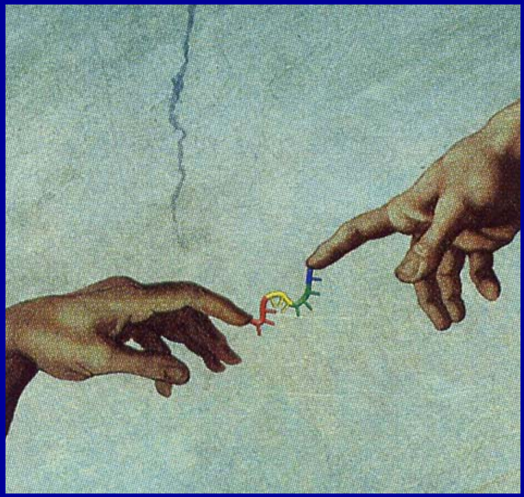
Imperial College London

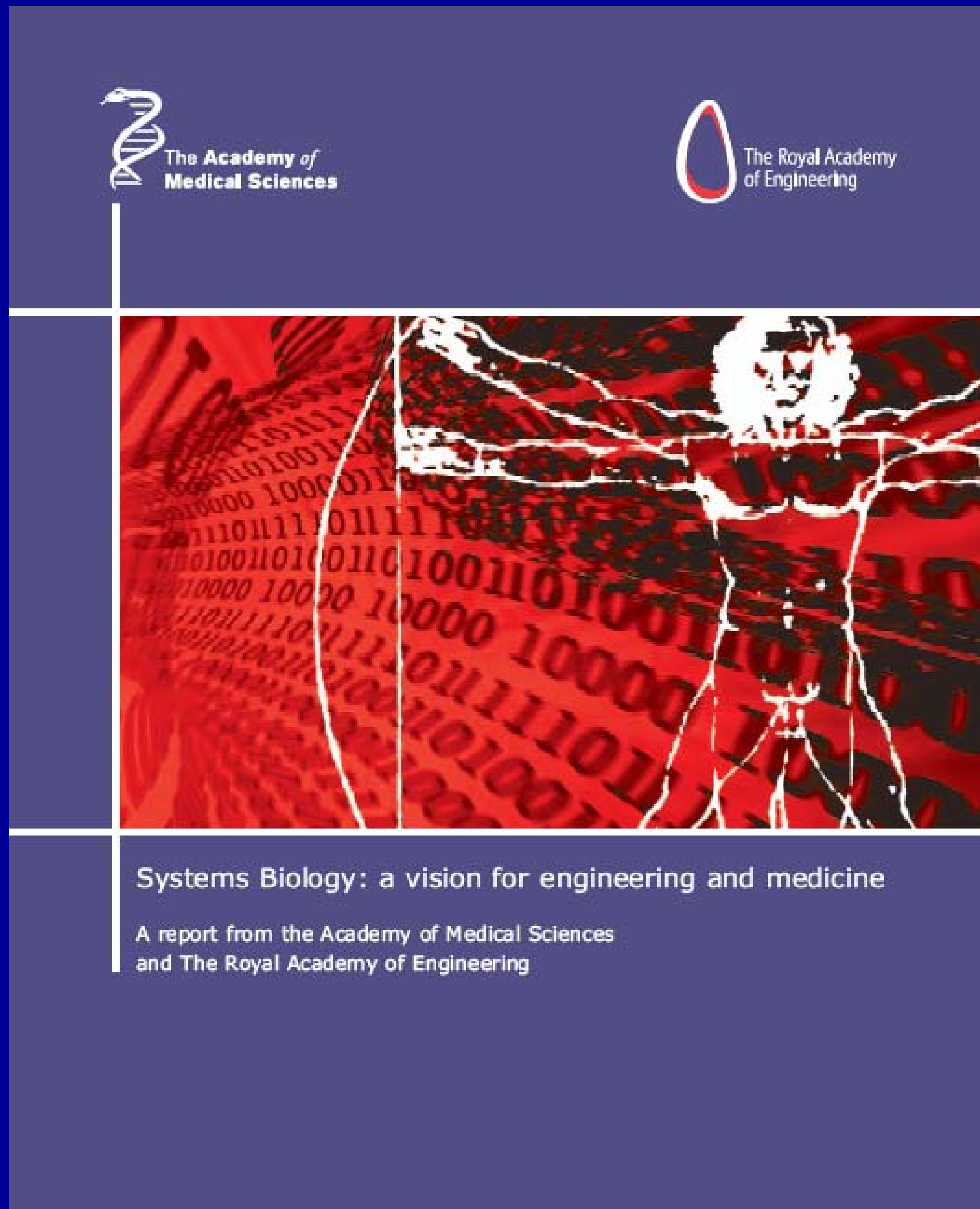


Synthetic Biology – State, Importance and Development

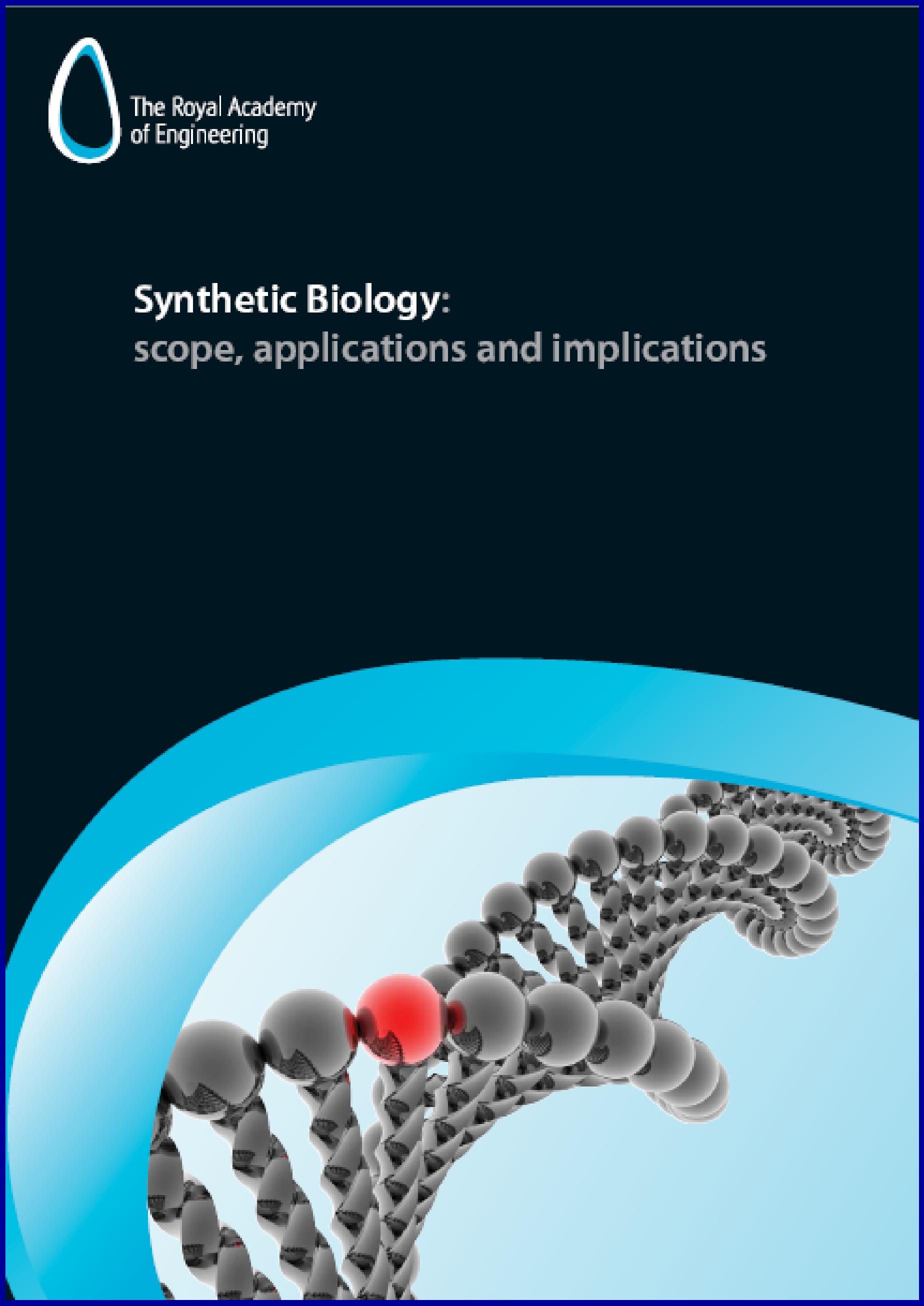
Professor Richard I Kitney

Chairman - The Institute of Systems and Synthetic Biology

Co-director – Centre for Synthetic Biology and Innovation



http://www.raeng.org.uk/policy/engagement/pdf/Systems_Biology_Report.pdf



http://www.raeng.org.uk/news/publications/list/reports/Synthetic_biology.pdf

Synthetic Biology

What is Synthetic Biology?

- Designing and making biological parts and systems that do not exist in the natural world using engineering principles

- Re-designing existing biological systems, again using engineering principles

Why now?

Why now?

- High speed DNA sequencing
- DNA synthesis
- Powerful computers
- Broadband networks
- The Internet
- The confluence of biology, engineering and physical science

Key Points

The endpoint of Synthetic Biology is industrialisation

The endpoint of analysing biological systems is Systems Biology

Synthetic Biology

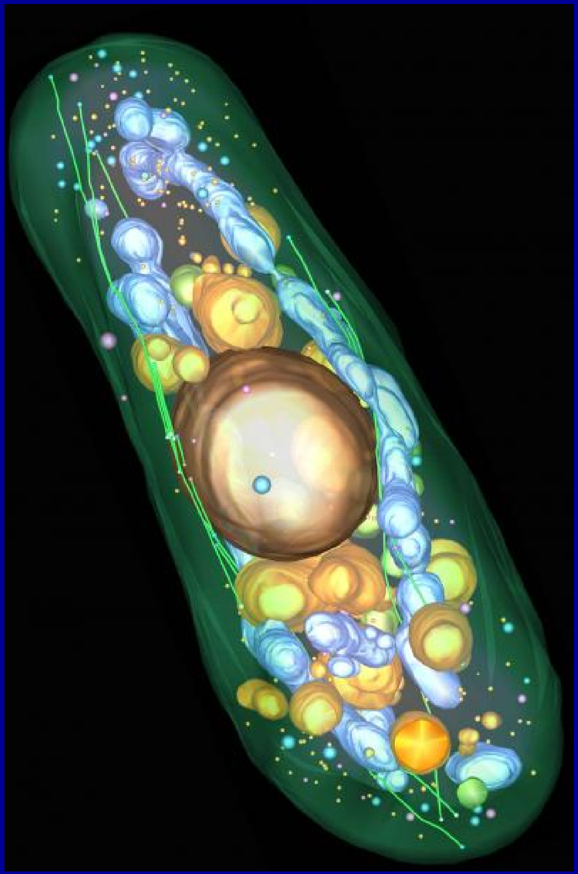
A Broad Church

- Bio nanotechnology
- Synthetic genomics
- Engineering

With Social Science and Ethics integrated part of the field

Four Approaches to Synthetic Biology

- Bottom Up
- Metabolic Engineering
- Chassis
- Parts, Devices and Systems



1. Bottom Up

Article Views
- Abstract
- Full Text (HTML)
- Full Text (PDF)
- Figures Only
- Supporting Online Material

VERSION HISTORY
- 319/5867/1215 (most recent)
- 1151721v1

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DOI: 10.1126/science.1151721

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RESEARCH ARTICLES

Complete Chemical Synthesis, Assembly, and Cloning of a *Mycoplasma genitalium* Genome

Daniel G. Gibson, Gwynedd A. Benders, Cynthia Andrews-Pfannkoch, Evgeniya A. Denisova, Holly Baden-Tillson, Jayshree Zaveri, Timothy B. Stockwell, Anushka Brownley, David W. Thomas, Mikkel A. Algire, Chuck Merryman, Lei Young, Vladimir N. Noskov, John I. Glass, J. Craig Venter, Clyde A. Hutchison, III, Hamilton O. Smith*

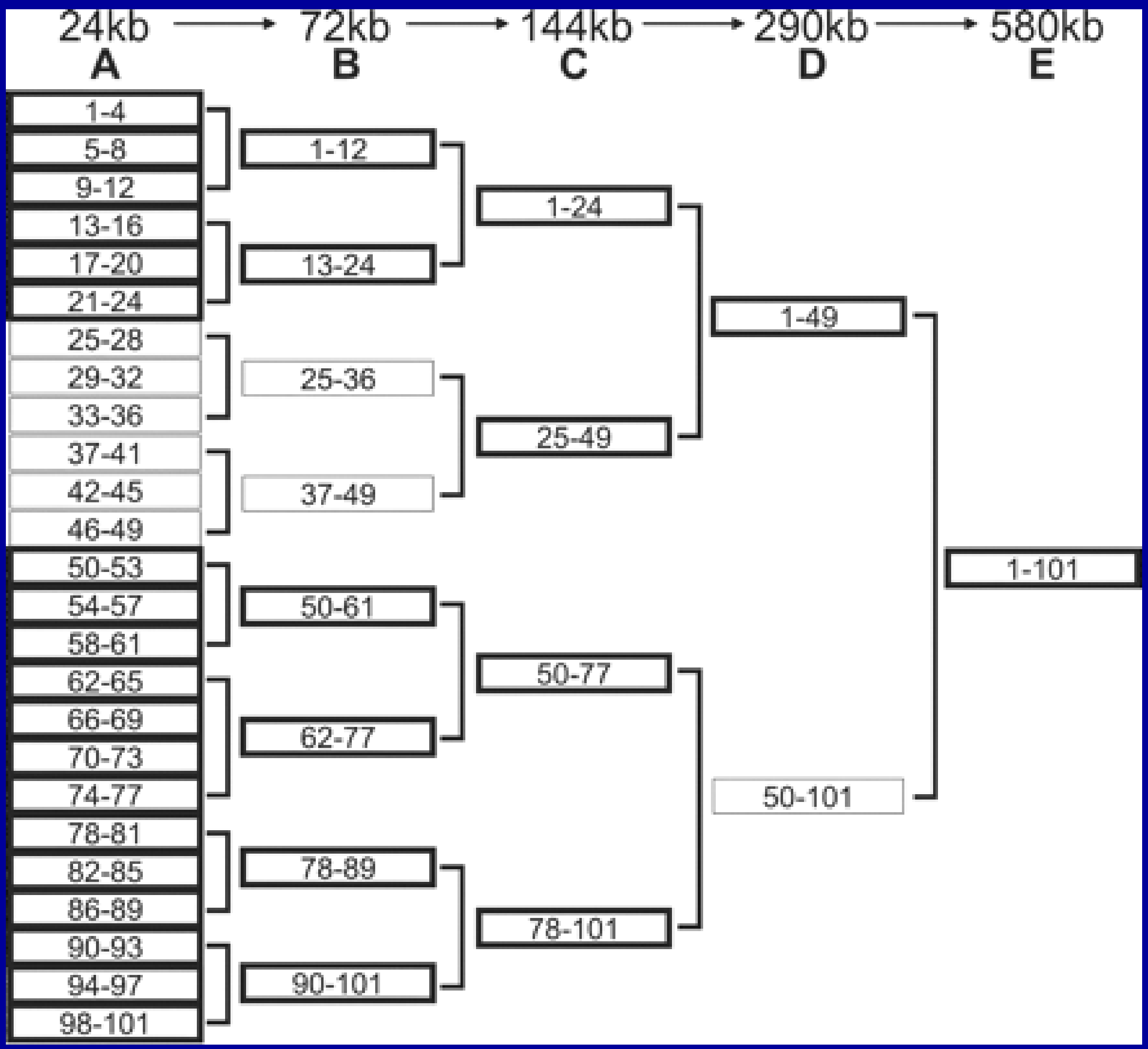
We have synthesized a 582,970–base pair *Mycoplasma genitalium* genome. This synthetic genome, named *M. genitalium* JCVI-1.0, contains all the genes of wild-type *M. genitalium* G37 except MG408, which was disrupted by an antibiotic marker to block pathogenicity and to allow for selection. To identify the genome as synthetic, we inserted "watermarks" at intergenic sites known to tolerate transposon insertions. Overlapping "cassettes" of 5 to 7 kilobases (kb), assembled from chemically synthesized oligonucleotides, were joined by in

Steps in the synthesis of a 583kbp M.Genitalium Genome

1. Overlapping “cassettes” of 5 to 7 kb were assembled from chemically synthesised oligonucleotides

2 Joined *in vitro* to produce intermediate assemblies of approximately 24kb, 72kb (1/8 genome) and 148kb (1/4 genome) – all cloned as bacterial artificial chromosomes (BACs) in E. coli

3. The complete synthetic genome was assembled using transformation associated recombination (TAR) cloning in yeast



BLUEHERON® BIOTECHNOLOGY

GENEART THE GENE OF YOUR CHOICE

DNA 2.0

2. Metabolic Engineering

Malaria

Artemisia

- Used by Chinese herbalists for more than 1000 years to treat Malaria
- 1972 - Tu Youyou discovered artemisinin in the leaves of the Artemisia Annua (annual wormwood)

Tu Youyou 屠呦呦 [sources / revisions]

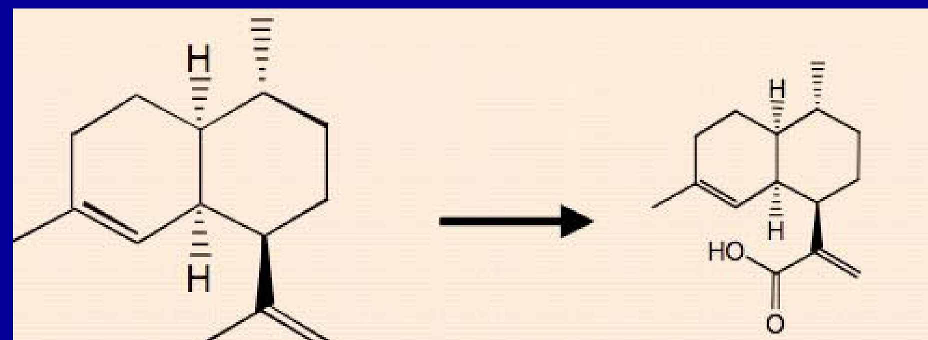
Chief Research Fellow of the Institute of Chinese Traditional Medicines at the Chinese Academy of Traditional Chinese Medicine

Born: 1930

Artemisia annua

Making Complex Drugs

Anti-malarial drug Artemesinin



Amyris Biotechnologies

Institute for OneWorld Health

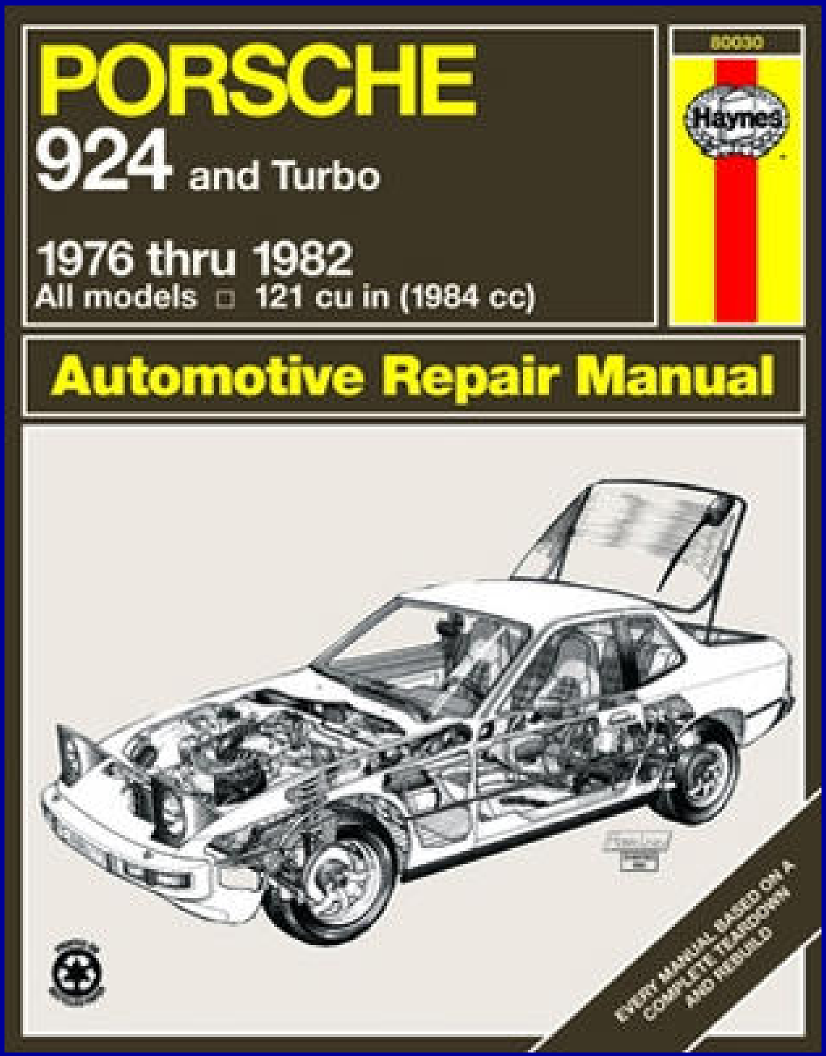
3. Chassis

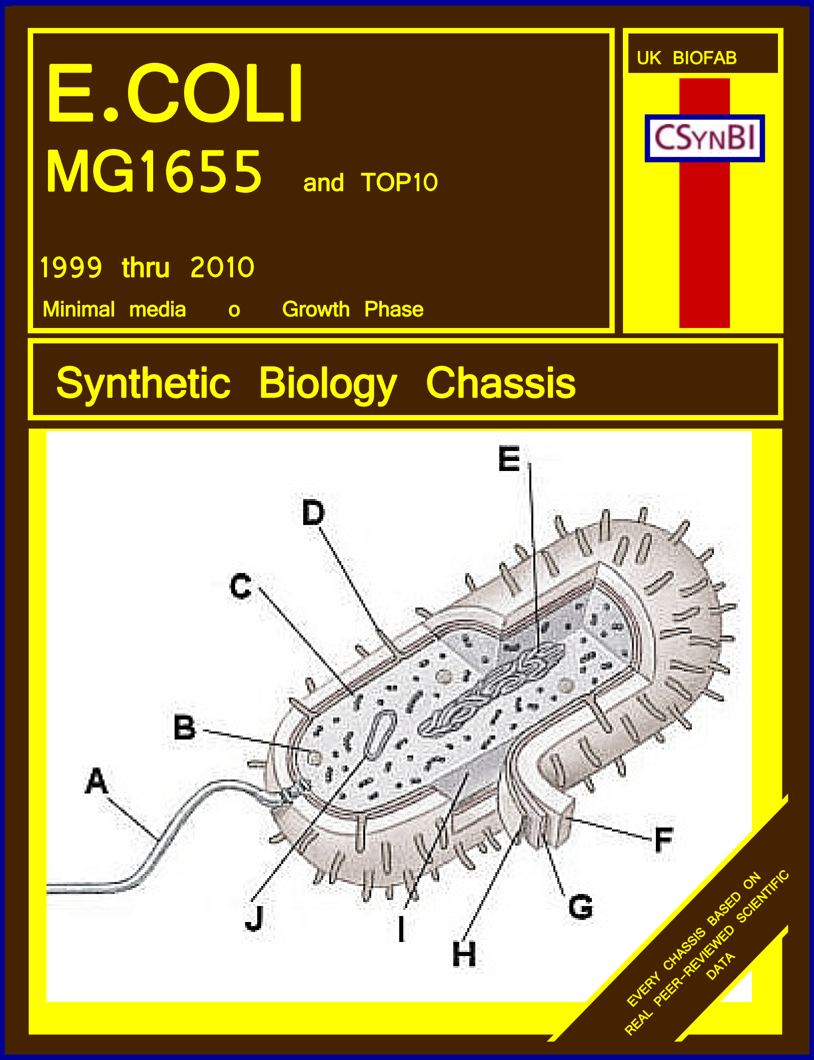
Chassis

- Natural Chassis
  - E. Coli
  - B. Subtilis
  - Mycoplasma
  - Yeast
  - P. putida
- Minimal Cells
  - achieving control

