

#### Synthetic biology

### challenges for the creation and modification of biological systems

Roman Jerala

# Interaction between life sciences and informatics

### Biological systems

- -Nervous system
- -Immune response
- -Metabolism
- -Genome
- -Species (evolution)...

concepts, examples...

#### **Informatics**

- -Neural networks
- -Genetic algorithms
- -Pattern recognition...

modeling, systems theory...

#### **Outline**

- What is synthetic biology
- Areas of application
- Information processing
- Case studies of synthetic biology projects
  - Negative feedback loop for cell signaling
  - Mutation independent HIV defense
  - DNA based scaffold Perspectives



#### Synthetic biology

 Biological engineering, constructive biology

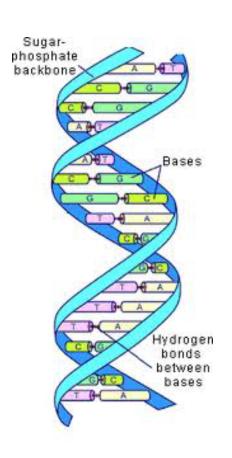


- Biology as technology "cell factory", better production methods
- Redesign biological systems to suit our purposes
- Synthetic systems to test our understanding of biological processes

# Genetic modification of organisms

- Classical breeding random genome modification, based on selection
- Recombinant DNA techniques 1970s, introduction of foreign DNA of defined sequence
   plasmids, viruses... Production of recombinant proteins (e.g. insulin), transgenic animals...
- Synthetic biology introduction of regulatory circuits, complete biosynthetic pathways, organisms based on synthetic genomes

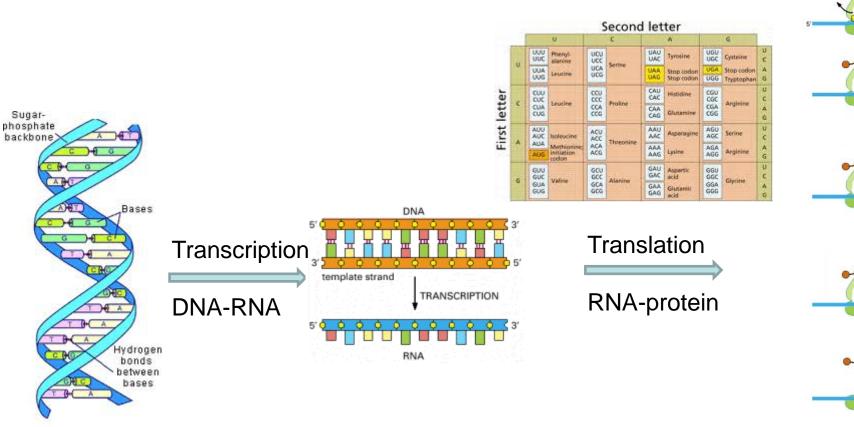
#### DNA as the carrier of information

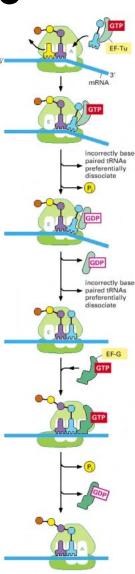


String of 4 different nucleotides: A, C, G, T Watson-Crick base pairing: A=T, G=C Semiconservative replication

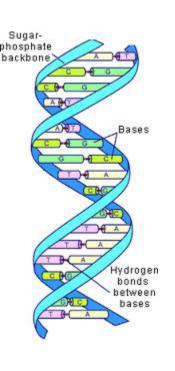
High information content at 1 bit/nm<sup>3</sup>

## DNA codes for the sequence of polypeptides based on the universal triplet code



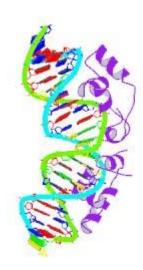


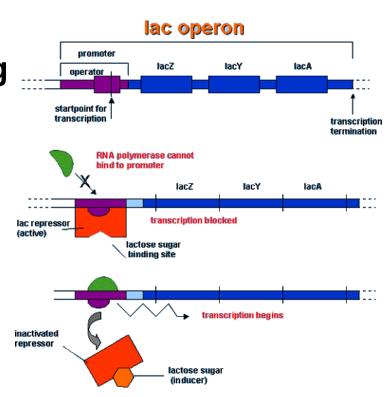
## DNA also stores the information to control the proces flow



