Synthetic Biology

Introducction to Synthetic Biology

E Navarro
A Montagud
P Fernandez de Cordoba
JF Urchueguía

Overview

- Introduction
- Synthetic biology evolution
- Some examples
- Biology and engineering. The paradigm of synthetic biology.
- Systems biology work
- Synthetic gene regulatory systems

What is synthetic biology?

- Biology: Science of life
- Synthetic: Said from a product produced by industrial methods

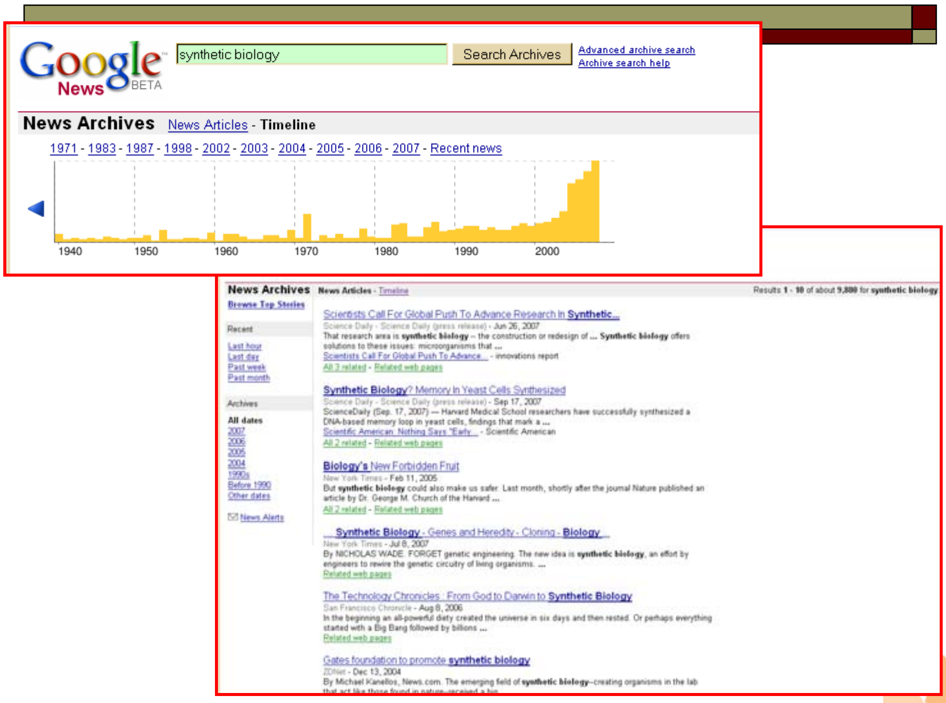
Produce life using artificial methods

Produce a part of a biological system by artificial methods

http://www.syntheticbiology.org

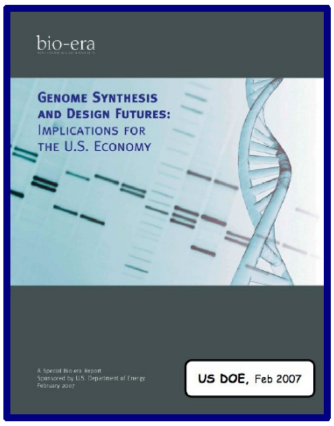
Synthetic Biology History

- 1953 –DNA discovery by Watson and Crick
- 1961 – Discovery of mathematical logic in gene regulation
- 1970 – First gene synthesized from scratch (alanine tRNA)
- 1978 – Nobel prize awarded to Werner Arber, Daniel Nathans and Hamilton Smith for the discovery of restriction enzymes
- 1978 (Boyer at UCSF) – A synthetic version of the human insulin gene was constructed and inserted into the bacterium *E. coli*.
- 1980 – Kary Mullis invents PCR
- 1991 – Affymetrix chip-based oligonucleotide synthesis
- 2003 – Creation of standardized parts libraries at MIT




Some think bank with tradition.
We think 150 years of innovation.
America drops, Asia shops
Special Report
Synthetic biology
Life 2.0
The new science of synthetic
Forbes
Architect of Life
Drew Endy aims to reinvent the biotechnology industry.
Synthetic biology
Playing demigods
Synthetic biology needs to be monitored, but not stifled
Economist, Aug 2006

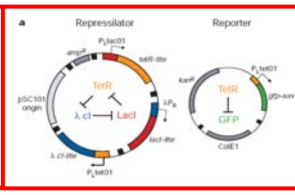

SCIENTIFIC AMERICAN.com
CURRENT ISSUE HIGHLIGHTS:
Uncommon Scents
The Dark Ages of the Universe
Technology | Space & Physics | Health | Mind | Nature | Biology | Archaeology &
News | Blog | In Focus | Ask the Experts | Podcasts | Gallery | Recreations | Magazine | SA
October 23, 2006
FEATURE ARTICLES
June 2006 issue
BIOTECHNOLOGY
Engineering Life: Building a Fab for Biology
Principles and practices learned from engineering successes can help transform biotechnology from a specialized craft into a mature industry
By The Bio FAB Group
TheScientist
MAGAZINE OF THE LIFE SCIENCES
Volume 20 | Issue 1 | Page 36
FEATURE
Is This Life?
Just What Is Synthetic Biology?
WIRED MAGAZINE
Issue 13.01 - January 2005
Life, Reinvented
Designs on life
Nature, Nov 2005
Biologists are crafting libraries of interchangeable DNA parts and assembling them inside microbes to create programmable, living machines
SYNTHETIC LIFE
Scientific American, May 2004

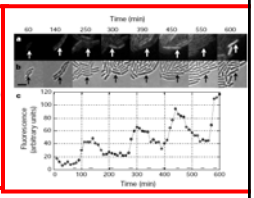


A synthetic oscillatory network of transcriptional regulators

Michael B. Elowitz & Stanislas Leibler

Departments of Molecular Biology and Physics, Princeton University, Princeton, New Jersey 08544, USA



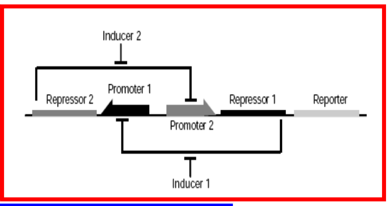


More than 600 cites!!

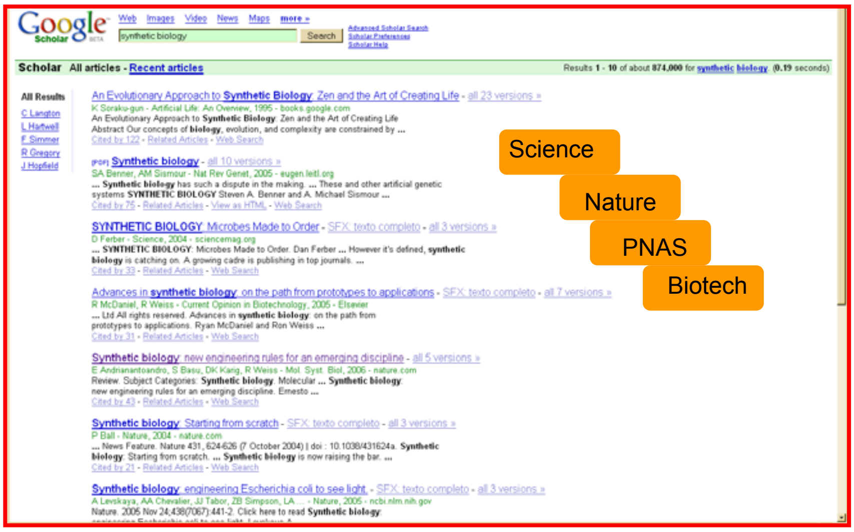
Construction of a genetic toggle switch in *Escherichia coli*

