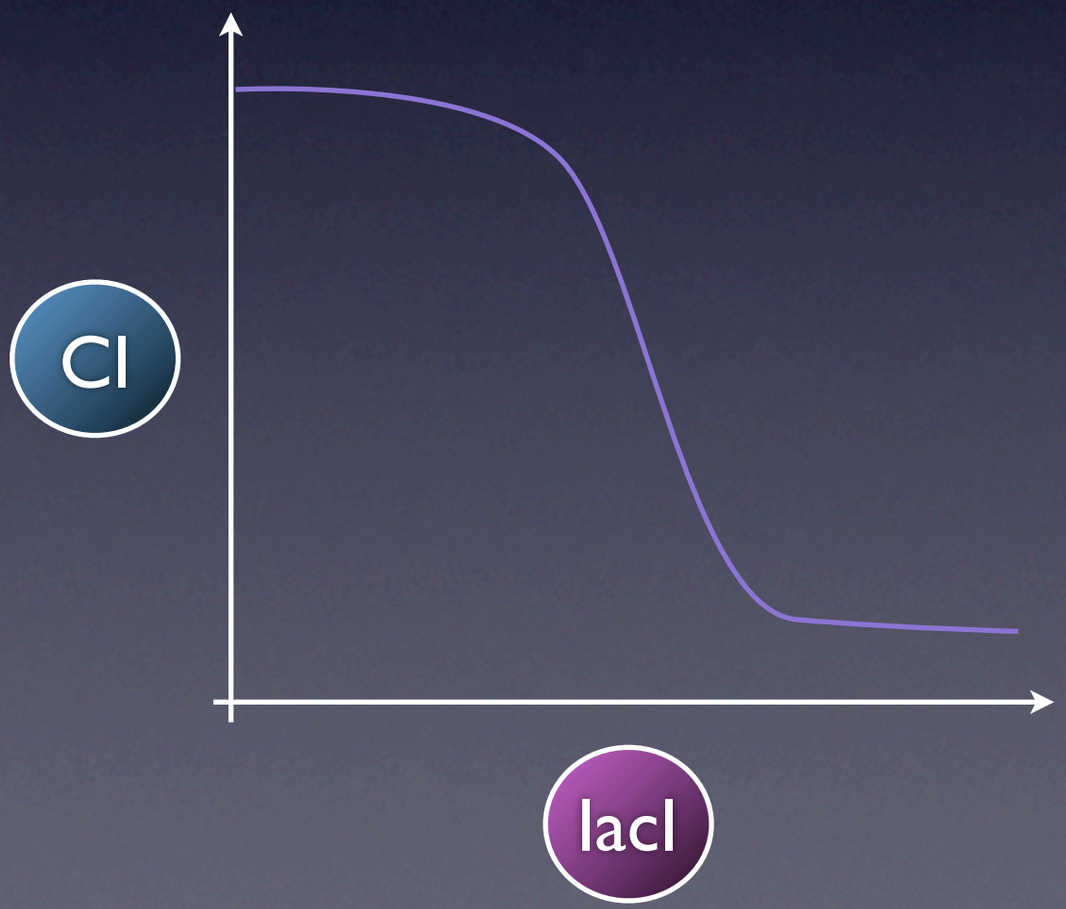

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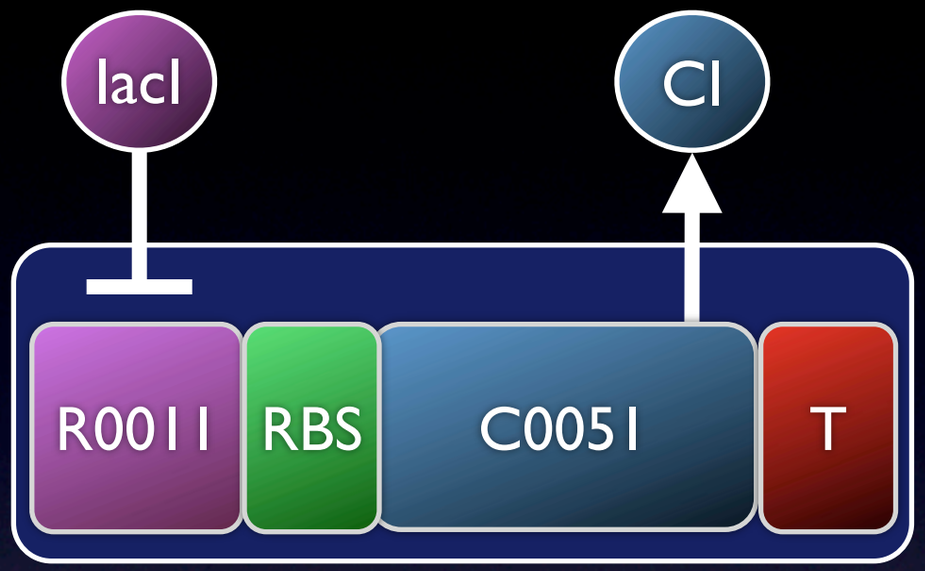