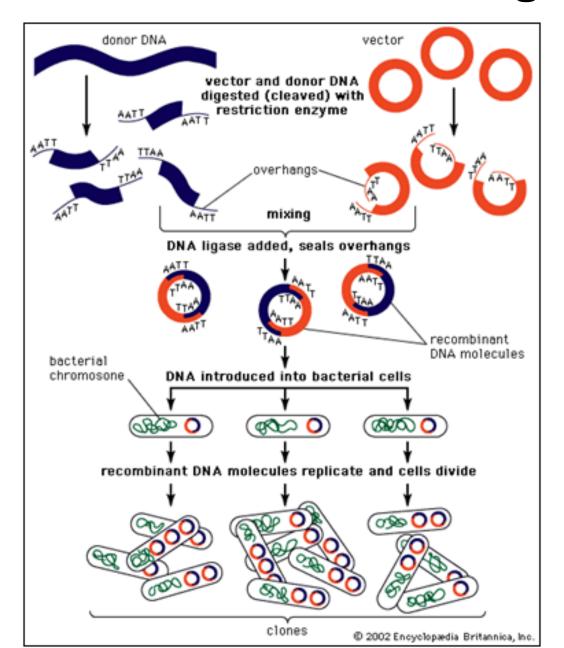
### Regulatory DNA binding proteins:

- -RNA polymerases
- repressors
- activators...



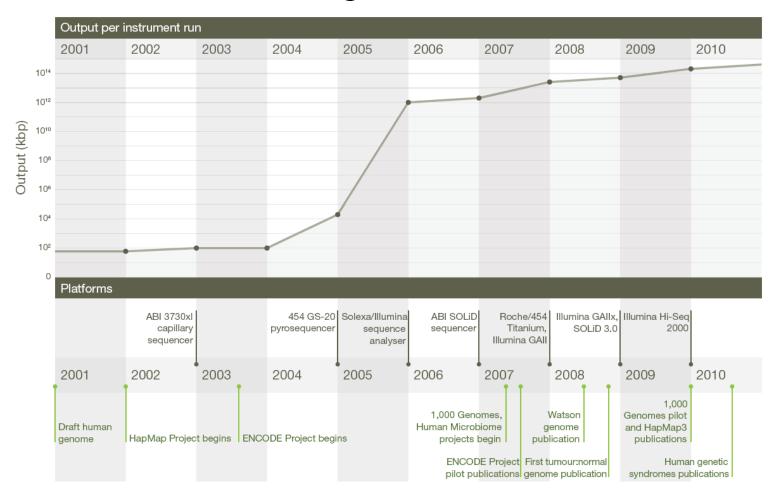


#### From DNA to modified organisms



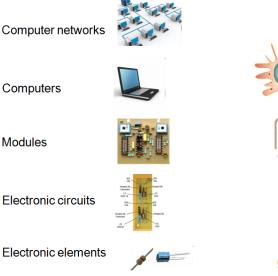
#### What's new?

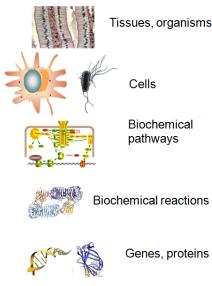
### New tools: DNA sequencing, DNA synthesis, Biochemical knowledge



#### **Engineering approach**

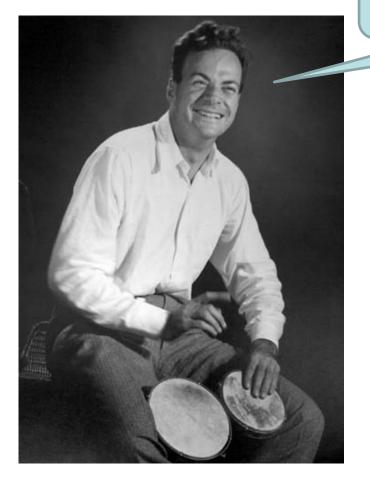
- Application of engineering principles into biological systems
- Important engineering principles:
  - Modularity
  - Abstraction
  - Reliability
  - Predictability
  - Standardization





# Synthetic biology as an investigative tool

"What I cannot create, I do not understand!"



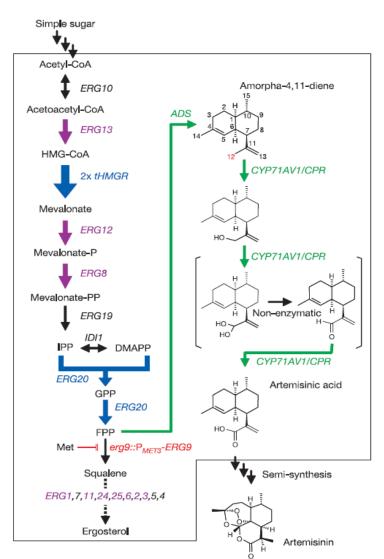
How and why are natural systems constructed as they are (functional requirements or evolutionary coincidence)?

Do we understand the function of all components of a device/system?

