#### Imperial College London



### Synthetic Biology – State, Importance and Development

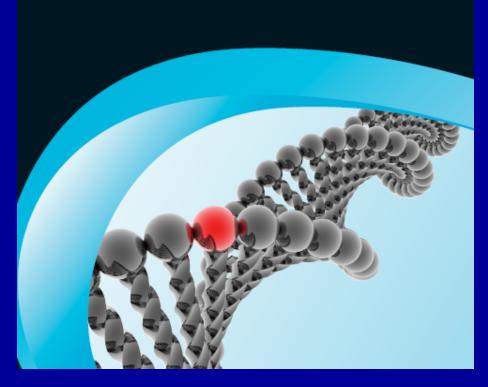
#### Professor Richard I Kitney Chairman - The Institute of Systems and Synthetic Biology Co-director – Centre for Synthetic Biology and Innovation



http://www.raeng.org.uk/policy/engagement/pdf/Systems\_Biology\_Report.pdf



Synthetic Biology: scope, applications and implications



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



The endpoint of Synthetic Biology is industrialisation

The endpoint of analysing biological systems is Systems Biology

## Synthetic Biology

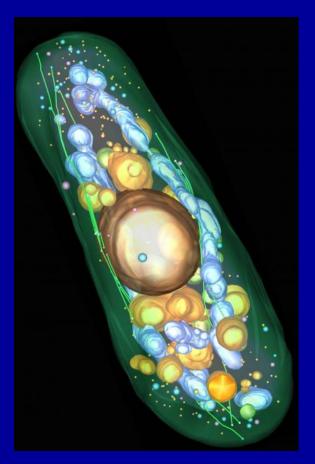
#### A Broad Church

- 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

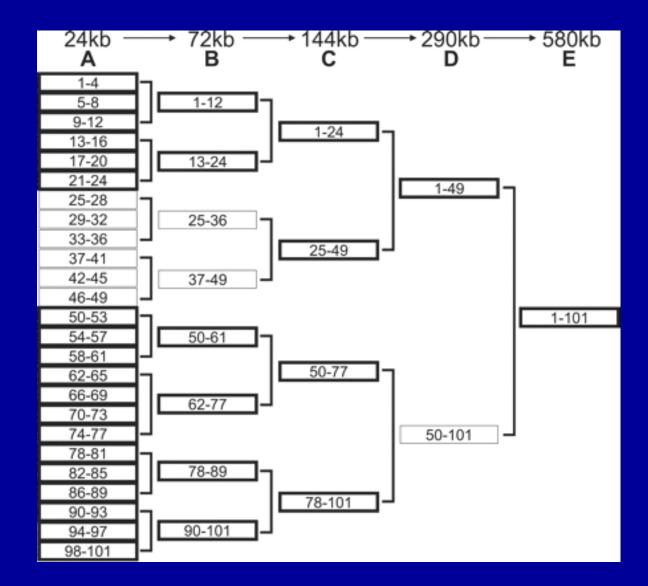
| Science AAA                 |  | GO> Advanced  |  |
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| MAAAS Mag                   |  |   |  |
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| Home > <u>Science Magaz</u> | <u>ine</u> > <u>29 February 2008</u> > Gibson et al. , pp. 1215 - 1220   |   |  |
| Article Views               | Originally published in Science Express on 24 January 2008 <pre></pre>   |   |  |
| Abstract                    | Science 29 February 2008:<br>Vol. 319. no. 5867, pp. 1215 - 1220   | lolecular Biology + Plant Biology<br>Renew or<br>ant Biology + Development + Biol |  |
| Full Text (HTML)            | DOI: 10.1126/science.1151721 Subscribe Today!  |   |  |
| → Full Text (PDF)           | RESEARCH ARTICLES  | CLICK HERE  |  |
| • Figures Only              | Complete Chemical Synthesis, Assembly, and Cloning of a  | oche Signaling Bioir  |  |
| Supporting Online           |  |   |  |
| Material                    | Daniel G. Gibson, Gwynedd A. Benders, Cynthia Andrews-Pfannkoch,   |   |  |
| VERSION HISTORY             | Evgeniya A. Denisova, Holly Baden-Tillson, Jayshree Zaveri, Timothy B. Stockwell,<br>Anushka Brownley, David W. Thomas, Mikkel A. Algire, Chuck Merryman, Lei Young, | ADVERTISEMENT   |  |
| 319/5867/1215 (most         | Vladimir N. Noskov, John I. Glass, J. Craig Venter, Clyde A. Hutchison, Ill,   | A BYERHOEMENT   |  |
| recent)                     | Hamilton O. Smith <sup>*</sup>   |   |  |
| ° 1151721v1                 | We have synthesized a 582,970-base pair Mycoplasma genitalium genome. This synthetic   |   |  |
| Article Tools               | genome, named <i>M. genitalium</i> JCVI-1.0, contains all the genes of wild-type <i>M. genitalium</i>  |   |  |
|                             | G37 except MG408, which was disrupted by an antibiotic marker to block pathogenicity and   |   |  |
| → Save to My Folders        | to allow for selection. To identify the genome as synthetic, we inserted "watermarks" at   |   |  |
| Download Citation           | intergenic sites known to tolerate transposon insertions. Overlapping "cassettes" of 5 to 7  | Submit  |  |
| Alert Me When               | kilobases (kb), assembled from chemically synthesized oligonucleotides, were joined by in  | Submit  |  |

# 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









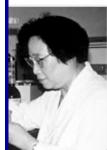
## 2. Metabolic Engineering

## 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

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

Academy of Born: 1930 [sources / revisions]

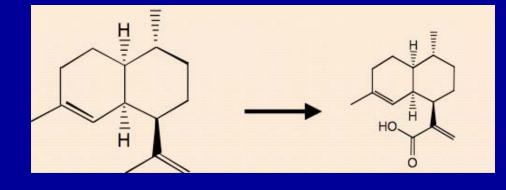


#### Making Complex Drugs

#### Anti-malarial drug Artemesinin









Amyris Biotechnologies



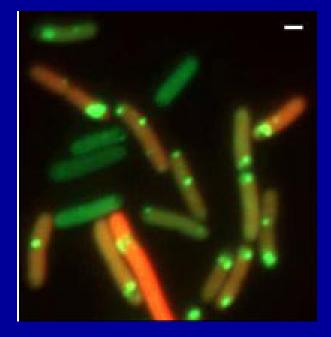
Institute for OneWorld Health

## 3. Chassis

#### Chassis

#### Natural Chassis

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



Developing chassis that are fit for purpose

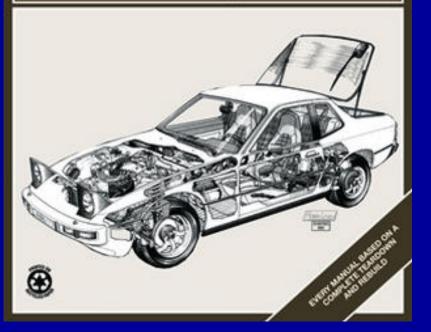
## **Chassis for Synthetic Biology**

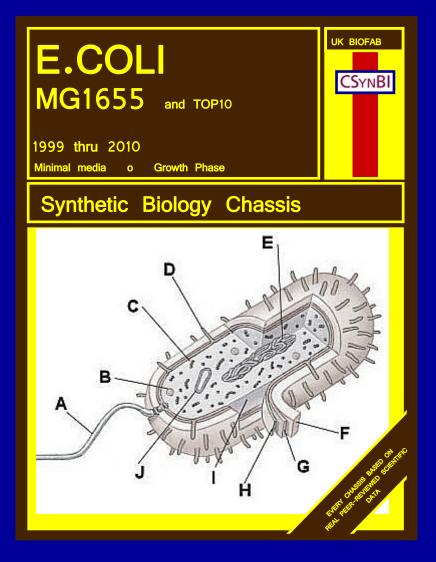
PORSCHE 924 and Turbo



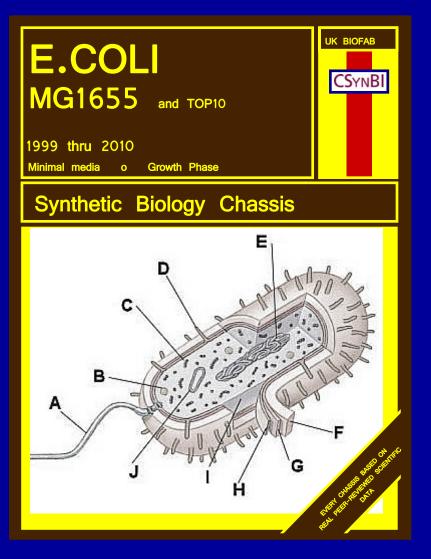
**1976 thru 1982** All models 
121 cu in (1984 cc)