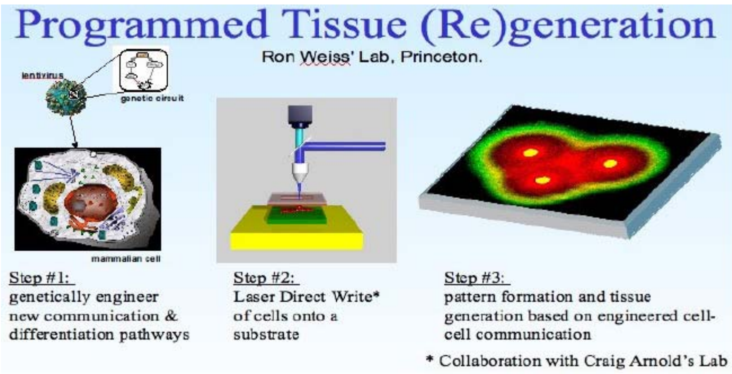
Timothy S. Gardner*†, Charles R. Cantor* & James J. Collins*†

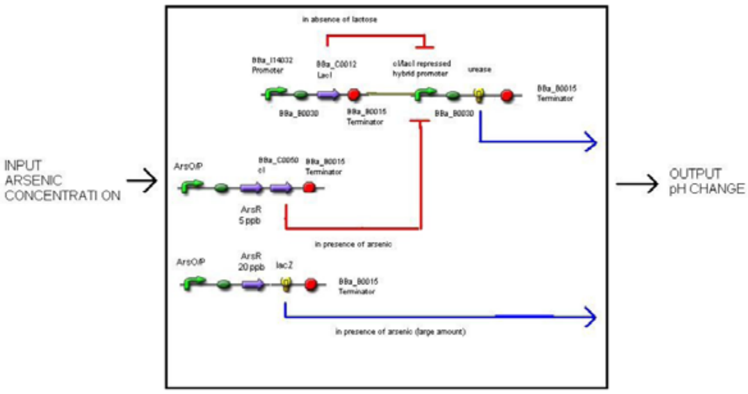
* *Department of Biomedical Engineering, † Center for BioDynamics and ‡ Center for Advanced Biotechnology, Boston University, 44 Cummington Street, Boston, Massachusetts 02215, USA*



More than 500 cites!!

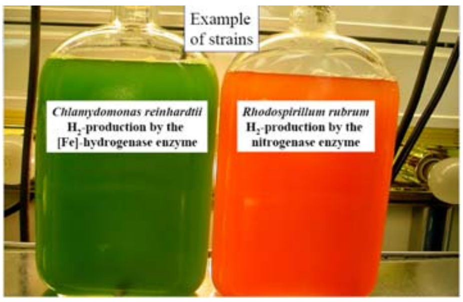

Google Scholar
Web Images Video News Maps more »
synthetic biology
Search
Scholar All articles - Recent articles
All Results
C Langton
L Hartwell
F Simmer
R Gregory
J Hopfield
An Evolutionary Approach to Synthetic Biology: Zen and the Art of Creating Life
Synthetic biology
SYNTHETIC BIOLOGY: Microbes Made to Order
Advances in synthetic biology: on the path from prototypes to applications
Synthetic biology: new engineering rules for an emerging discipline
Synthetic biology: Starting from scratch
Synthetic biology: engineering Escherichia coli to see light
Science
Nature
PNAS
Biotech


Programmed Tissue (Re)generation
Ron Weiss' Lab, Princeton.
lentivirus
genetic circuit
mammalian cell
Step #1:
genetically engineer new communication & differentiation pathways
Step #2:
Laser Direct Write* of cells onto a substrate
Step #3:
pattern formation and tissue generation based on engineered cell-cell communication
* Collaboration with Craig Arnold's Lab

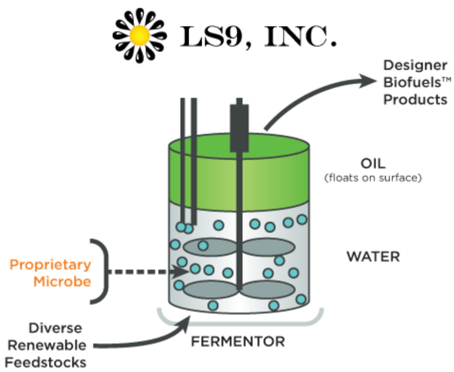


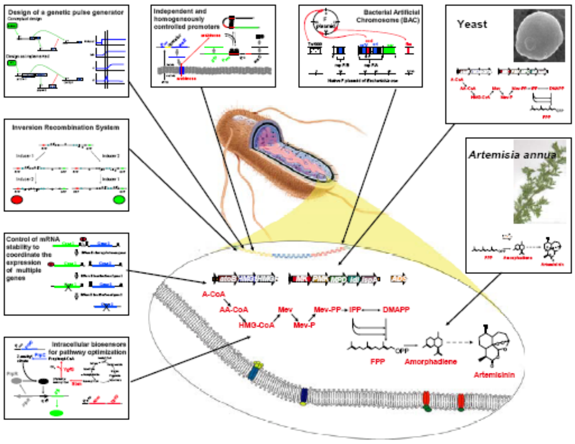
Arsenic Biosensor, iGEM 2006, Edinburgh Team

Biofuels



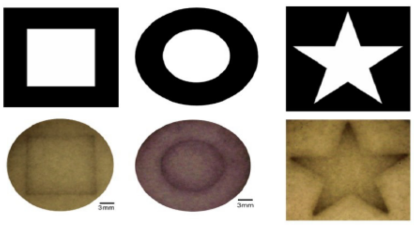
Green algae and photosynthetic bacteria could operate with a solar energy conversion efficiency to H2as high as ~10% and ~6%, respectively, provided that specific barriers are overcome.





Drugs from bugs

JD keasling

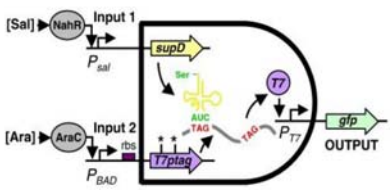


Bacterial edge detector

J Tabor

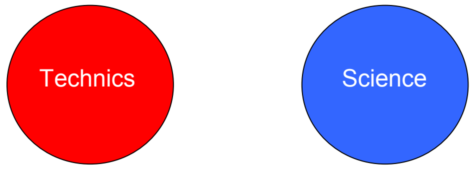
And gate

JC Anderson



What is engineering?

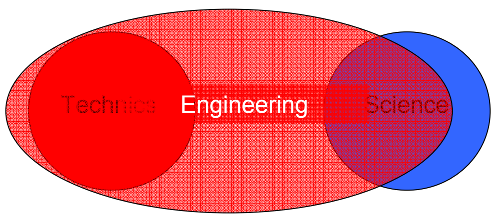
Engineer → Engine → Ingenious



A model is not true or false, a model is more or less useful

What is engineering?

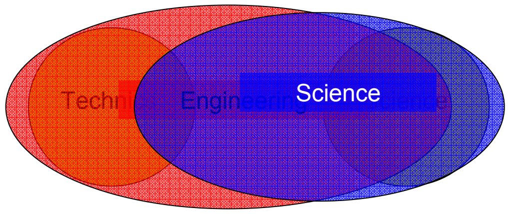
Engineer → Engine → Ingenious



A model is not true or false, a model is more or less useful

What is engineering?