# Synthetic biology to design

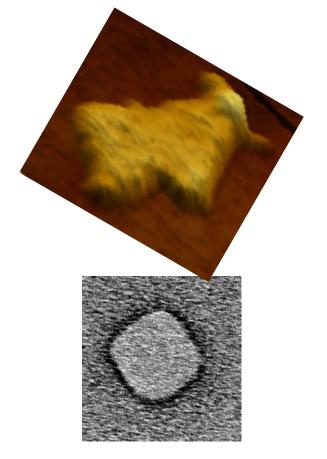
# Heterologous production:

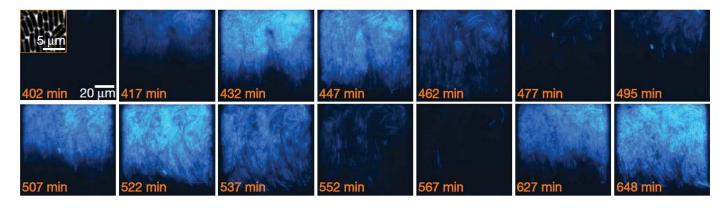
- -Artemisinin
- -Biofuel
- -Spider silk...



#### Transcending the nature:

- -Bioanomaterials
- -Information processing
- -Oscillators
- -Engineered cell signaling...

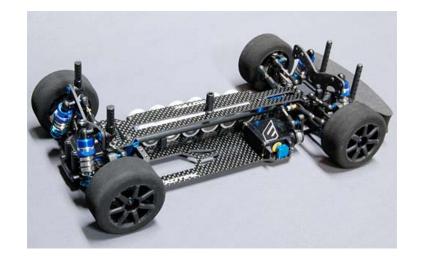




# Different approaches of synthetic biology

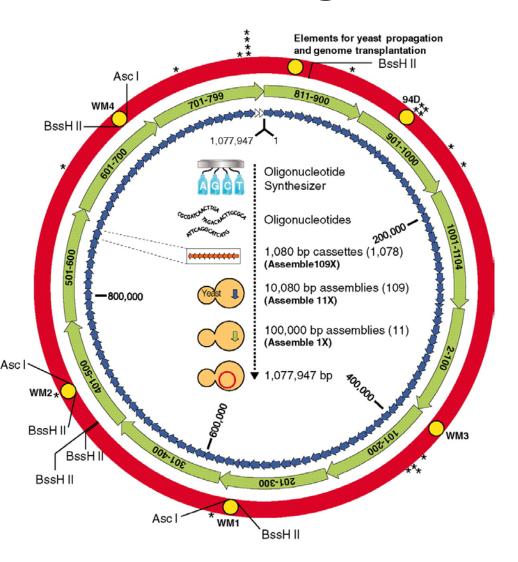
 Building on the existing chasis (modification of functional biological systems)  (Re)construction of complete genomes
 -synthetic genome, genome transplantation, rebooting cells
 -creation of new minimal selfreplicating systems

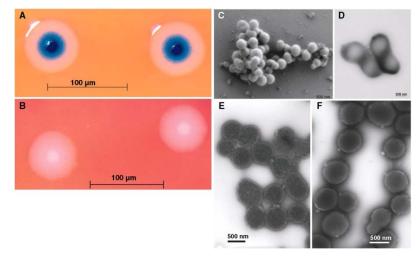






## The assembly of a synthetic *M. mycoides* genome in yeast



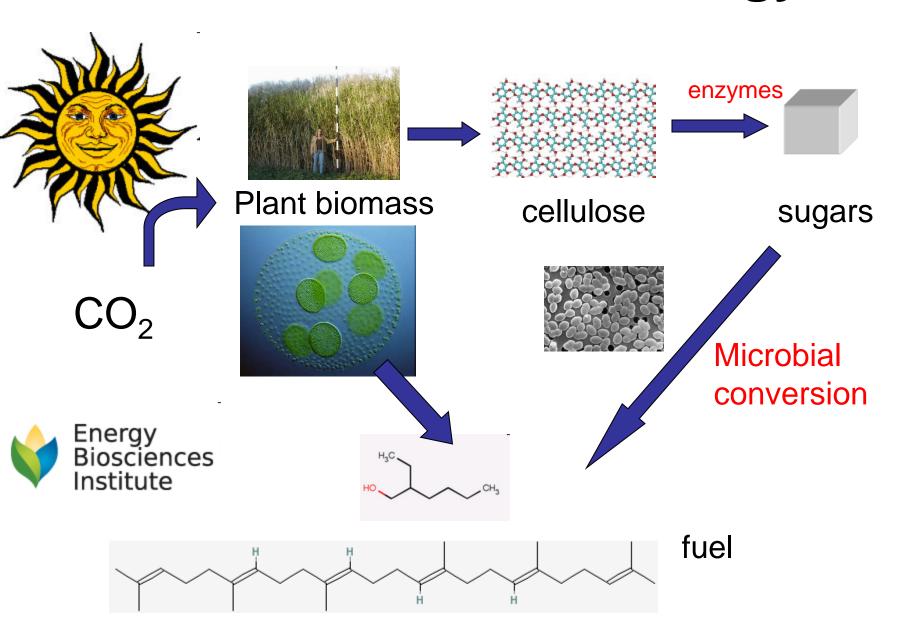




# Areas of application of synthetic biology

- Medicine
- Renewable sources of energy
- New materials & bionanomaterials
- Information processing
- Biosensors
- Bioremediation...