#### **Automotive Repair Manual**





## 1<sup>st</sup> Generation Synthetic Biology





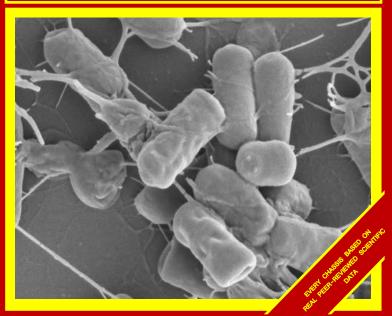
## 2<sup>nd</sup> Generation Synthetic Biology

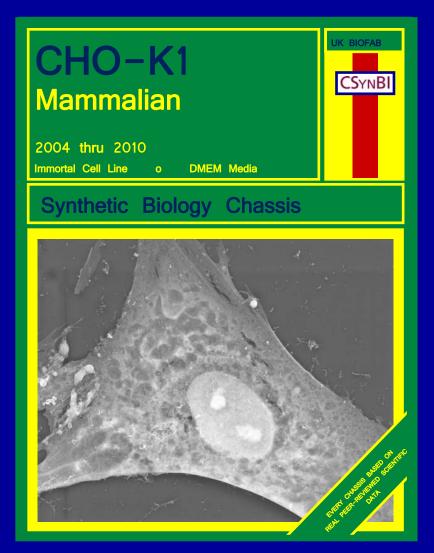
B.SUBTILIS Gram Positive UK BIOFAB

2006 thru 2010

Sporulation-capable o Growth Phase

#### Synthetic Biology Chassis





#### **Relevance of Current Chassis**

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

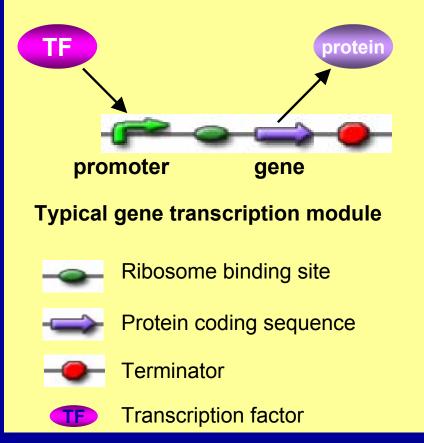
Industrial-scale biosynthesis

Ease of re-engineering

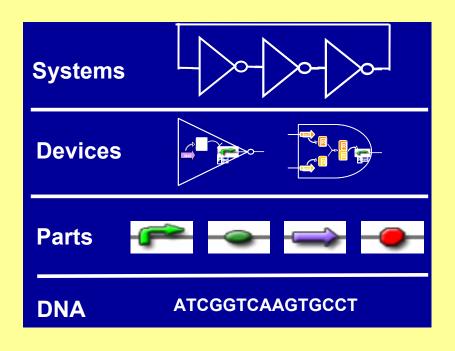
## 4. Parts, Devices and Systems

## **Engineering v Biology**

#### Modularity, Characterisation, Standardisation



#### 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**

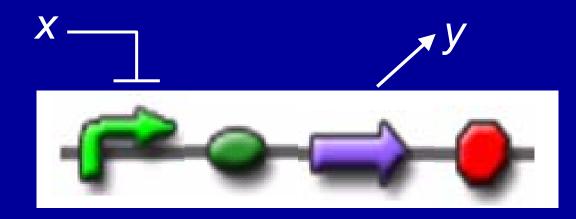
# To engineer biology it needs to be broken down into parts

#### The Engineering Approach



Modelling

### An inverter described using BioBrick icons

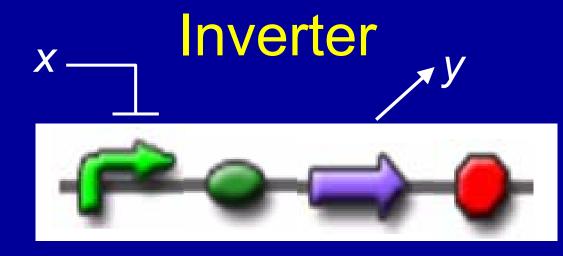


#### Promoter

Gene

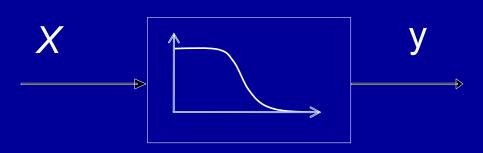
 $\frac{dy}{dt} = \frac{\beta x^n}{K^n + x^n} - \gamma y$ 

γ Protein degradation rate *X* Input repressor protein *n* Hill constant
β Protein synthesis rate



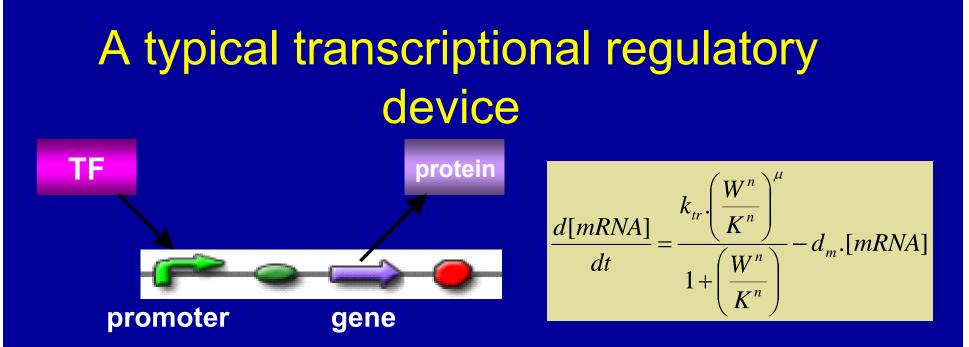
### Promoter





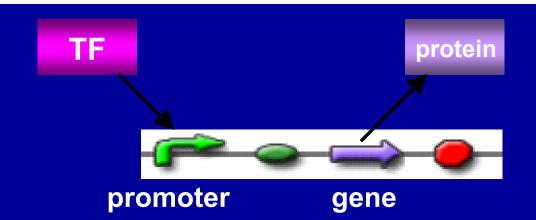
| X (Input<br>Repressor) | Y (Output<br>Protein) |
|------------------------|-----------------------|
| 1                      | 0                     |
| 0                      | 1                     |

1: High Concentration 0: Low Concentration



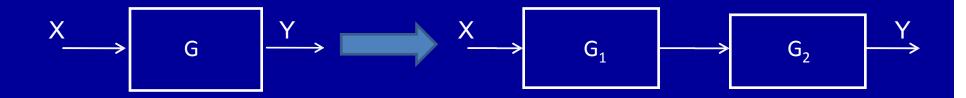
### Currently ODEs are mainly used for modelling in Synthetic Biology