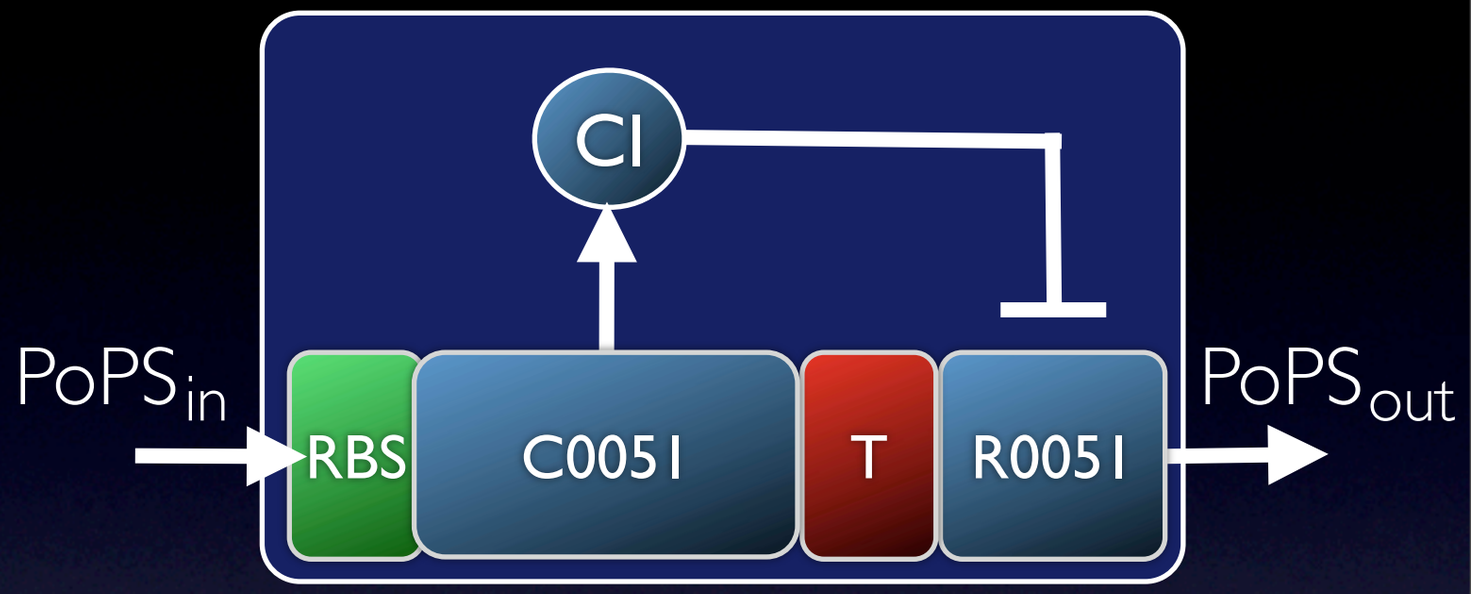

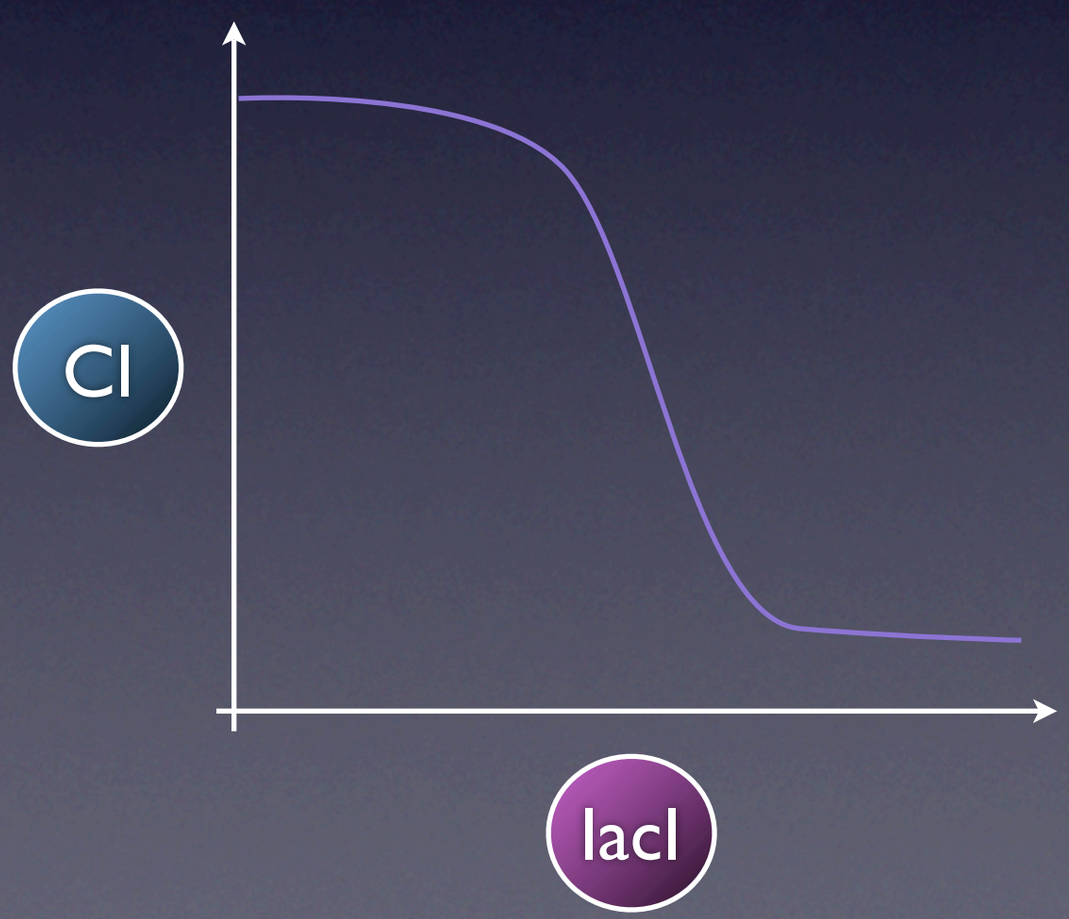

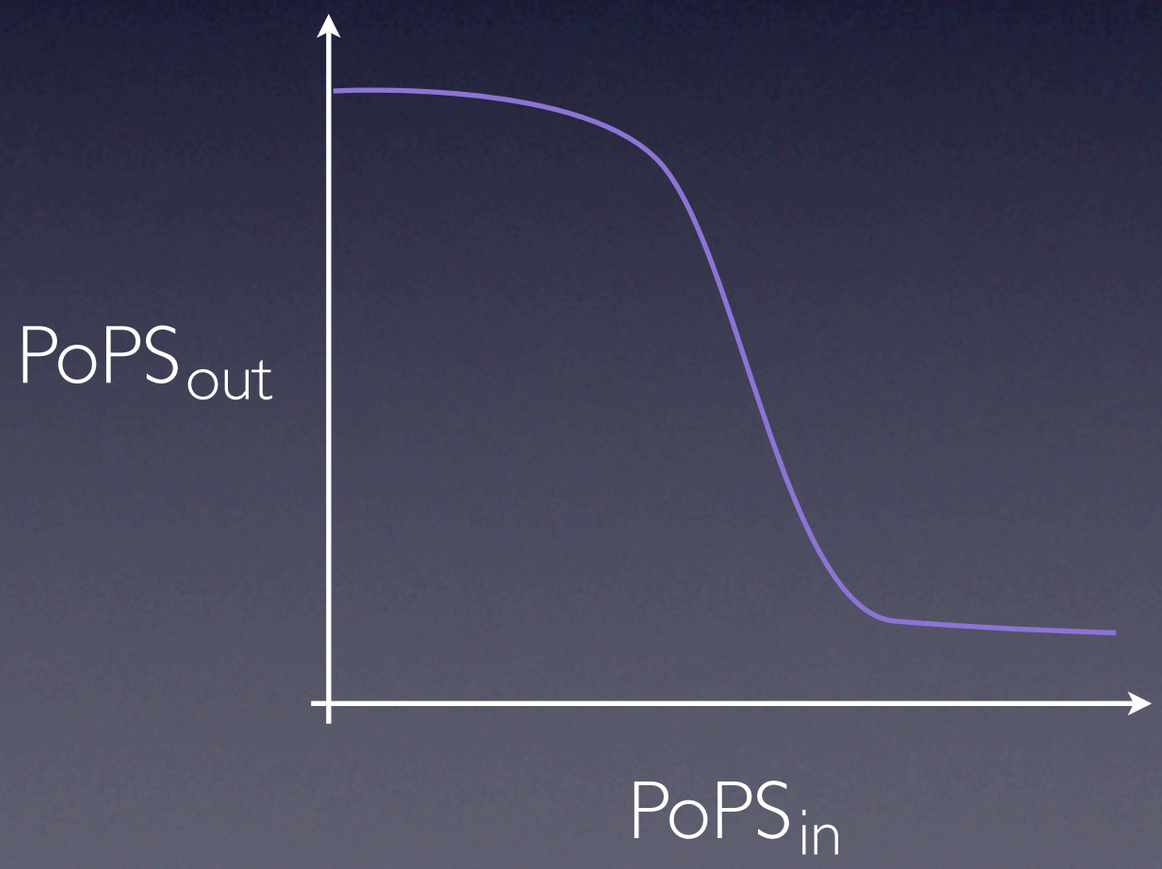

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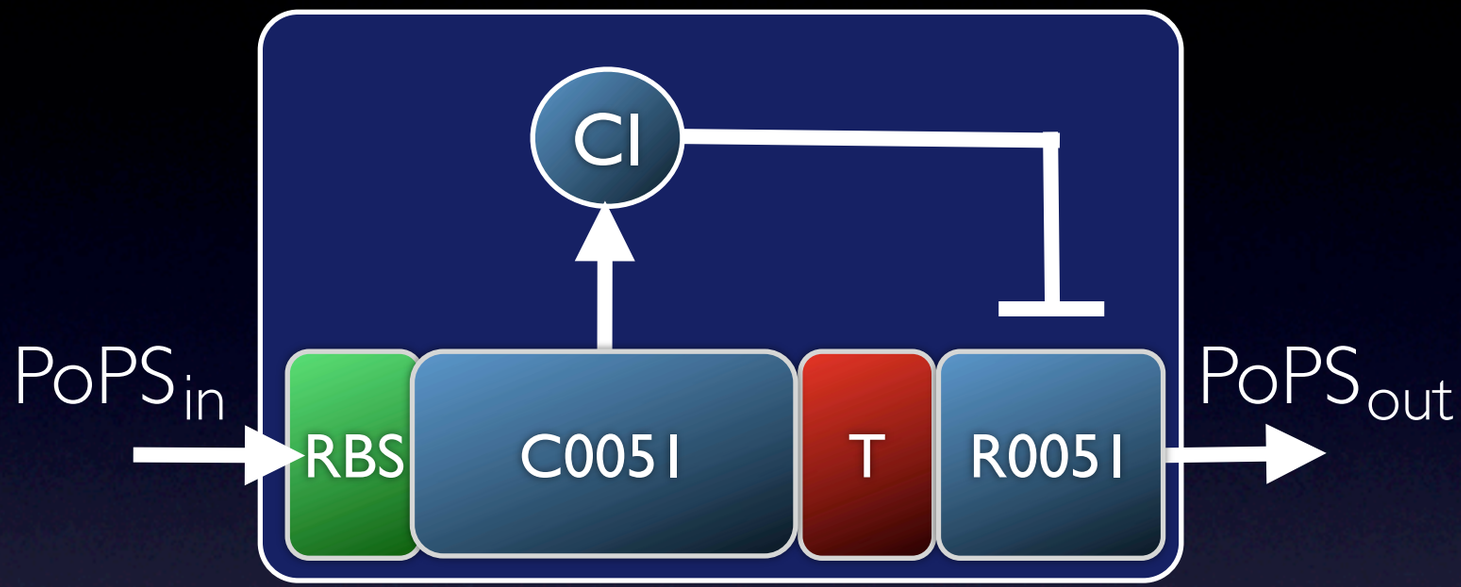