Developing chassis that are fit for purpose

Chassis for Synthetic Biology





1st Generation Synthetic Biology

UK BIOFAB

CSynBI

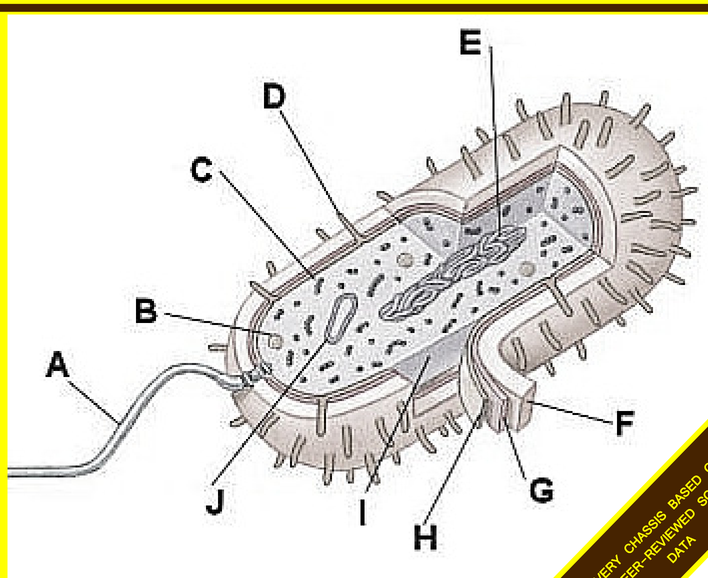
E.COLI

MG1655 and TOP10

1999 thru 2010

Minimal media o Growth Phase

Synthetic Biology Chassis



EVERY CHASSIS BASED ON REAL PEER-REVIEWED SCIENTIFIC DATA

UK BIOFAB

CSynBI

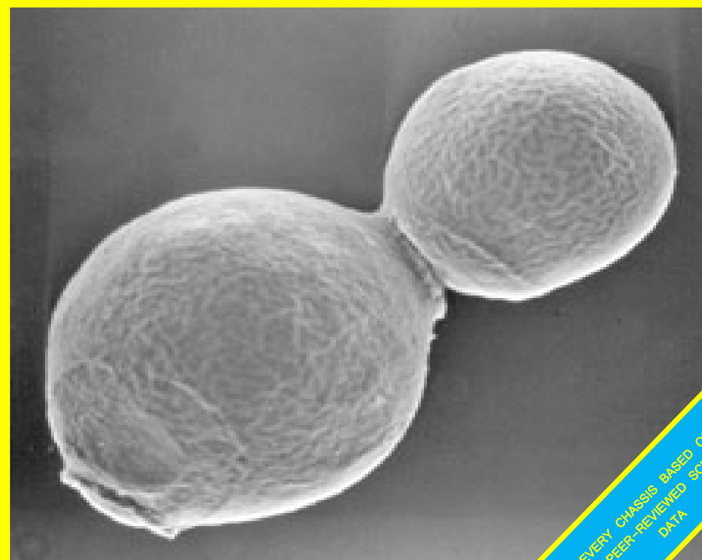
YEAST

S.CEREVISIAE

2002 thru 2010

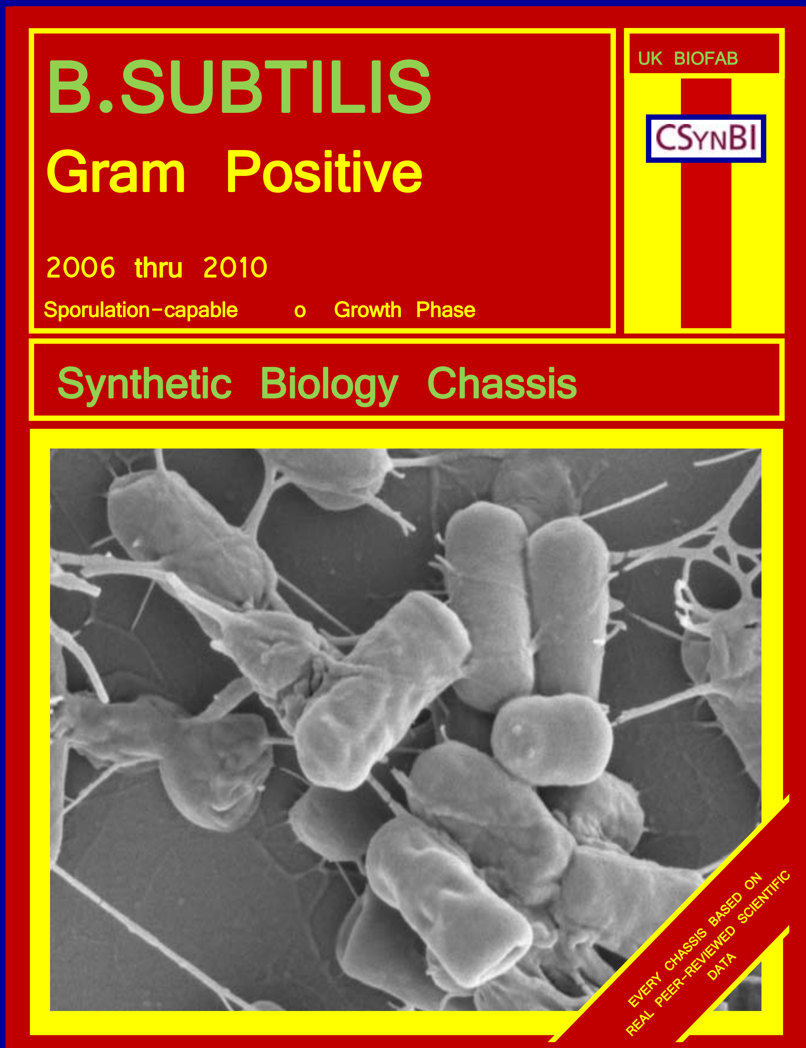
Synthetic Defined Media o Growth Phase

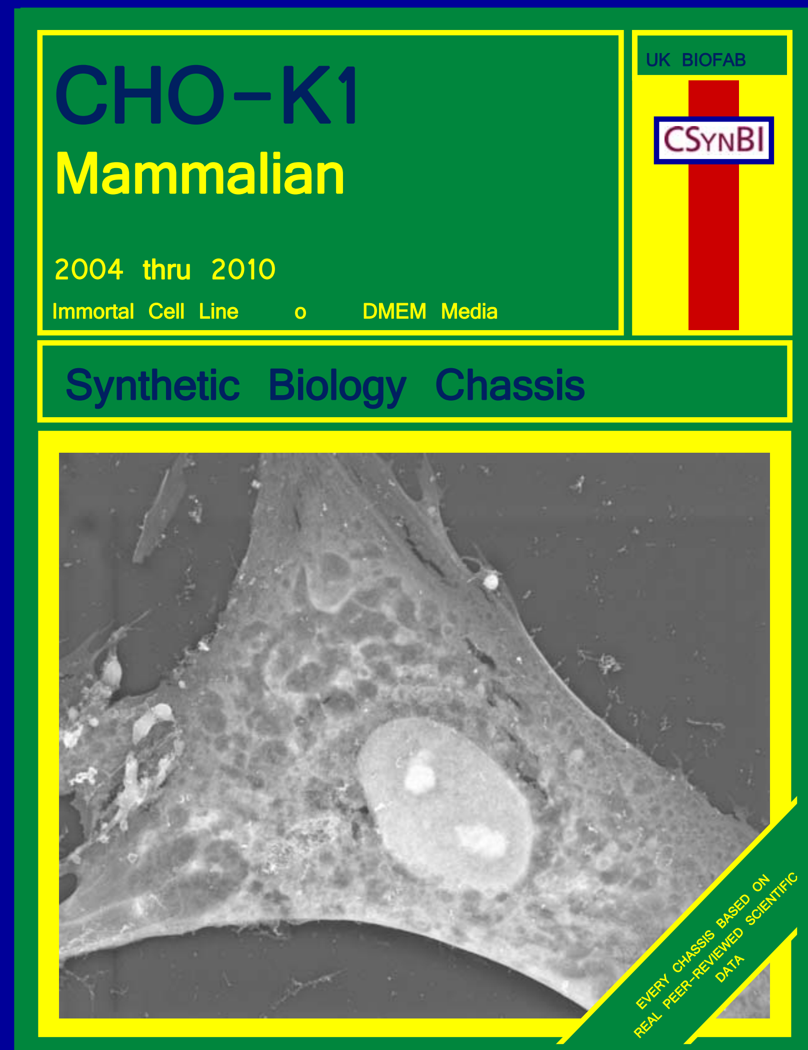
Synthetic Biology Chassis



EVERY CHASSIS BASED ON REAL PEER-REVIEWED SCIENTIFIC DATA

2nd Generation Synthetic Biology





Relevance of Current Chassis

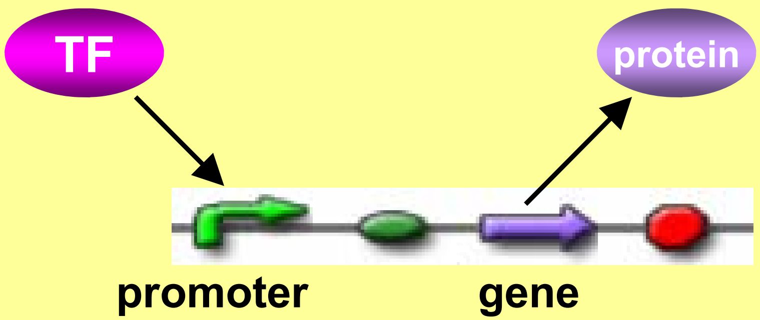
E.coli	Advanced molecular cloning Industrial-scale application
B.subtilis	Commonly used in industry Well-understood genetic regulation
S.cerevisiae	Major industrial organism Extensively characterised
CHO-K1 cells (+ others)	Easy to use immortal mammalian cell line Good transfection efficiency

Industrial-scale biosynthesis — Ease of re-engineering

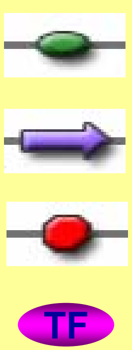
4. Parts, Devices and Systems

Engineering v Biology

Modularity, Characterisation, Standardisation



Typical gene transcription module



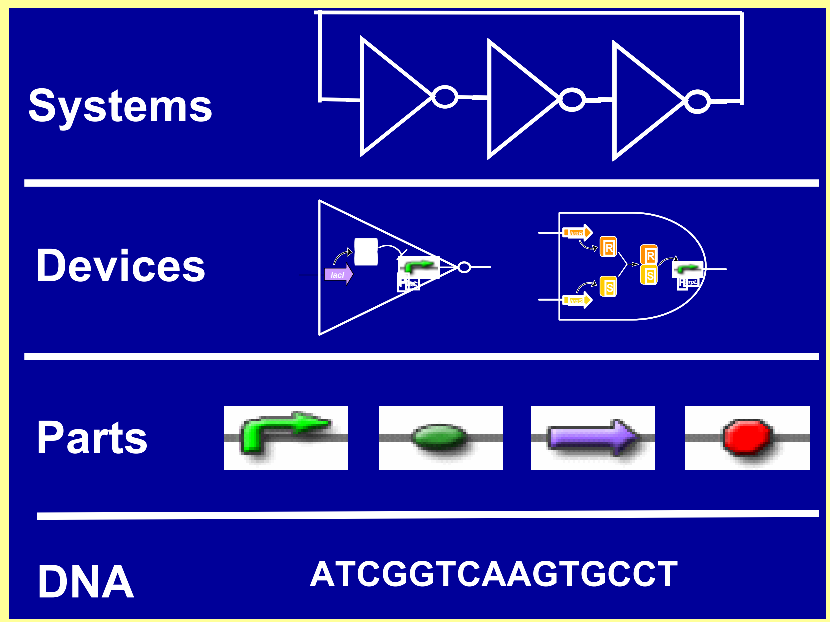
Ribosome binding site

Protein coding sequence

Terminator

Transcription factor

A hierarchy for synthetic biology



Systematic Design

The basis of all engineering - parts, devices and systems

The Engineering Approach to Design

- Abstraction
- Decoupling
- Standardisation

The Engineering Approach to Design in Synthetic Biology

Engineering systems are built from a hierarchy

- Parts
- Devices
- System

- At each level the characteristics of the Part, Device or System are well defined and reproducible

- In engineering the aim is to build a system on the basis of devices which comprise standard parts

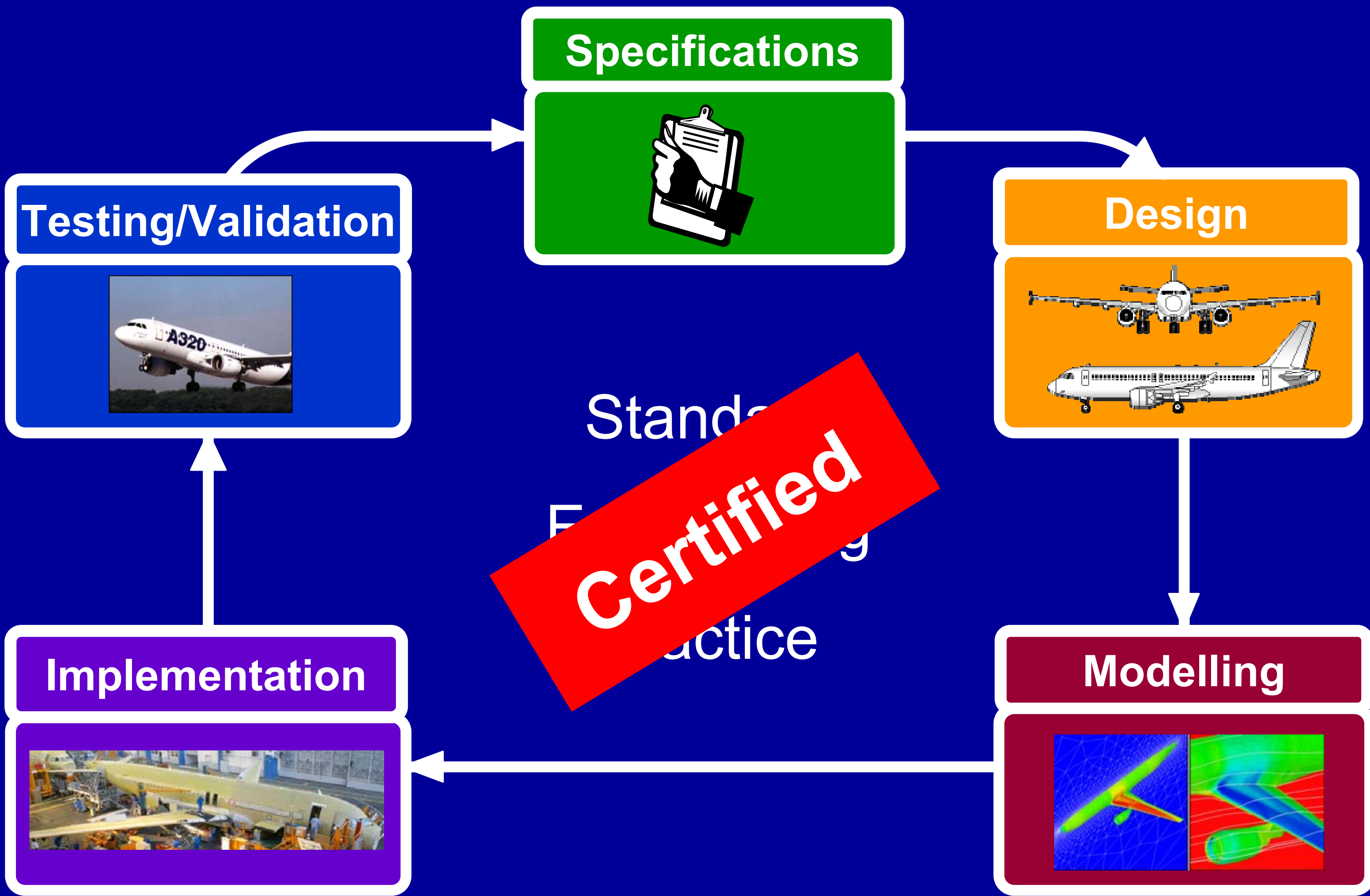
Synthetic Biology: aims to build applications from Biobricks

- Parts – encode biological functions (ie often modified DNA)
- Devices – made from a collection of parts and encode human-defined functions (eg logic gates)
- Systems – perform tasks, eg counting

Engineering Biology

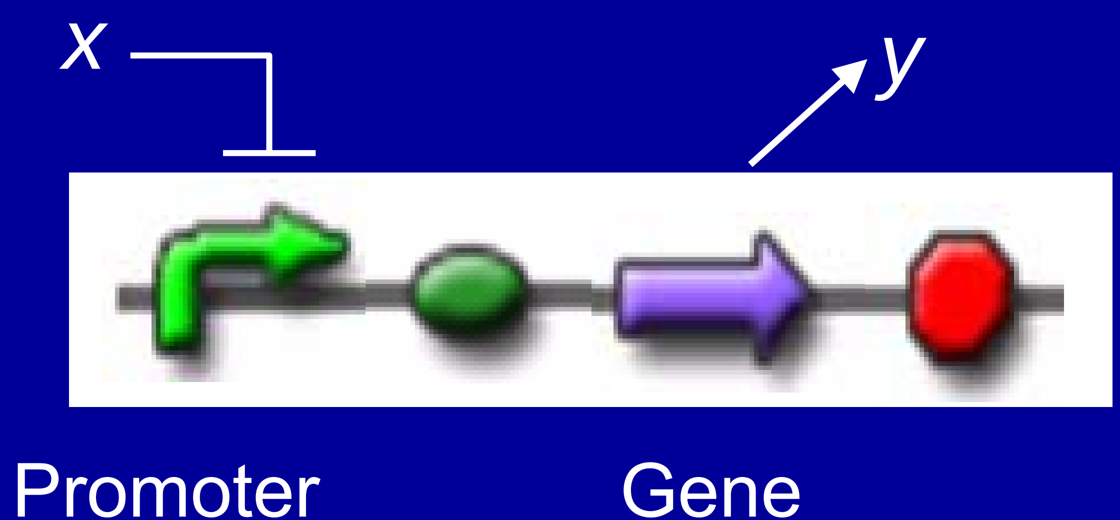
To engineer biology it needs to be broken down into parts