This becomes cumbersome as the complexity of the systems increases



### What is required is the application of Systems Theory

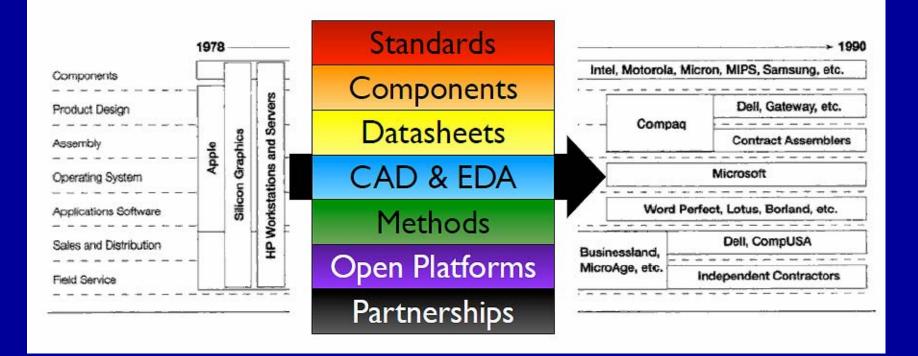
Modularisation



and, the application of Transform Methods

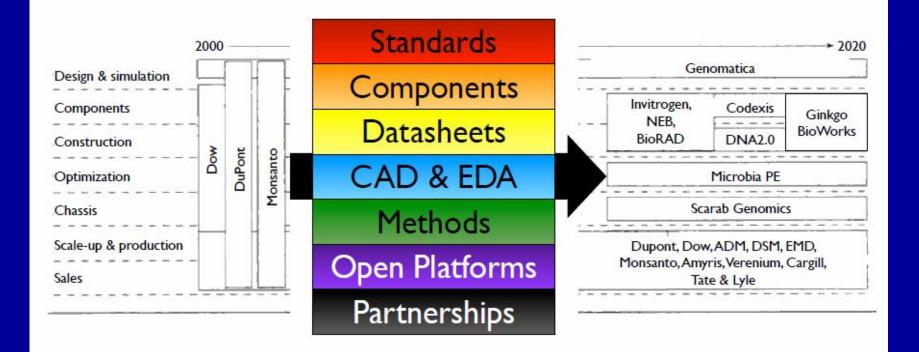
### The Evolution of Industrial Approaches

### Computing - circa 1980

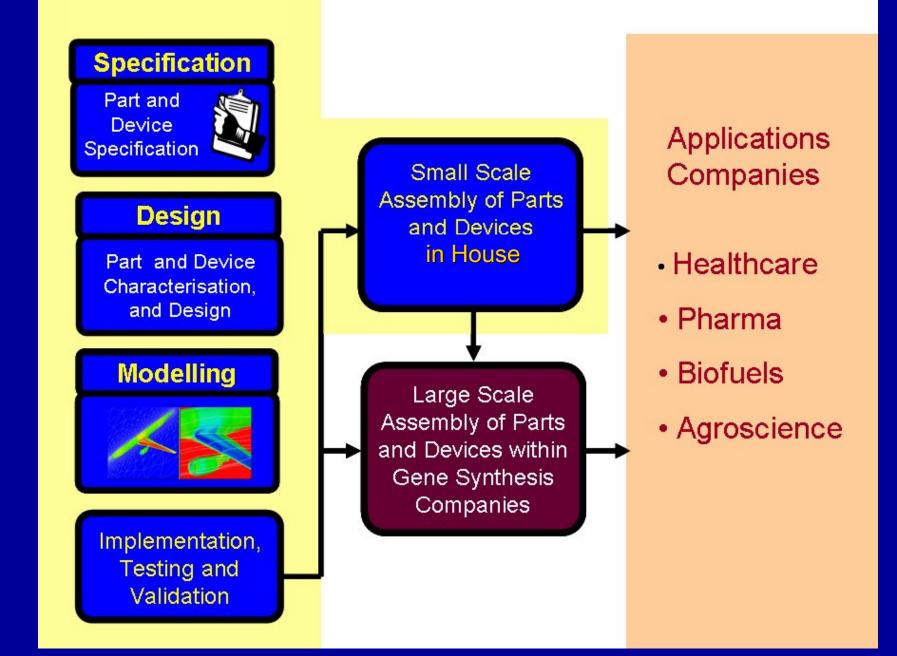


New foundational tools catalysed revolutionary transitions in computer technology, creating new industries and huge opportunities The Innovator's Solution - CM Christensen and M E Raynor – HBSP - 2003

### **Biotech is Next**



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



### Biobrick BBa\_F2620



#### **BBa\_F2620**



Authors: Barry Canton (bcanton@mit.edu) Anna Labno (labnoa@mit.edu)

Last Update: 5 October 2006

3OC<sub>6</sub>HSL → PoPS Receiver http://parts.mit.edu/registry/index.php/Part:BBa\_F2620

#### Description

A transcription factor (LuxR, BBa\_C0062) that is active in the presence of cell-cell signaling molecule  $30C_{e}HSL$  is controlled by a TetR-regulated operator (BBa\_R0040). Device input is  $30C_{e}HSL$ . 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 30C<sub>6</sub>HSL, exogenous

 Output Swing:
 21±3 to 590±9 GFP molecules cfu<sup>-1</sup> s<sup>-1</sup>

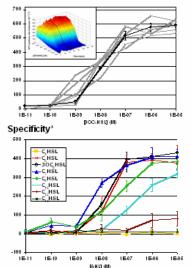
 Switch Point:
 10 nM 30C<sub>6</sub>HSL, exogenous

 LH Response:
 9.7 min (t<sub>son</sub>), 17 min (t<sub>son</sub>)

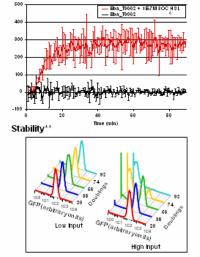
#### Key Components

BBa\_R0040: TetR-regulated operator BBa\_C0062: IuxR ORF BBa\_R0062: LuxR-regulated operator

#### Transfer Function\*



#### Response Time\*



# Signaling Devices

 Constraint
 Stability (low/high input)

 336/9449 ribosomes cfu<sup>-1</sup>
 Genetic: >92/74 m

 5040/141600 charged tRNA cfu<sup>-1</sup> s<sup>-1</sup>
 Performance: >92/74 m

#### Translational: Compatibility

Demand (low/high input)

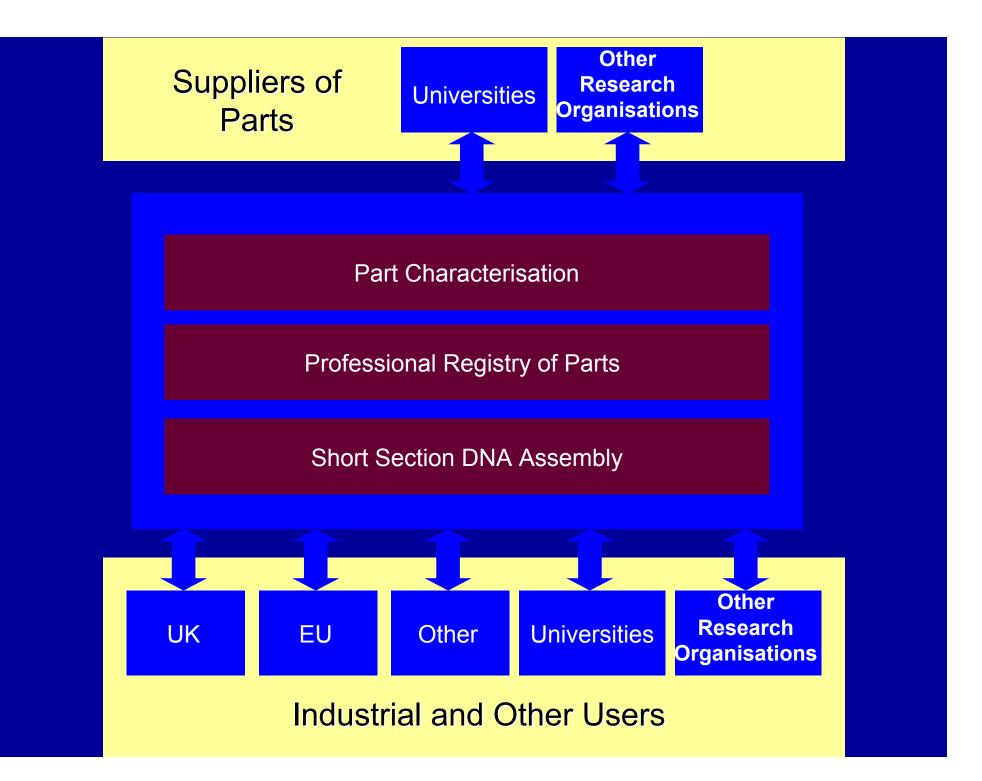
Chassis: Compatible with MC4100, MG1655, and DH5α Plasmids: Compatible with pSB3K3 and pSB1A2 Devices: Compatible with E0240, E0430 and E0434 Crosstalk with systems containing TetR (C0040) Signaling: Crosstalk with input molecules similar to 30C<sub>0</sub>HSL Performance: >92/74 replication events\* Conditions (abridged) Output: Indirect via BBa\_E0240 Vector: pSB3K3 Chassis: MG1655 Culture: Supplemented M9, 37°C