#### Renewable sources of energy



## Microbial production of raw materials

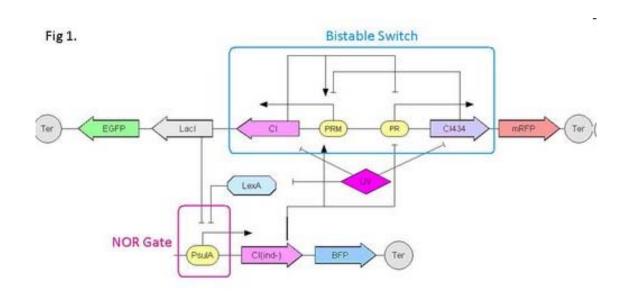
Production of 1,3-propanediol, source of Sorona polymers from renewable sources (37%)

- DuPont, invested 400 M\$ in 7 years
- Modification of 19 genes of *E.coli*, introduction of 8 genes from yeast and *Klebsiella*
- Production 135 g/l; 90% carbon yield, production >100,000 T/year
- The first billion dollar nonmedical application of SB

#### Information processing

DNA contains high density of information

DNA processes information near thermodynamic limit: 10<sup>19</sup> ops/J vs. 10<sup>9</sup> for computers



# Media for information processing in biological systems

- Genetic circuits: repressors, activators...
   (tens of minutes)
- Protein modification: phosphorylation, ubiquitination, proteolysis... (seconds)
- Membrane potential: neuronal networks (milliseconds) ...

#### Information processing in cells

## Organisms are massively parallel information processing systems

#### Advantages:

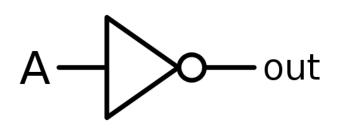
- Complex networks
- Adaptive systems
- Self-replication and selfassembly
- Highly efficient
- Robustness

#### **Drawbacks**:

- Unknown and complex structure ot the network
- Slow (compared to electronic devices)

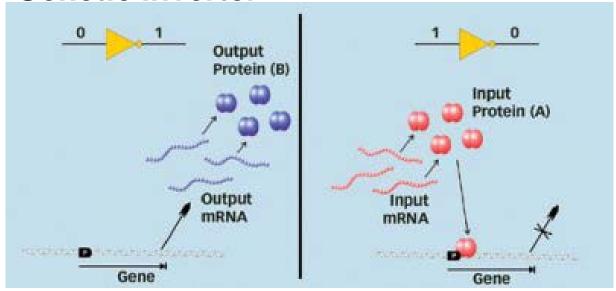
#### Discrete logical functions

#### **INVERTER** (logical negation):

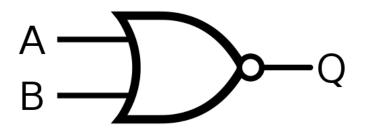


INPUT	ОИТРИТ
Α	NOT A
0	1
1	0

#### **Genetic inverter**

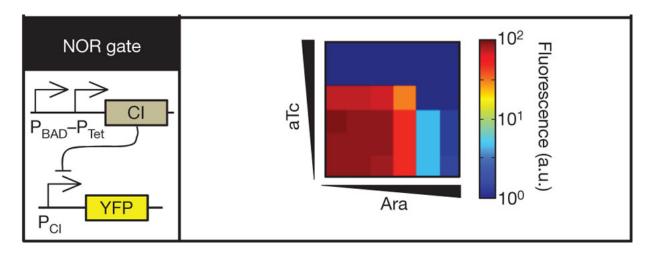


#### **NOR** operator



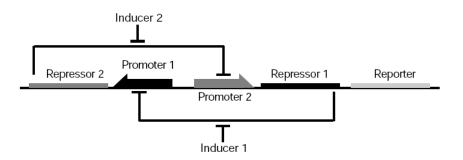
INPUT		OUTPUT
Α	В	A NOR B
0	0	1
0	1	0
1	0	0
1	1	0