The Engineering Approach



Modelling

An inverter described using BioBrick icons



$$\frac{dy}{dt} = \frac{\beta x^n}{K^n + x^n} - \gamma y$$

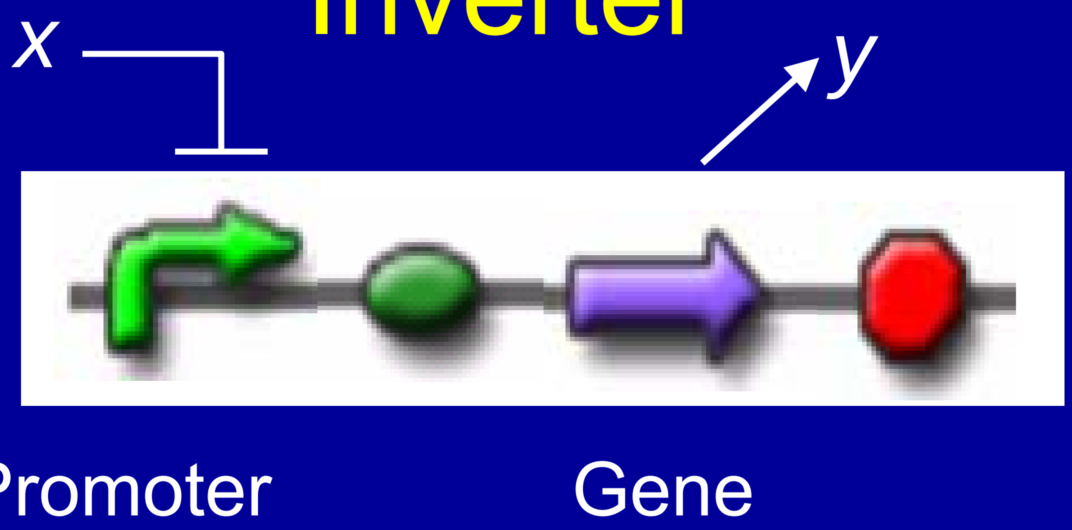
γ Protein degradation rate

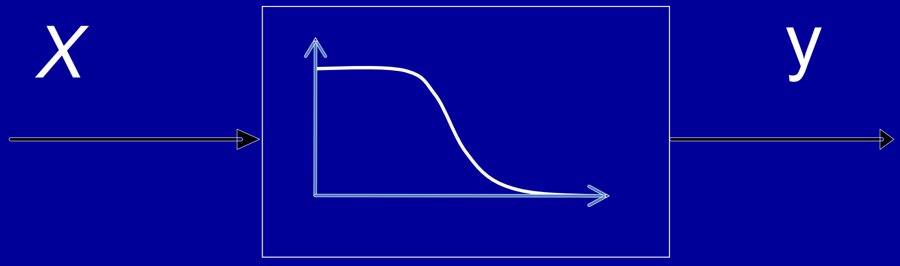
x Input repressor protein

n Hill constant

β Protein synthesis rate

Inverter

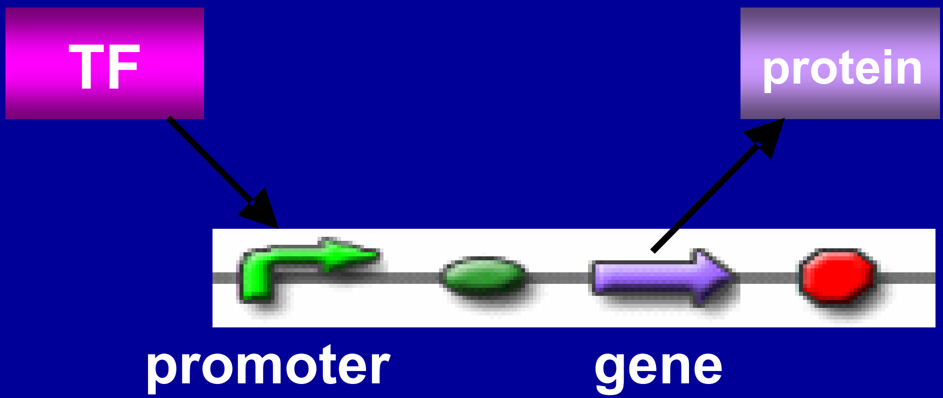




X (Input Repressor)	**Y (Output Protein)**
1	0
0	1

1: High Concentration
0: Low Concentration

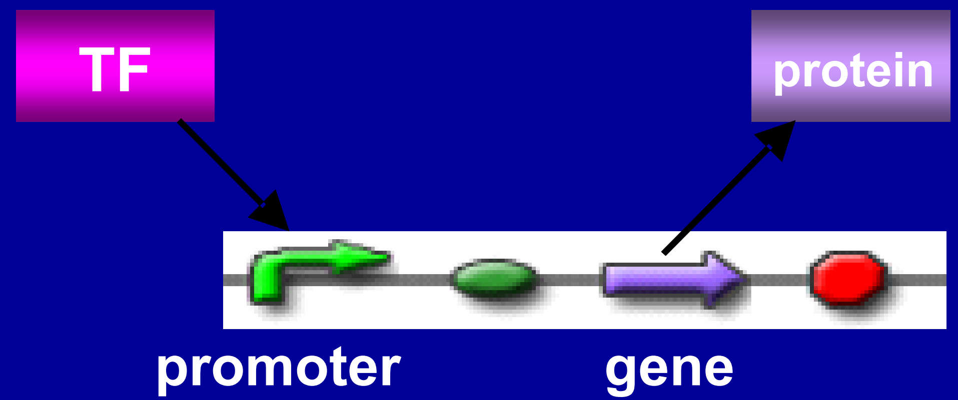
A typical transcriptional regulatory device



$$\frac{d[mRNA]}{dt} = \frac{k_{tr}.\left(\frac{W^n}{K^n}\right)^{\mu}}{1+\left(\frac{W^n}{K^n}\right)} - d_m.[mRNA]$$

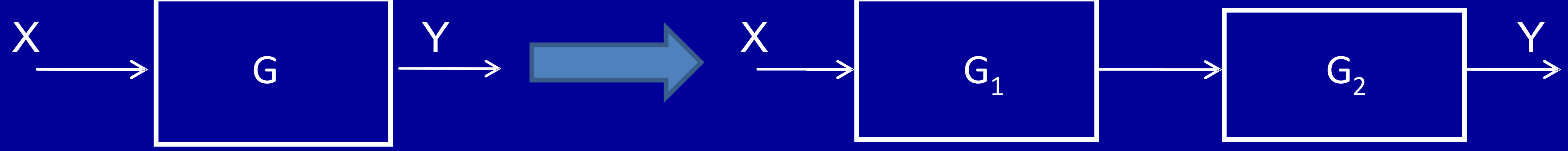
Currently ODEs are mainly used for modelling in Synthetic Biology

This becomes cumbersome as the complexity of the systems increases



What is required is the application of Systems Theory

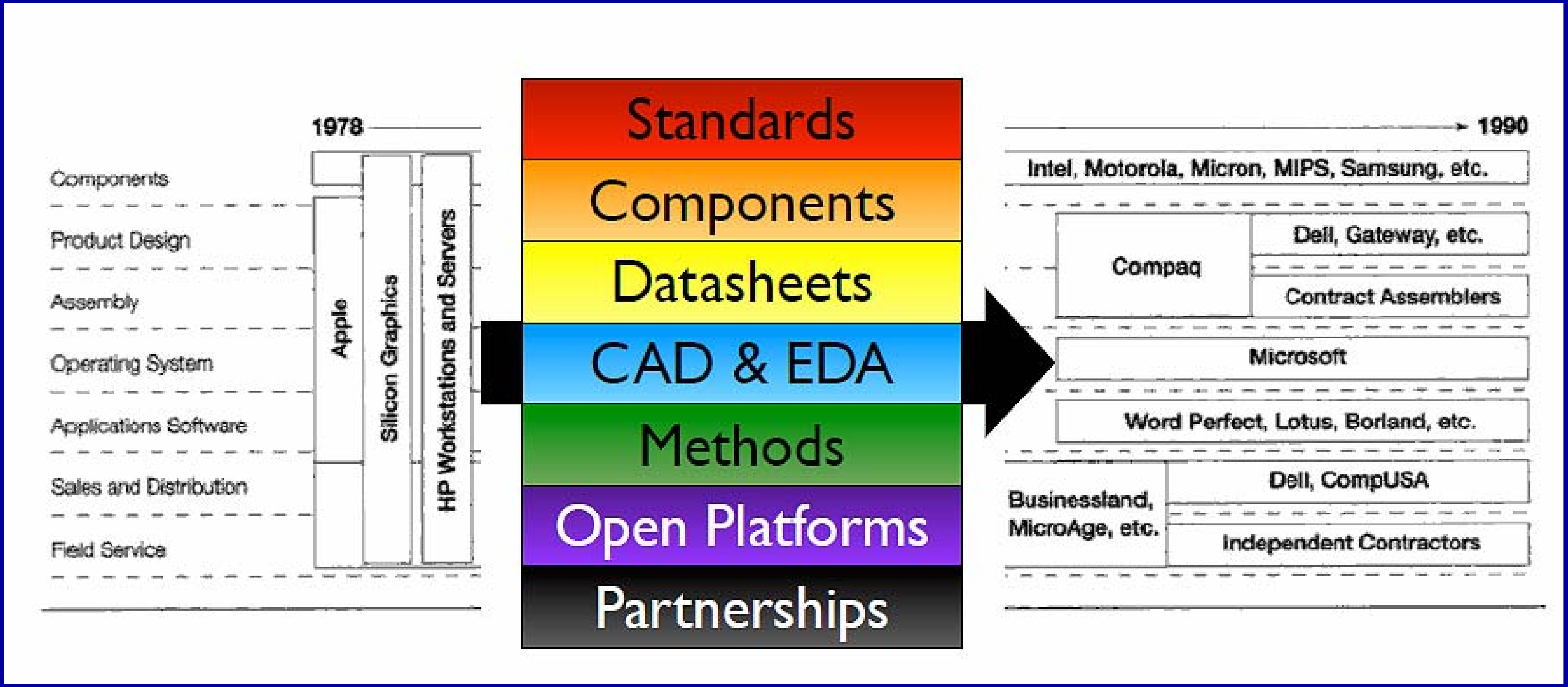
Modularisation



and, the application of Transform Methods

The Evolution of Industrial Approaches

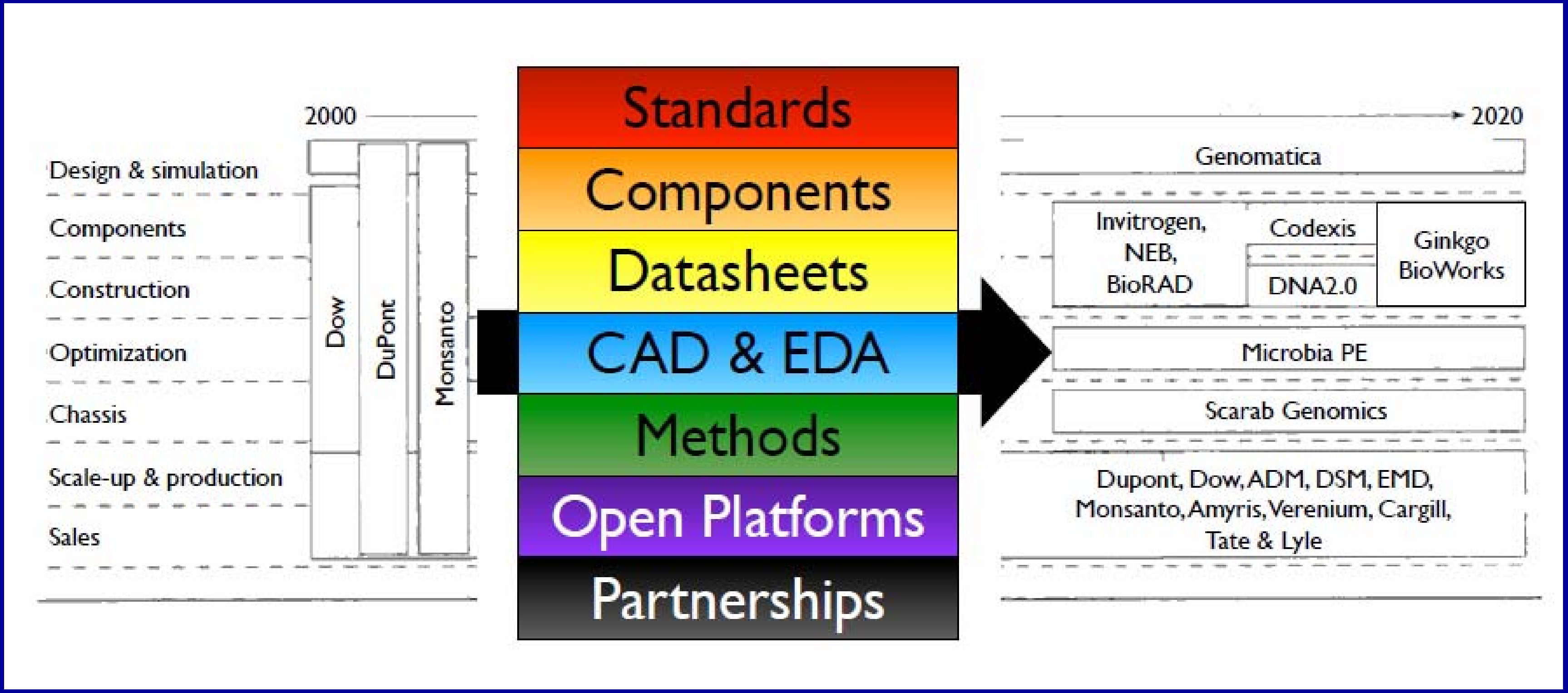
Computing - circa 1980



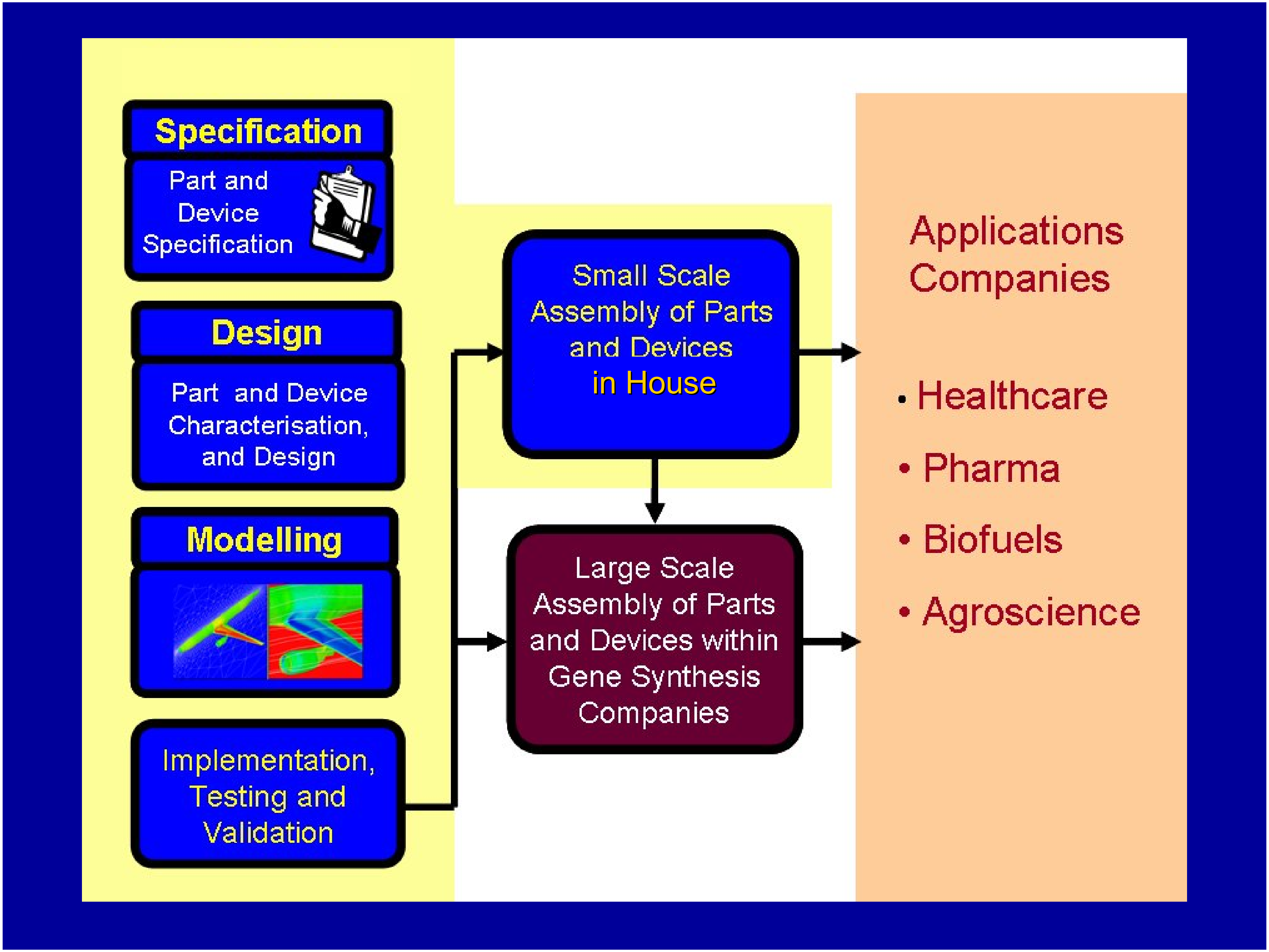
New foundational tools catalysed revolutionary transitions in computer technology, creating new industries and huge opportunities

The Innovator's Solution - CM Christensen and M E Raynor – HBSP - 2003

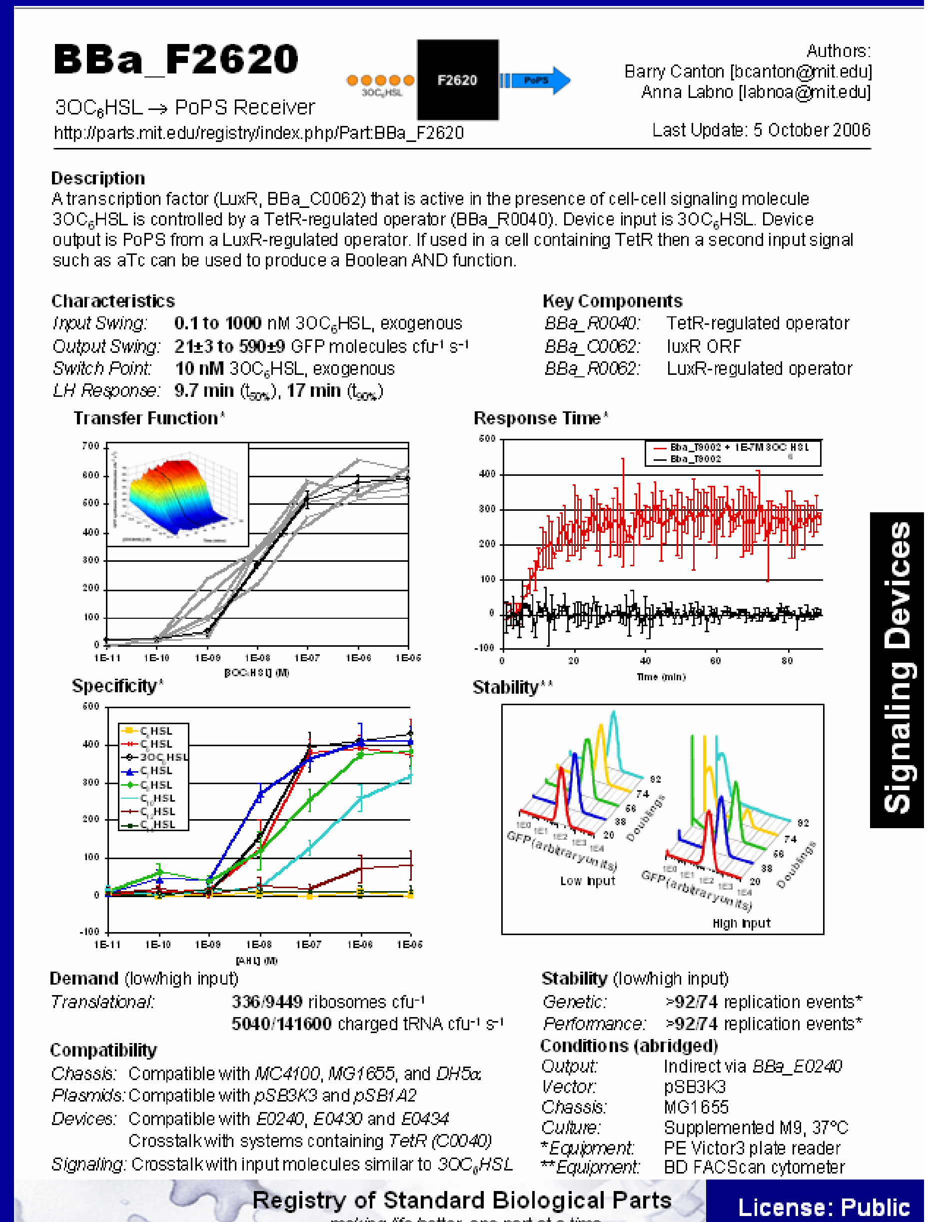
Biotech is Next



Poised for similar revolutionary reorientation from few successful vertical organisations to many partnered and enabling industries


Specification
Part and Device Specification
Design
Part and Device Characterisation, and Design
Modelling
Implementation, Testing and Validation
Small Scale Assembly of Parts and Devices in House
Large Scale Assembly of Parts and Devices within Gene Synthesis Companies
Applications Companies
• Healthcare
• Pharma
• Biofuels
• Agroscience

Biobrick BBa_F2620



BBa_F2620

$3OC_6HSL$ → PoPS Receiver

http://parts.mit.edu/registry/index.php/Part:BBa_F2620

Authors:
Barry Canton [bcanton@mit.edu]
Anna Labno [labnoa@mit.edu]

Last Update: 5 October 2006

Description

A transcription factor (LuxR, BBa_C0062) that is active in the presence of cell-cell signaling molecule $3OC_6HSL$ is controlled by a TetR-regulated operator (BBa_R0040). Device input is $3OC_6HSL$. Device output is PoPS from a LuxR-regulated operator. If used in a cell containing TetR then a second input signal such as aTc can be used to produce a Boolean AND function.