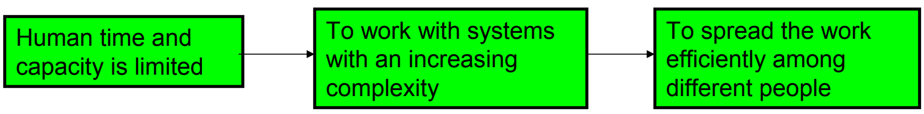
Engineer → Engine → Ingenious



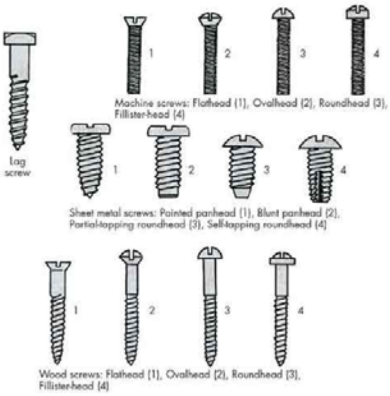
A model is not true or false, a model is more or less useful

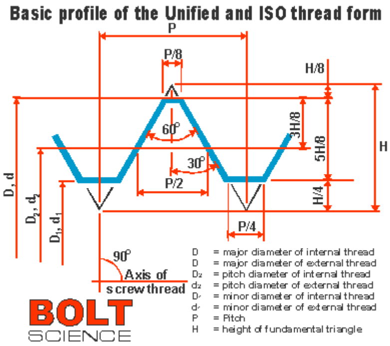
Advances in engineering

- The first change in engineering was the scientific methods and the development of basic science.
- The three basic changes which allows the industrial revolution and the increase in complexity of the different engines were:
  - Standardization
  - Decoupling
  - abstraction



Standaritzation





Interoperability, reusability

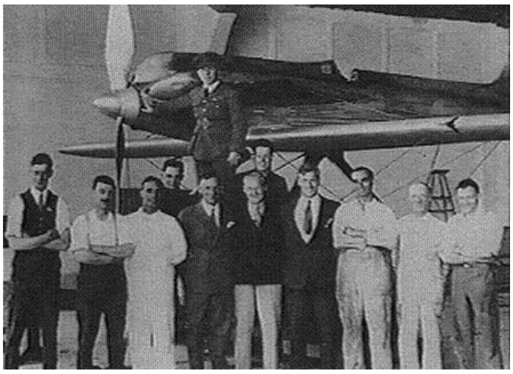
Decoupling

Rules insulating design process from details of fabrication

Abstraction/modularity

- Biology is one of the most complicated machinery that we know
- Synthetic biology applies all this procedures to biology.
- Mechanical Engineering, Electrical Engineering and electronics were all at the stage where they were “too complicated”.

Science ⟷ Engineering



Why now?

Recombinant DNA
Cloning-Directed evolution

High throughput technologies
(NMR, microarrays, automation)