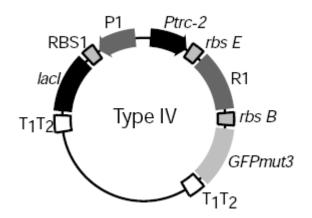
#### Genetic NOR gate



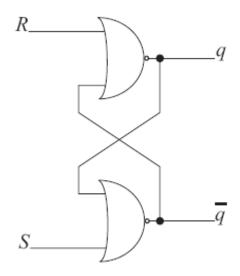
# Construction of a memory cell from two NOR gates

Bistable - toggle switch

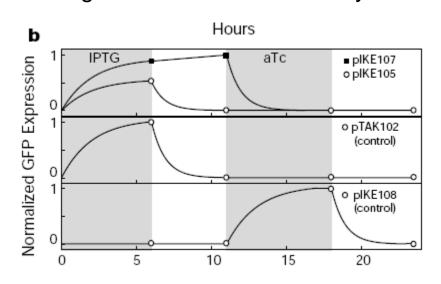




Gardner et al., Nature 2000

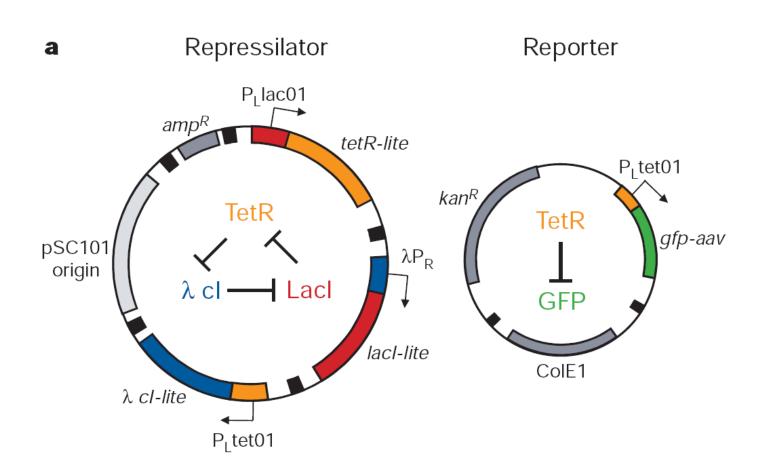


Logical outline of RS memory cell

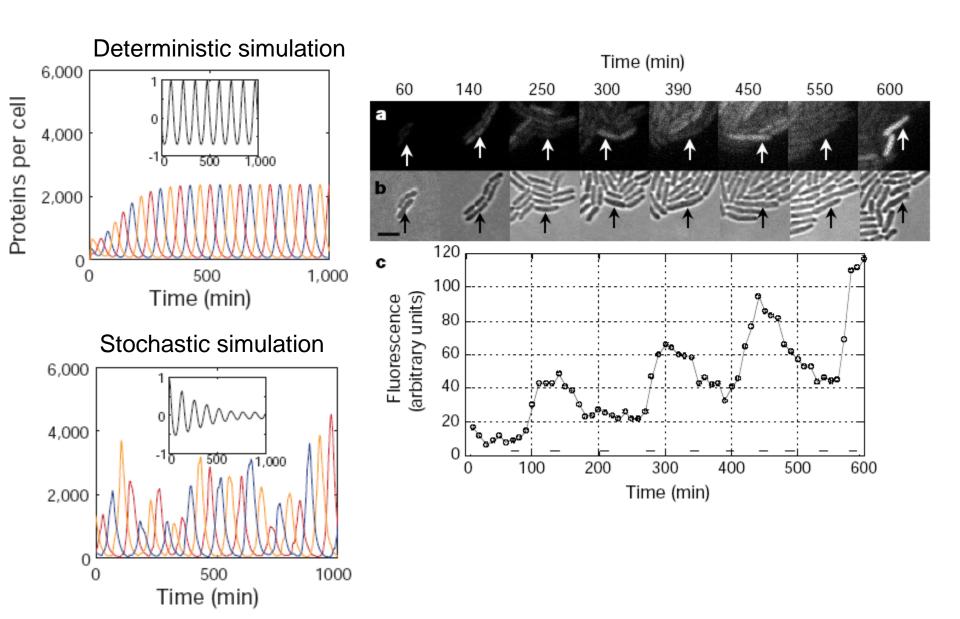


#### Repressilator

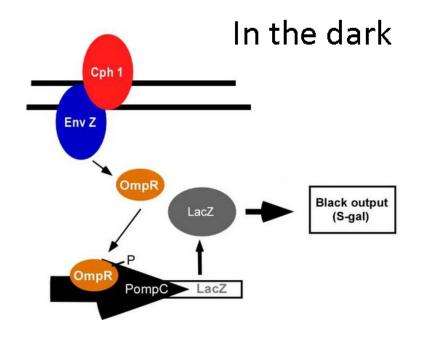
- Cyclic negative feedback loop
- Consists of 3 circularly connected repressors (+ reporter)