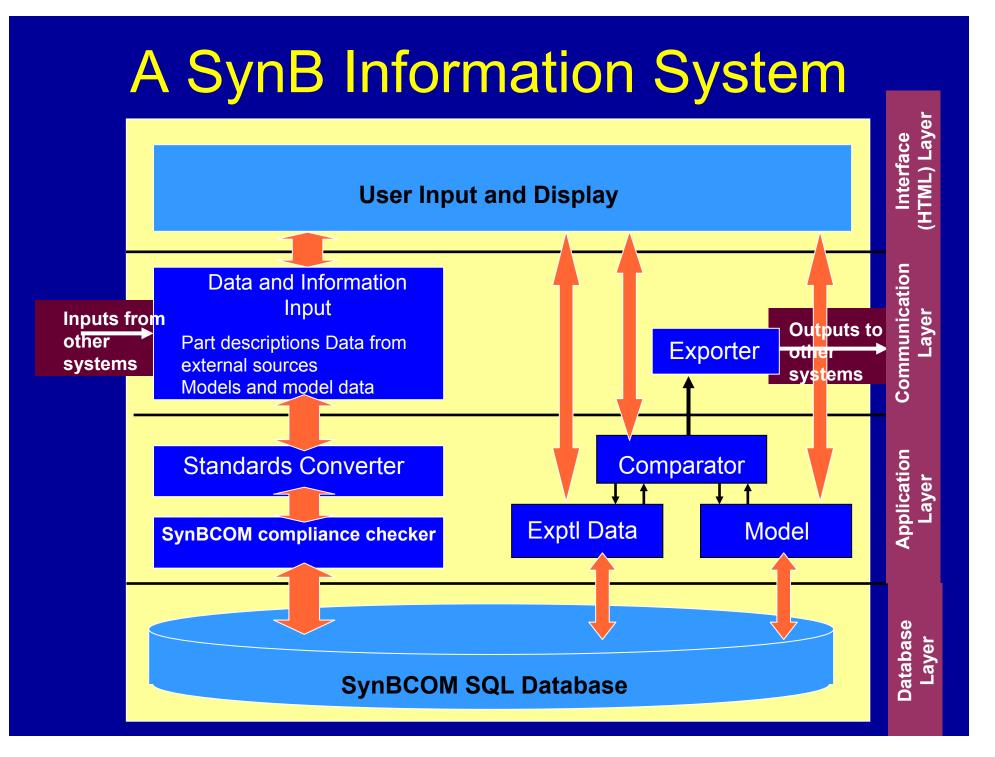
Culture: Supplemented M9, 37°C \*Equipment: PE Victor3 plate reader #\*Equipment: BD FACScan cytometer