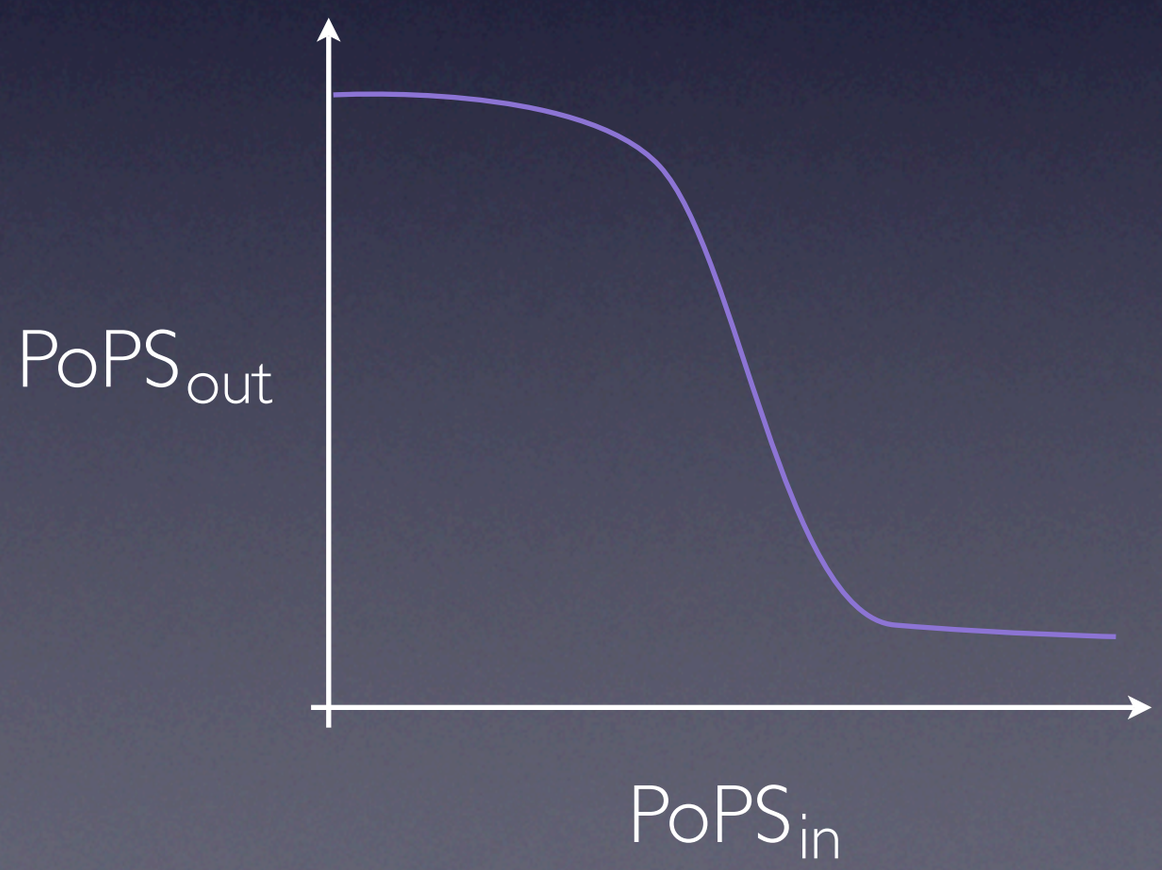

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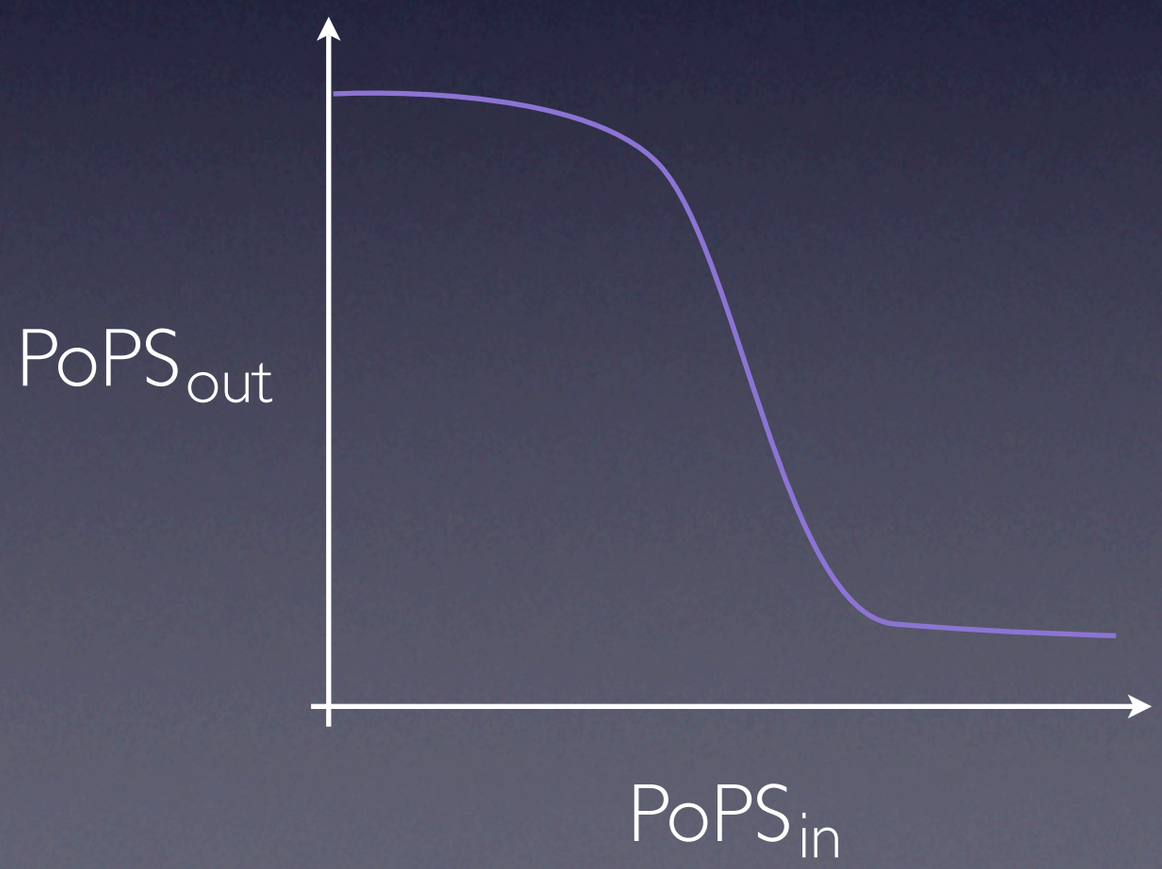