Characteristics

Input Swing: **0.1 to 1000** nM $3OC_6HSL$, exogenous
Output Swing: **21±3 to 590±9** GFP molecules cfu^{-1} s^{-1}
Switch Point: **10 nM** $3OC_6HSL$, exogenous
LH Response: **9.7 min** ($t_{50\%}$), **17 min** ($t_{90\%}$)

Key Components

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

Transfer Function*

Response Time*

Specificity*

Stability**

Low Input
High Input

Demand (low/high input)

Translational: **336/9449** ribosomes cfu^{-1}
5040/141600 charged tRNA cfu^{-1} s^{-1}

Stability (low/high input)

Genetic: **>92/74** replication events*
Performance: **>92/74** replication events*

Compatibility

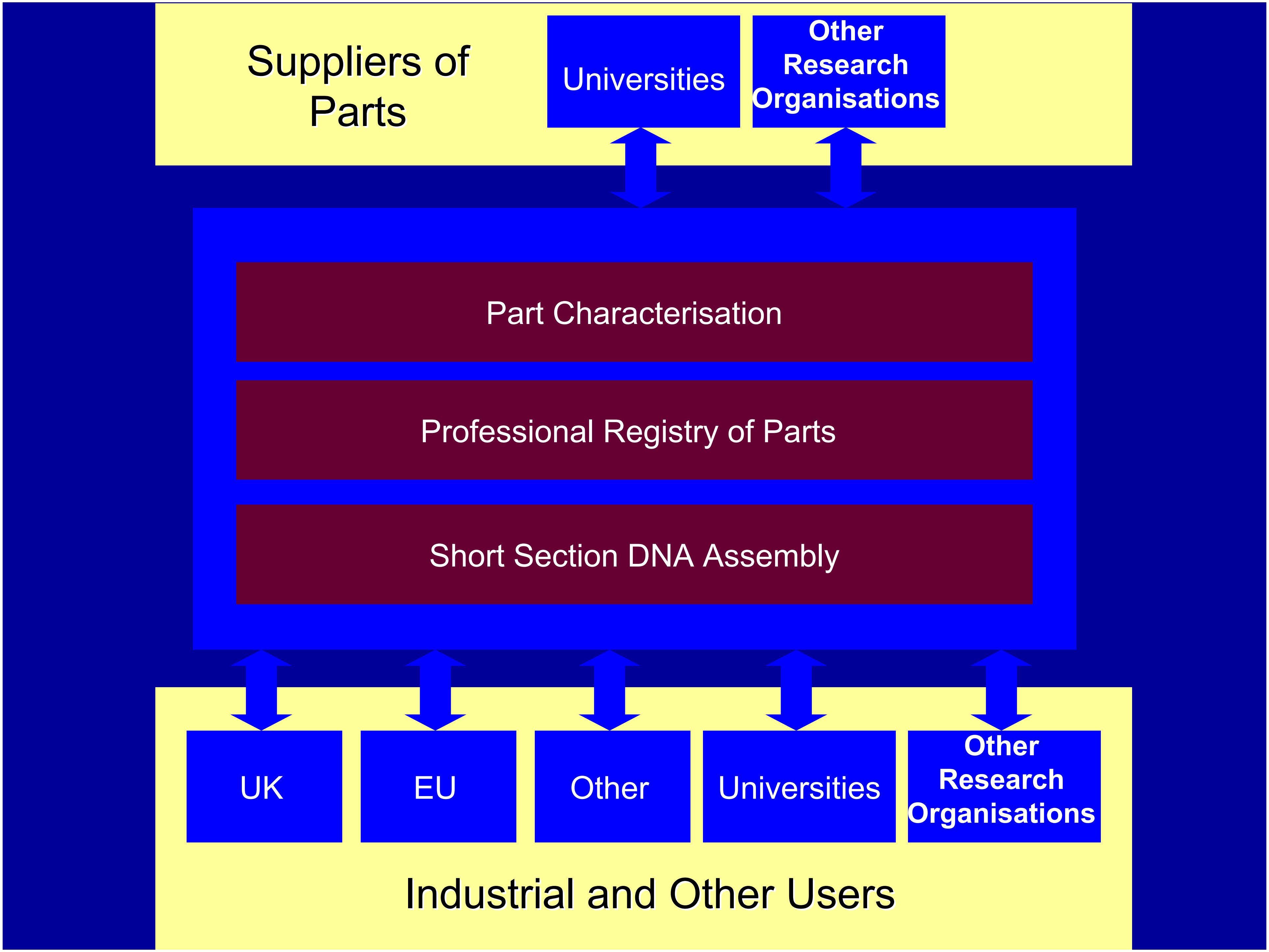
Chassis: Compatible with *MC4100*, *MG1655*, and *DH5α*
Plasmids: Compatible with *pSB3K3* and *pSB1A2*
Devices: Compatible with *E0240*, *E0430* and *E0434*
Crosstalk with systems containing *TetR (C0040)*
Signaling: Crosstalk with input molecules similar to $3OC_6HSL$

Conditions (abridged)

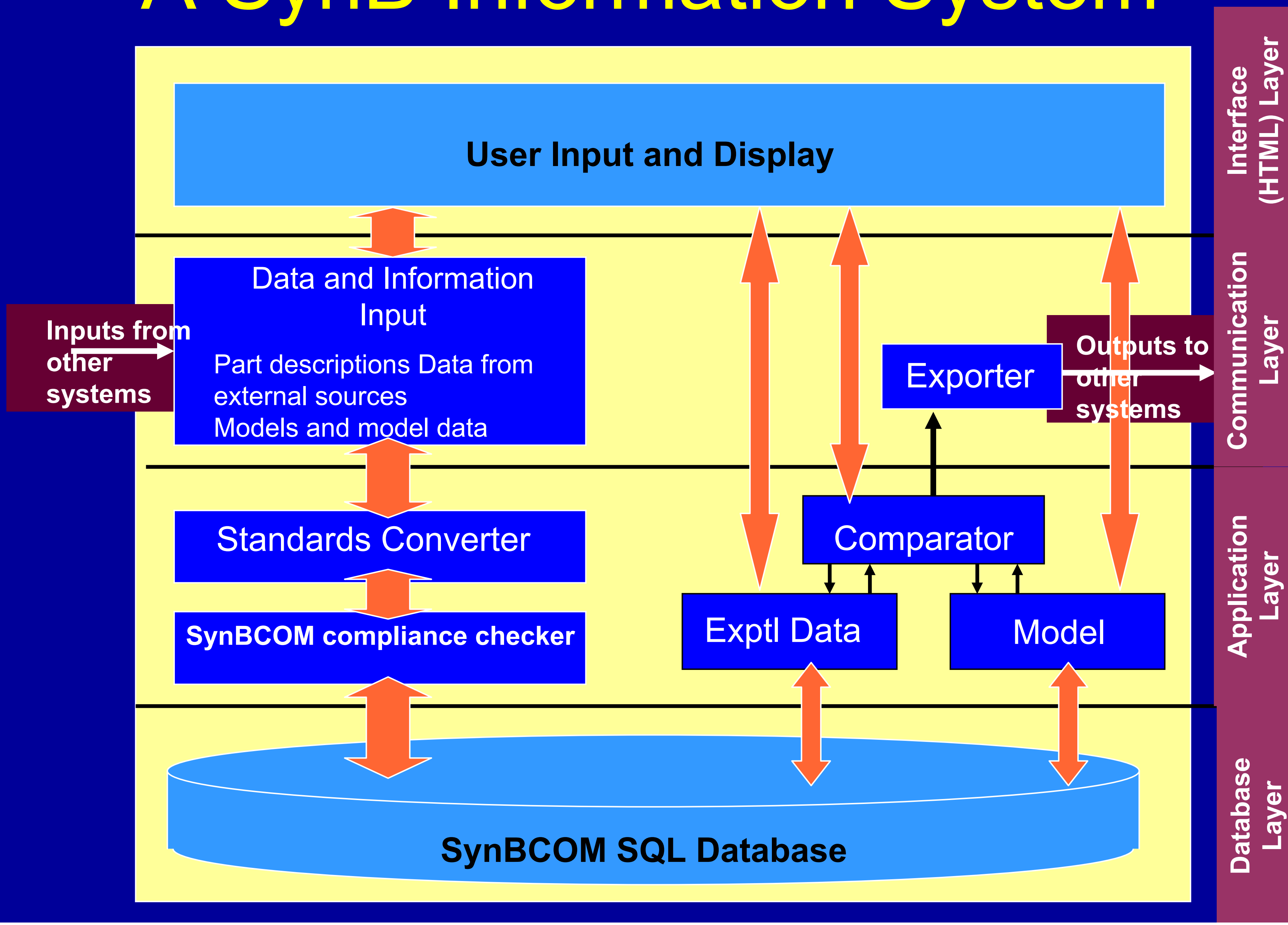
Output: Indirect via *BBa_E0240*
Vector: pSB3K3
Chassis: MG1655
Culture: Supplemented M9, 37°C
**Equipment:* PE Victor3 plate reader
***Equipment:* BD FACScan cytometer

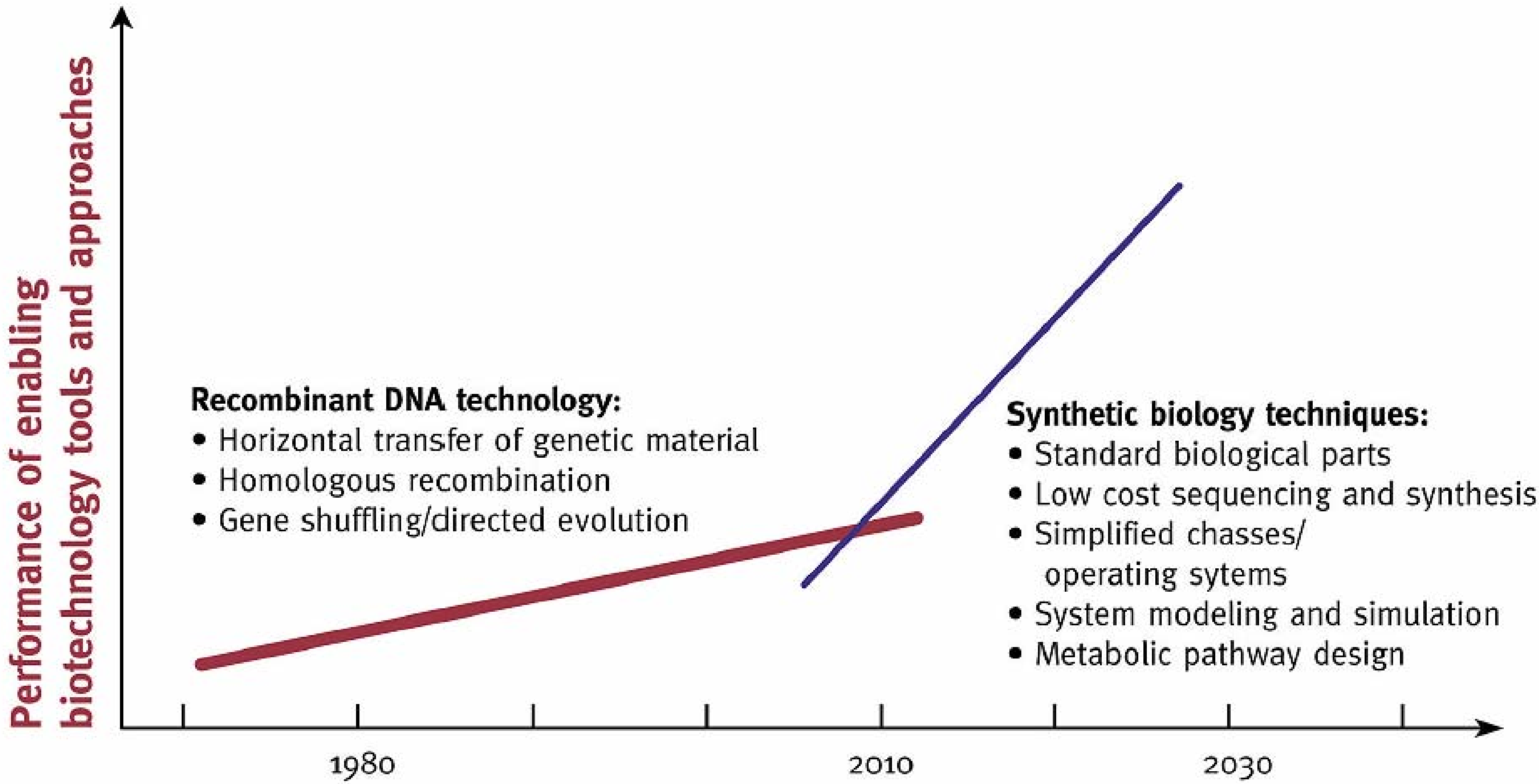
Registry of Standard Biological Parts
making life better, one part at a time

Signaling Devices


Suppliers of Parts
Universities
Other Research Organisations
Part Characterisation
Professional Registry of Parts
Short Section DNA Assembly
UK
EU
Other
Universities
Other Research Organisations
Industrial and Other Users

A SynB Information System

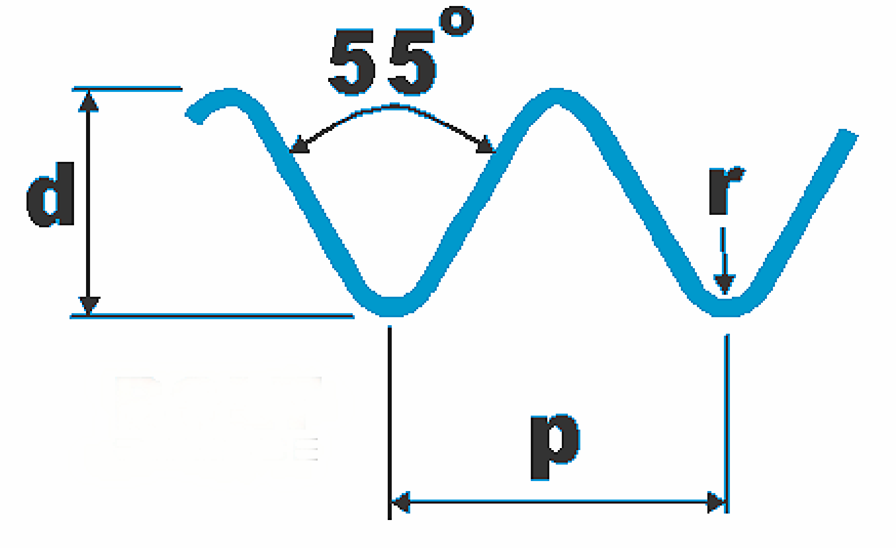




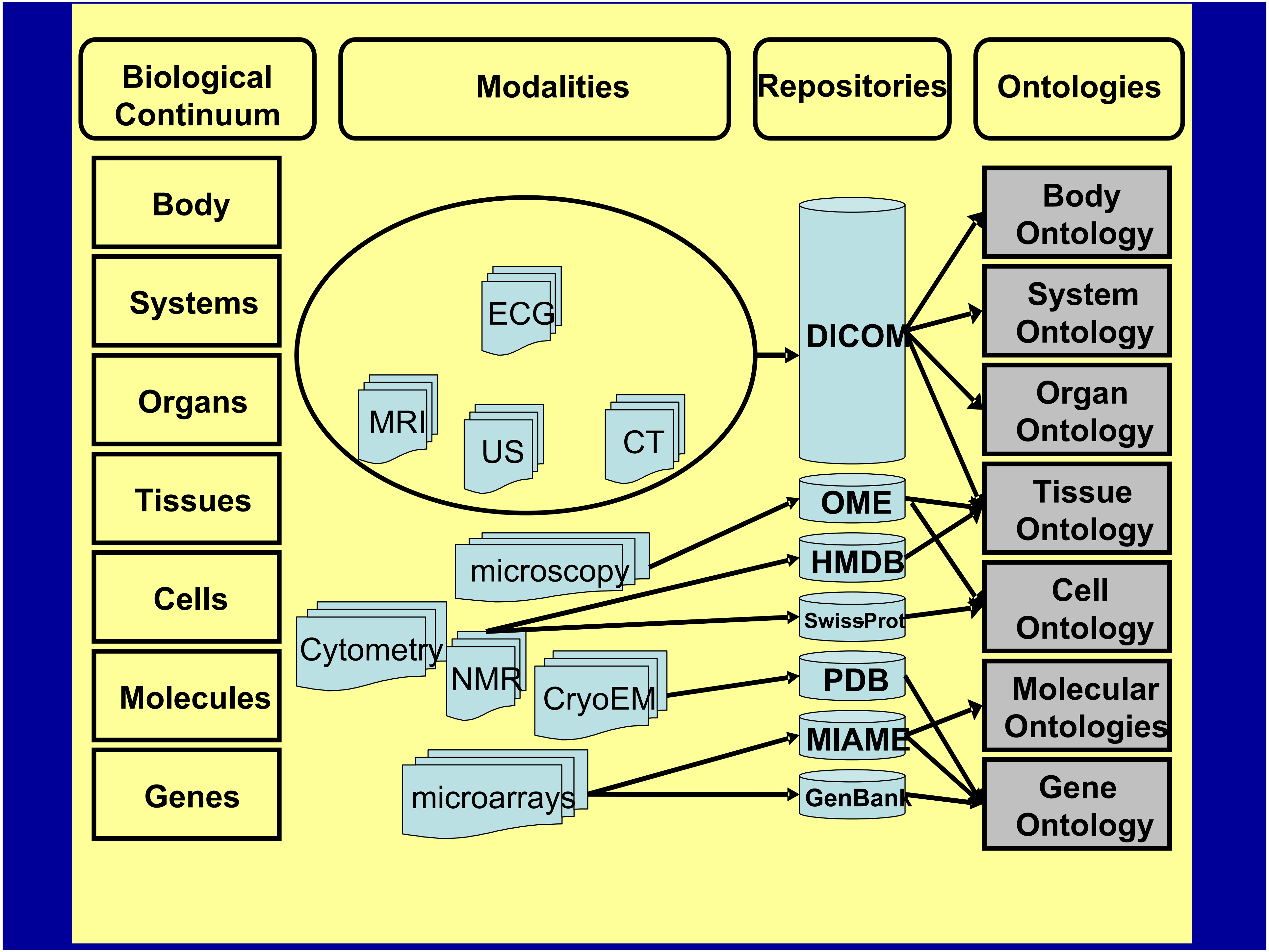
Genome Synthesis and Design Futures - Special Bio-era Report – US DoE 2007

Standards

The Whitworth Thread



The first standard thread – Sir Joseph Whitworth 1841



Biological Continuum
Modalities
Repositories
Ontologies
Body
Systems
Organs
Tissues
Cells
Molecules
Genes
ECG
MRI
US
CT
microscopy
Cytometry
NMR
CryoEM
microarrays
DICOM
OME
HMDB
SwissProt
PDB
MIAME
GenBank
Body Ontology
System Ontology
Organ Ontology
Tissue Ontology
Cell Ontology
Molecular Ontologies
Gene Ontology

DICOM

Digital Imaging and Communications in Medicine

NEMA, Suite 1752
1300 North 17th Street
Rosslyn, VA 22209
Ph: (703) 841-3285
http://dicom.nema.org

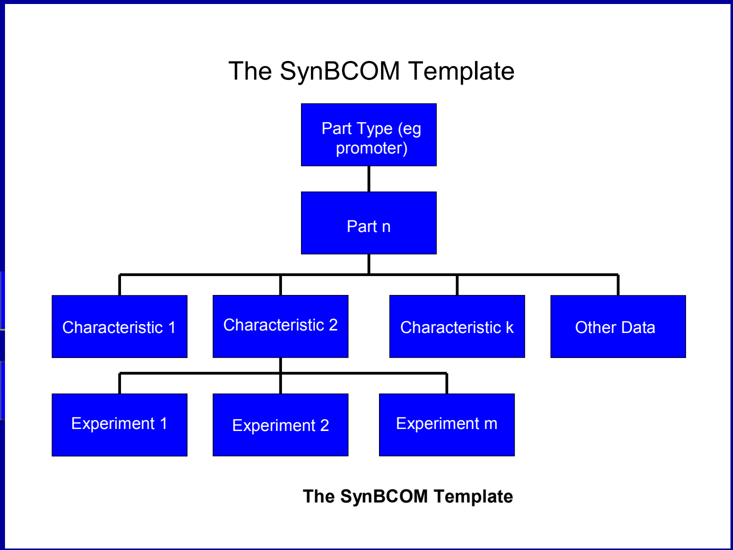
http://medical.nema.org/

Based on the DICOM standard for medical images
http://medical.nema.org/

Machine readable to allow programmes to collate, search and update the information contained where appropriate

Parts will be ontologically organised to aid design

Parts will be defined by their characteristics, which are determined by experiments and data which will be associated with the part



Synthetic Biology's Engineering Principles

Characterisation, Standardisation and Automation

Characterisation:

- Of parts and their parameters and characteristics
- To produce models and improve understanding
- To aid design and prediction

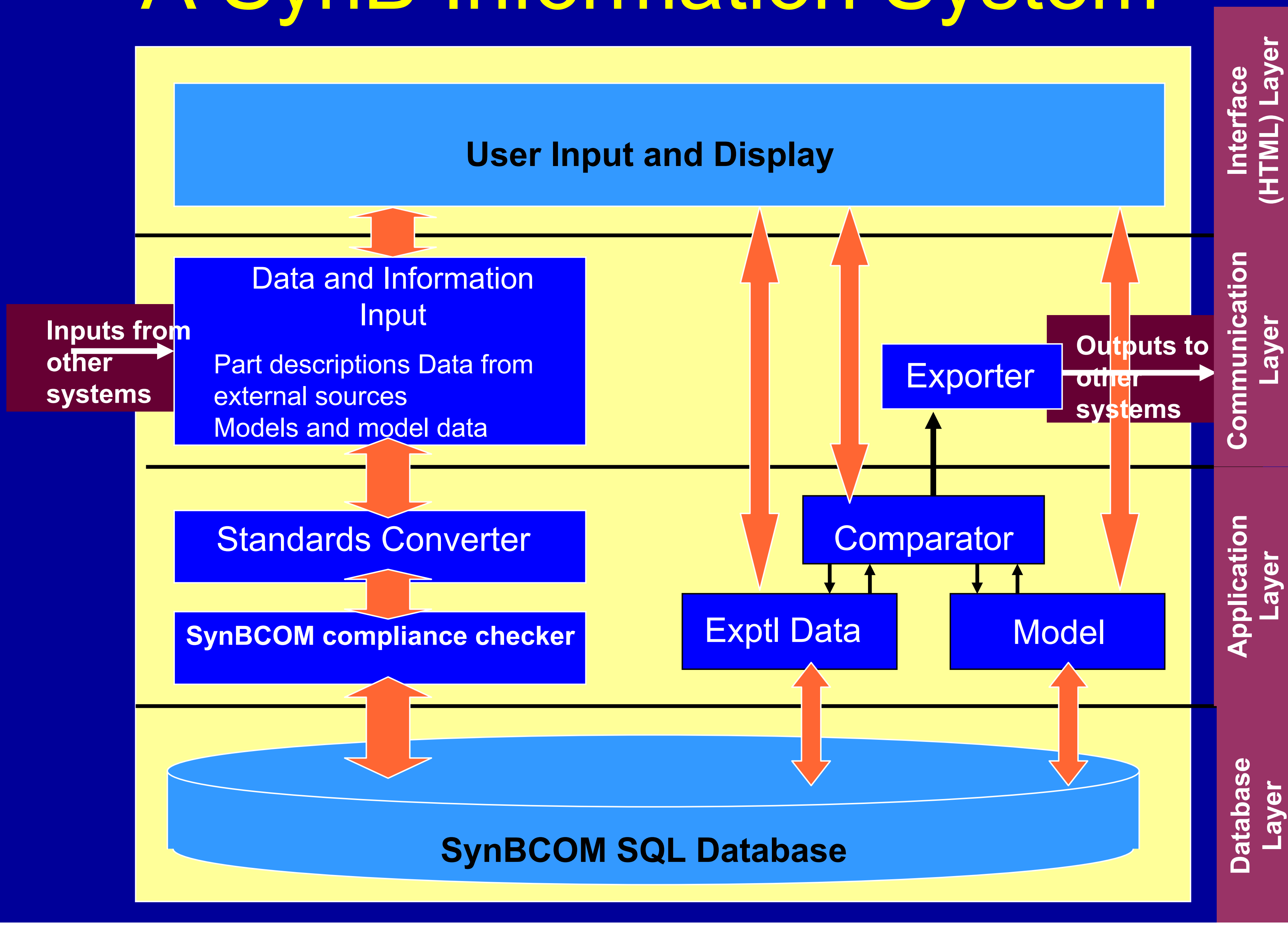
Standardisation

- Of many part types to ensure correct part inter-connectivity, function and insulation
- Of part ontology and documentation