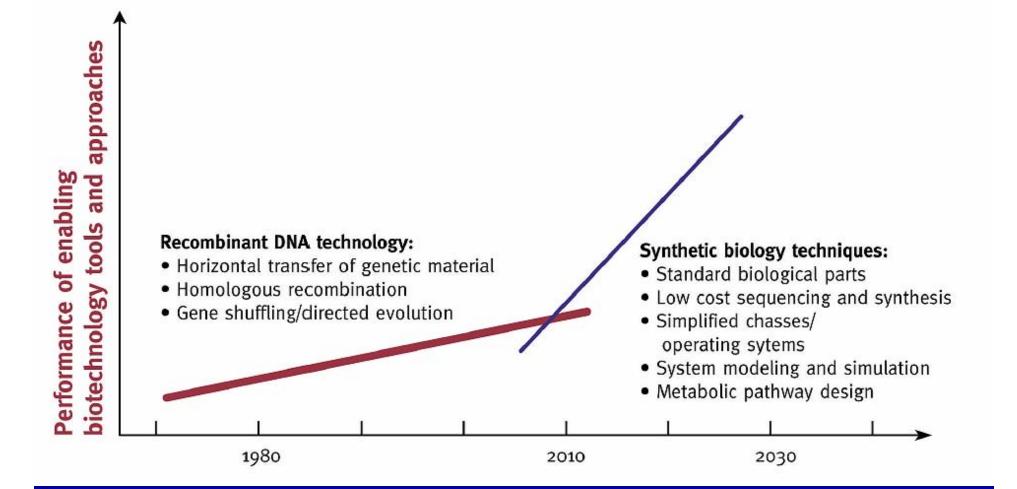
>92/74 replication events\*

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

License: Public



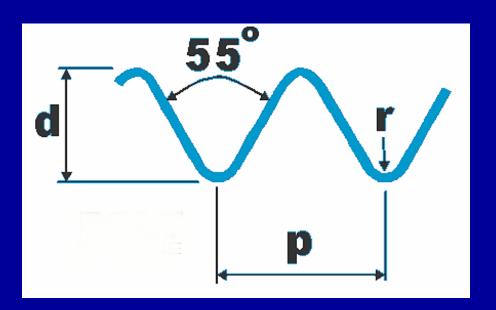




#### Genome Synthesis and Design Futures - Special Bio-era Report - US DoE 2007

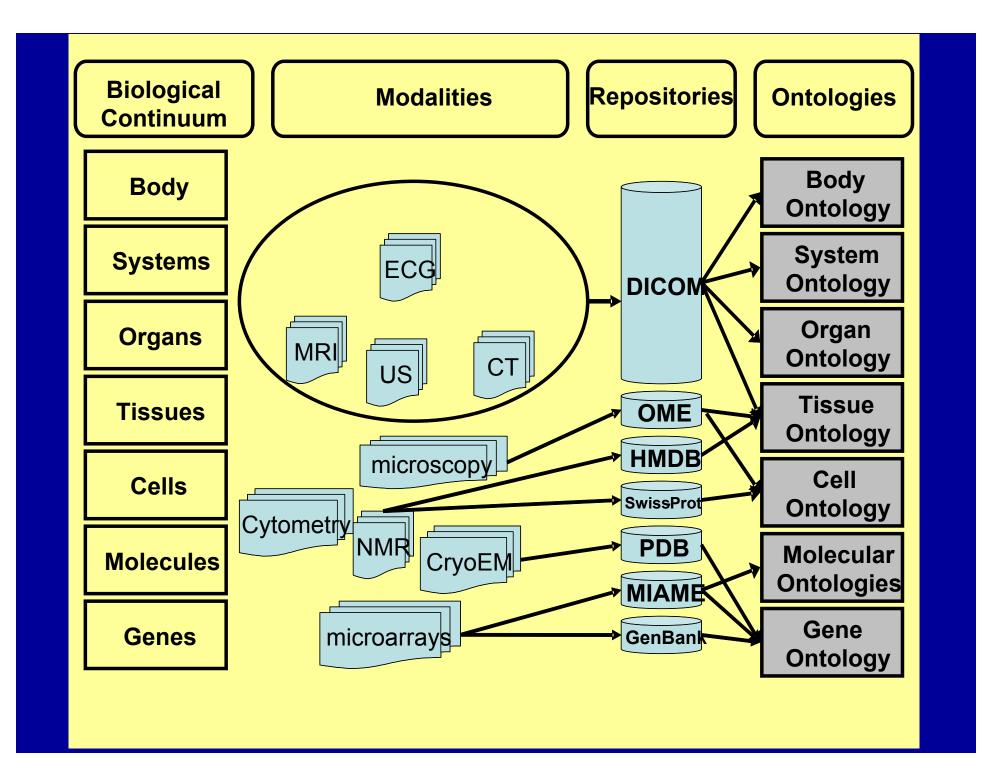
### Standards

### **The Whitworth Thread**





## The first standard thread – Sir Joseph Whitworth 1841





Digital Imaging and Communications in Medicine

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

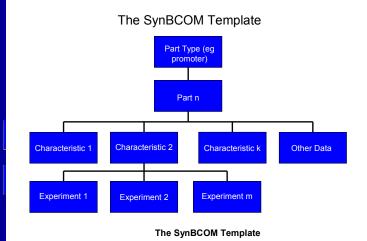
### http://medical.nema.org/

#### 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:**

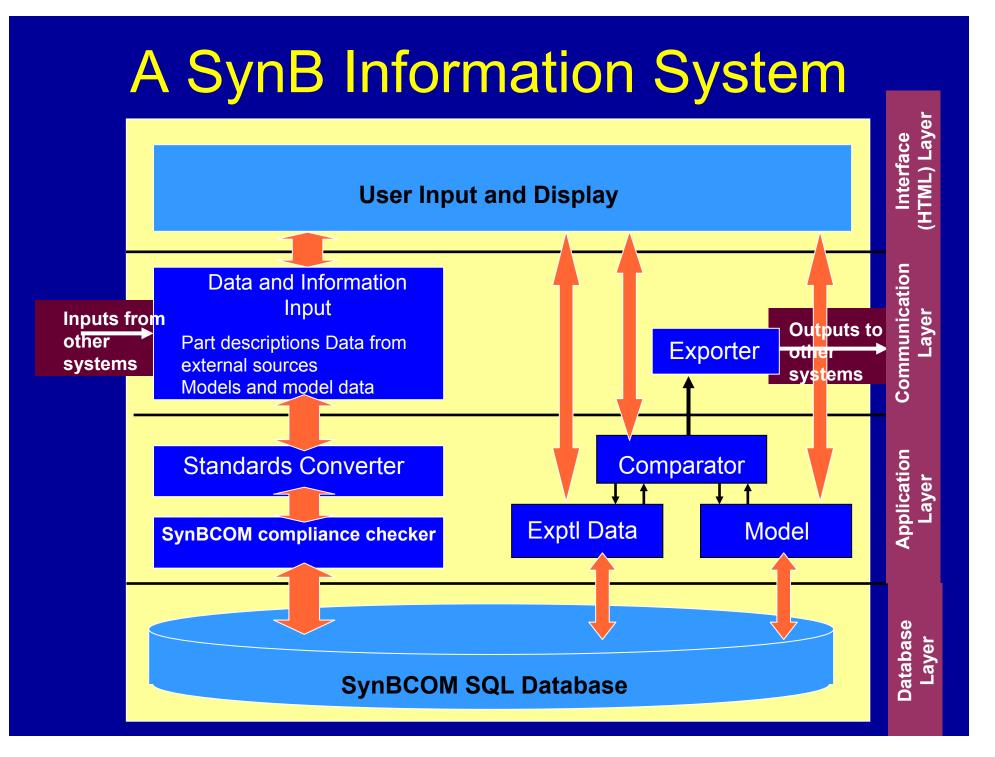
- Of parts and their parameters and characteristics To produce models and improve understanding
- To aid design and prediction

#### 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



Developing a Registry of standard, composable models

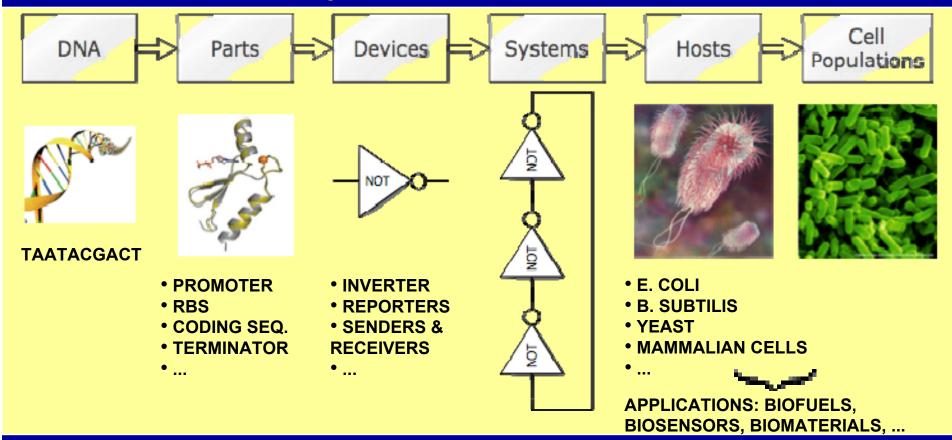
### **Combining parts**



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**



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

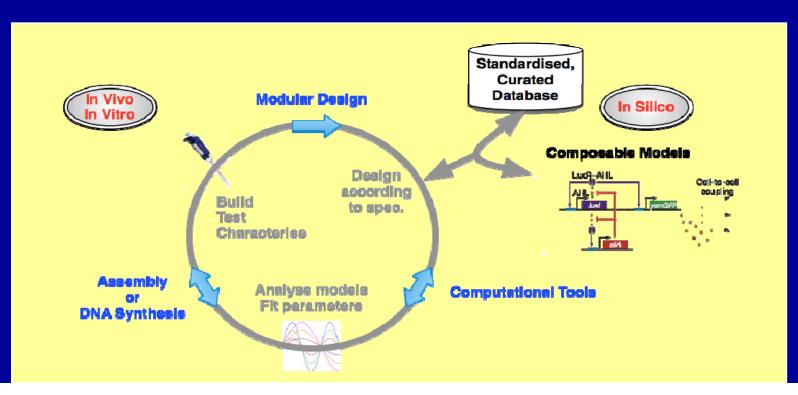
#### What is needed? Targeted dynamic **Definition of the design objectives** phenotypic (behaviour, robustness, yield) behaviour Model-based Possible design alternatives Specific DNA Biological realisation & testing computer Param, estim, & modulation Experimental results sequences simulations Robustness & sensitivity anal. C.A.D Standardised, Curated Database

- 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 <u>registry of standard, composable models</u> together with an appropriate <u>synthetic biology C.A.D.</u> environment