DNA sequencing
DNA synthesis

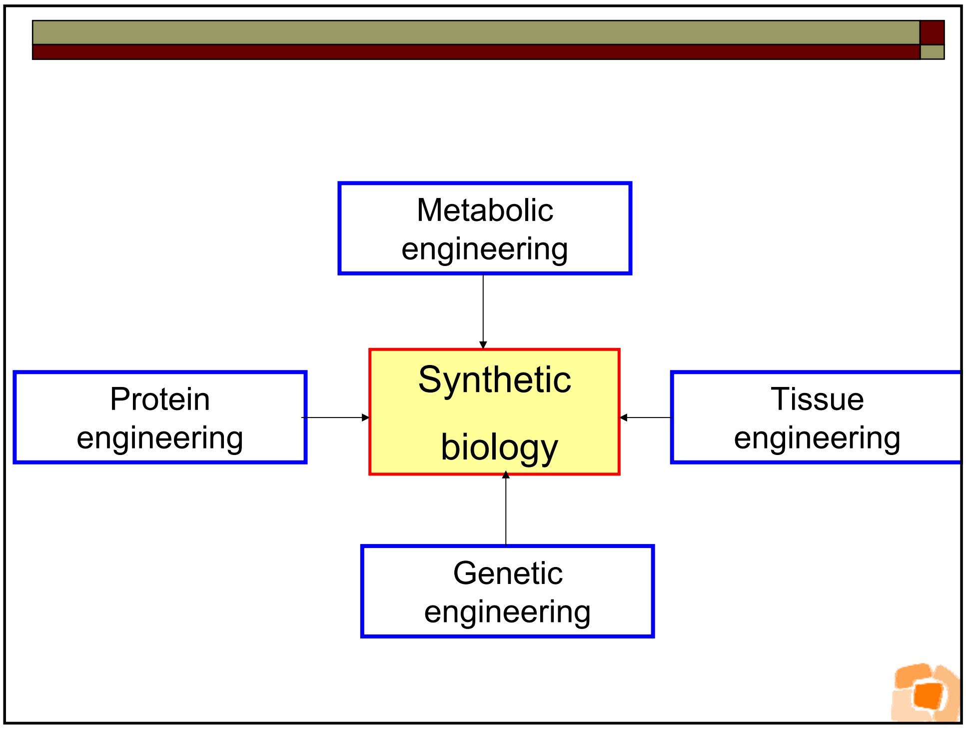
Computational
Modelling

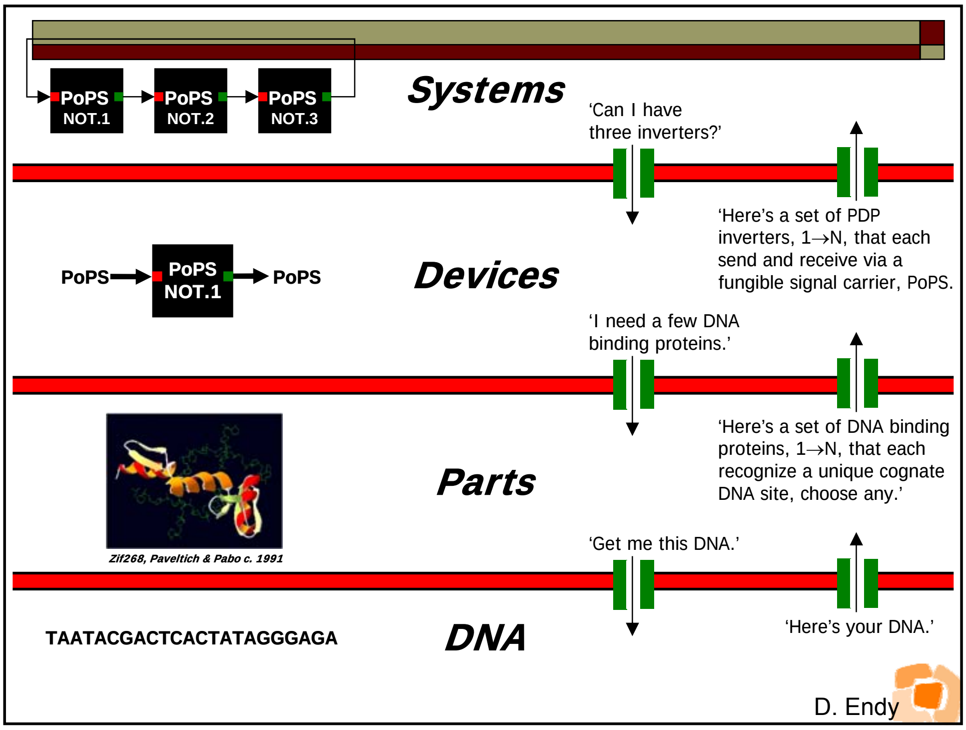
Protein engineering

Tissue engineering

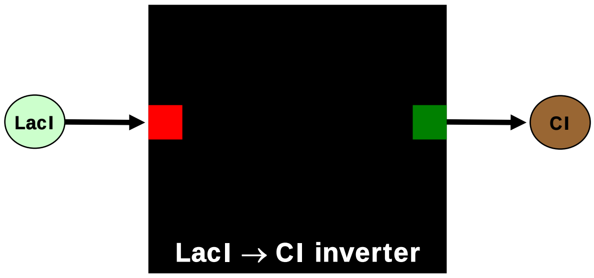
Genetic engineering

Metabolic engineering

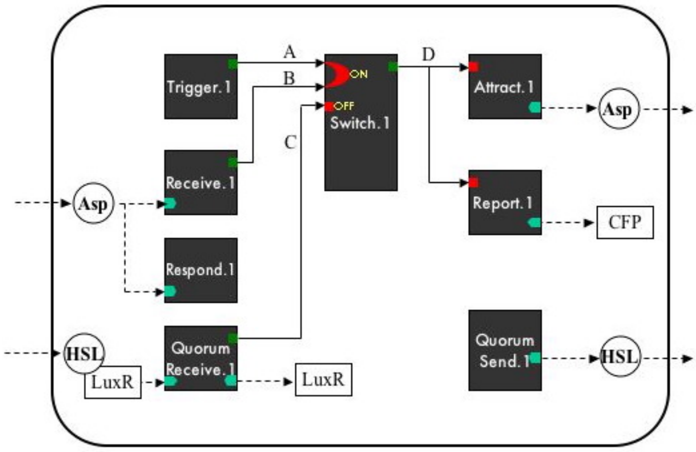

Metabolic engineering
Protein engineering
Synthetic biology
Tissue engineering
Genetic engineering


PoPS NOT.1
PoPS NOT.2
PoPS NOT.3
Systems
'Can I have three inverters?'
'Here's a set of PDP inverters, 1→N, that each send and receive via a fungible signal carrier, PoPS.'
PoPS
PoPS NOT.1
PoPS
Devices
'I need a few DNA binding proteins.'
'Here's a set of DNA binding proteins, 1→N, that each recognize a unique cognate DNA site, choose any.'
Zif268, Paveltich & Pabo c. 1991
Parts
'Get me this DNA.'
'Here's your DNA.'
TAATACGACTCACTATAGGGAGA
DNA
D. Endy

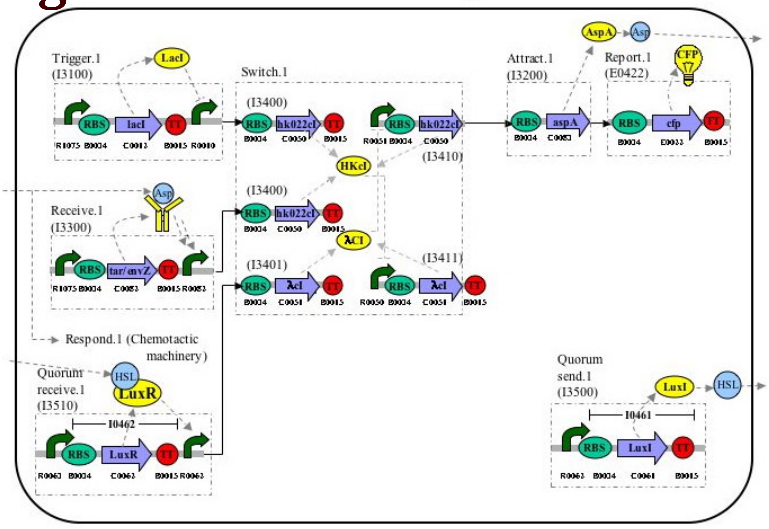
Devices



Device-Level System Diagram



Parts- and Device-Level System Diagram

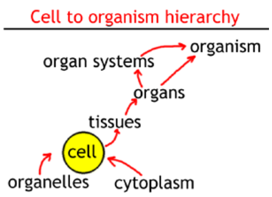


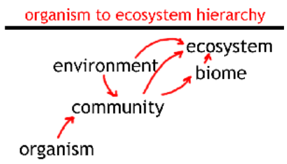
Standariztion

- Standard cell host
- Standard culture conditions
- Standard measurement
- Standard functional composition
- Standard DNA composition

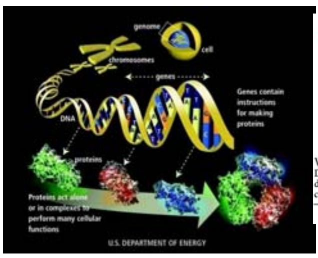
Can we work in such a way?

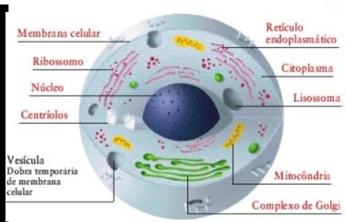
1- Biology is hierarchical

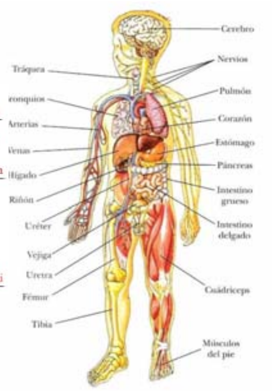




2- Biology is Modular

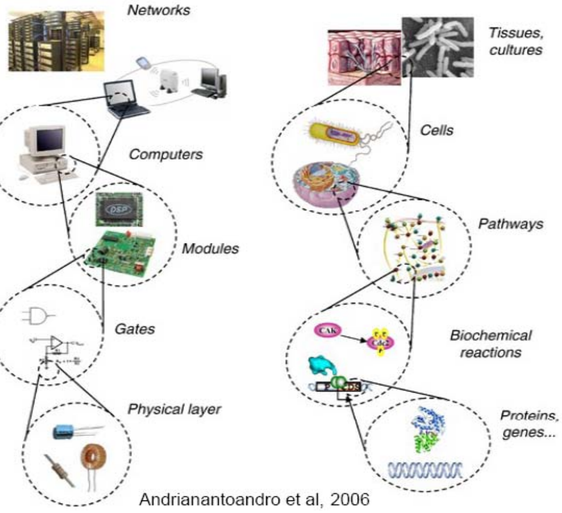






Hierarchy and Modular (recurrent) organization in some standard way allows biology to be understandable and synthetic biology to be possible.

In some way some process of evolution are based on this: adding properties to a system



Andrianantoandro et al, 2006

Applications

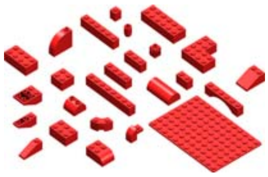
- Biofuels
- Biomaterials
- Biosensors
- Drug development
- Nanotechnologies.

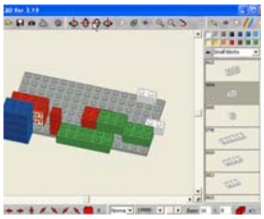
iGEM

iGEM - The international Genetically Engineered Machine competition

iGEM addresses the question: Can simple biological systems be built from standard, interchangeable parts and operated in living cells? Or is biology simply too complicated to be engineered in this way?

Pure engineering approach





It began in 2003 with several student from MIT trying to play with an oscillator

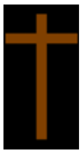
Last year more than 50 teams from universities all over the world participate in that competition

And sometimes they can purely engineer biology

Differences with standard engineering

Evolution and mutation

Death

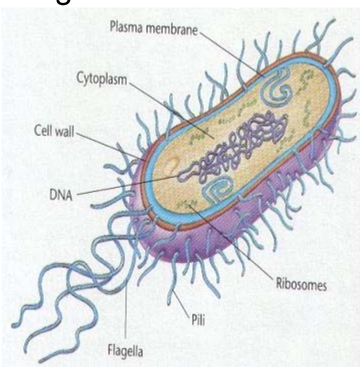


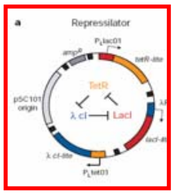
Noise interference cross talk

If we can solve this problems we can have the most powerful chemical factory

Chassis

All this machinery is going to be inserted in a living organism





To insert a system inside an organism could produce an undesired interference between both

Madigan, M.T. Martinko, and J. Parker. 2003.
Biology of Microorganisms.
Prentice Hall Upper Saddle River, NJ

Systems Biology

Attempts to describe the living systems as a hole.