Automation

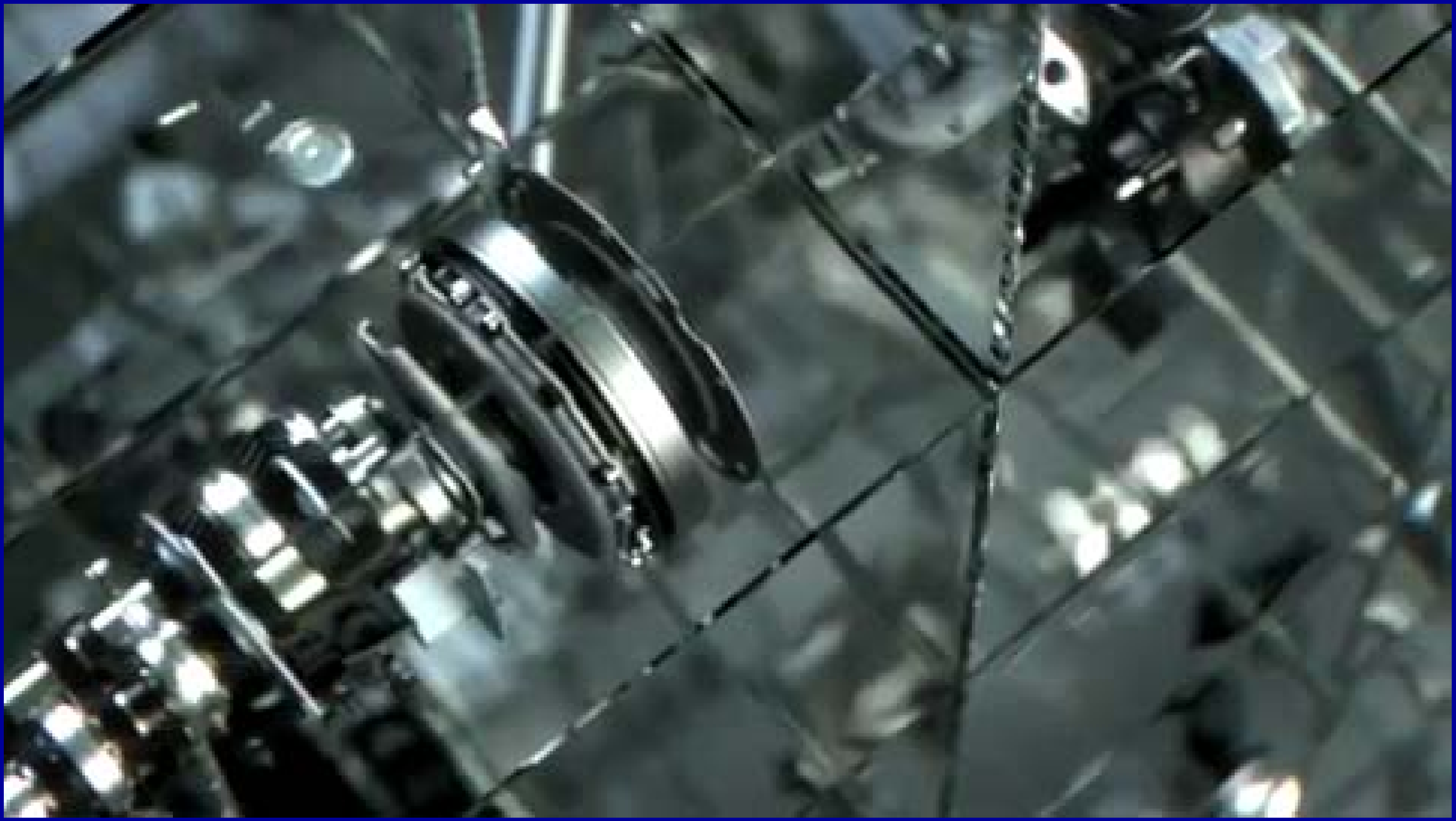
- Increase throughput
- Reduce researcher 'waiting' time
- Use of tools to speed up both design and lab processes

A SynB Information System



Developing a Registry of standard, composable models

Combining parts



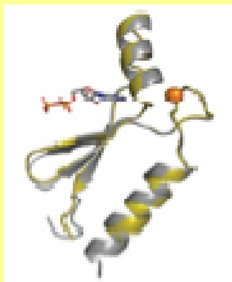
To predict the behaviour of complex systems built from many parts, we need to have:

1. *mechanisms to compose* part models into a system model
2. *predictive, composable models* for the parts

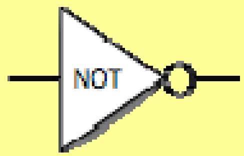
Complex Systems & Abstractions

DNA ⇒ Parts ⇒ Devices ⇒ Systems ⇒ Hosts ⇒ Cell Populations

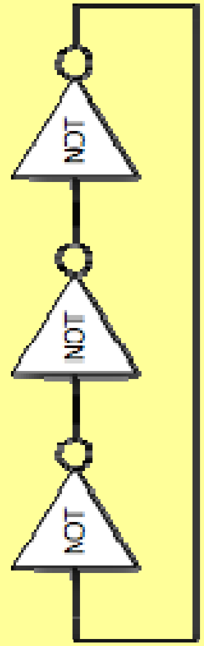
TAATACGACT

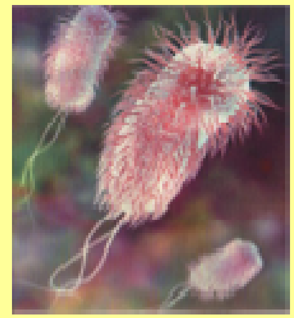


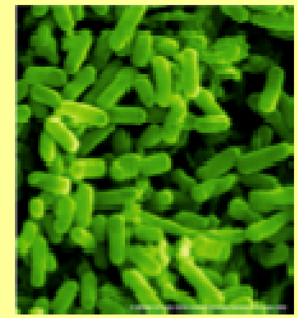
- PROMOTER
- RBS
- CODING SEQ.
- TERMINATOR
- ...



- INVERTER
- REPORTERS
- SENDERS & RECEIVERS
- ...







- E. COLI
- B. SUBTILIS
- YEAST
- MAMMALIAN CELLS
- ...

APPLICATIONS: BIOFUELS, BIOSENSORS, BIOMATERIALS, ...

To predict the behaviour of complex systems built from many parts, we need to have: predictive, composable models for the parts mechanisms to compose part models into a system model

Current tools

- There are already many *systems biology model repositories* (e.g., Biomodels, CellML model repository, Open Wetware repository, Java web simulation online, ModelDB, etc.) and *model analysis and design tools* available.

- However, these repositories and tools lack some of the important features of a *proper SynB C.A.D. framework*

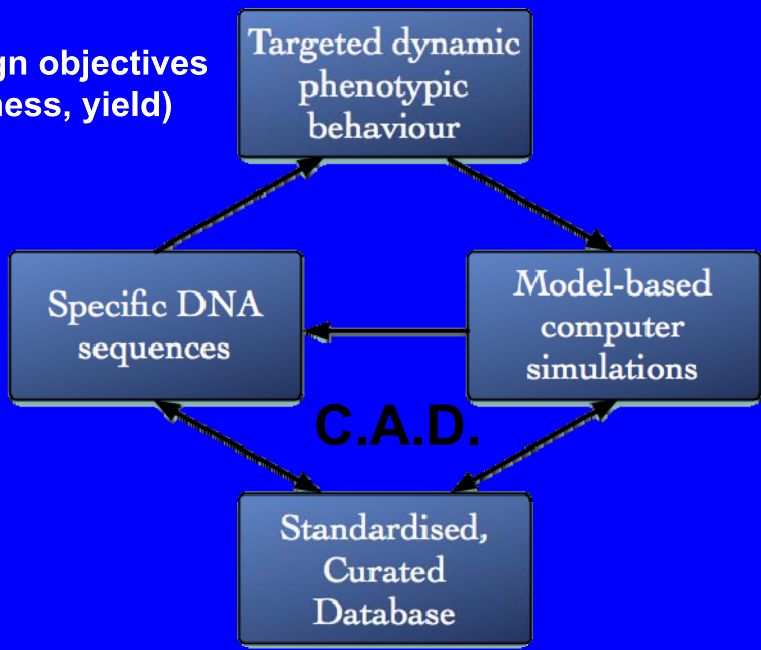
- They hardly support the modular building process used to create complex systems from the interconnection of parts and forming an integral part of the engineering cycle

- They do not provide a unified C.A.D. environment with access to composable and reusable mathematical models

What is needed ?

Definition of the design objectives
(behaviour, robustness, yield)

- Biological realisation & testing
- Experimental results



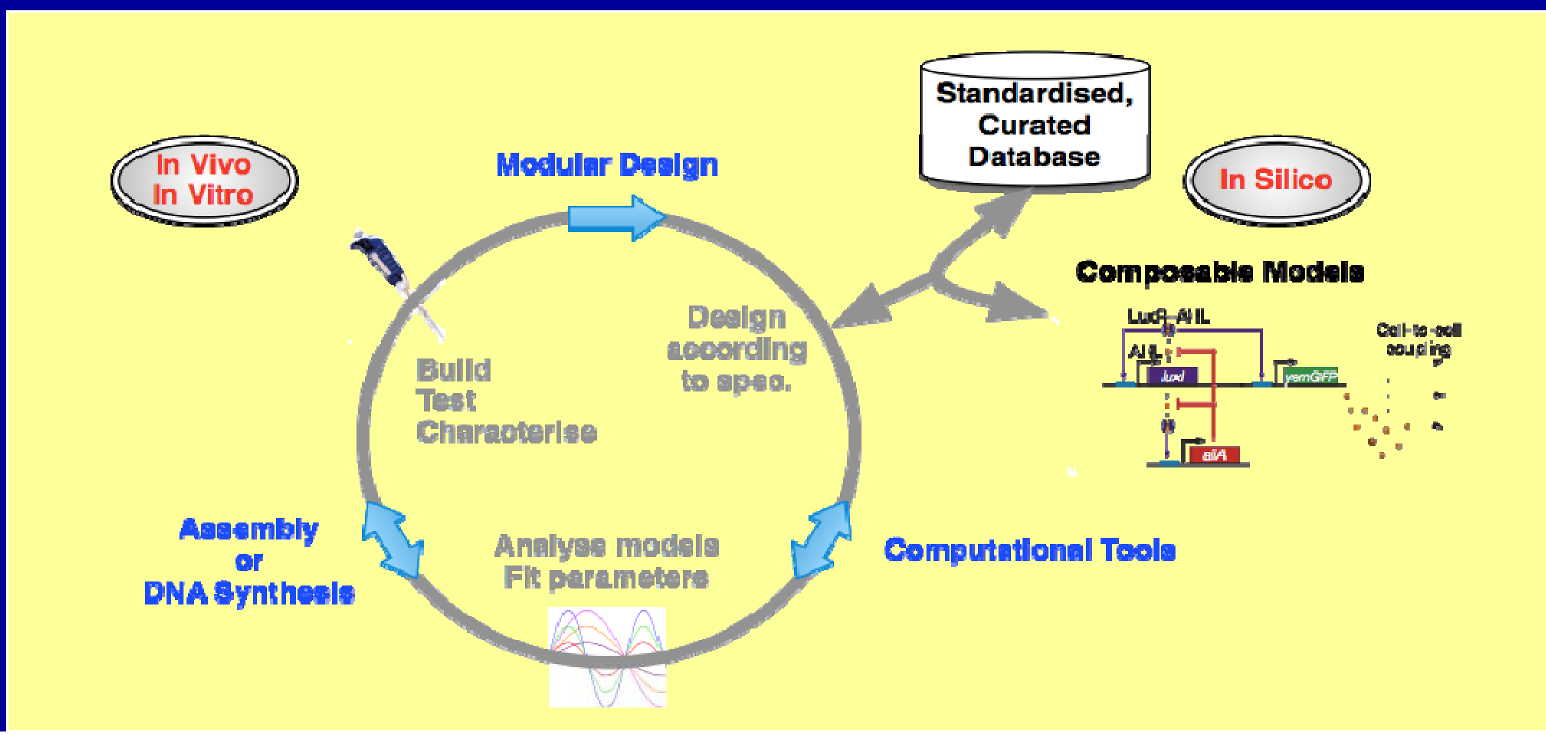
- Possible design alternatives
- Param. estim. & modulation
- Robustness & sensitivity anal.

- A *modular in silico C.A.D.* framework allowing:
- Easy design, simulation, and *composition* of *SynB models*
- Direct robustness and sensitivity analysis of models
- Seamless integration with a standardised & curated database:
  - search & annotation of part models based on design spec
  - search & modulation of model parameters
  - automated DNA sequence prediction & *de novo* synthesis

CAD and Professional Model Registry

In parallel with increasing the number of available parts and characterising them professionally, a logical extension would be to build a *registry of standard, composable models* together with an appropriate *synthetic biology C.A.D.* environment

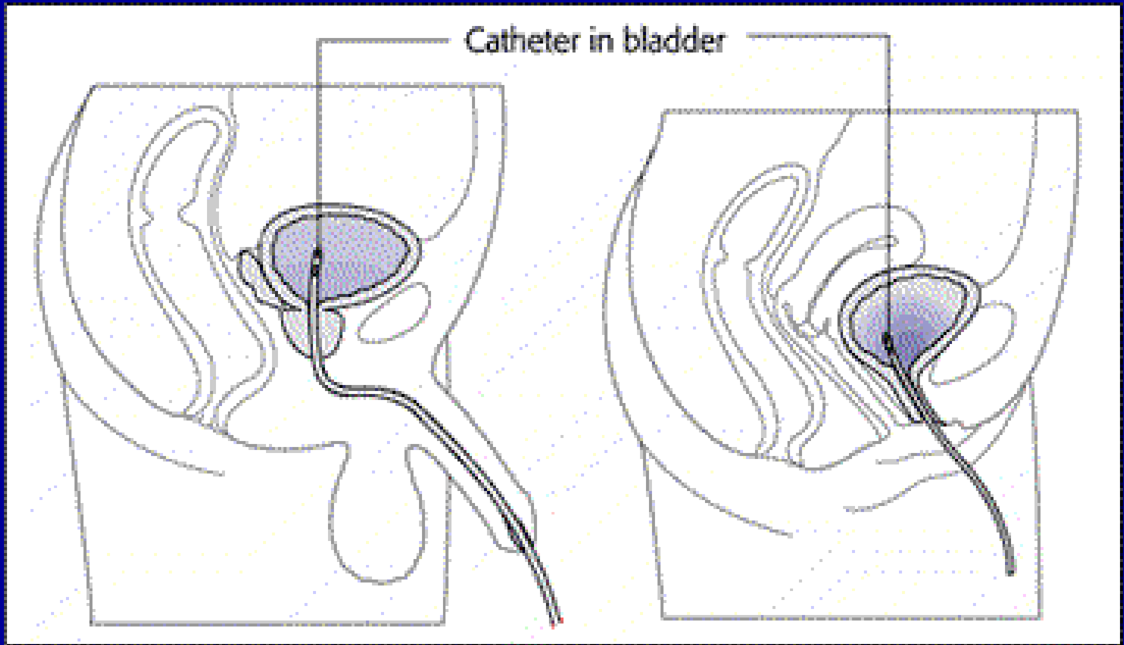
Engineering design cycle



Example 1 – Urinary Tract Infection (UTI)

The Problem

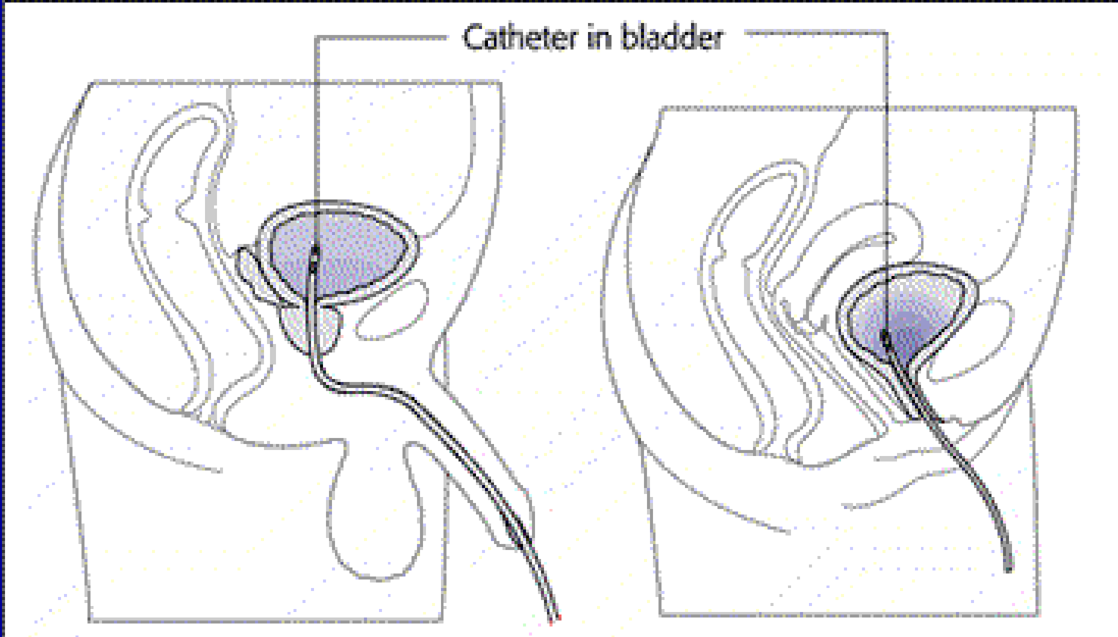
Infections take the form of a biofilm that creeps up the catheter into the urethra



Male and female lower urinary tract

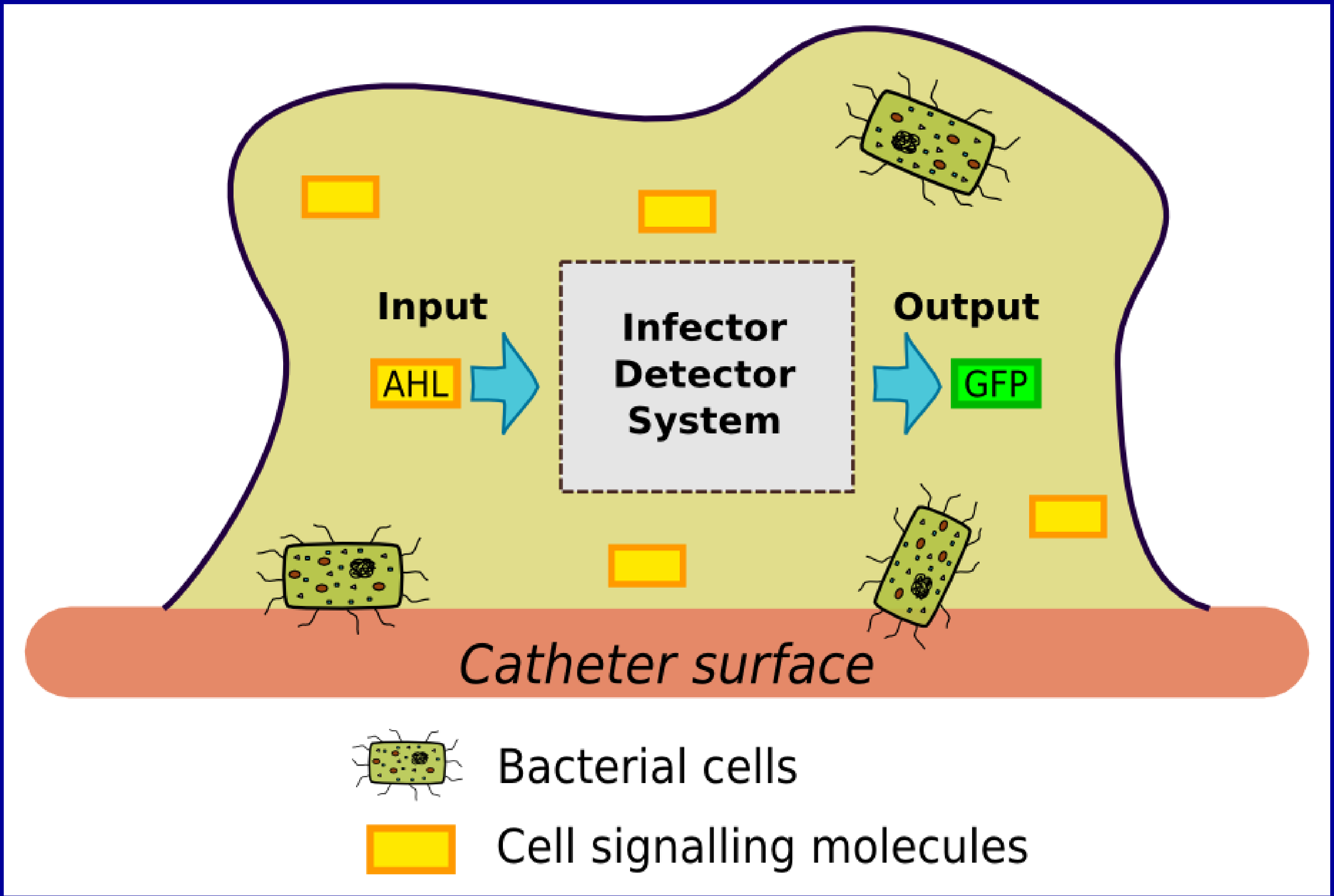
Our Aim

To design a genetically engineered machine which detects the presence of biofilm infection on urinary catheters