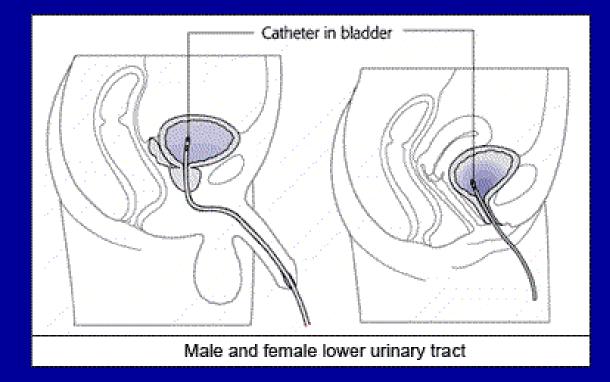
### Engineering design cycle



### Example 1 – Urinary Tract Infection (UTI)

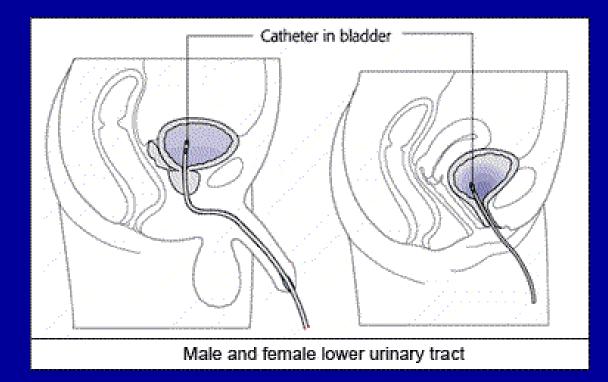
### The Problem

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

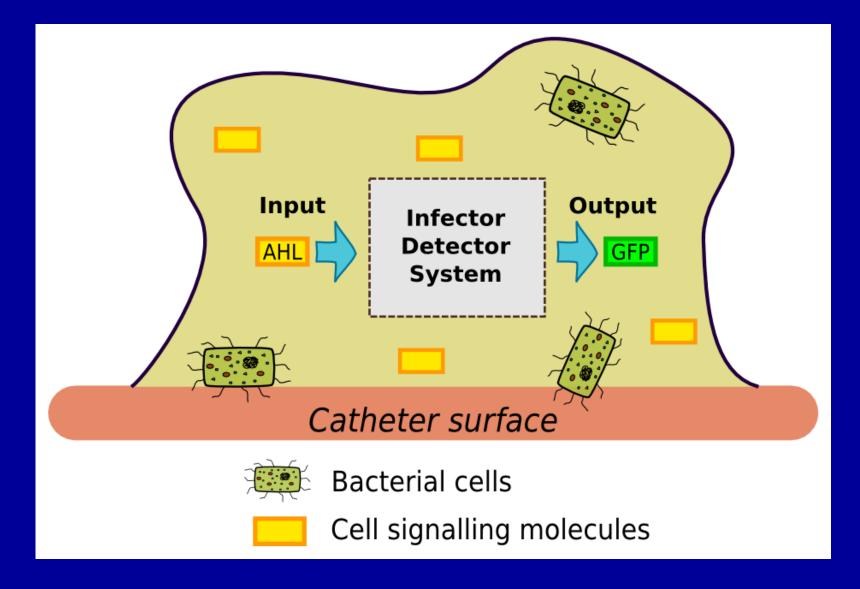


### Our Aim

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



### **Our Detection Strategy**



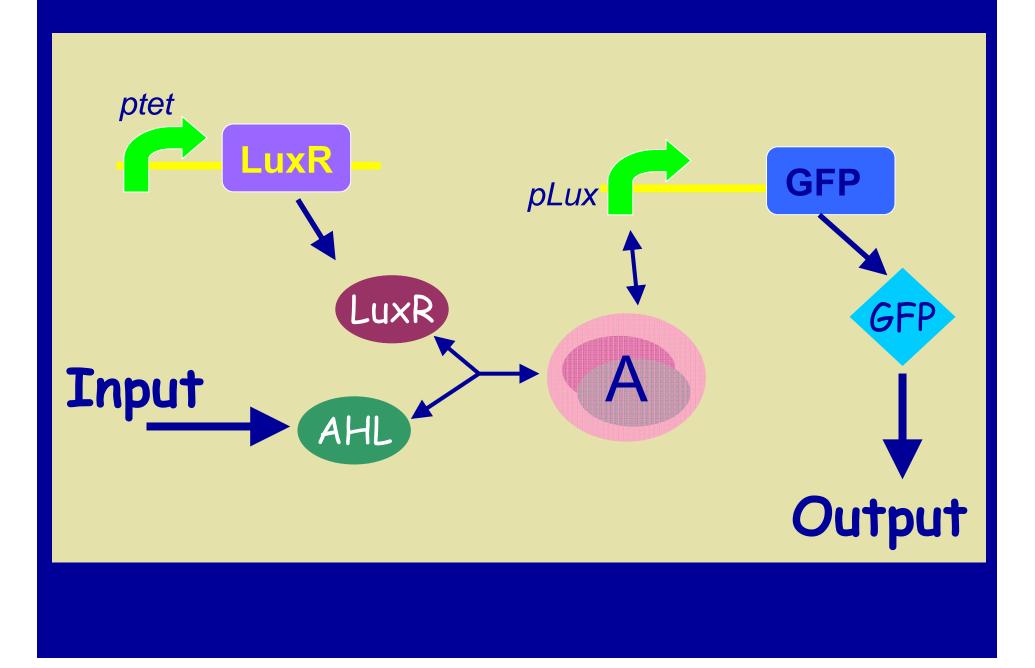
### Urinary Tract Infection Detector – a three stage device

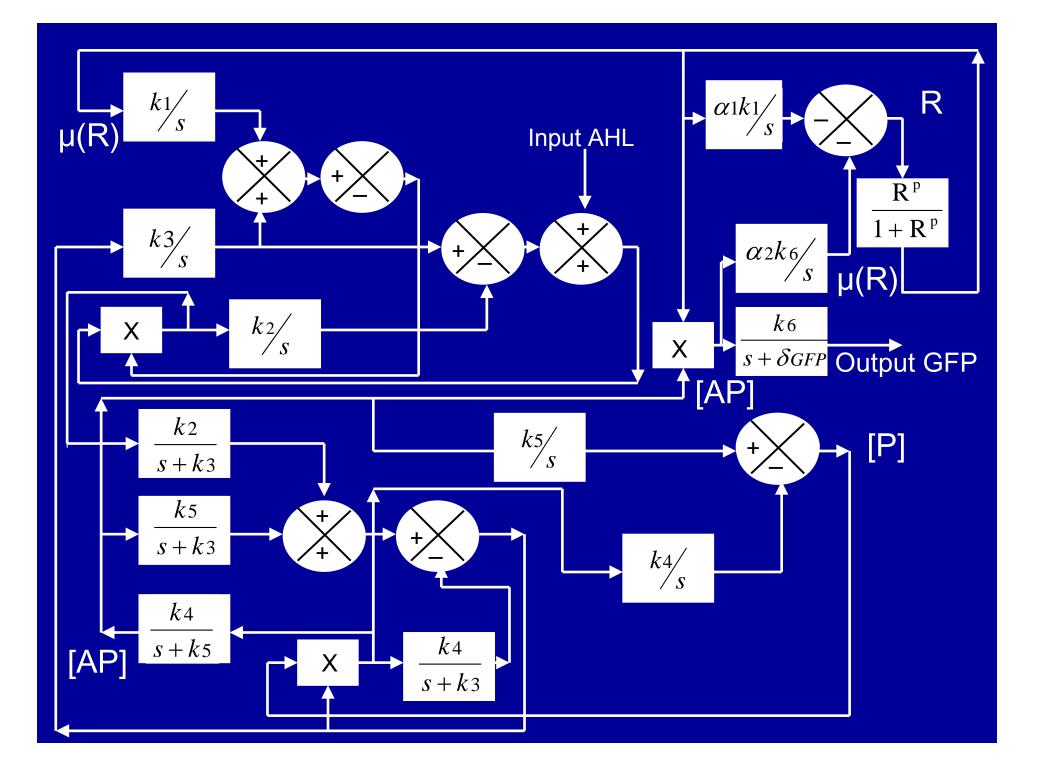


| Detector | Amplifier | Indicator |
|----------|-----------|-----------|
|----------|-----------|-----------|