#### Theory and experiment



#### **Bacterial photolitography**

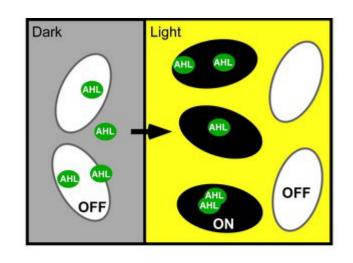


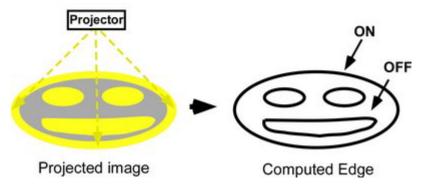


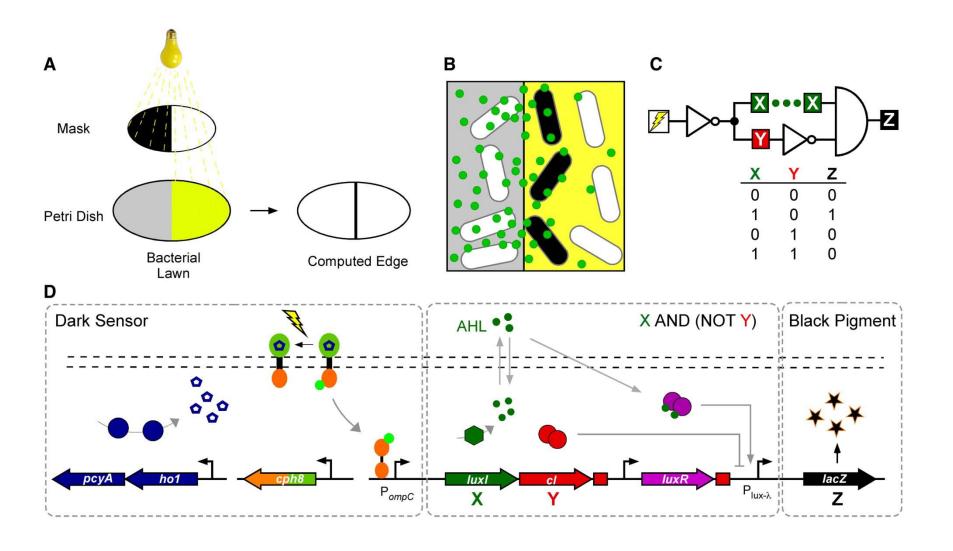
University of Texas iGEM2005

Levskaya, et al., (2005) Engineering E. coli to see light, Nature, **438**: 441-442.