Male and female lower urinary tract

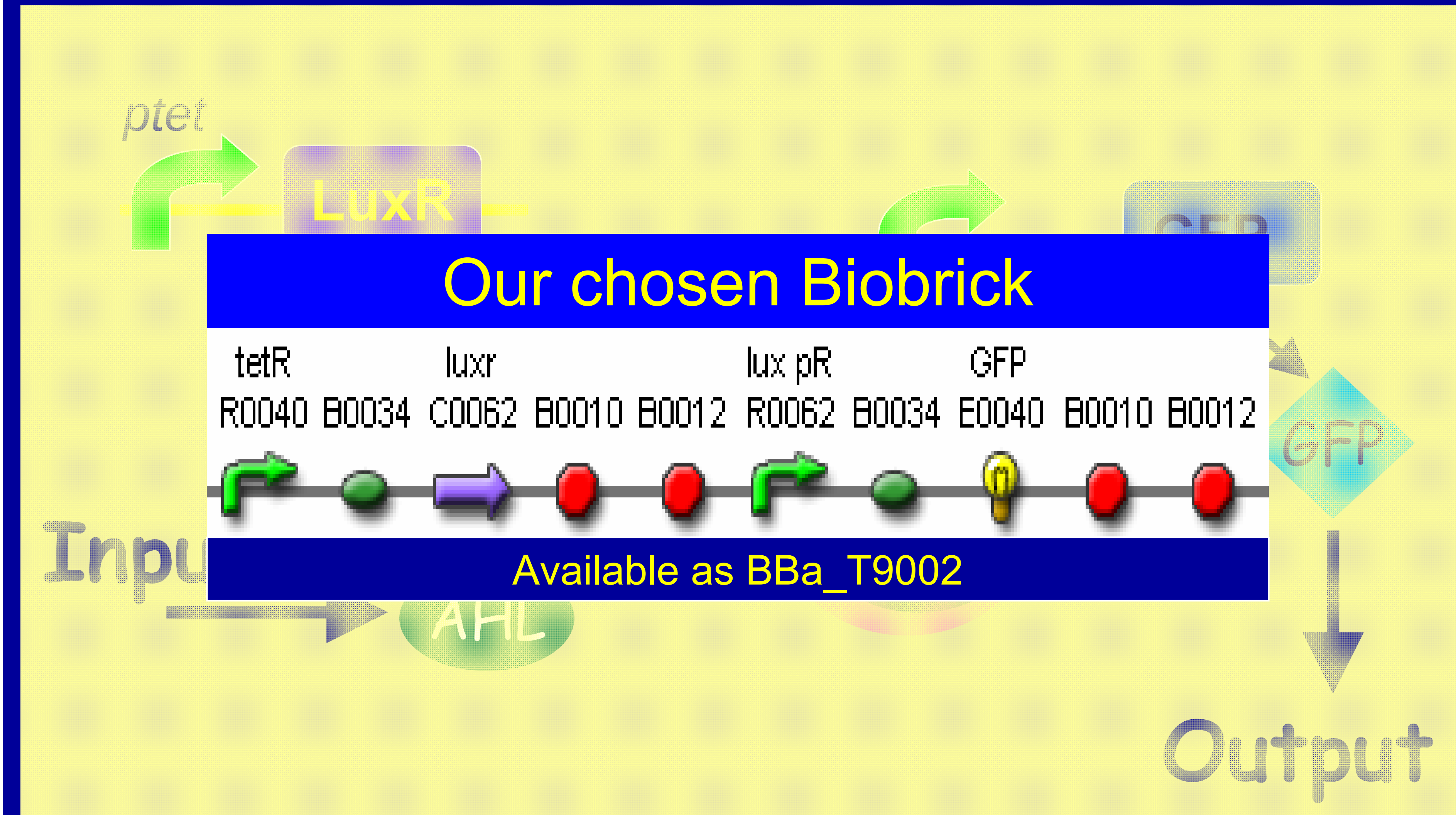
Our Detection Strategy

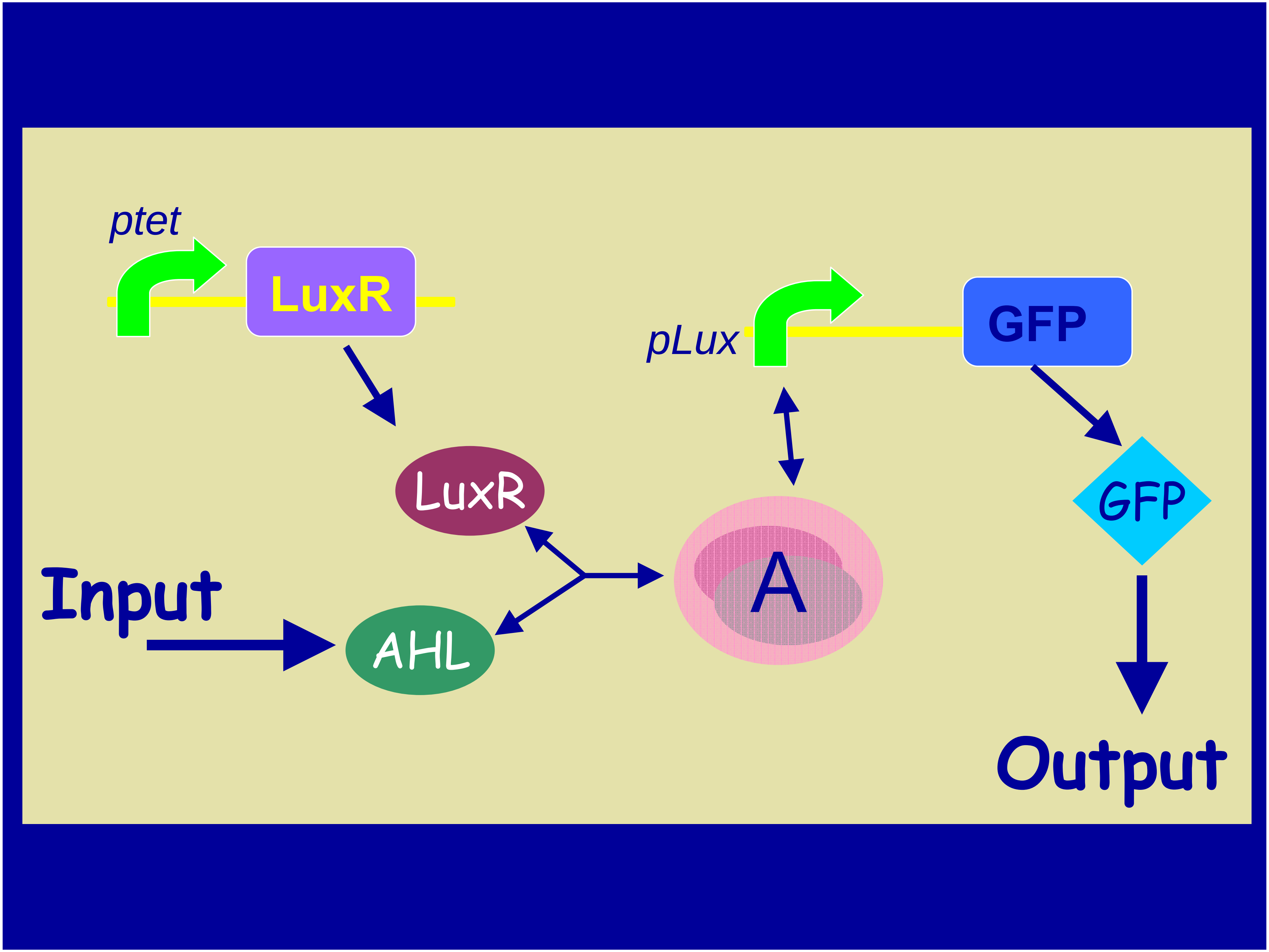


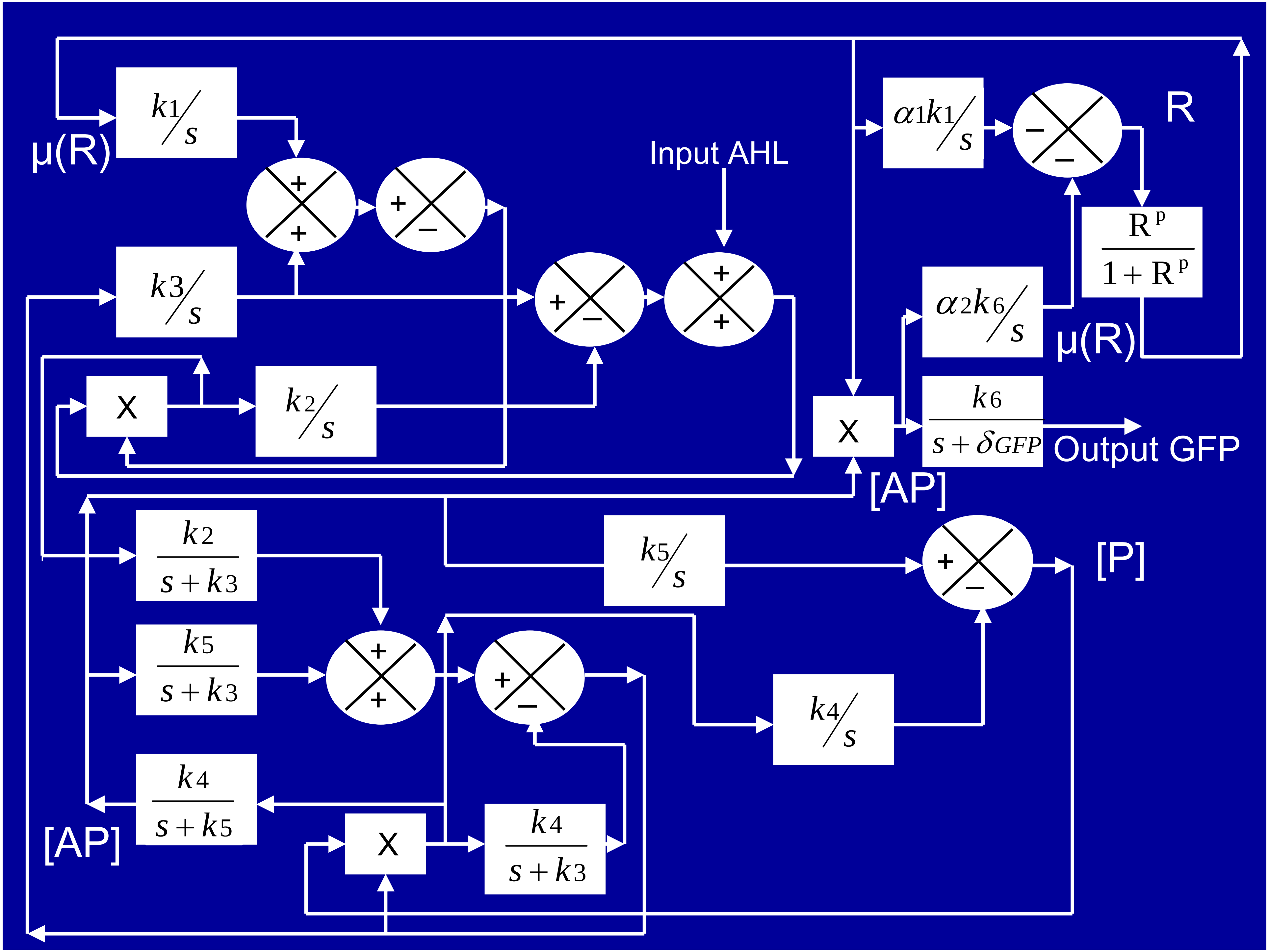
Urinary Tract Infection Detector – a three stage device

Detector	Amplifier	Indicator

The Biochemical Network – the basis of Infector Detector



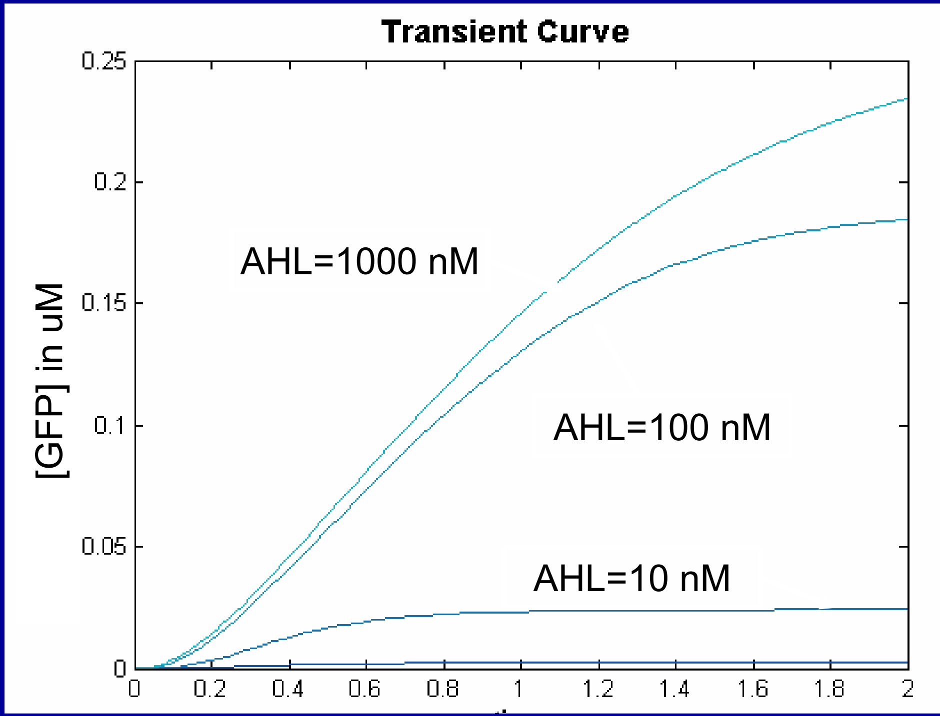

ptet
LuxR
pLux
GFP
LuxR
GFP
Input
AHL
A
Output


μ(R)
k1/s
k3/s
X
k2/s
Input AHL
α1k1/s
R
R^p / (1 + R^p)
α2k6/s
μ(R)
X
k6 / (s + δGFP)
Output GFP
[AP]
k2 / (s + k3)
k5/s
[P]
k5 / (s + k3)
k4/s
k4 / (s + k5)
[AP]
X
k4 / (s + k3)

Typical Simulations

General Behaviour:

- Slow uptake
- Saturation after few hours (Resources exhausted)
- The higher the input (AHL) , the higher the output (GFP)

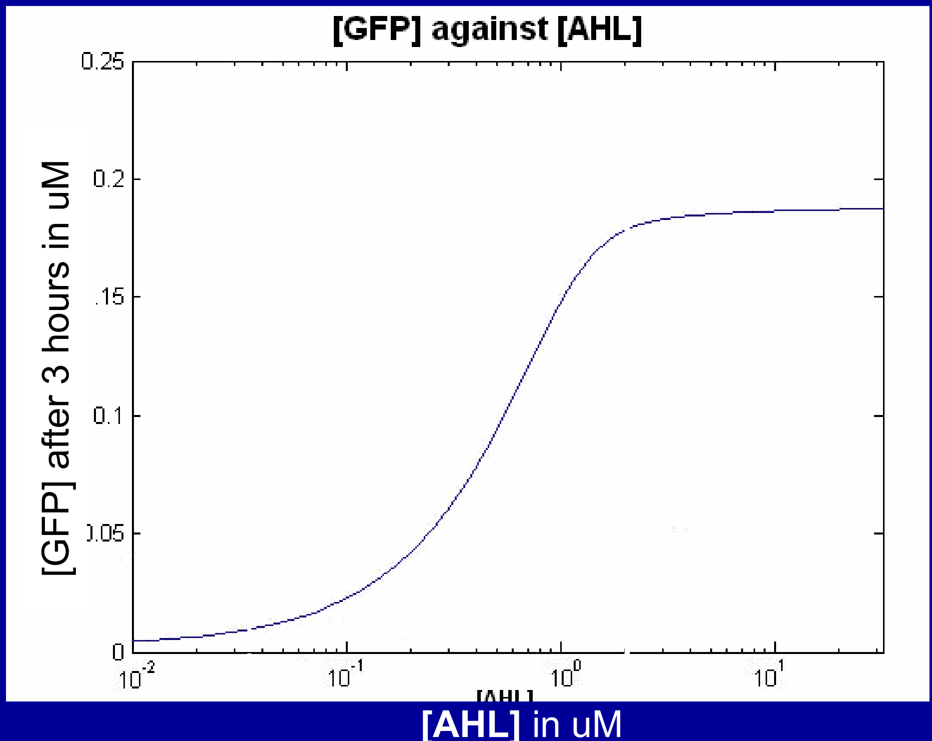


Time in hours

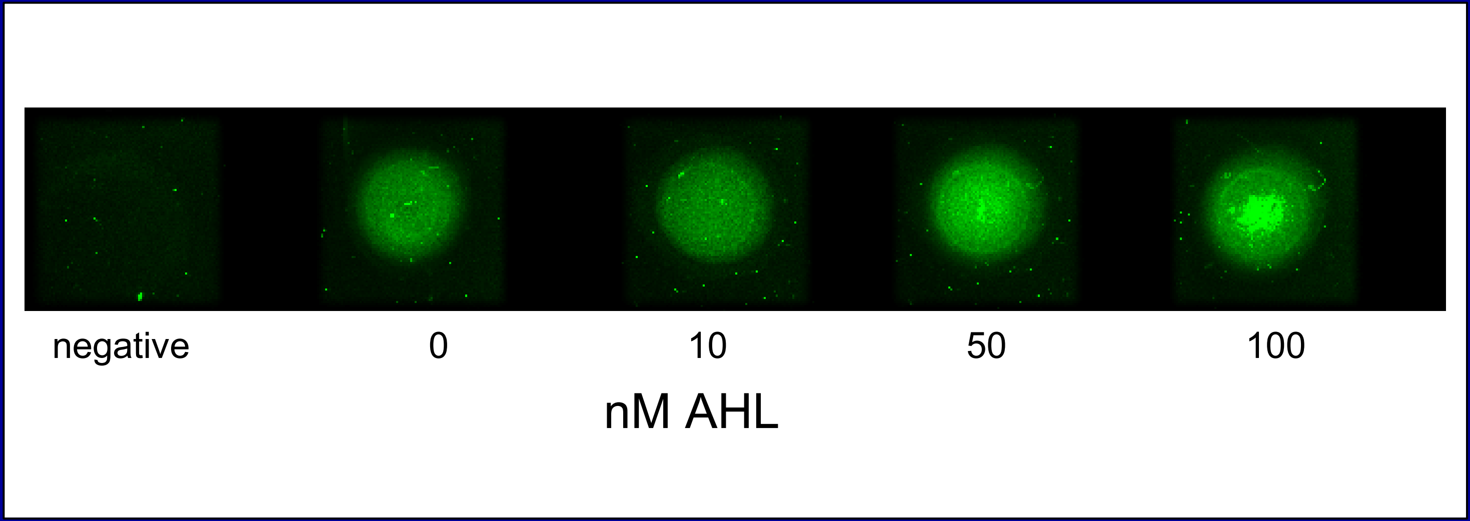
Transfer Function

GFP vs AHL

- Similar to F2620 in vivo
- Below T1 : No detection
- Above T2: Saturation

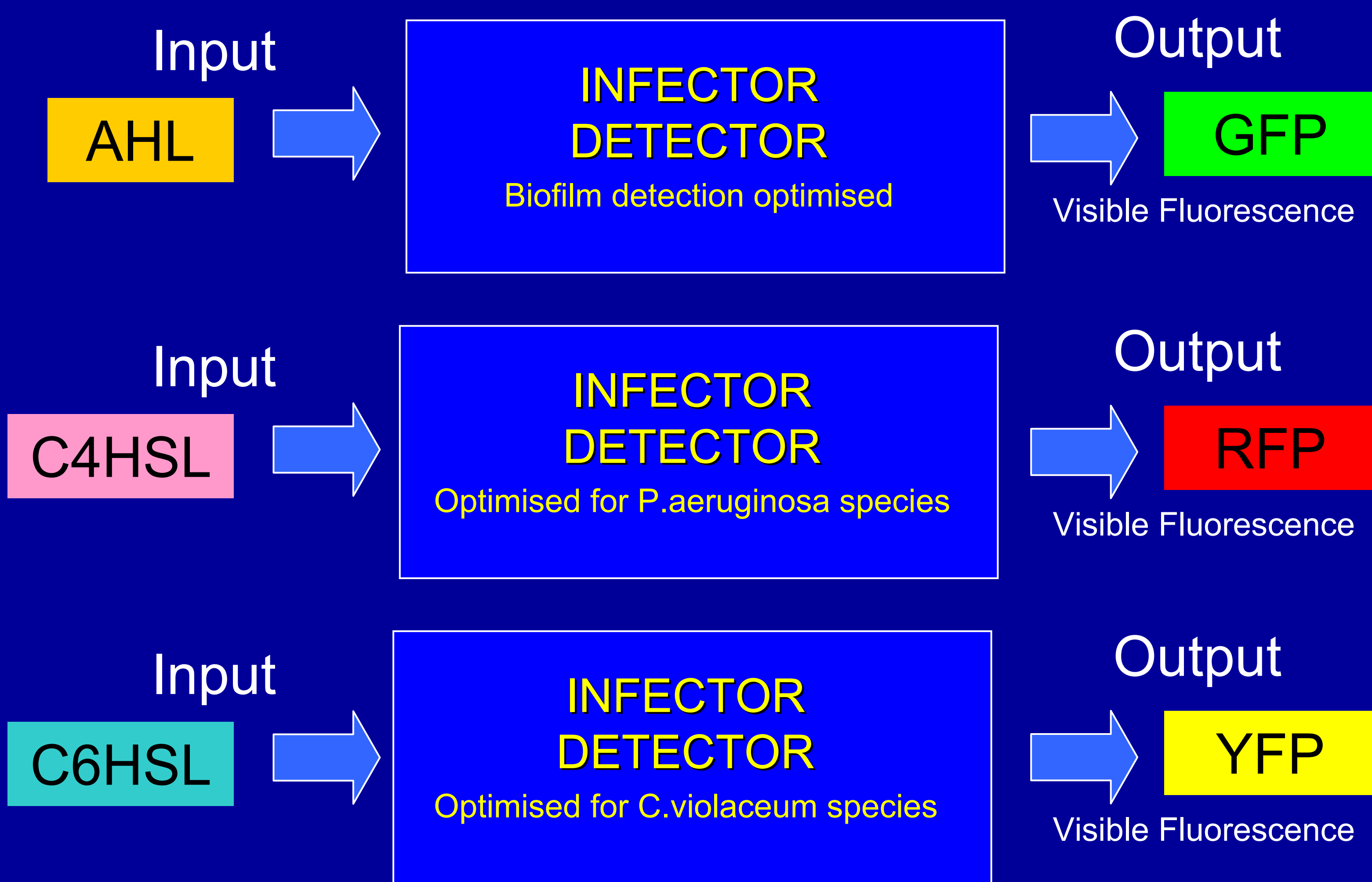


Testing Infector Detector on Agarose



Agarose drops with Infector Detector detecting different concentrations of AHL

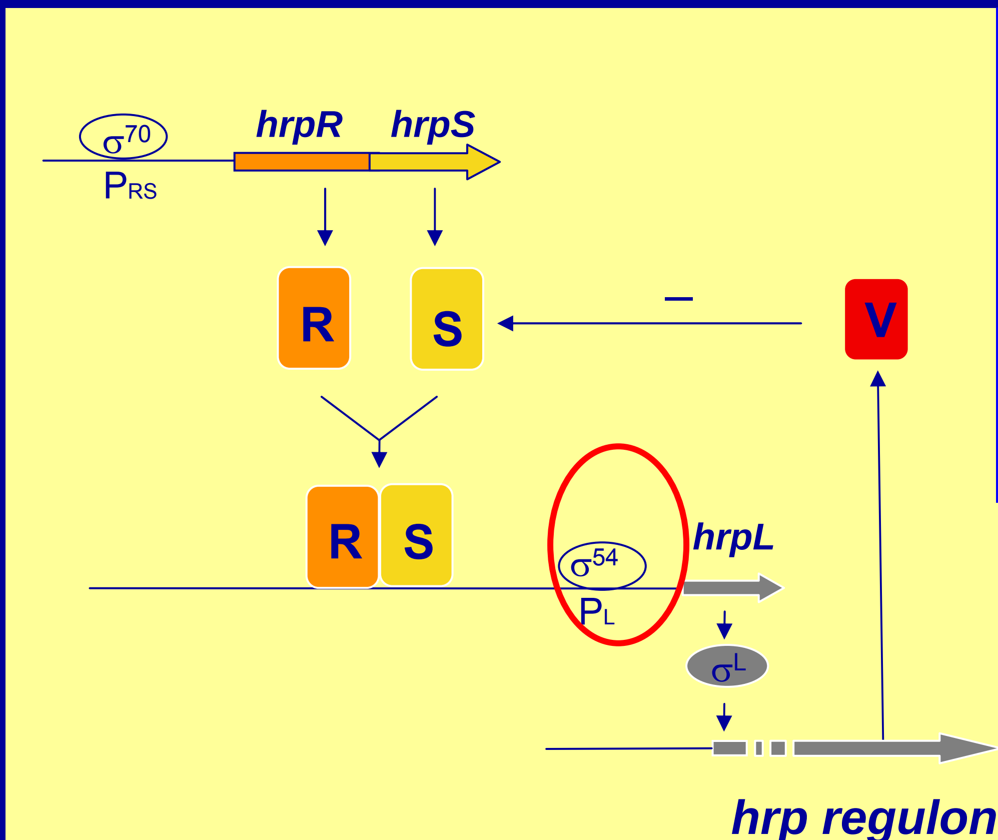
Ongoing Work: Customisation



Example 2 – Logic Gates

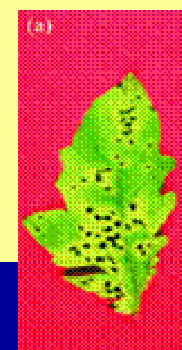
The hrp gene regulation system – a great system for modular biologically-based logical devices