### The Biochemical Network – the basis of Infector Detector



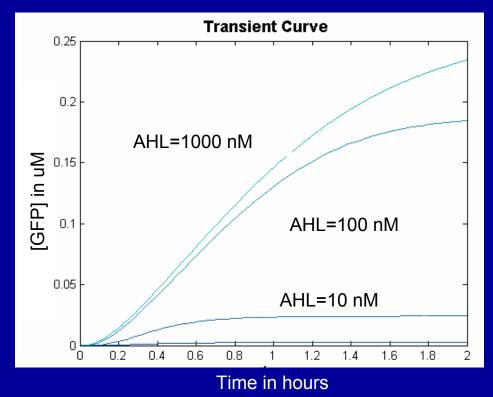




### **Typical Simulations**

### **General Behaviour:**

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

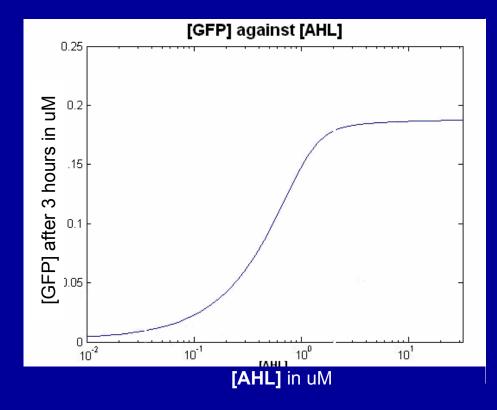


### **Transfer Function**

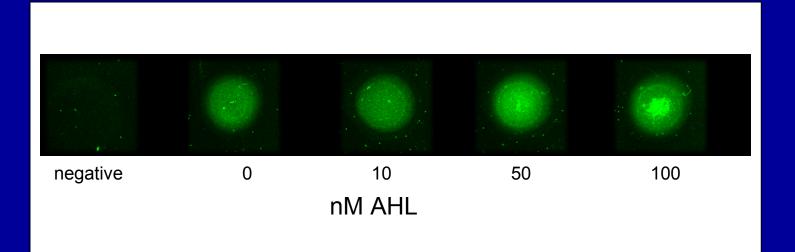


### **GFP vs AHL**

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

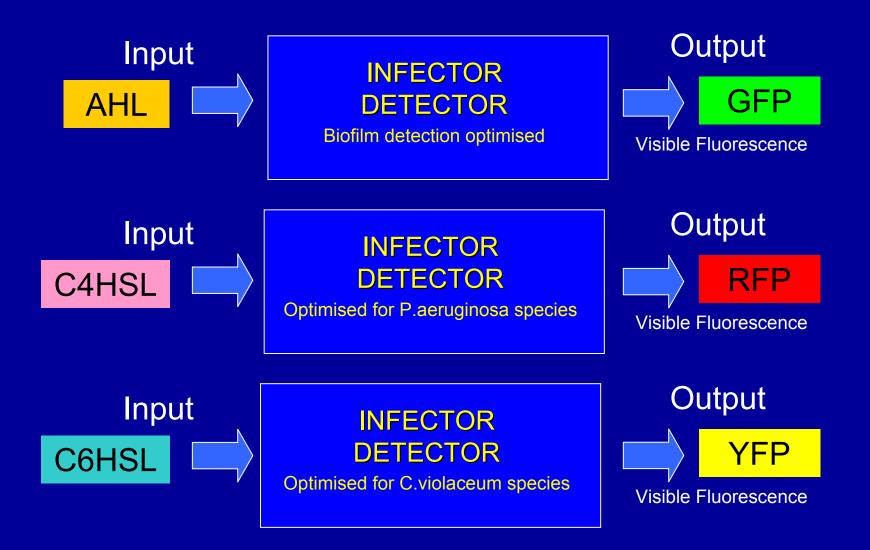


### **Testing Infector Detector on Agarose**



Agarose drops with Infector Detector detecting different concentrations of AHL

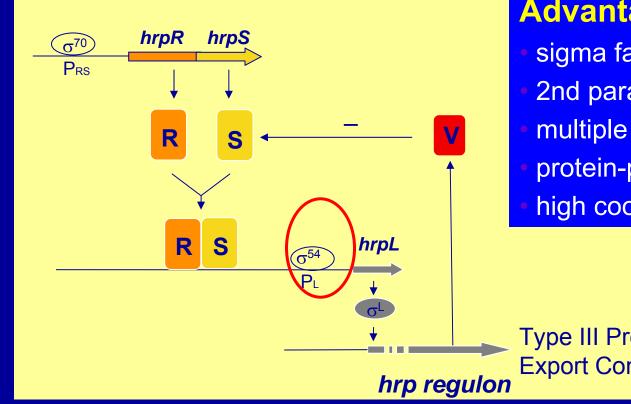
### **Ongoing Work: Customisation**



# 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

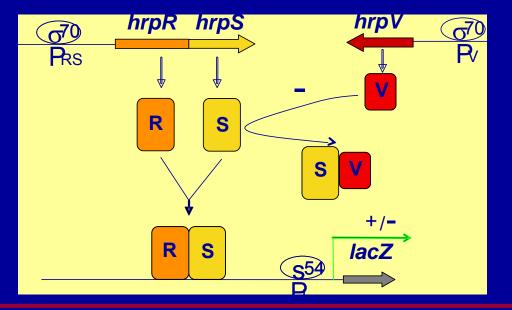
Type III Protein **Export Complex** 



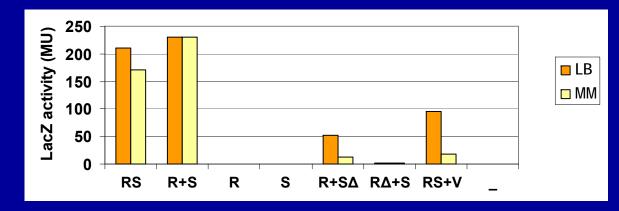
Pseudomonas syringae hrp regulatory system

## **Biological Experimental Results**

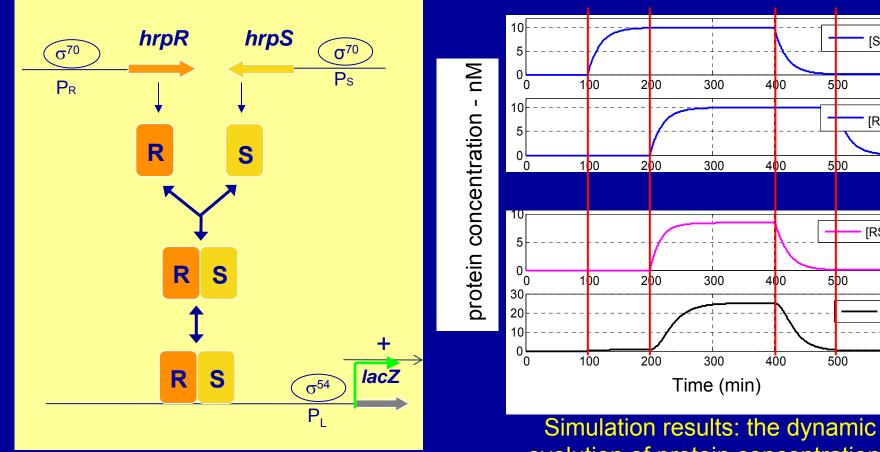
Identifying regulation mechanism for *hrpL* promoter activity



*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



evolution of protein concentrations

 $[S^T]$ 

 $[R^{T}]$ 

[RS]

600

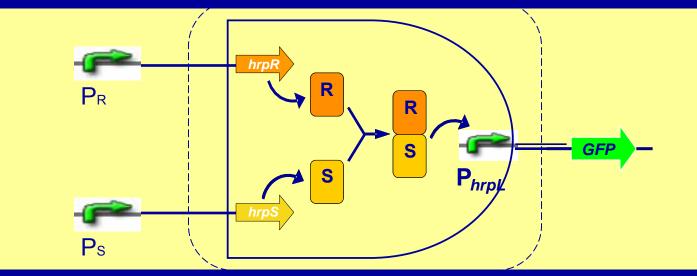
600

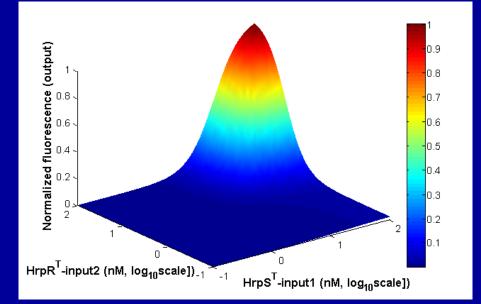
600

600

Ζ

## 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 19<sup>th</sup> Century



### Modern examples of natural dyes in the Mysore market in India



A.D. 1856 . . . . . . Nº 1984.

Dyeing Fabrics.

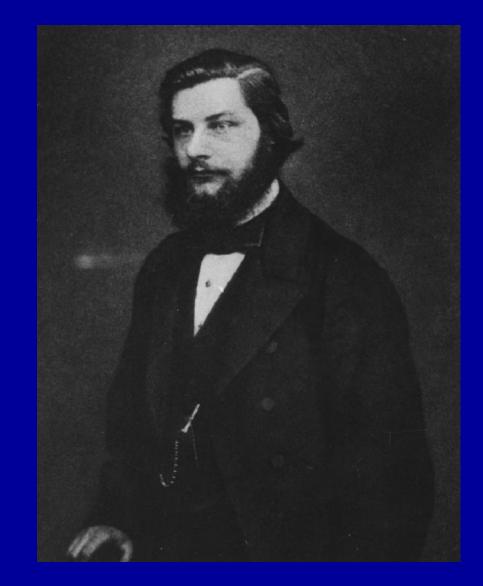
LETTERS PATENT to William Henry Perkin, of King David Fort, in the Parish of Saint George in the East, in the County of Middlesex, Chemist, for the Invention of "PROPUEISE & New Colonise Matter ros District wirt & Linke on Puerts Colon Stores or Sain, Corros, Ween, on orners Materials."