Life not depend only on the expression of a single gen but also in the combination of expressions of different gens.

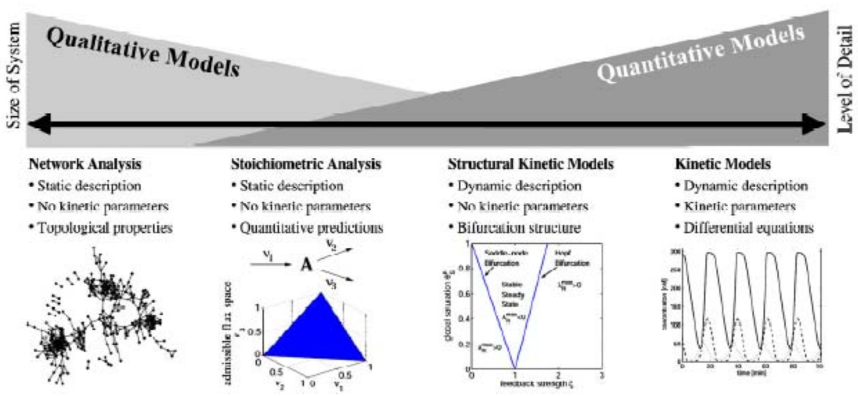
Life is a very complex machine → As we increase the complexity of a system it depends more in the protocols of its interactions than on its individual components

Systems biology has developed several mathematical tools in order to be able to improve the knowledge of biological systems

It is useful for synthetic biology

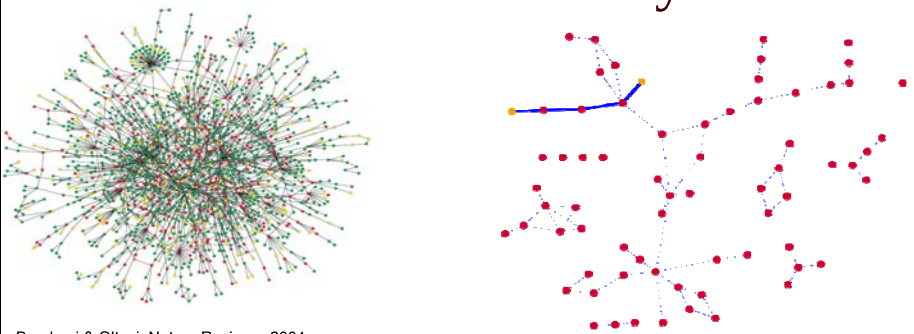
- It "understand" the chassis
- Model biological systems
- Databases and information

Models in systems biology

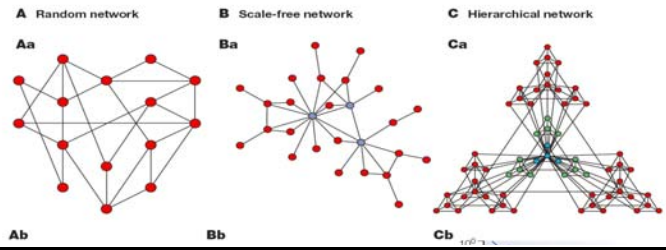


Steuer R. 2007, *Photochemistry*

Network analysis



Barabasi & Oltvai, Nature Reviews, 2004

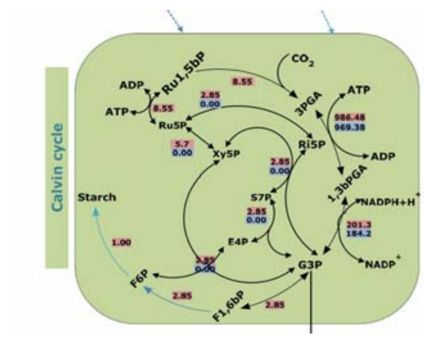


Stoichiometric analysis

	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	R_{10}	V_m	V_{growth}	A_{up}	D_{up}	F_{up}	H_{up}
A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B	1	−1	0	0	−1	0	0	−1	0	0	0	−1	0	0	0	0
C	0	2	−1	0	0	0	0	0	0	0	0	0	0	0	0	0
D	0	0	1	−1	0	1	0	0	0	0	0	0	0	0	0	0
E	0	0	0	0	1	−1	0	0	0	0	0	0	0	0	0	0
F	0	0	0	0	0	1	−1	0	0	0	0	0	0	0	0	0
G	0	0	0	0	0	0	0	1	−1	0	0	0	0	0	0	0
H	0	0	0	0	0	0	0	0	1	−1	0	−2	0	0	0	0
I	0	0	0	0	1	0	0	0	0	0	−1	0	0	0	0	0
$A_{external}$	−1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
$D_{external}$	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0
$F_{external}$	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0
$H_{external}$	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1

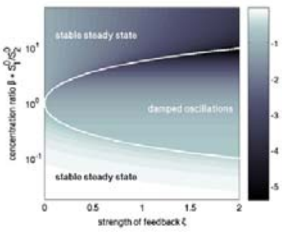
$$\times \begin{bmatrix} R_1 \\ R_2 \\ R_3 \\ R_4 \\ R_5 \\ R_6 \\ R_7 \\ R_8 \\ R_9 \\ R_{10} \\ V_m \\ V_{growth} \\ A_{up} \\ D_{up} \\ F_{up} \\ H_{up} \end{bmatrix} = \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{bmatrix}$$

FBA
EFM
ROOM



Structural kinetic models

- describes the possible dynamics of metabolic systems, as well as the stability and robustness of metabolic states, and concomitantly identifies the relevant interactions and parameters governing the dynamic properties of the system.



Bridge between structural models and dynamic models

Steuer R. 2007, *Photochemistry*

Kinetic Models

- They need more parameters than the other approach.
- Nowadays it is not possible to use them to describe the whole system, but they could give accurate information of a part of the system.

Kinetic Models

The basic models are based on non linear diferential equations.

Hypotesis:

- The diffusion is not importat (not explicit spatial dependence)
- The variables are a continuous functions on time (no stochasticity).

Regulatory model

$$\frac{d\ Z}{dt} \qquad \frac{\frac{S^{n}}{K^{n}}}{1 \quad \frac{S^{n}}{K^{n}}} \qquad Z$$

- M has a value of 0 or 1
- K the Hill constant (the value of the signal that yield 50% response)
- n the Hill coefficient (the slope of the response)
- Beta is the decay constant of the reporter protein
- Gamma is the basal gene expresion
- Alpha sigma dependent gene expresion (gamma=a alpha)

Computer tools

- A common lenguage has been developed to be able to make the work reusable allowing the comunication between research groups:

SBML http://www.sbml.org

cellML http://www.cellml.org

Functions

- **Document and store** the structure of physiological and biochemical reaction networks
- Store values and math associated with the network dynamics
- Do **not** provide commands or instructions for building, simulating, or analyzing networks.
- As mozilla is able to read html, there are several programs able to read these languages (mark-up languages).