- *hrp* (hypersensitive response and pathogenicity)



Advantages

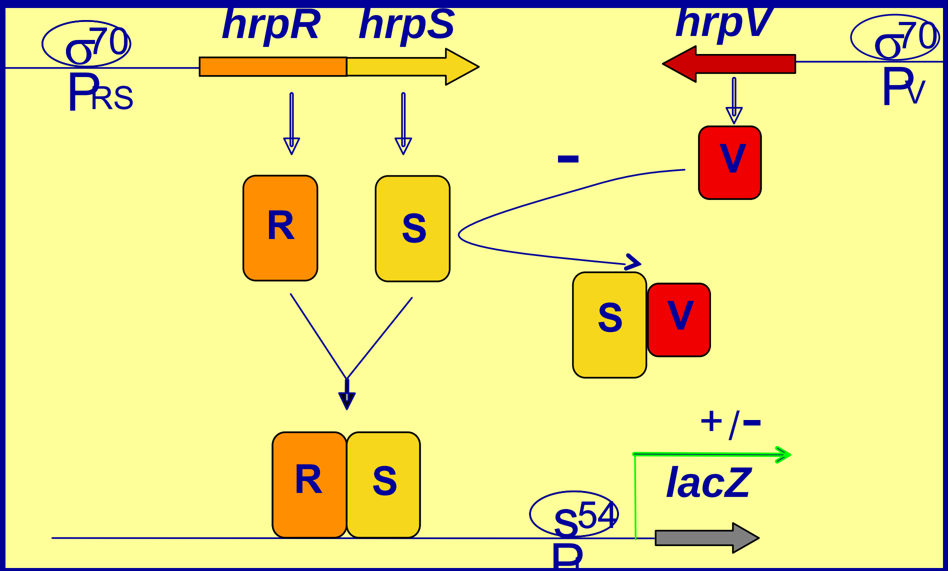
- sigma factor 54
- 2nd paradigm of gene activation
- multiple regulation factors
- protein-protein interactions
- high cooperatively



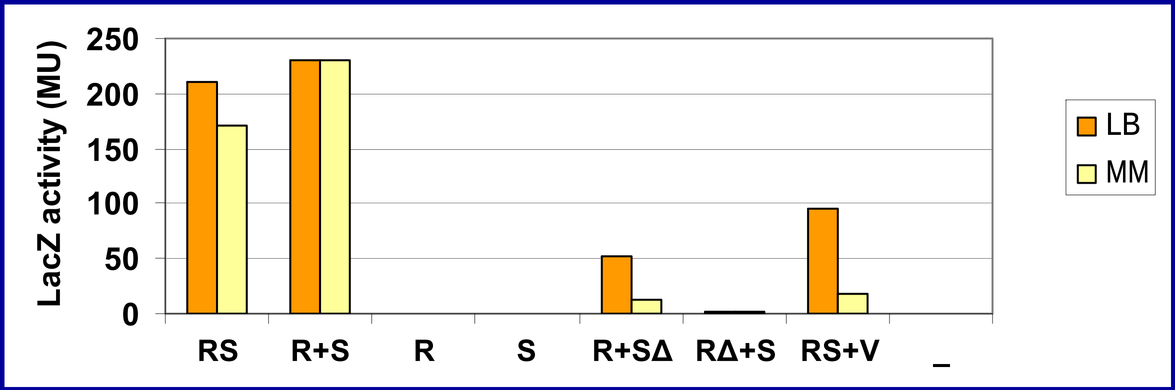
Pseudomonas syringae hrp regulatory system

Biological Experimental Results

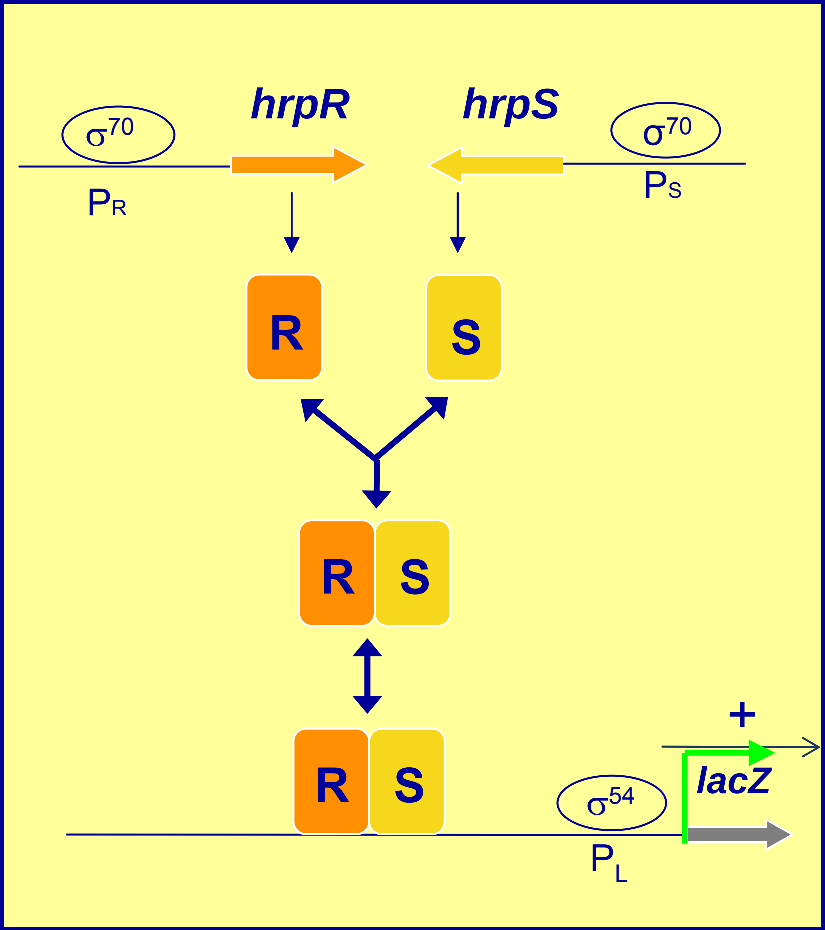
Identifying regulation mechanism for *hrpL* promoter activity

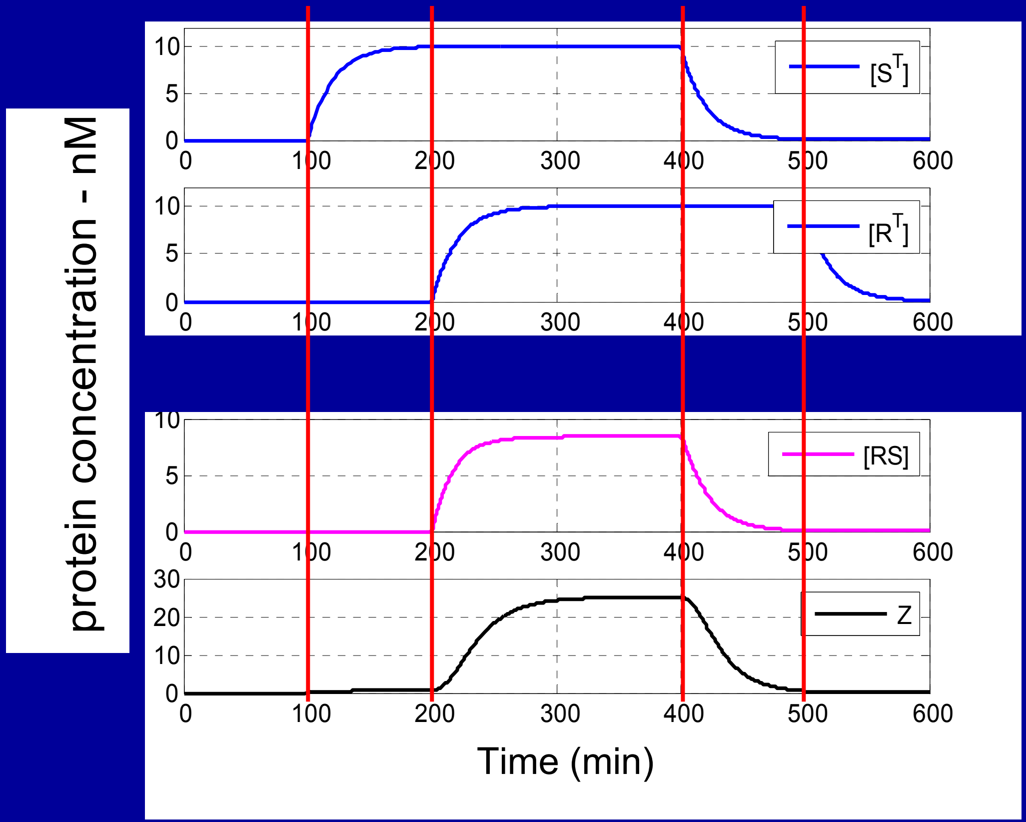


In vivo expression in *E.Coli* (MC4100 Ø*hrpL-lacZ*) of various *hrp* constructs in *cis* (RS) or *trans* (R+S) or individually(R, S).



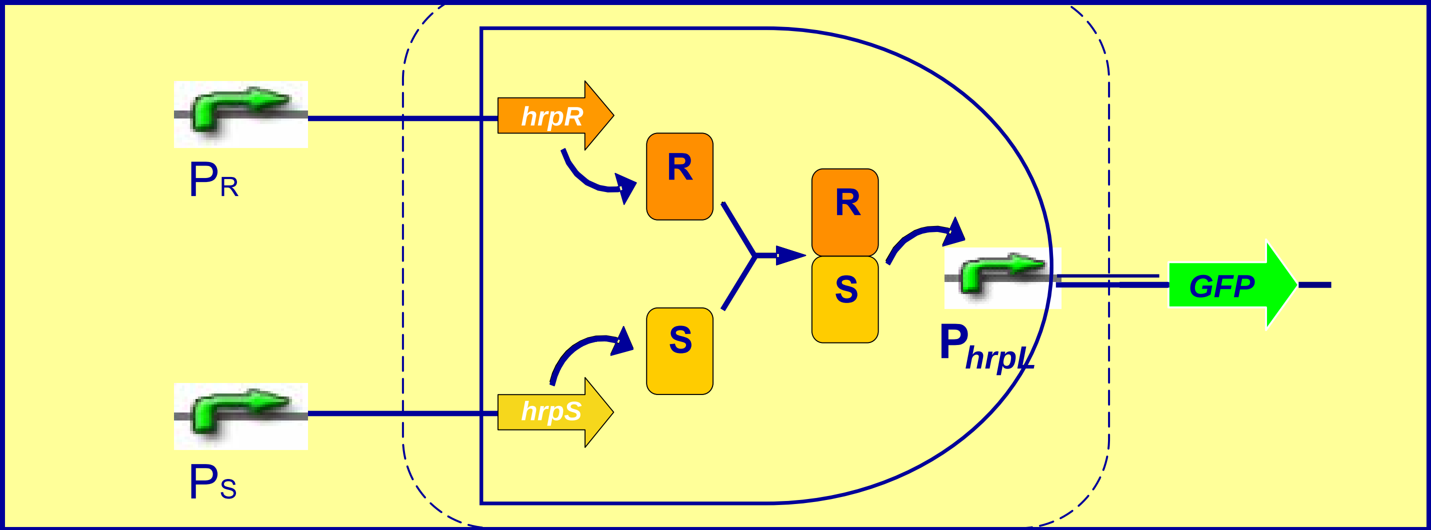
Modelling Case1: *hrpL* regulated by 2 factors

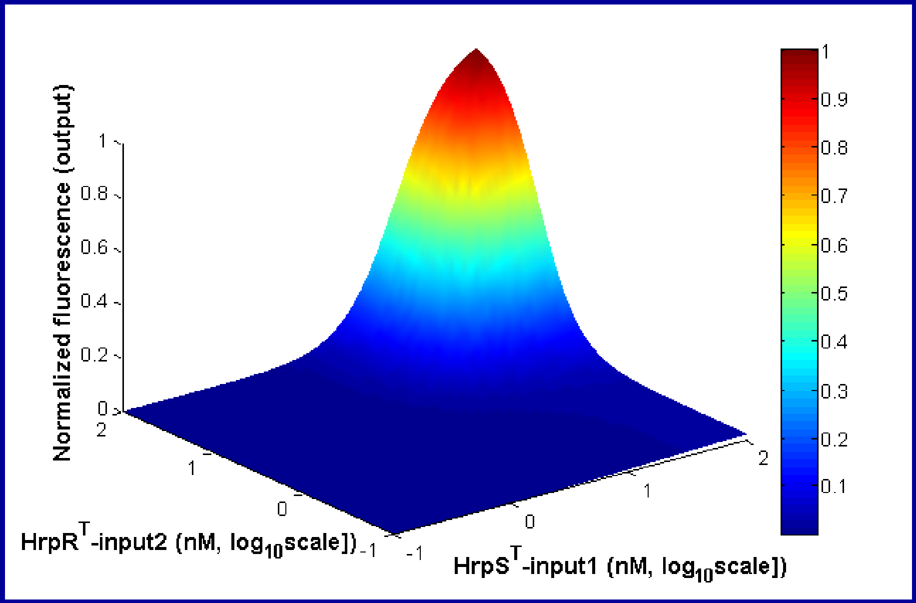




Simulation results: the dynamic evolution of protein concentrations

A Modular AND Gate



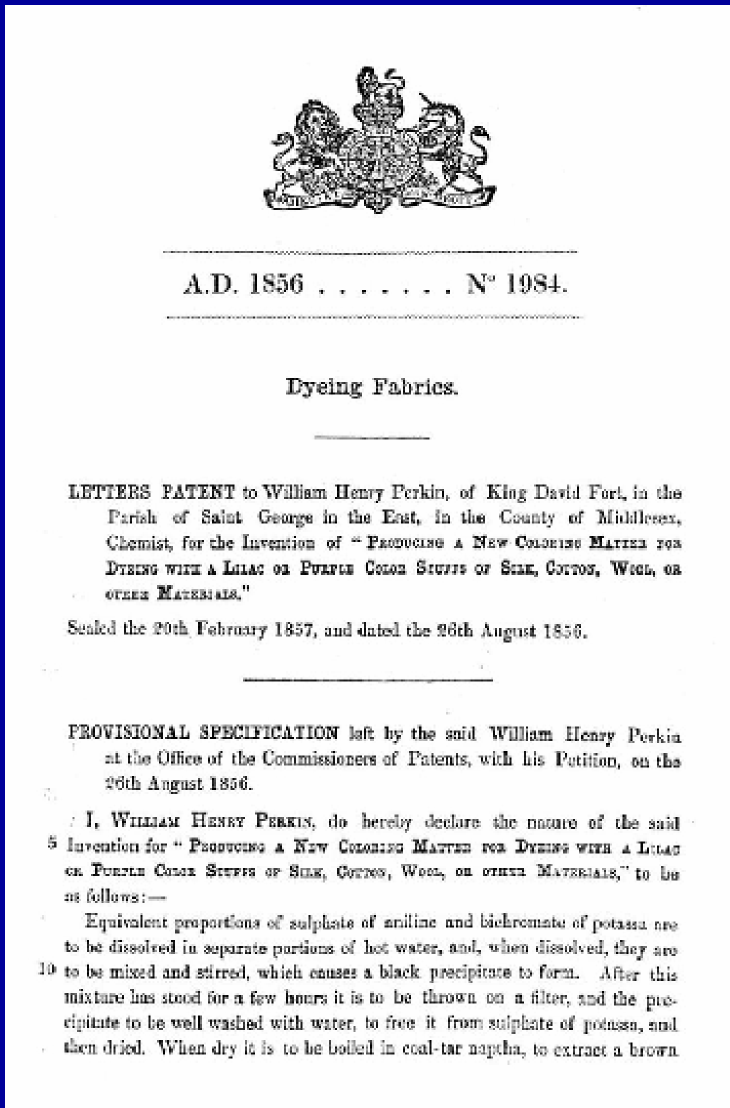


PR	PS		pHrpL
0	0		0
0	1		0
1	0		0
1	1		1

Logic Gates are the basic building blocks of all digital devices - counters, microprocessors, computers

There are strong parallels with Synthetic Chemistry in the 19th Century

Modern examples of natural dyes in the Mysore market in India



A.D. 1856 . . . . N° 1984.

Dyeing Fabrics.

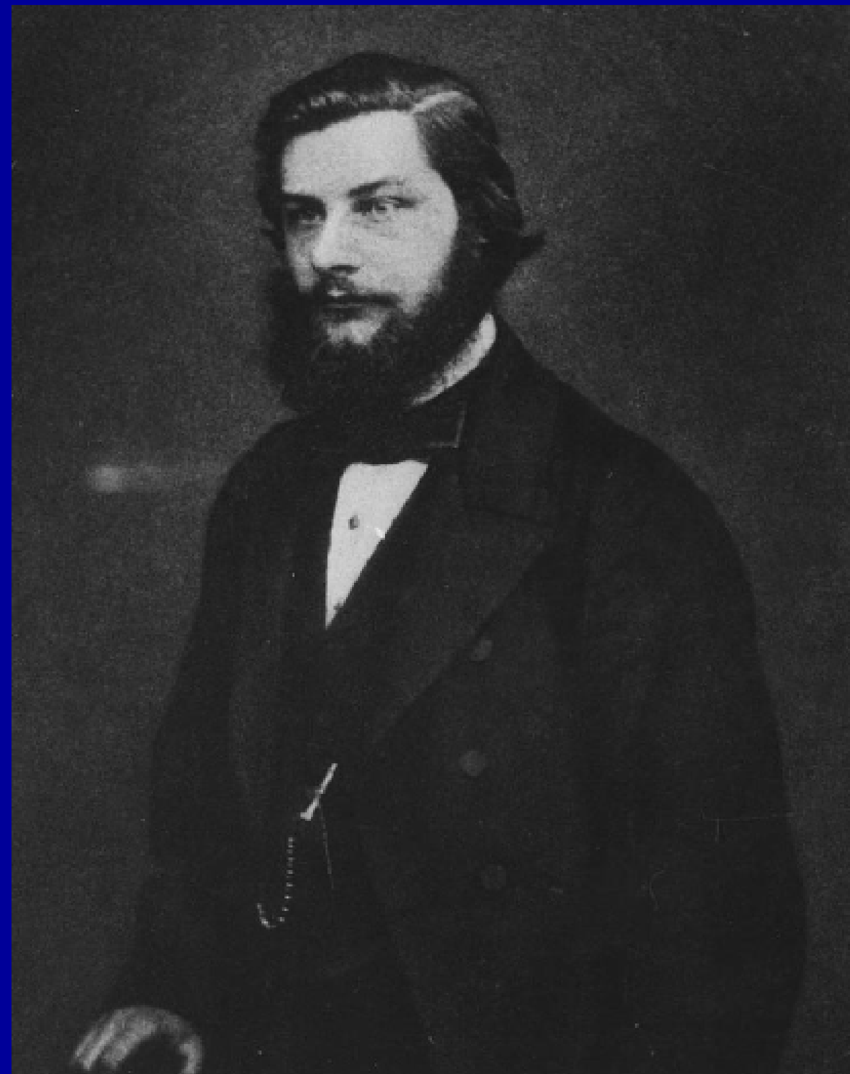
LETTERS PATENT to William Henry Perkin, of King David Fort, in the Parish of Saint George in the East, in the County of Middlesex, Chemist, for the Invention of "PRODUCING A NEW COLORING MATTER FOR DYEING WITH A LILAC OR PURPLE COLOR STUFFS OF SILK, COTTON, WOOL, OR OTHER MATERIALS."

Sealed the 20th February 1857, and dated the 26th August 1856.

PROVISIONAL SPECIFICATION left by the said William Henry Perkin at the Office of the Commissioners of Patents, with his Petition, on the 26th August 1856.