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

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

Equivalent proportions of sulphste of nulline and biehromate of potassa are to be dissolved in separate partians of hot water, and, when dissolved, they are

10 to be mixed and stirred, which causes a black precipitote 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 suphate of poinsa, 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



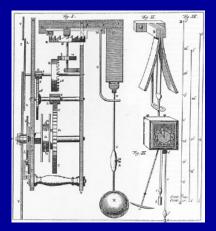
# Synthetic Rubber

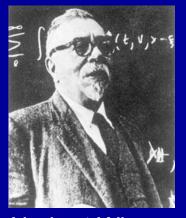
### Analogue Age

## Digital Age

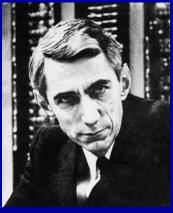
## **Biological Age**

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

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

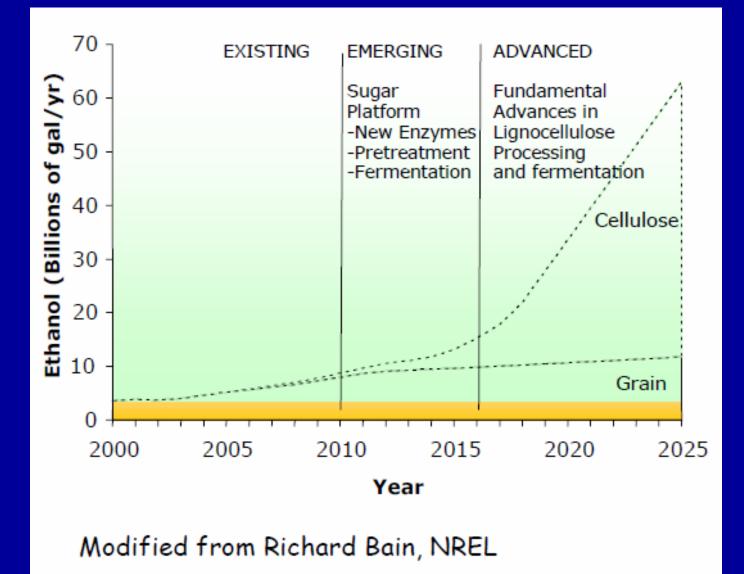
# **Some Industrial Examples**

The objective of synthetic biology is the industrialisation of biology

Engineering microorganisms to make Bio-diesel



# A DoE (US) Ethanol Vision



# Example: Halophile energy from desalination



### Halobacterium halobium

Thrives in waste brine from desalination

Engineered to produce isobutanol biopetrol from sunlight and CO<sub>2</sub>

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

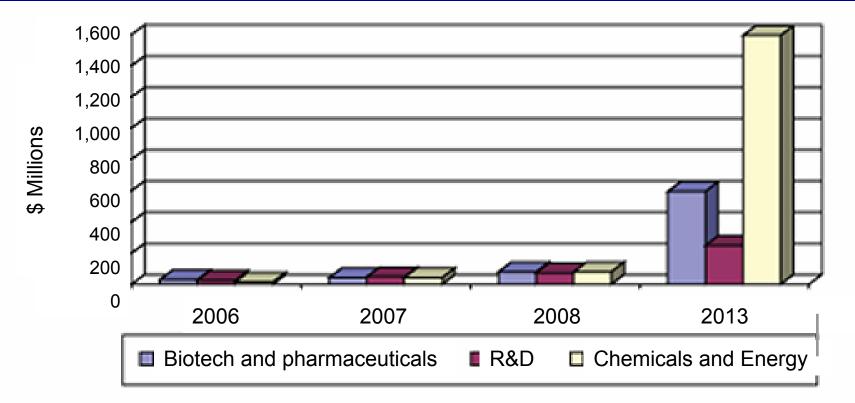
Bind – Detect – Signal

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<br>Areas       |
|-----------------|---|--|
| Medical devices | Tissue<br>Engineering/Biomaterials                            | Medical Devices/implants               |
| Pharmaceutical  | Diagnostics/Biomarkers  | Pharmaceutical                         |
|                 | Molecular imaging   | Medical Contrast<br>agents/imaging     |
|                 | DNA Vaccines  | Infectious diseases                    |
|                 | Drug synthesis (Improving<br>synthesis of existing<br>agents) | Pharma/ Bioprocessing<br>/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<br>etc       |

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



Source: BCC Research

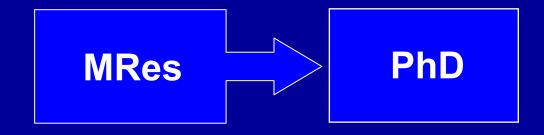
Report ID:BIO066A, Published: June 2009, Analyst: John Bergin

# **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



discussion view source history team













earch

### iGEM 2009 Jamboree

October 31 to November 2, 2009 Massachusetts Institute of Technology

> Quick links: Team abstracts Team websites Schedule Campus Map iGEM 2009 Jamboree results



### Add your iGEM 2009 publicity, photos, & publications 🌟



### About iGEM

What is iGEM?

- Previous iGEM competitions
- iGEM Headquarters
- Frequently Asked Questions
- iGEM Press Kit
- Join the iGEM Mailing List
- Sponsor iGEM

#### **iGEM Start to Finish**

- Calendar of events
- Start a team
- Requirements
- iGEM 2009 Registration
- Spring workshops
- Summer News & Events
- The Jamboree

# iGEM The Need for European Funding

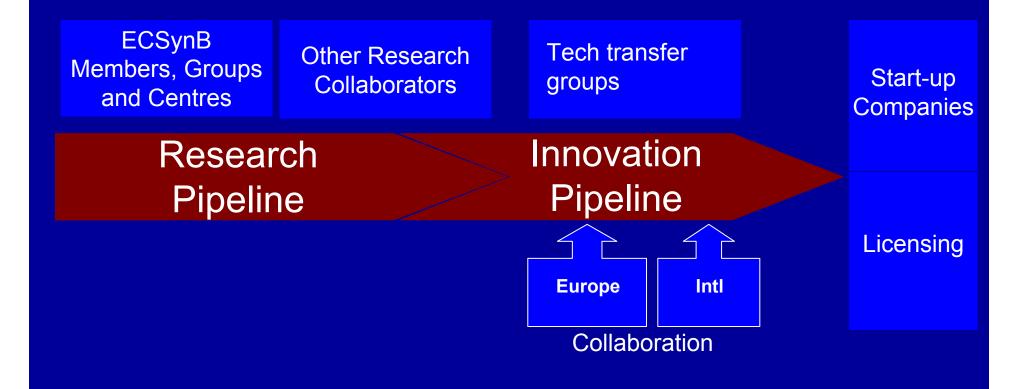
# Establishing European Collaboration in Synthetic Biology

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

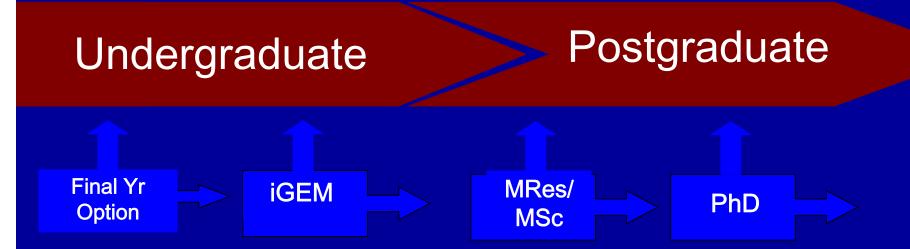
# ECSynB - European Consortium for Synthetic Biology

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



## **The Education Pipeline**



# The End



This paper was produced for a meeting organized by Health & Consumers DG and represents the views of its author on the subject. These views have not been adopted or in any way approved by the Commission and should not be relied upon as a statement of the Commission's or Health & Consumers DG's views. The European Commission does not guarantee the accuracy of the data included in this paper, nor does it accept responsibility for any use made thereof.