Example (in SBML)

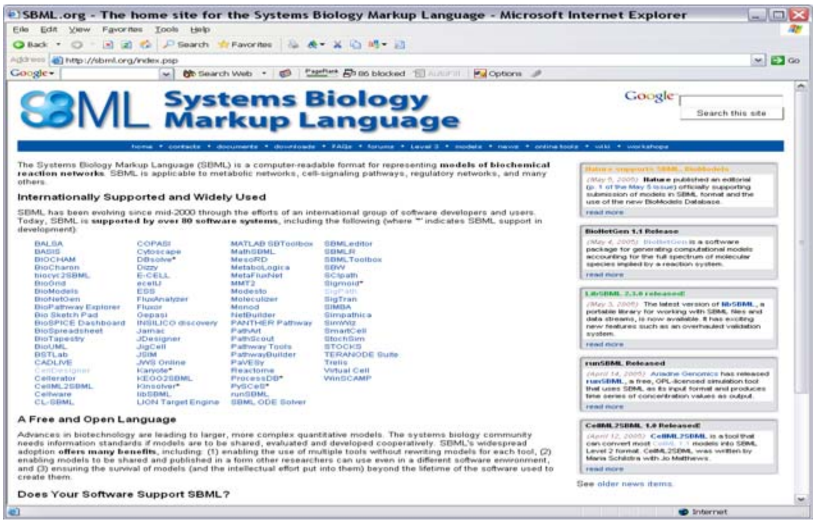
```
< reaction id="R1" reversible="false" >
    < listOfReactants >
        < speciesReference species="Sout" />
    </ listOfReactants >
    < istOfProducts >
        < speciesReference species="Sin" />
    </ listOfProducts >
    < kineticLaw >
        <math xmlns=http://www.w3.org/1998/Math/MathML >
                < ci > v_1 </ ci >
        </ math >
        < listOfParameters >
                < parameter id="v_1" value="100" />
        </ listOfParameters >
    </ kineticLaw >
</ reaction >
```

Characteristics of SBML

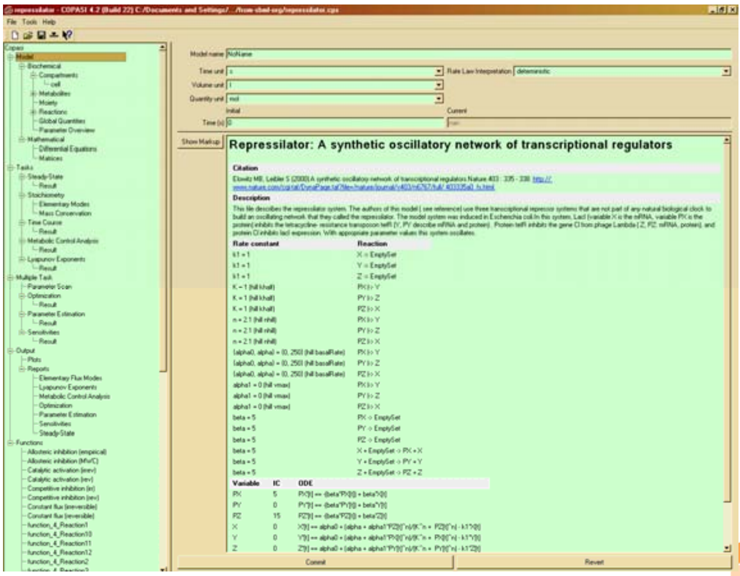
- Many top-level types, little nesting
  - Units, Compartment, Species, Parameter, Reaction, Rule, Function, Event
- Non-modular structure
  - Next SBML ‘Level’ (3) will introduce modularity
- Emphasis on reactions
- Some math implicit
  - Explicit rate equations; implicit integration
  - Implicit concentration conversion between compartments
- Compartments are physical containers for species
  - Spatial dimensions (volume, surface)

Characteristics of CellML

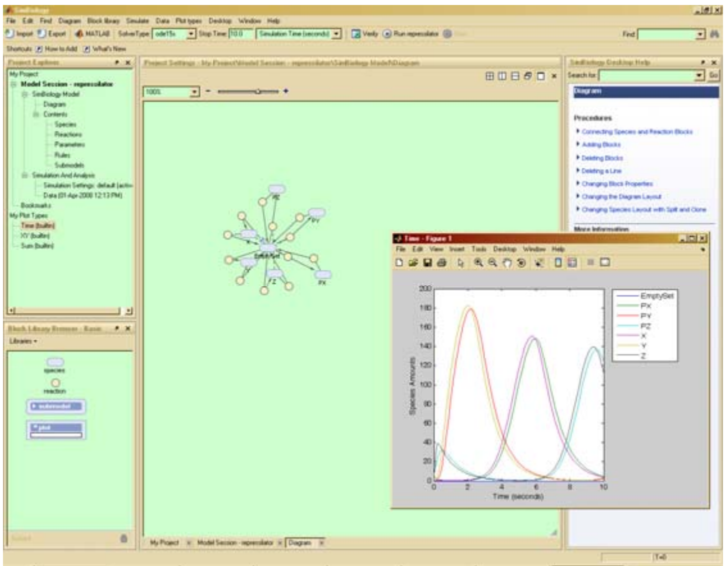
- Few top-level types, extensive nesting
  - Units, Component, Connection, Group, Import
- Highly modular structure
  - Nesting of components in groups
- Emphasis on model organization
- All math explicit
  - Explicit rate equations; explicit integration
  - Explicit concentration conversion in transport processes
- Groups may specify physical or conceptual containment
  - No spatial dimensions



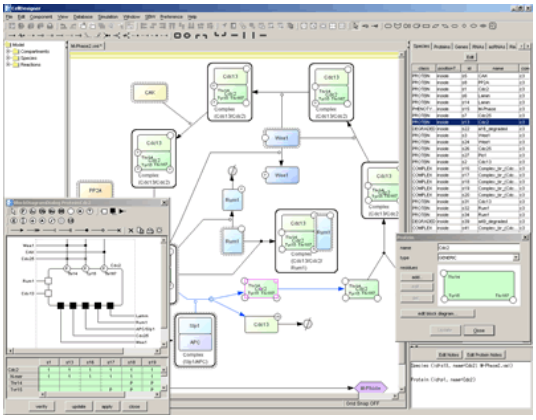
Copasi



Simbiology



Cell designer



Databases

KEGG

PUMA2 -- Evolutionary Analysis of Metabolism

InterPro

BRENDA

BioModels

NCGR

swissprot

UniProt

United States
National Library of Medicine
National Institutes of Health

Single Nucleotide Polymorphism

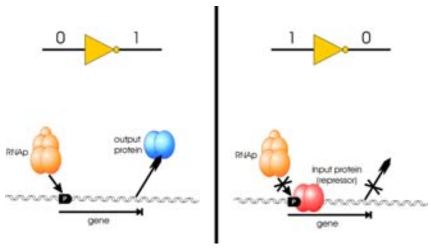
OMIM
Online Mendelian Inheritance in Man

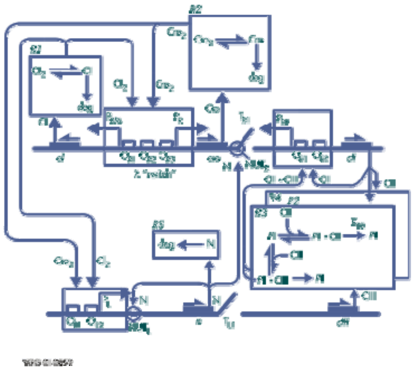
Johns Hopkins University

METACYC™

http://www3.oup.co.uk/nar/database

Regulatory networks





Modeling life as an information system

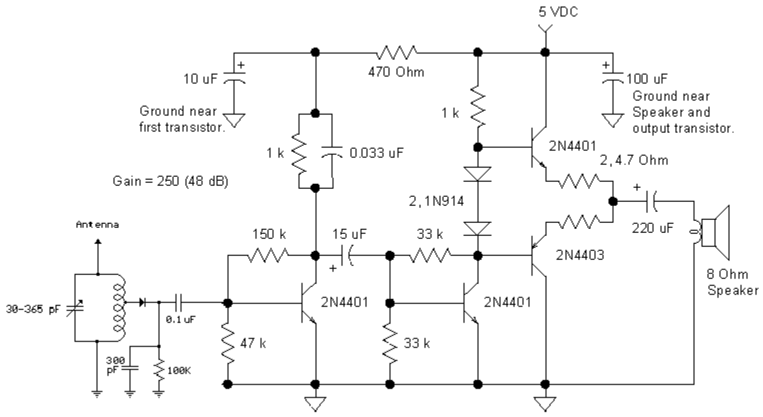
http://www.ee.princeton.edu/people/Weiss.php