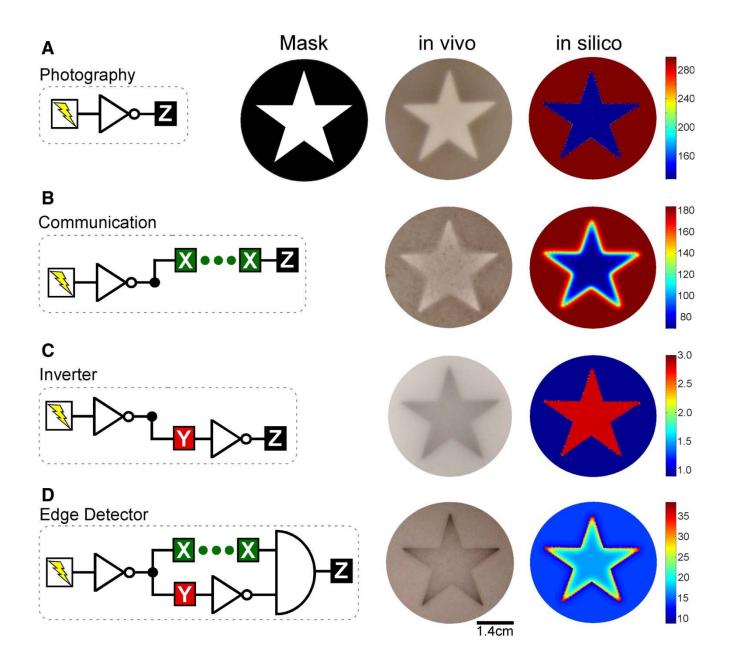
#### **Bacterial edge detector**



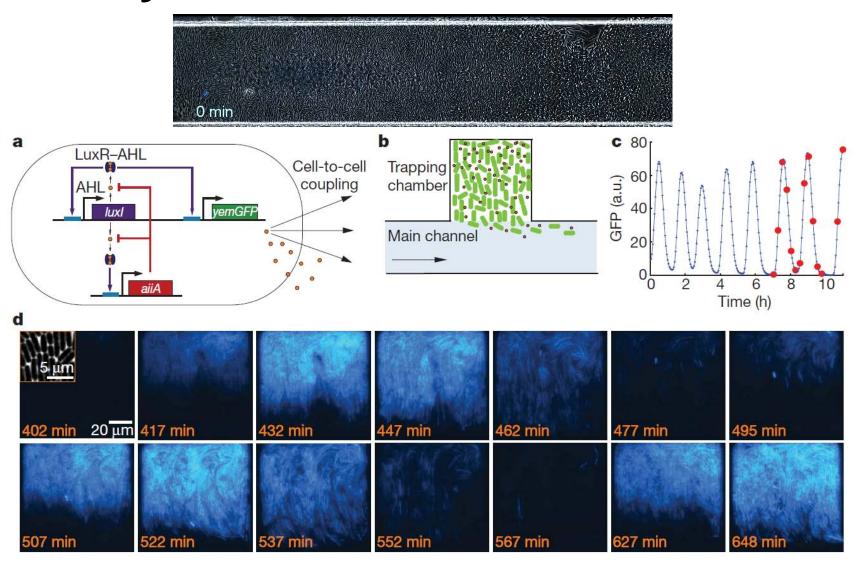




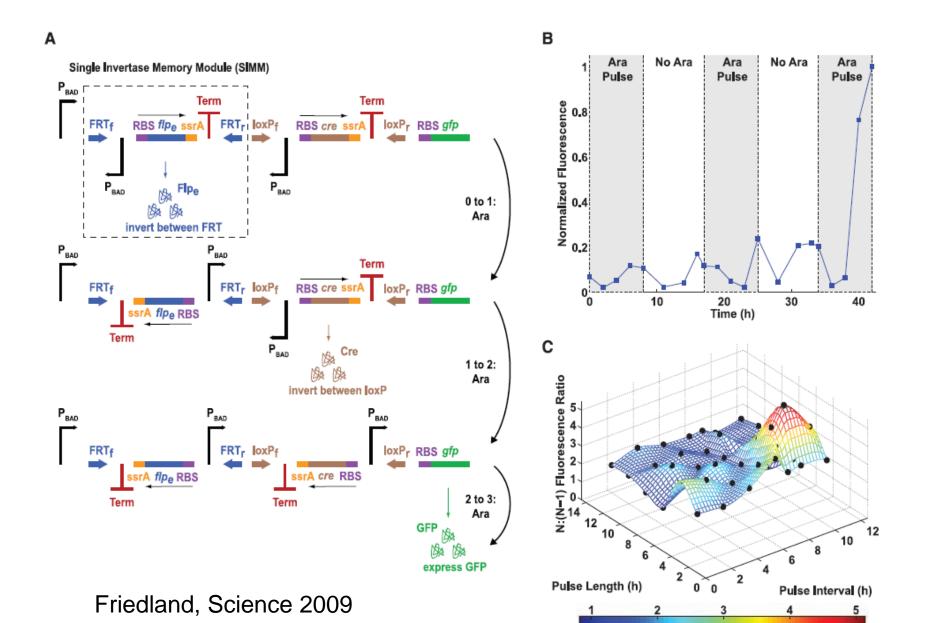
Tabor et al., Cell (2009) A synthetic Genetic Edge Detector Program



#### Synchronous bacterial clock



#### Synthetic gene network that counts



# Experimentally achievable complexity of artificial oscillators and logic circuits

Based on available and well characterised parts: repressors:

lacl

**TetR** 

λC

Lack of the standardized and well characterised parts

Only one type of 3-membered repressilator has been implemented experimentally Peciliarities of natural parts requires fine tuning

#### **iGEM**

## international geneticaly engineered machines competition



- 2004: meeting of five USA teams (MIT, BU, Caltech, UT Austin, Princeton)
- 2005: first international meeting: 13 teams, 2 european (ETH and Cambridge), no formal awards or judges
- 2006: 37 teams, Grand Prize
- 2007: 54 teams
- 2008: 84 teams
- 2009: 121 teams
- 2010: 130 teams