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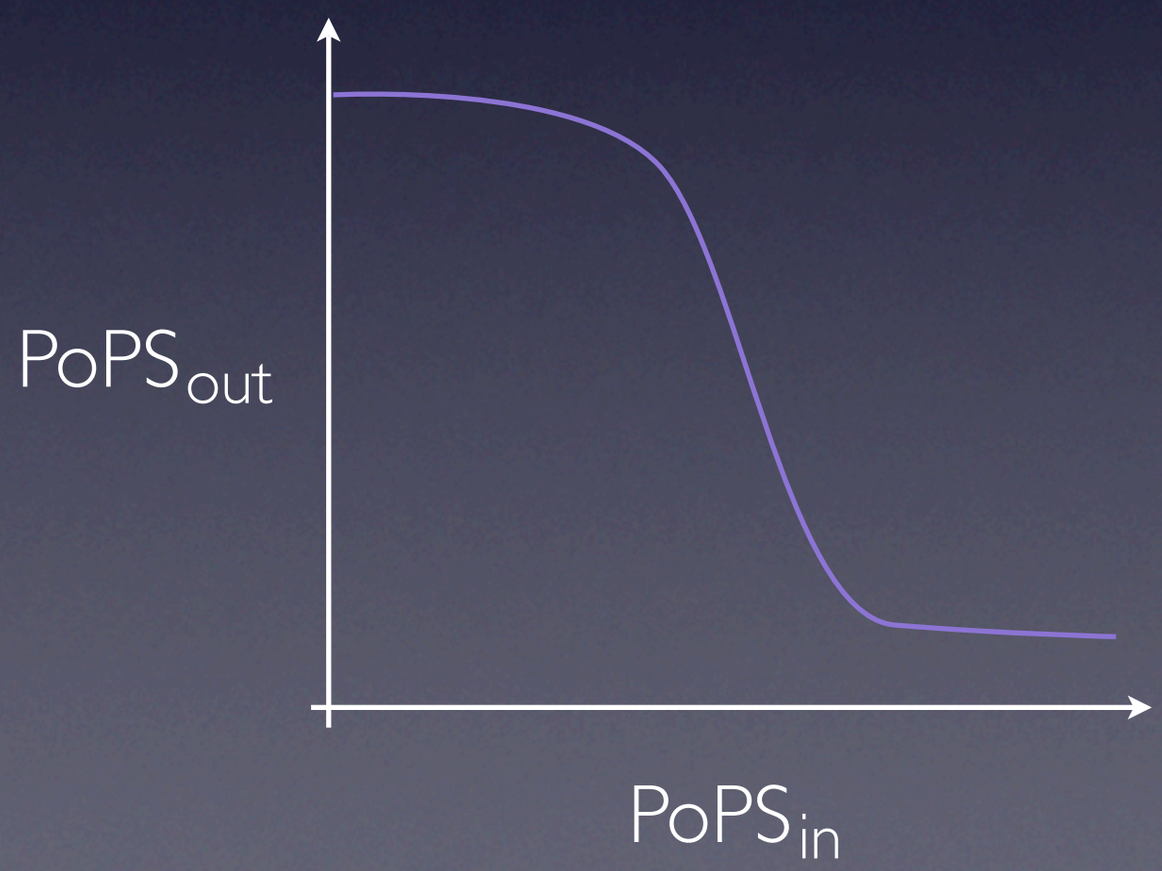
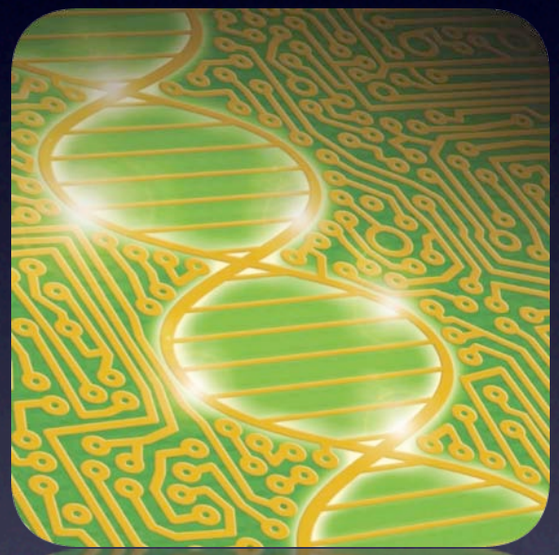


The iGEM Competition



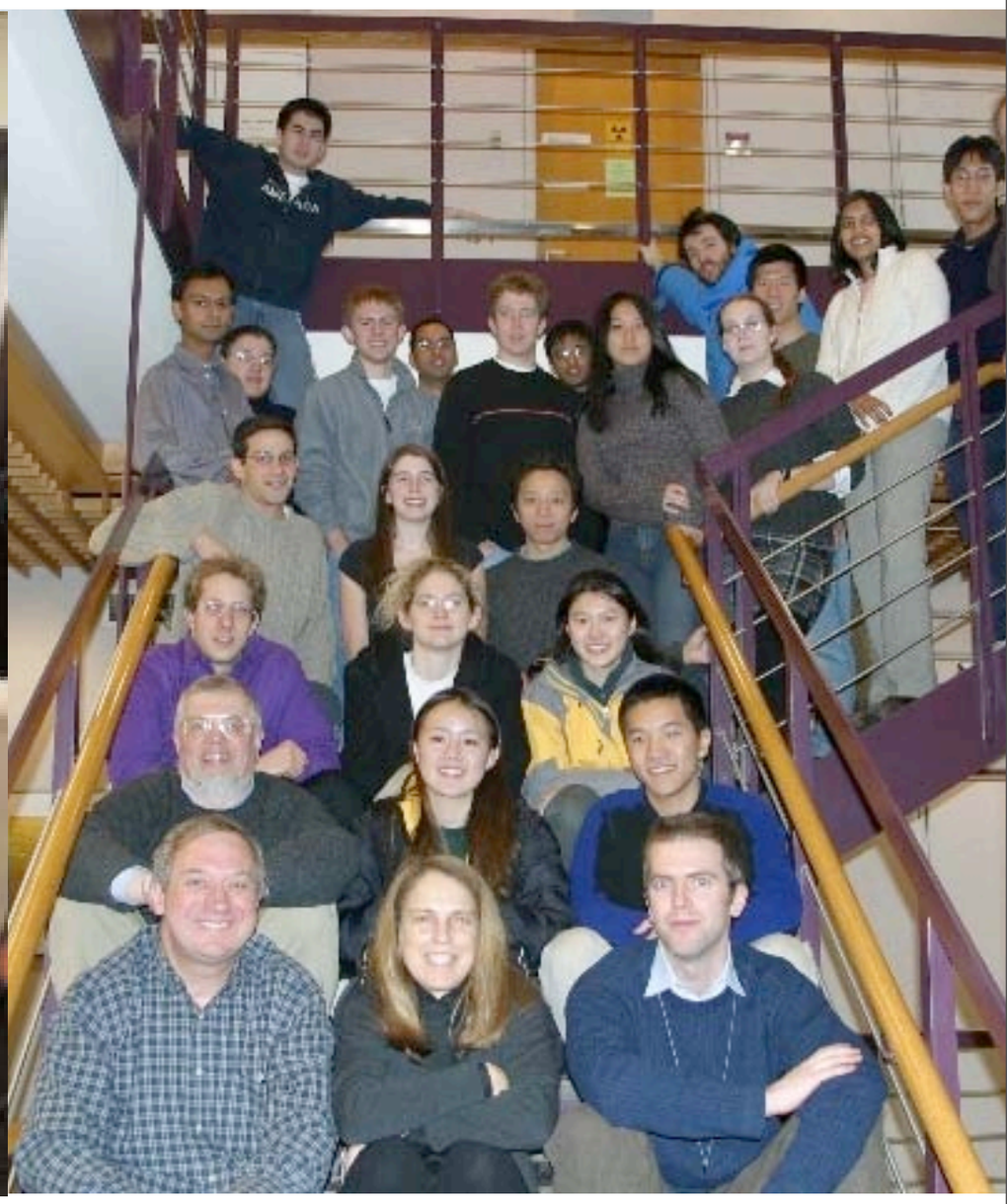
An Engineering Question

"Can simple biological systems be built from standard, interchangeable parts and operated in living cells?

Or, is biology so complex that each case is unique?"

MIT IAP 2003/4

Independent Activities Program
1 class - 30 Students



SBC 2004

IAP 2003
30 students

5 US Universities
BU, MIT, UT Austin, Caltech, Princeton

iGEM 2005

13 Universities

BU, Caltech, Cambridge, ETH Zurich, Harvard, MIT, Oklahoma, Penn State, Princeton, Toronto, UC Berkeley, UCSF, UT Austin

IAP 2003
30 students

SBC 2004
5 US Teams

Designs on life

Earlier this month, students from around the world locked horns in competition. Their challenge was to build functioning devices out of biological parts. Erika Check finds out how they got on.

Even if you're thinking big, you usually have to start small. Especially, as a group of Swiss students found, when big means counting to infinity. The team was drawing up a blueprint for the world's first counting machine made entirely of biological parts. Although they had their sights on loftier numbers, they opted to go no higher than two. If the plan worked, it would be a proof-of-principle for a much larger tallying device.

The group, from the Federal Institute of Technology (ETH) in Zurich, was one of 17 teams unveiling their projects at the first international Intercollegiate Genetically Engineered Machine (iGEM) competition, held at the Massachusetts Institute of Technology (MIT) in Cambridge on 5 and 6 November. The event attracted students from all over the world to design and build machines made entirely from biological components such as genes and proteins. They drew up grand designs for bacterial Etch-a-Sketches, photosensitive t-shirts, thermometers and sensors. And if none of the designs succeeded completely, that was more because of the limitations of the nascent science of synthetic biology than any lack of enthusiasm, creativity or hard work.

Synthetic biology aims to merge engineering approaches with biology. Researchers working at the most basic level are copying simple biological processes, such as the production of a protein from a gene. They break the process down into its component elements, such as a gene and the pieces of DNA and other molecules that control its activity. They then string these elements together to build a module they know will behave in a particular way — say, oscillate between producing and not producing a protein, or produce a protein that can switch another module on or off.