How Intel Engineers Cope

Complex man-made devices are modeled and designed on multiple levels, each level may use different modeling techniques:

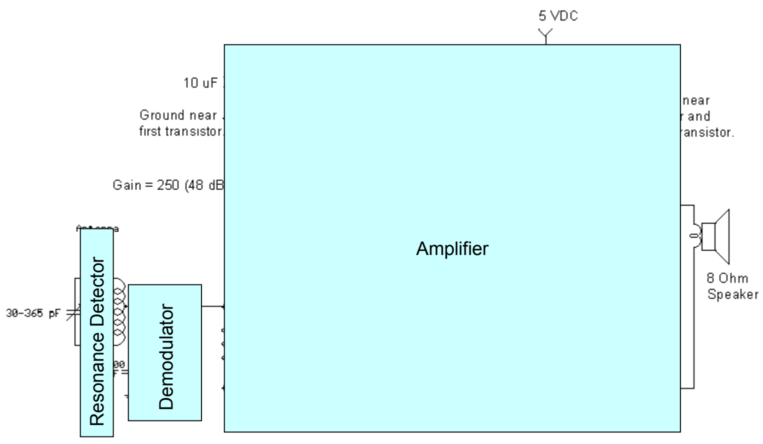
Transistor Characteristics	Fundamental Protein Chemistry
↓	↓
Basic Logic Gates	Basic Enzyme Rate Characteristics
↓	↓
Small Gate Modules	Small Enzyme Motifs
↓	↓
Hierarchy of functional modules	Hierarchy of functional modules
↓	↓
Top Level Module	Top Level Module

Functional Motif Identification



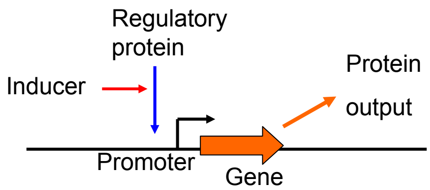
27 components

Functional Motif Identification



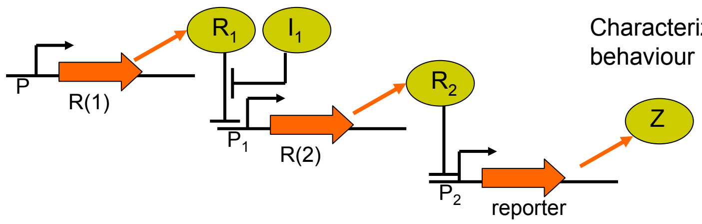
$$\frac{d[Z]}{dt} = \gamma + \frac{\alpha\left(\frac{S^n}{K^n}\right)^{\mu}}{1+\left(\frac{S^n}{K^n}\right)} - \beta[Z] \quad \xrightarrow[\text{State}]{\text{Steady}} \quad [Z]_{ss} = \frac{\alpha}{\beta} + \left(a + \frac{\alpha\left(\frac{S^n}{K^n}\right)^{\mu}}{1+\left(\frac{S^n}{K^n}\right)} \right)$$

$$S = \frac{[R_T]}{K_i^{n_i} + [I]^{n_i}}$$



Regulatory protein	Regulated promoters	Inducer
TetR	$P_{tet,}P_{LtetO}$	tetracycline
LacI	P_{lac}, P_{lacO}, P_{trc}	Lactose, IPTG
cI	P_L,P_R, P_{RM},P_{luxOR}	
LuxR	P_{lux}, P_{luxOR}	AHL

Two steps cascade



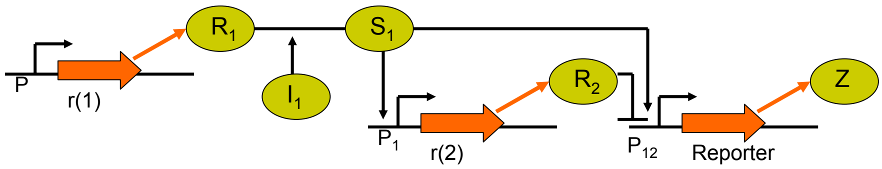
Characterize the behaviour R_2/P_2

$$\frac{d[R_2]}{dt} = a_1\alpha_1 + \frac{\alpha_1\left(\frac{[I_1]^n}{K_1{}^n}\right)^{\mu}}{1+\left(\frac{[I_1]^n}{K_1{}^n}\right)} - \beta_2[R_2] \longrightarrow [R_2] = \frac{\alpha_1}{\beta_2}\left(a_1 + \frac{\left(\frac{[I_1]^n}{K_1{}^n}\right)^{\mu}}{1+\left(\frac{[I_1]^n}{K_1{}^n}\right)}\right)$$

$$\frac{d[Z]}{dt} = a_2\alpha_2 + \frac{\alpha_2}{1+\left(\frac{[R_2]^n}{K_2{}^n}\right)} - \beta[Z] \qquad [Z] = \frac{\alpha_2}{\beta}\left(a_2 + \frac{1}{1+\left(\frac{[R_2]^n}{K_2{}^n}\right)}\right)$$

Rosenfeld et al. Science 307 2005

Pulse generating network



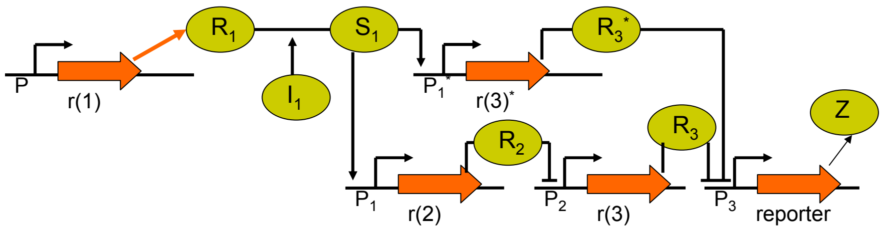
$$\frac{d[R_2]}{dt} = \frac{\alpha_1 s^{n_1}}{1+s^{n_1}} - \beta_2[R_2] \qquad [R_2]_{ss} = \frac{\alpha_1}{\beta_2}H$$

$$H = \frac{s^{n_1}}{1+s^{n_1}};\, s = \frac{S_1}{K_1}$$

$$\frac{dZ}{dt} = \frac{\alpha_{12}}{1+\left(\frac{[R_2]}{K_2}\right)^{n_2}}\frac{s^{n_1}}{1+s^{n_1}} - \beta[Z] \qquad [Z]_{ss} = \frac{\alpha_{12}}{d}\frac{K_2^{n_2}H}{K_2^{n_2}+[R_2]_{ss}^{n_2}}$$

Basu et al. PNAS 2004

Concentration band detector



$$[Z]_{ss} = \frac{\alpha_3}{\beta} \frac{K_3^{n_3}}{K_3^{n_3} + ([R_3]_{ss} + [R_3^*]_{ss})^{n_3}}$$

$$[R_2]_{ss} = \frac{\alpha_1}{\beta_2} \frac{s^{n_1}}{1+s^{n_1}}$$

$$[R_3]_{ss} = \frac{\alpha_2}{\beta_3} \frac{K_2^{n_2}}{K_2^{n_2} + [R_2]_{ss}^{n_2}}$$