I, WILLIAM HENRY PERKIN, do hereby declare the nature of the said Invention for "PRODUCING A NEW COLORING MATTER FOR DYEING WITH A LILAC OR PURPLE COLOR STUFFS OF SILK, COTTON, WOOL, OR OTHER MATERIALS," to be as follows:—

Equivalent proportions of sulphate of aniline and bichromate of potassa are to be dissolved in separate portions of hot water, and, when dissolved, they are to be mixed and stirred, which causes a black precipitate to form. After this mixture has stood for a few hours it is to be thrown on a filter, and the precipitate to be well washed with water, to free it from sulphate of potassa, and then dried. When dry it is to be boiled in coal-tar naptha, to extract a brown



William Henry Perkin -1856, the production of synthetic quinine from benzene

Aspirin 1897

Chemist Felix Hoffmann, at Bayer in Germany

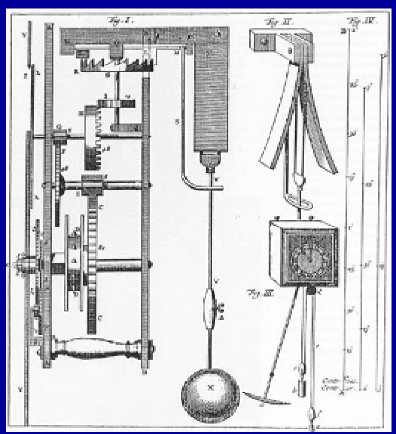
Synthetic Rubber

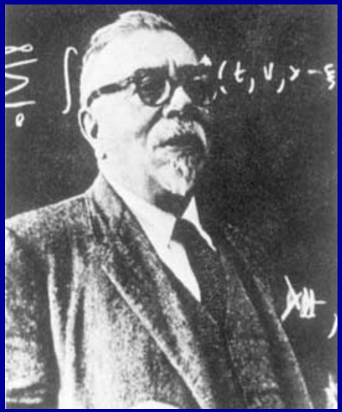
Analogue Age

Digital Age

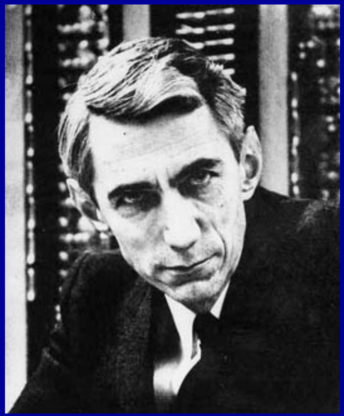
Biological Age

Huygens
Pendulum Clock
1656. Accurate to
better than 1
minute per day

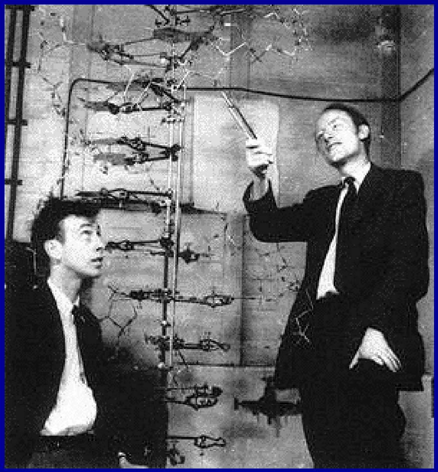




Norbert Wiener



Claude Shannon



Nature 409, 860 - 921 (2001)

Initial sequencing and analysis of the human genome

International Human Genome Sequencing Consortium

The human genome holds an extraordinary trove of information about human development, physiology, medicine and evolution. Here we report the results of an international collaboration to produce and make freely available a draft sequence of the human genome. We also present an initial analysis of the data, describing some of the insights that can be gleaned from the sequence.

A New Industrial Revolution in the Making (?)

Synthetic Biology promises a shift comparable in importance to the ICT revolution with the power to revolutionise many sectors of the economy including:

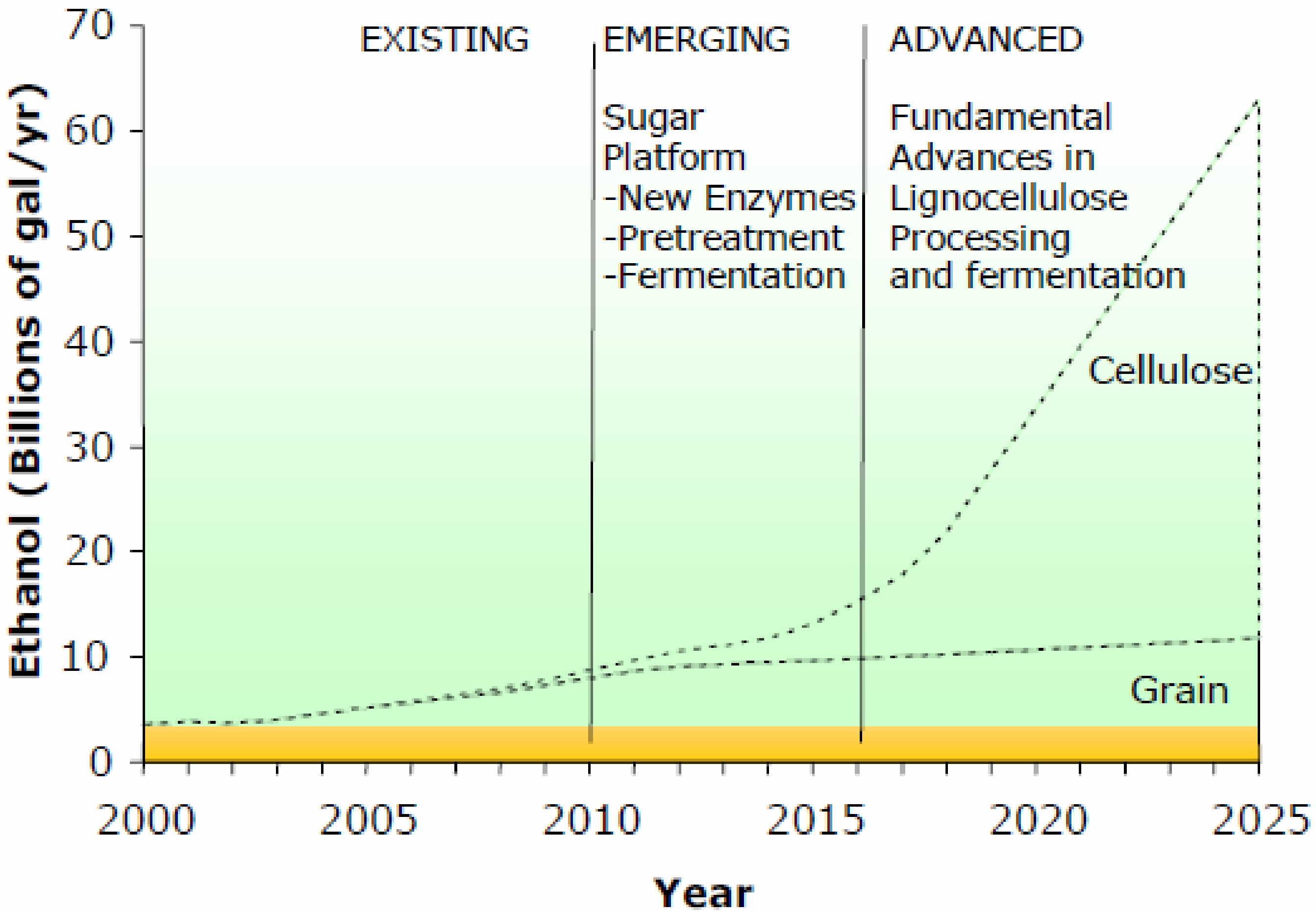
- Biofuels
- Biomaterials
- Medicines/Drugs/Vaccines
- Biosensors

Some Industrial Examples

The objective of synthetic biology is the industrialisation of biology

Engineering micro-organisms
to make Bio-diesel

A DoE (US) Ethanol Vision



Modified from Richard Bain, NREL

Example: Halophile energy from desalination

Halobacterium halobium

Thrives in waste brine from desalination

Engineered to produce isobutanol biopetrol from sunlight and CO_2

Provides an local source of energy for desalination

Example: Heavy-metal biosensors for water

Arsenic, Antimony, Lead

Small molecules that are expensive to detect

Natural proteins can bind these

Microbial two-component signalling systems are modular

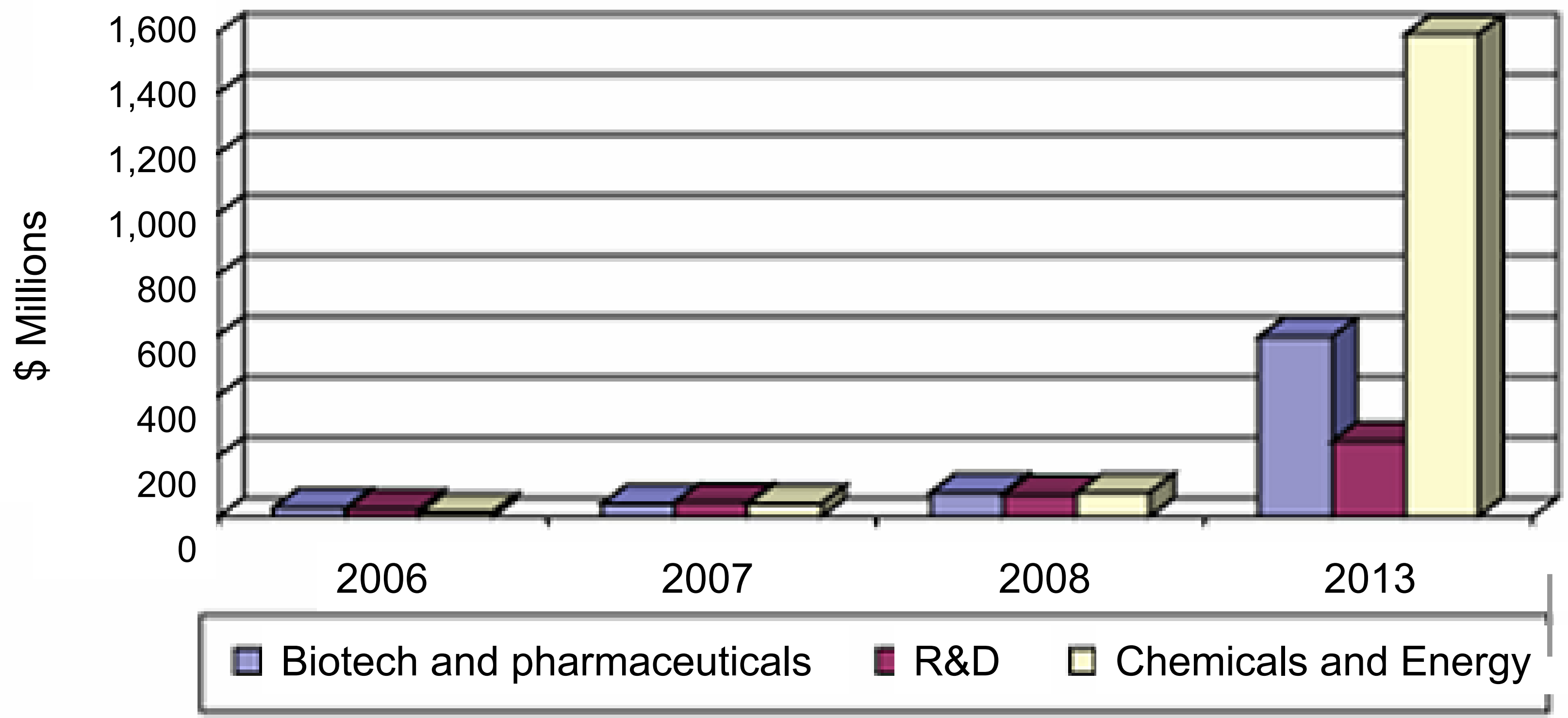
Bind – Detect – Signal

What microbes /organisms can be safely added to points in the water supply?

- Coliform bacteria – *E.coli*, *Citrobacter*
- Algae, pond weed plants

Market	Segment	Associated Products and Areas
Medical devices	Tissue Engineering/Biomaterials	Medical Devices/implants
Pharmaceutical	Diagnostics/Biomarkers	Pharmaceutical
	Molecular imaging	Medical Contrast agents/imaging
	DNA Vaccines	Infectious diseases
	Drug synthesis (Improving synthesis of existing agents)	Pharma/ Bioprocessing /Biosynthesis
	Pharma-Cosmetic	Biosynthesis
Agroscience	Pesticide/Toxicity testing	
	Plant Breeding/Crop Yield	
	Food Quality Monitoring	Food Packaging
	Nutrition	Biosynthesis
Utilities	Environmental Monitoring	Water Supply/Bioterrorism etc

SUMMARY FIGURE
GLOBAL VALUE OF SYNTHETIC BIOLOGY MARKET BY INDUSTRY 2006-2013
($ MILLIONS)



Source: BCC Research

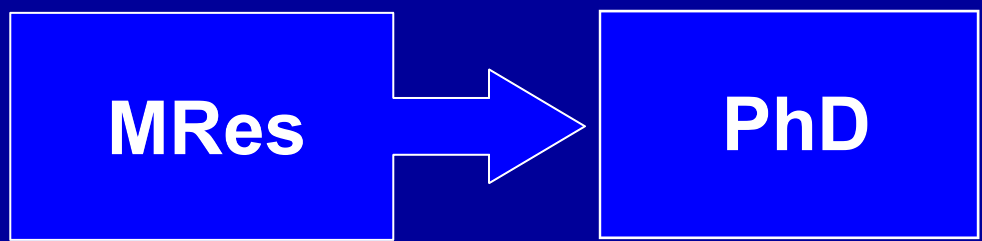
Report ID:BIO066A, Published: June 2009, Analyst: John Bergin

Education and Training

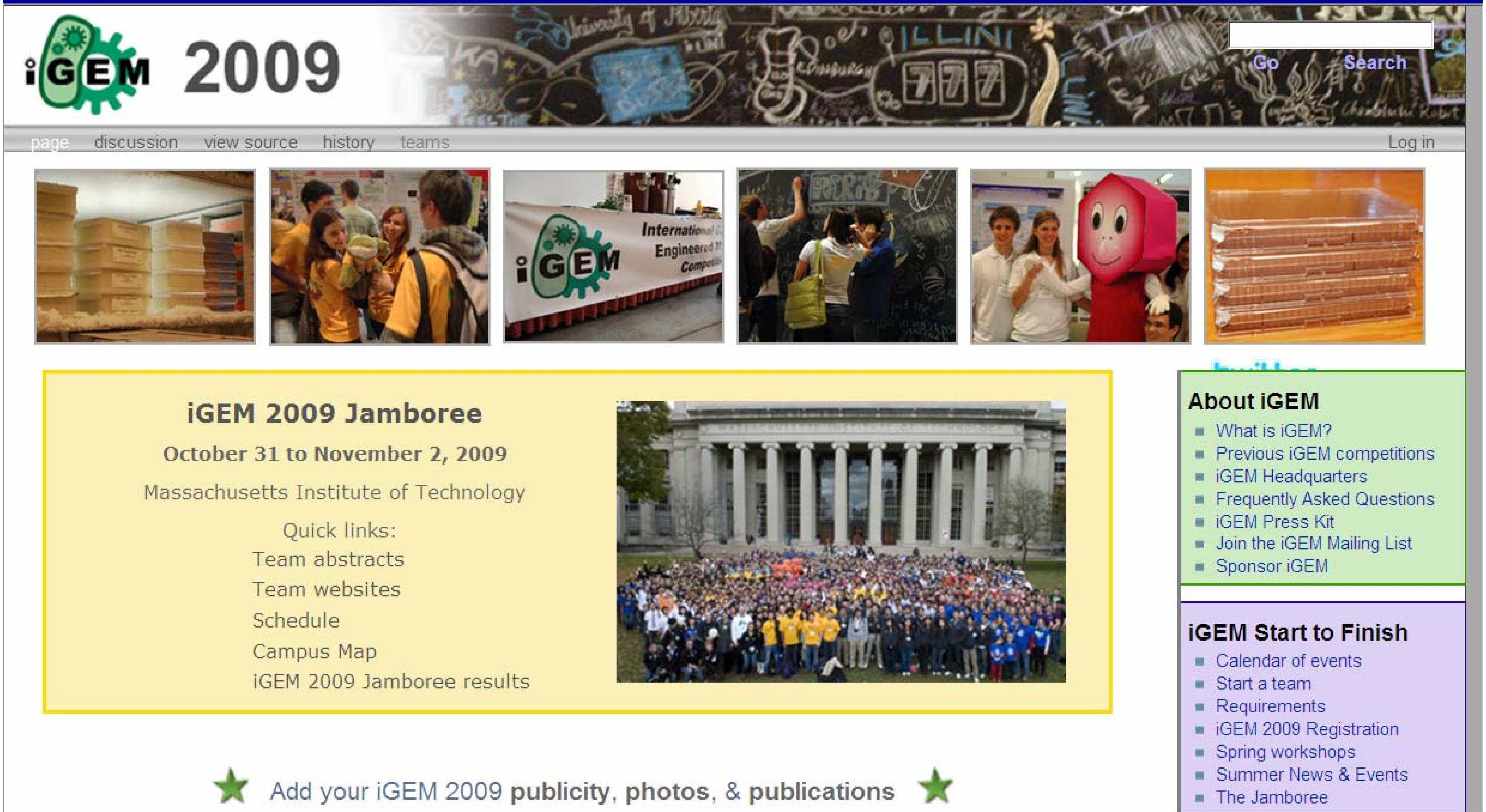
Undergraduate Training

- Final Year course in Synthetic Biology typically 15 students from engineering + 15 from biology
- iGEM (the international Genetically Engineered Machines Competition) – run by MIT

Graduate Training



- The Imperial College (IoSSB) MRes started October 2008
- Ongoing PhD Programme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iGEM
The Need for European Funding

Establishing European Collaboration in Synthetic Biology

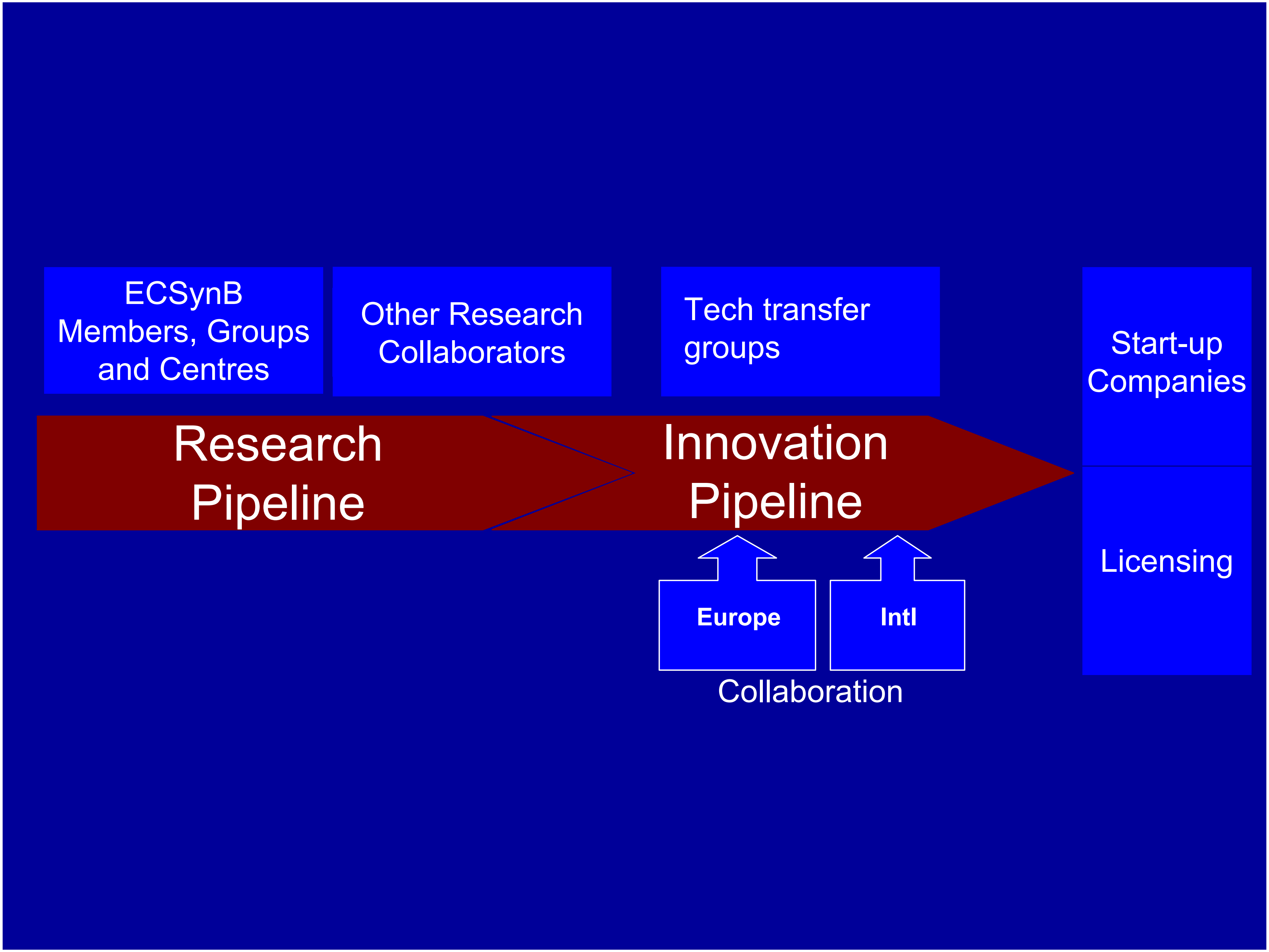
- What is required is leading European academic groups to work with industry
- Establishing a European Consortium
- Hub

ECSynB - European Consortium for Synthetic Biology

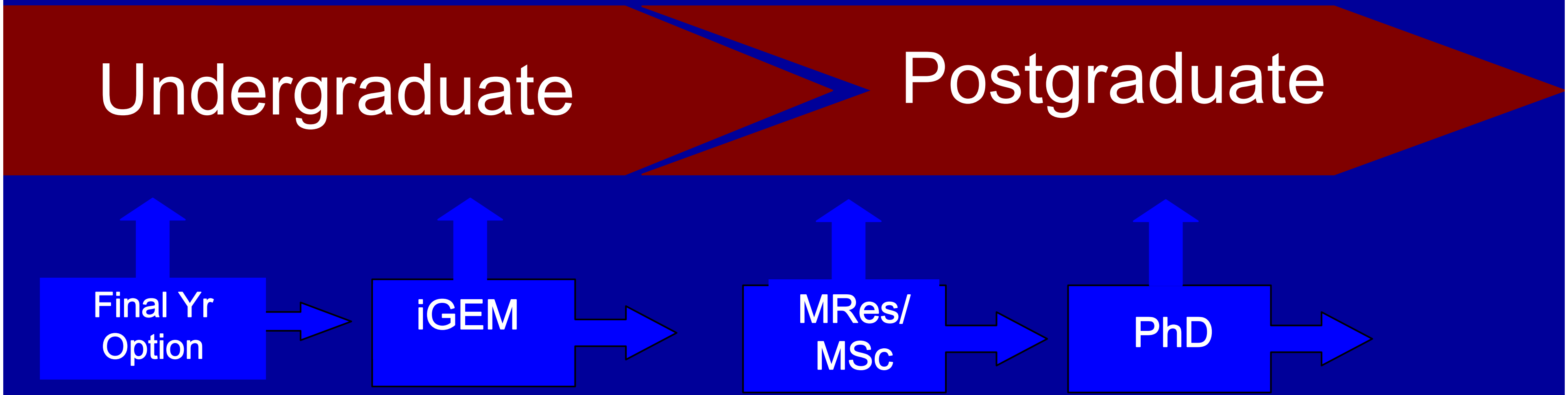
Phase 1. Undertake an audit of European Research Activity (academic and industrial) – 6 months

Phase 2. Undertake a more general audit to develop a strategic plan for Europe (use Tessy and other reports)

Phase 3. Identify Grand Challenges


ECSynB Members, Groups and Centres
Other Research Collaborators
Tech transfer groups
Start-up Companies
Research Pipeline
Innovation Pipeline
Licensing
Europe
Intl
Collaboration

The Education Pipeline



The End