It is these kinds of components — oscillators and switches — that engineers order from suppliers and link together to build more complex electronic circuits and machines. Synthetic biologists are trying to develop a similar armoury of biological components, dubbed BioBricks, that can be inserted into any genetic circuit to carry out a particular function. Scientists at MIT have established a Registry of Standard Biological Parts, a catalogue of BioBricks that theoretically

Bidding for glory: teams from the ETH in Zurich (top), Cambridge, UK, (bottom right) and Massachusetts at the first international Intercollegiate Genetically Engineered Machine competition.

from the ground up. To do so, they have commandeered a time-honoured engineering tradition: the student competition. The iGEM event began life as a project class for MIT students in 2003. Last year, it was thrown open to other US universities, and this year it went international. The organizers hope to attract 30 to 50 teams next year, including some from Asia.

Competitive culture

Much like the robot competitions that tap into students' desire to build something cool, the iGEM jamborees fire the participants' natural curiosity — hopefully encouraging biologists to learn something from engineers, and vice

selection of designs. Students from the University of Cambridge, UK, tried to make a circuit that could control the movement of *Escherichia coli* bacteria. They aimed to engineer the bacteria to contain a switch governing their sensitivity to the sugar maltose. With the switch off, the microbes would ignore the sugar. Tripping the switch would make the bacteria sensitive to the sugar and induce them to move towards it. In the end, the group — like almost every other entrant — had trouble completing assembly of its genetic parts in time.

Many of the other students also tackled problems related to bacterial communication and motion. The team from Pennsylvania

iGEM 2006

37 Universities

IAP 2003
30 students

SBC 2004
5 US Teams

iGEM 2005
13 Teams

IET Synthetic Biology

http://www.ietdl.org/IET-STB

IAP 2003
30 students

iGEM 2006

37 Universities

Univeristy of Calgary, Canada
Missouri Western State Univeristy, USA
UCSF, USA
UC Berkeley, USA
Caltech, USA
UC Irvine, USA
University of Arizona, USA
University of Oklahoma, USA
University of Texas, USA
Prairie View A&M Univeristy, USA
Rice University, USA
National Polytechnical Institute, Mexico
Mississippi State Univeristy, USA
Purdue University, USA
University of Michigan, USA
Univeristy of Toronto, Canada
McGill University, Canada
Boston University, USA
MIT, USA
Harvard University, USA
Brown University, USA
Cold Spring Harbor Laboratory, USA
Princeton University, USA
Duke University, USA
Davidson College, USA
Penn State University USA
Team Latin America
University of Edinburgh, Scotland
University of Cambridge, England
Imperial College, England
Freiburg University, Germany
ETH Zurich, Switzerland
University of Ljubljana, Slovenia
UPV-UV Valencia, Spain
EGE University, Turkey
Chungbuk National University, South Korea
Tokyo Institute of Technology, Japan
Chiba Univeristy, Japan
NCBS Bangalore, India
Team Africa
© J. R. Brown, iGEM 200

IET Synthetic Biology

http://www.ietdl.org/IET-STB

iGEM 2007

57 Universities - 750 Participants

IAP 2003
30 students

SBC 2004
5 US Teams

iGEM 2005
13 Teams

iGEM 2006
35 Teams

Europe
9 Teams

Bologna
ETH Zurich
Freiburg
Ljubljana
Naples
Paris
St Petersburg
Turkey
Valencia

Asia
8 Teams

Bangalore
Chiba
Peking
Taipai
Tianjin
Tokyo
Tsinghua
USTC

North America
30 Teams

Alberta
Berkeley
Boston
Brown
Calgary
Caltech
CSHL
Davidson
Duke
Missouri
Harvard
Lethbridge
McGill
Mississippi
MIT
Michigan
Minnesota
Penn State
Prairie View
Princeton
Purdue
Rice
Stanford
Toronto
UCSF
Utah
Virginia
Virginia Tech
Waterloo
Wisconsin

UK
4 Teams

Cambridge
Edinburgh
Glasgow
Imperial

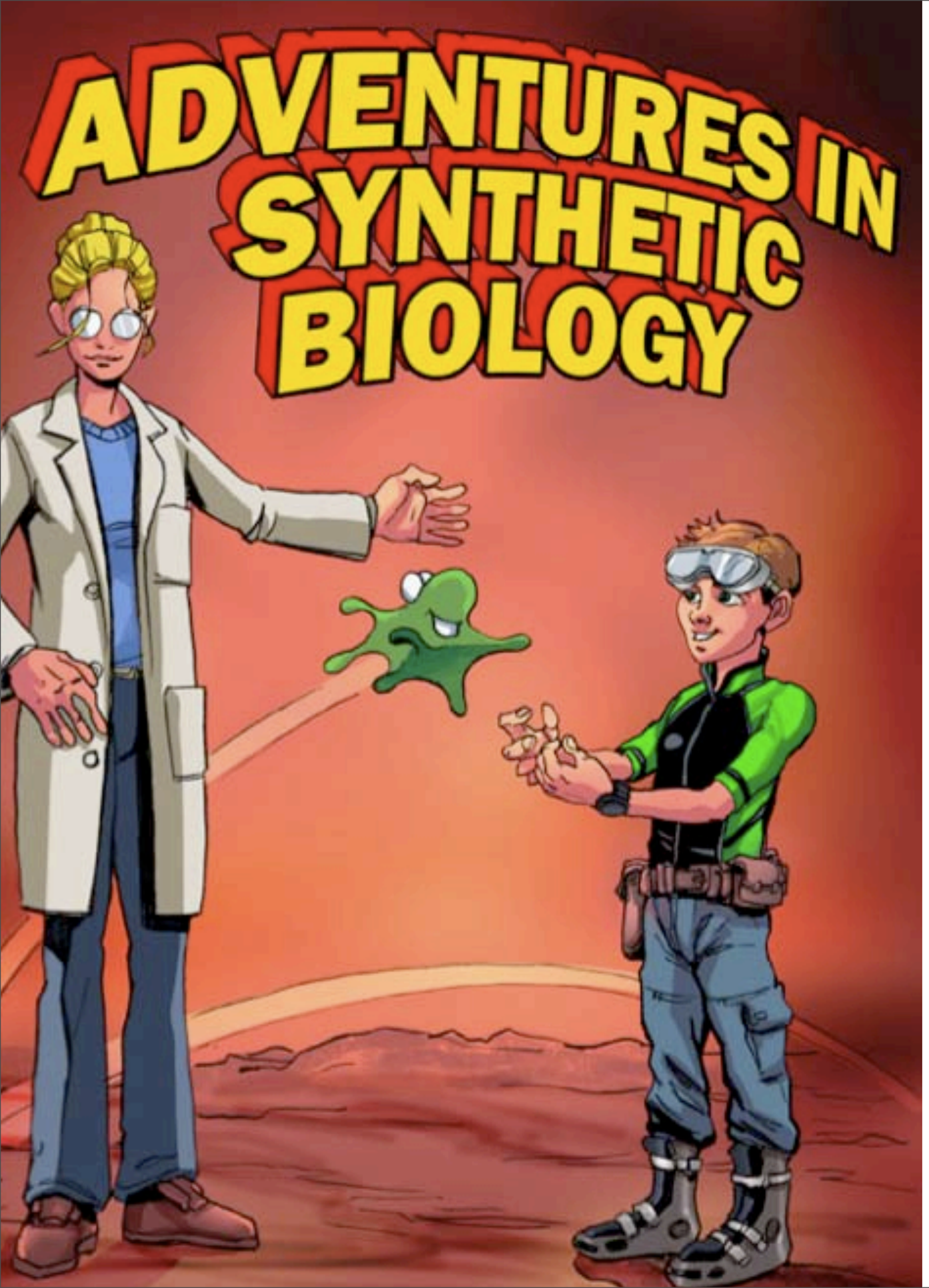
Rest of World

Melbourne
Mexico
Colombia

iGEM 2007

57 Universities

750 Participants



iGEM 2008

100 teams

Design Brief

"Design and test a simple biological system from standard, interchangeable parts and operate it in living cells."

Synthetic Biology

University of Cambridge

www.synbio.org.uk

iGEM Synthetic Biology competition

This year the Cambridge iGEM team worked on novel systems for intercellular communication. This included new peptide signalling systems, outer membrane pores, transcription activator components and Gram positive chassis systems. For more information see the team wiki. The Cambridge team received Gold Awards and a prize for best BioBrick.

UT Austin - Bacterial Photofilm - iGEM 2005

Engineering *Escherichia coli* to see light

These smart bacteria 'photograph' a light pattern as a high-definition chemical image.