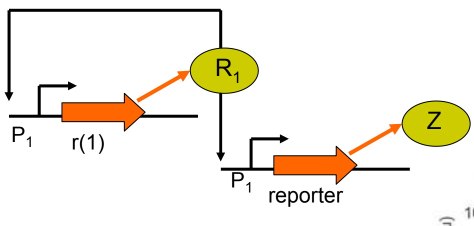
$$[R_3^*]_{ss} = \frac{\alpha_1^*}{\beta_3} \frac{s^{n_1}}{1+s^{n_1}}$$

$$S_{low} = K_1; S_{high} = K_1 \sqrt[n_2]{\frac{\alpha_2 K_2}{\alpha_1 - \beta_2 K_2}}$$

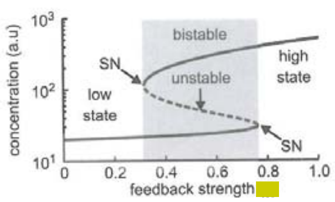
$$\alpha_1 > 2K_2\beta_2$$

Basu et al. PNAS 2004

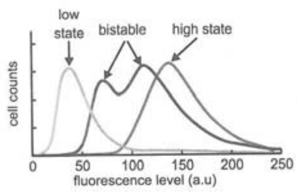
Bistable Network



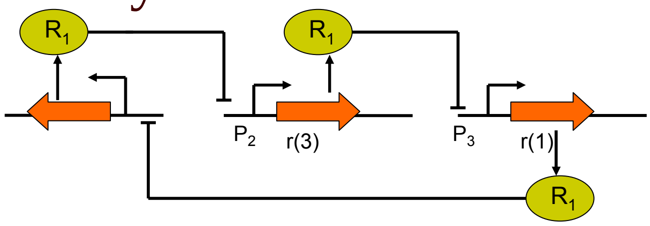
$$\frac{d[R_1]}{dt} = a\alpha + b\frac{\alpha[R_1]^{n_1}}{K^{n_1}[R_1]^{n_1}} - \beta[R_1]$$



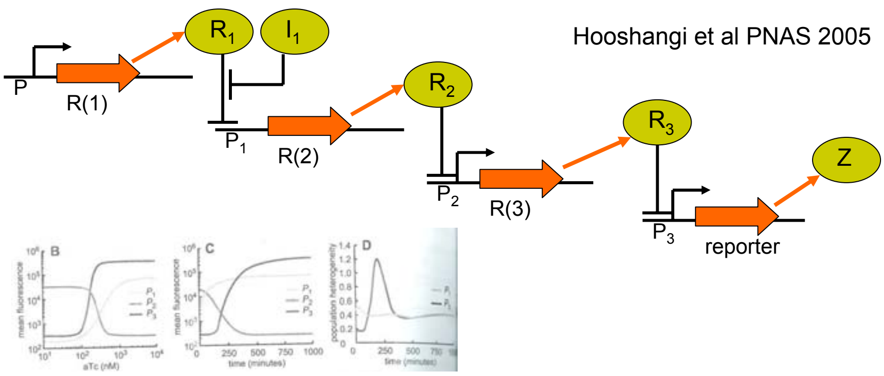
a=0.1; n=3; k=5



Oscilatory networks



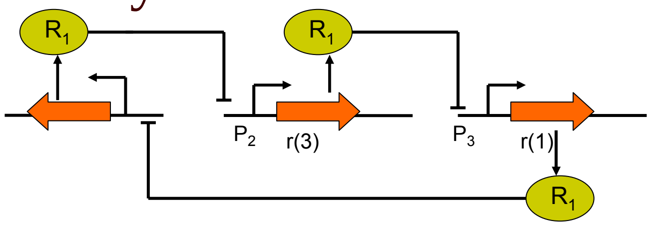
Elowitz et al, Nature 2000



Hooshangi et al PNAS 2005

The Hill coeficient of the system increases as we increase the numer of cascade systems → Increase the sensitivity to the input signal

Oscilatory networks

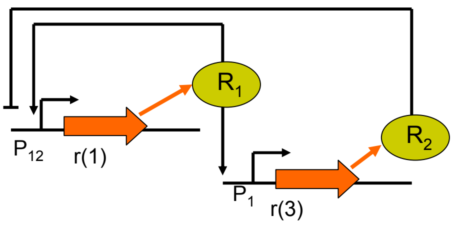


$$\frac{d[M]}{dt} = a\alpha_{tr} + \frac{\alpha_{tr}\left(\frac{S^n}{K^n}\right)^{\mu}}{1+\frac{S^{\mu}}{K^{\mu}}} - \beta_M[Z] \qquad \longrightarrow \qquad \frac{dm_i}{d\tau} = a\kappa + \frac{\kappa}{1+r_j^n} - m_i$$

$$\frac{d[Z]}{dt} = \alpha_{tr}[M] - \beta[Z] \qquad\qquad \frac{dr_i}{dt} = \varepsilon(m_i - r_i)$$

Separation of transcription and translation introduces response delays

Robust oscillatory networks



Vilar et al. PNAS 2002

Think about this…

Science

Biological systems are very complex.

Study of simple parts of that systems and increase difficulty gradually.

Engineering

Some of the engineering knowledge is present on simple biological systems.

Be able to design and build simple machines with a desired function.

Bibliography

- This presentation has been partially based on:
  - V. Rouilly introduction course in synthetic biology. http://openwetware.org/wiki/Imperial_College/Courses/Spring2008/Synthetic_Biology
  - M Kærn and R Weiss: Synthetic gene regulatory systems. System modeling in cell biology from concepts to nuts and bolts.
- Additional material:
  - D Endy. Foundations for engineering biology. doi:10.1038/nature04342
  - SA Benner, A M Sismour. Synthetic Biology doi:10.1038/nrg1637
  - E Andrianantoandro, S Basu1, DK Karig, R Weiss. Synthetic biology: new engineering rules for an emerging discipline. doi:10.1038/msb4100073.
  - Extreme genetic engineering, an introduction to synthetic biology. ETC group report.
  - R McDaniel, R Weiss. Advances in synthetic biology: on the path from prototypes to applications. DOI:10.1016/j.copbio.2005.07.002.
  - M Heinemann, S Panke.Synthetic biology—putting engineering into biology. doi:10.1093/bioinformatics/btl469.
  - A Kuznetsov, M Kærn, N Kopell. Syncrony in a population of hysteresis-based genetic oscillators. SIAM J appl. Math. 65:392-425, 2005.
  - www.openwetware.org

Bibliography

- Some general additional information:

  - PL Luisi,F Ferri, P Stano. Approaches to semi-synthetic minimal cells: a review. Doi: 10.1007/s00114-005-0056-z.
  - FJ Isaacs, DJ Dwyer, JJ Collins. RNA synthetic biology. doi:10.1038/nbt1208.
  - GM church. From systems biology to synthetic biology. doi:10.1038/msb4100007.
  - MA O'Malley, A Powell, JF Davies, J Calvert. Knowledge-making distinctions in synthetic biology. Doi:10.1002/bies.20664.
  - D Sprinzak, MB Elowitz. Reconstruction of genetic circuits. doi:10.1038/nature04335.
  - CA Voigt. Genetic parts to program bacteria. doi: 10.1016/j.copbio.2006.09.001.
  - LY Chan, S Kosuri, D Endy. Refactoring bacteriophage T7. doi:10.1038/msb4100025.
  - P Ball. Synthetic biology for nanotechnology. http://dx.doi.org/10.1088/0957-4484/16/1/R01.
  - J Pleiss. The promise of synthetic biology. doi: 10.1007/s00253-006-0664-3
  - J Pereto, J Catala, The renaissance of synthetic biology. doi:10.1162/biot.2007.2.2.128
  - H Saito et al.Towards constructing synthetic cells: RNA/RNP evolution and cell-free translational systems in giant liposomes. http://ieeexplore.ieee.org/xpls/abs_all.jsp?arnumber=4420868 .
  - SP Radko et al.The synthesis of artificial genome as the basis of synthetic biology. doi:10.1134/S1990750807040014