We have designed a bacterial system that is switched between different states by red light. The system consists of a synthetic sensor kinase that allows a lawn of bacteria to function as a biological film, such that the projection of a pattern of light on to the bacteria produces a high-definition (about 100 megapixels per square inch), two-dimensional chemical image. This spatial control of bacterial gene expression could be used to 'print' complex biological materials, for example, and to investigate signalling pathways through precise spatial and temporal control of their phosphorylation steps.

Plants and some bacteria use a class of protein photoreceptors known as phytochromes to control phototaxis, photosynthesis and the production of protective pigments[1–3]. Photoreceptors are not found in enterobacteria, such as *Escherichia coli*, so we created a light sensor that functions in *E. coli* by engineering a chimaera that uses a phytochrome from a cyanobacterium.

A phytochrome is a two-component system that consists of a membrane-bound, extracellular sensor that responds to light and an intracellular response-regulator[3]. The response-regulators of most phytochromes do not have DNA-binding domains and do not directly regulate gene expression, so we fused a cyano-

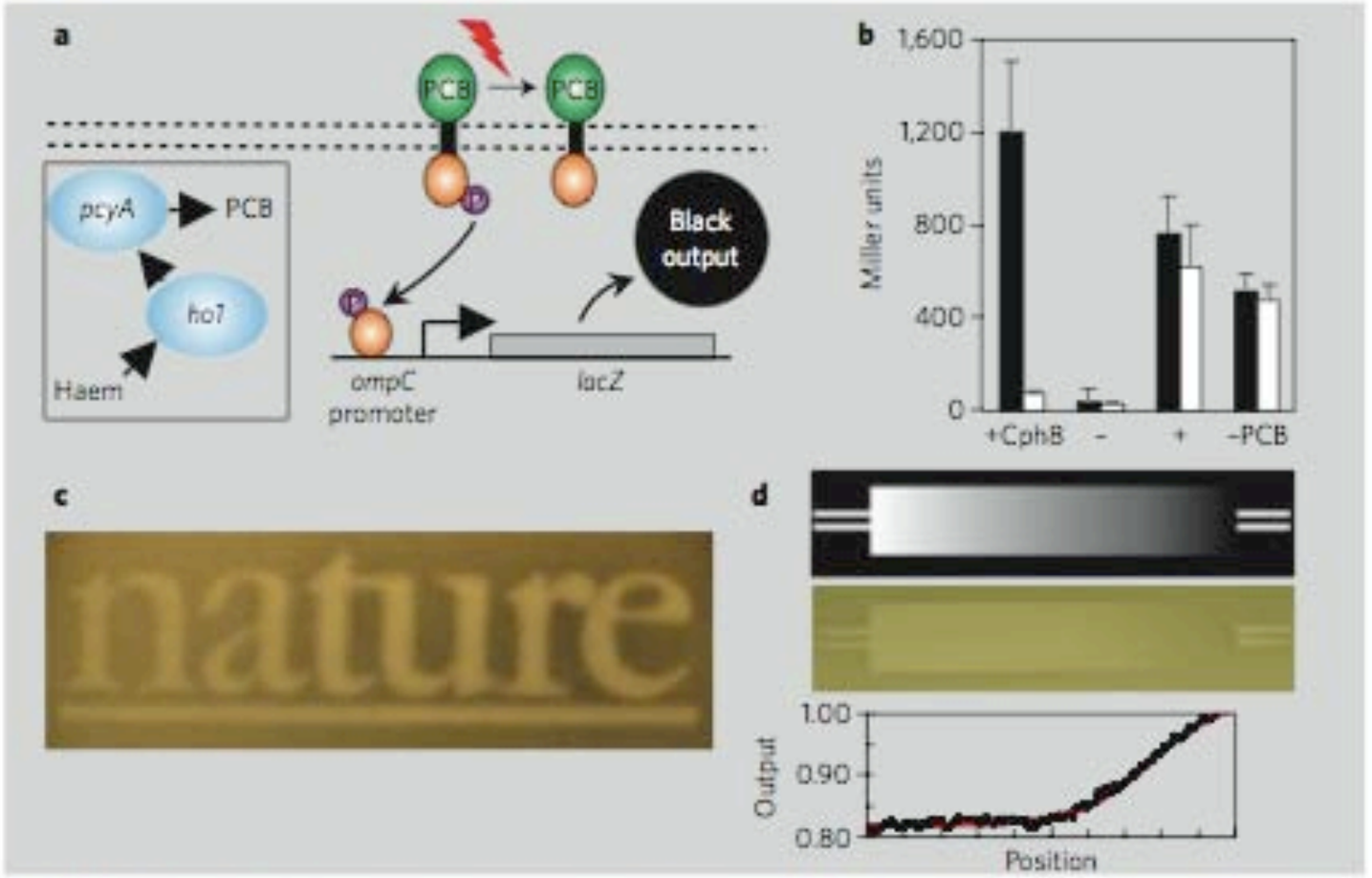
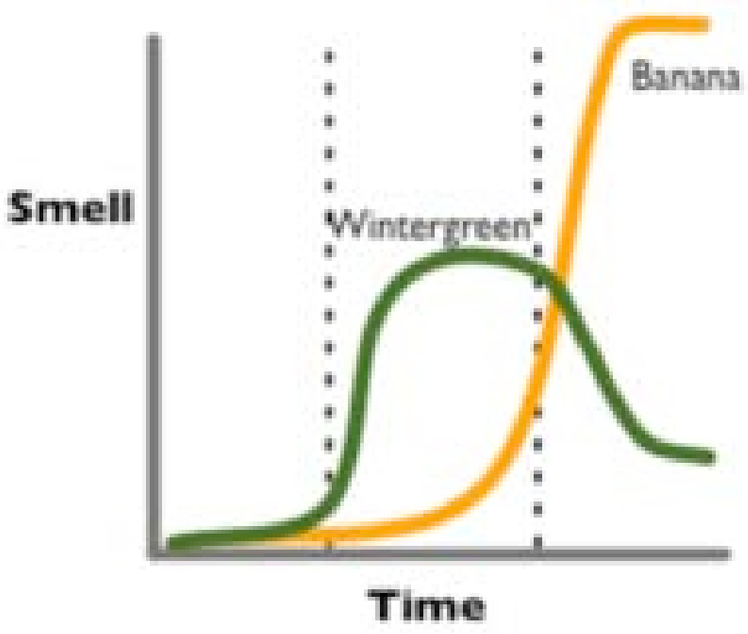
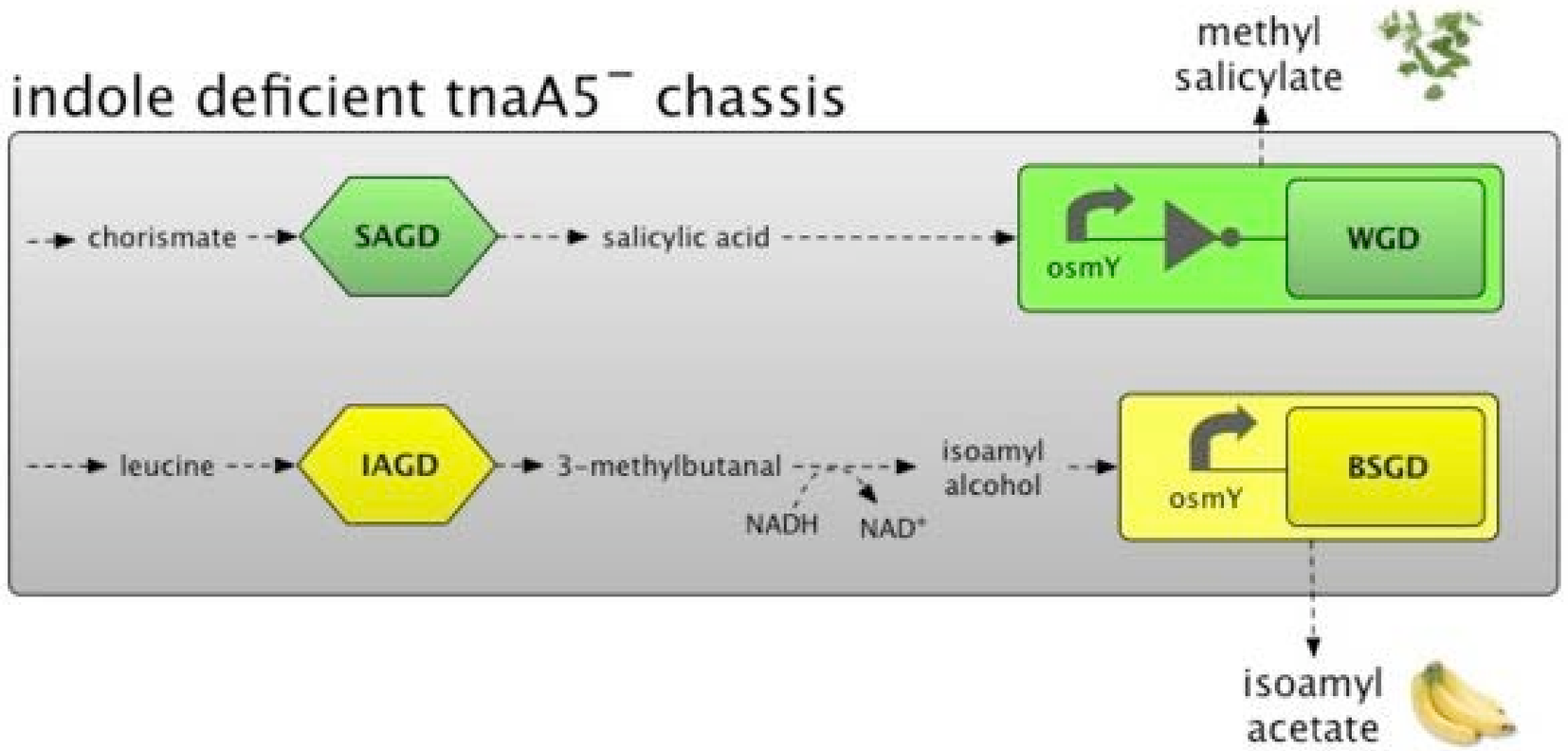


Figure 1 | Light imaging by engineered *Escherichia coli*. **a,** The chimaeric light receptor Cph8 contains the photoreceptor from Cph1 (green) and the histidine kinase and response-regulator from EnvZ–OmpR (orange); inset, conversion of haem to phycocyanobilin (PCB), which forms part of the photoreceptor. Red light drives the sensor to a state in which autophosphorylation is inhibited (right), turning off gene expression. For details of genes, see text. **b,** Miller assay showing that Cph8 is active in the dark (black bars) in the presence of PCB and inactive in the light (white bars). There is no light-dependent activity in the absence of Cph8 (−) and there is constitutive activity when only the histidine kinase domain of EnvZ is expressed (+), or when the PCB metabolic pathway is not included (− PCB). **c,** When an image is projected on to a bacterial lawn, the *LacZ* reporter is expressed only in the dark regions. **d,** Transfer function of the circuit. As the intensity of the light is increased by using a light


eau d’e coli
mit igem
2006


Smell
Wintergreen
Banana
Time


indole deficient tnaA5¯ chassis
methyl salicylate
chorismate
SAGD
salicylic acid
osmY
WGD
leucine
IAGD
3-methylbutanal
NADH
NAD+
isoamyl alcohol
osmY
BSGD
isoamyl acetate

Edinburgh - Arsenic Detector - iGEM 2006

The field test device

- A test tube could contain all the necessary components: Freeze dried bacteria, growth medium, indicator powder, Ampicillin salt, etc…

- These tubes could then be given to local villagers to monitor their own water quality themselves
- A good alternative to the widely used Gutzeit method

www.Macteria.co.uk

The iGEM Competition

Undergraduate teams compete to design biological systems from standard parts

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Pulls together students from different disciplines
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New educational model in an exciting new field, placing students at the cutting edge of research

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New educational model in an exciting new field, placing students at the cutting edge of research

Allows students to make a valuable contribution to the research field & community, while in pursuit of their own goals

www.igem.org

iGEM 2007 Wiki

International Genetically Engineered Machine Competition

Go Search

article discussion edit history Create an account or log in

teams jamboree participate help about registry

Peking wins the Grand Prize

the Peking iGEM 2007 team triumphantly hoists the Grand Prize BioBrick trophy

iGEM?

Hundreds of undergraduates all over the world spend their summer making Synthetic Biology a reality by participating in the annual International Genetically Engineered Machine competition.

iGEM through the years

- 2008
- 2007
- 2006

Learn More

Results of the Jamboree

sat & sun, nov 3-4

iGEM 2007 is now officially concluded! Congratulations to all!

- Results
- See the medal winners
- Media (including links to videos and

calendar

Jamboree roster + fees due	*fri 12 oct 12*
iGEM wiki frozen + parts postmarked	*fri 26 Oct 07*
Jamboree!	*sat-sun 3-4 nov 07*
Registry + BioBrick	*sun-tue*

Cambridge Philosophical Society &
Cambridge Synthetic Biology Society
One Day Symposium

Synthetic Biology:
Molecular Bioengineering for the 21st Century

Monday 3rd December 2007

Starts 9am, Pippard Lecture Theatre,
Cavendish Laboratory, Department of Physics.

Speakers include:

Ron Weiss, Alfonso Jaramillo, Georg Seelig,
Jim Haseloff, James Brown, Cambridge iGEM 2007 team

IET BioSysBio 2008

20 - 22 April 2008, Imperial College London

Synthetic Biology, Systems Biology and Bioinformatics

- Home
- Abstract Submission
- Registration
- Programme
- Organising Committee
- Scientific Advisory Board
- Sponsorship
- BioSysBio 2007
- Venue
- Contact Us

Abstract Submission Deadline:
9th December 2007

Registration opens early January

Speakers include:
Tom Knight, Adam Arkin & Jaroslav Stark

www.biosysbio.com

Summary

Rapid emergence & development of sequencing, synthesis and computational tools & technologies have made possible the rational engineering of biological systems

One must apply the engineering principles that have underpinned the development of mechanical and electrical engineering fields in order to deal with biological complexity

Synthetic Biology looks set to contribute to future improvements in the microbial, plant and animal cell engineering that are clearly needed for the renewable technologies of the 21st century

Thank Yous

Jim Haseloff

Jim Ajioka

Gos Micklem

Duncan Rowe

Randy Rettberg

Tom Knight

Drew Endy

Cambridge iGEM 2005/6/7

www.synbio.org.uk

Resources for learning assembly techniques

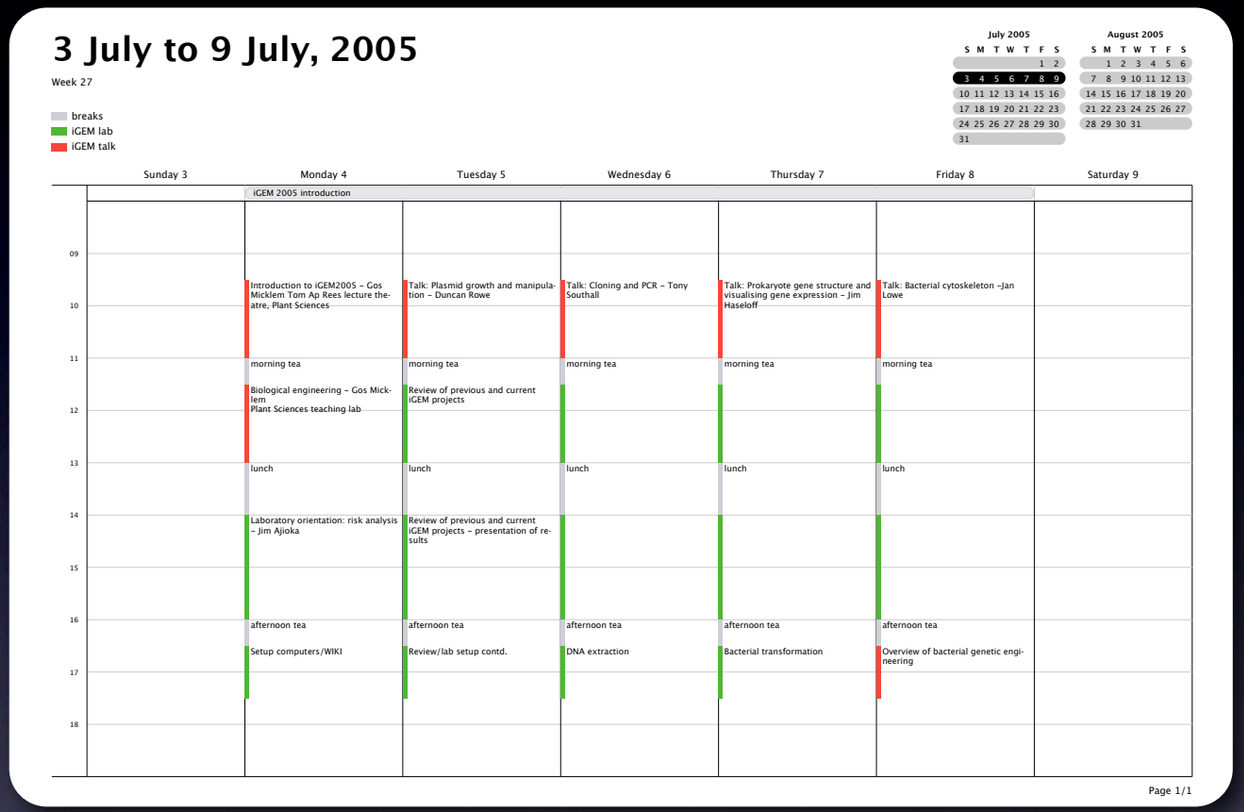
- protocols
- video podcasts
  - lecture material
  - how-to-do-it info
- local sources of expertise
- external links

iGEM2008 recruitment

Biologists & Engineers

Jim Haseloff, Department of Plant Sciences

2 week crash course in Synthetic Biology



3 July to 9 July, 2005

Week 27

breaks
iGEM lab
iGEM talk

July 2005

S	M	T	W	T	F	S
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31						

August 2005

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28	29	30	31			

	Sunday 3	Monday 4	Tuesday 5	Wednesday 6	Thursday 7	Friday 8	Saturday 9
		iGEM 2005 introduction					
09		Introduction to iGEM2005 – Gos Micklem Tom Ap Rees lecture theatre, Plant Sciences	Talk: Plasmid growth and manipulation – Duncan Rowe	Talk: Cloning and PCR – Tony Southall	Talk: Prokaryote gene structure and visualising gene expression – Jim Haseloff	Talk: Bacterial cytoskeleton –Jan Lowe	
10		morning tea	morning tea	morning tea	morning tea	morning tea	
11		Biological engineering – Gos Micklem Plant Sciences teaching lab	Review of previous and current iGEM projects				
12		lunch	lunch	lunch	lunch	lunch	
13		Laboratory orientation: risk analysis – Jim Ajioka	Review of previous and current iGEM projects – presentation of results				
14		afternoon tea	afternoon tea	afternoon tea	afternoon tea	afternoon tea	
15		Setup computers/WIKI	Review/lab setup contd.	DNA extraction	Bacterial transformation	Overview of bacterial genetic engineering	
16							
17							
18							

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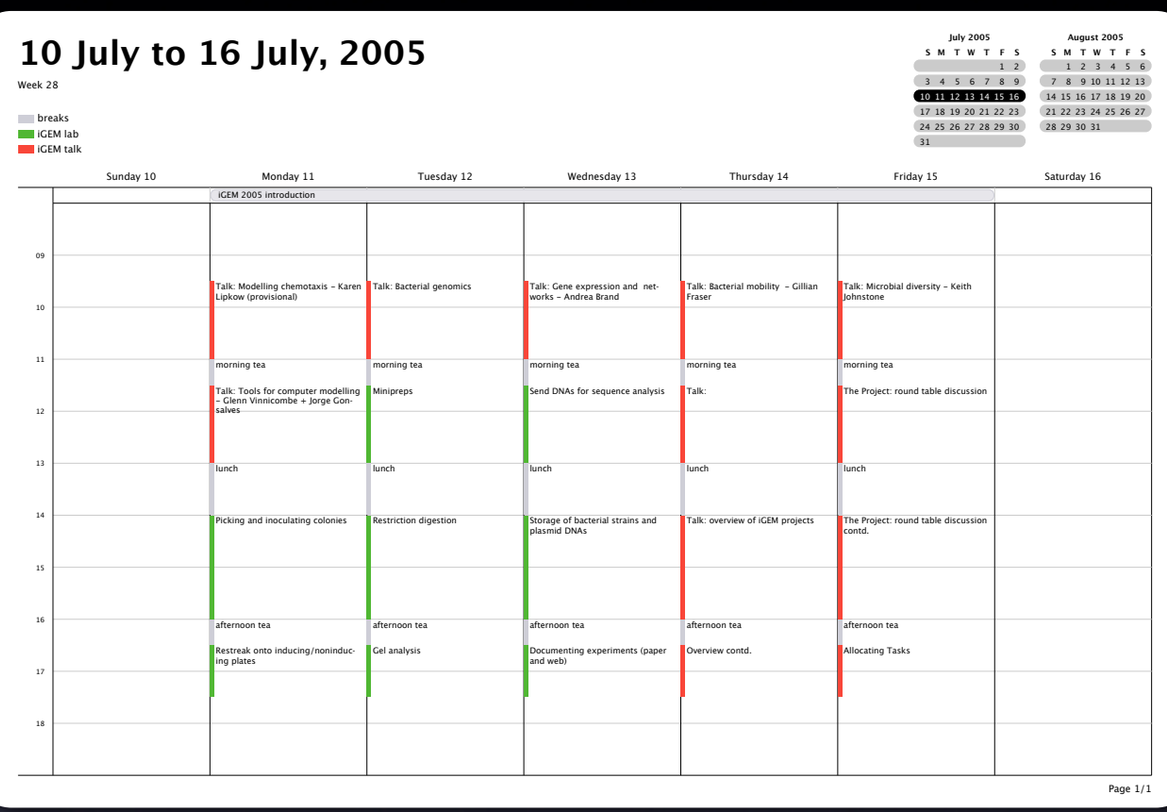

10 July to 16 July, 2005

Week 28

breaks
iGEM lab
iGEM talk

July 2005

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21	22	23	24	25	26	27
28	29	30	31			

	Sunday 10	Monday 11	Tuesday 12	Wednesday 13	Thursday 14	Friday 15	Saturday 16
		iGEM 2005 introduction					
09		Talk: Modelling chemotaxis – Karen Lipkow (provisional)	Talk: Bacterial genomics	Talk: Gene expression and networks – Andrea Brand	Talk: Bacterial mobility – Gillian Fraser	Talk: Microbial diversity – Keith Johnstone	
10		morning tea	morning tea	morning tea	morning tea	morning tea	
11		Talk: Tools for computer modelling – Glenn Vinnicombe + Jorge Gonsalves	Minipreps	Send DNAs for sequence analysis	Talk:	The Project: round table discussion	
12		lunch	lunch	lunch	lunch	lunch	
13		Picking and inoculating colonies	Restriction digestion	Storage of bacterial strains and plasmid DNAs	Talk: overview of iGEM projects	The Project: round table discussion contd.	
14		afternoon tea	afternoon tea	afternoon tea	afternoon tea	afternoon tea	
15		Restreak onto inducing/noninducing plates	Gel analysis	Documenting experiments (paper and web)	Overview contd.	Allocating Tasks	
16							
17							
18							

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July 2008
Lecture course and brain storming

July-August-September: Practical laboratory

Composition of the 2008 Team

Student team:
10 UROP summer studentships + externally funded students

Support:
James Brown, Department of Plant Sciences
James Godman, Department of Plant Sciences
Dr. Duncan Rowe, Department of Genetics
Dr. Alex Kabla, Department of Engineering

Faculty:
Dr. Jim Ajioka, Department of Pathology
Dr. Jim Haseloff, Department of Plant Sciences
Dr. Gos Micklem, Cambridge Computational Biology Institute
Dr. Jorge Goncalves, Department of Engineering
Dr. Lorenz Wernisch, MRC Biostatistics Unit

Benefits

- engineers & biologists working together
- open-ended learning
- project management and teamwork
- national & international scientific exchange
- scientific presentations
- scientific publication
- practical training in Synthetic Biology

iGEM, Synthetic Biology & Me

Graduate Student in Synthetic Biology
Haseloff Lab, Dept. of Plant Sciences

iGEM, Synthetic Biology & Me

Graduate Student in Synthetic Biology
Haseloff Lab, Dept. of Plant Sciences

3rd Yr Engineer
Systems & Control + Biology Options

iGEM, Synthetic Biology & Me

Graduate Student in Synthetic Biology

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iGEM 2005 Cambridge Team Member

3rd Yr Engineer

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iGEM, Synthetic Biology & Me

Graduate Student in Synthetic Biology
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Completed MEng
Electrical Engineering - continued iGEM Project

iGEM 2005 Cambridge Team Member

3rd Yr Engineer
Systems & Control + Biology Options

iGEM, Synthetic Biology & Me

Graduate Student in Synthetic Biology
Haseloff Lab, Dept. of Plant Sciences

iGEM 2006 Ambassador
based at MIT's Registry, supporting 10 teams

Completed MEng
Electrical Engineering - continued iGEM Project

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Synthetic Biology