#### Participation of world's best universities at iGEM competition



1	Harvard Univ
2	Stanford Univ
3	Univ California - Berkeley
4	Univ Cambridge
5	Massachusetts Inst Tech (MIT)
6	<u>California Inst Tech</u>
7	Columbia Univ
8	Princeton Univ
9	<u>Univ Chicago</u>
10	Univ Oxford
11	Yale Univ
12	Cornell Univ
13	Univ California - Los Angeles
14	Univ California - San Diego
15	<u>Univ Pennsylvania</u>
16	<u> Univ Washington - Seattle</u>
17	Univ Wisconsin - Madison
18	Univ California - San Francisco
19	Tokyo Univ
20	Johns Hopkins Univ
21	Univ Michigan - Ann Arbor
22	Univ Coll London
23	Kyoto Univ
24	Swiss Fed Inst Tech - Zurich
24	Univ Toronto
26	Univ Illinois - Urbana Champaign
27	Imperial Coll London
28	Univ Minnesota
29	Washington Univ - St. Louis
30	Northwestern Univ
31	New York Univ
32	<u>Duke Univ</u>
32	Rockefeller Univ
34	Univ Colorado - Boulder
35	Univ British Columbia
36	Univ California - Santa Barbara

	,
37	Univ Maryland
38	Univ North Carolina - Chapel Hill
39	Univ Texas - Austin
40	Univ Manchester
41	Univ Texas Southwestern Med Center
42	Pennsylvania State Univ
42	Univ Paris 06
42	Vanderbilt Univ
45	Univ Copenhagen
46	Univ California - Irvine
47	Univ Utrecht
48	Univ California - Davis
49	Univ Paris 11
50	Univ Southern California
51	Karolinska Inst Stockholm
52	Univ Pittsburgh - Pittsburgh
53	Univ Zurich
54	Rutgers State Univ - New Brunswick
55	Univ Edinburgh
55	Univ Munich
57	Tech Univ Munich
58	Univ Florida
59	Australian Natl Univ
60	McGill Univ
61	Univ Bristol
62	Carnegie Mellon Univ
62	Ohio State Univ - Columbus
64	Univ Oslo
65	Hebrew Univ Jerusalem
65	Purdue Univ - West Lafayette
67	Univ Heidelberg
68	Osaka Univ
68	Univ Helsinki
70	Moscow State Univ
71	Brown Univ
71	Uppsala Univ

73	Ecole Normale Super Paris
73	Univ Melbourne
73	Univ Rochester
76	Univ Leiden
77	Univ Arizona
77	Univ Sheffield
79	Tohoku Univ
79	Univ Utah
81	King's Coll London
82	Univ Nottingham
83	Boston Univ
83	Case Western Reserve Univ
83	Michigan State Univ
86	Stockholm Univ
87	<u>Univ Basel</u>
88	Texas A&M Univ
89	McMaster Univ
90	Univ Goettingen
91	Univ Birmingham
92	Indiana Univ - Bloomington
93	<u>Aarhus Univ</u>
93	Arizona State Univ - Tempe
95	Univ Virginia
96	Univ Freiburg
97	<u>Lund Univ</u>
97	Rice Univ
97	<u>Univ Bonn</u>
97	Univ Sydney

## Registry of BioBricks

Registry of Parts: several 1000 parts available

#### Types of parts:

- Promotors
- •RBS
- Terminators
- Protein coding sequences
- Plasmids
- Composite parts

### Types of devices:

- Protein generators
- Reporters
- Inverters
- Receivers and senders
- Measurement devices
- •..

## **Examples of iGEM projects**

Engineering cellular signaling

Antiviral device

PAMP, TLR, dning cascade

Modes

Bignaling cascade

And Mignaling cascade

ILR\_symm

ILR\_symm

ILR\_symm

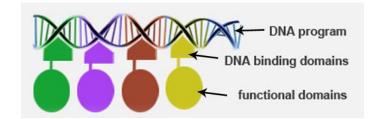
ILR\_symm

Indiana

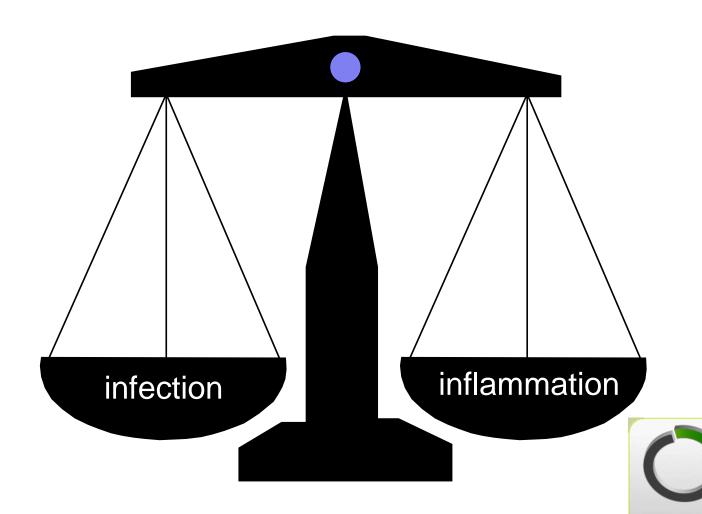
India



**DNA-based scaffold** 



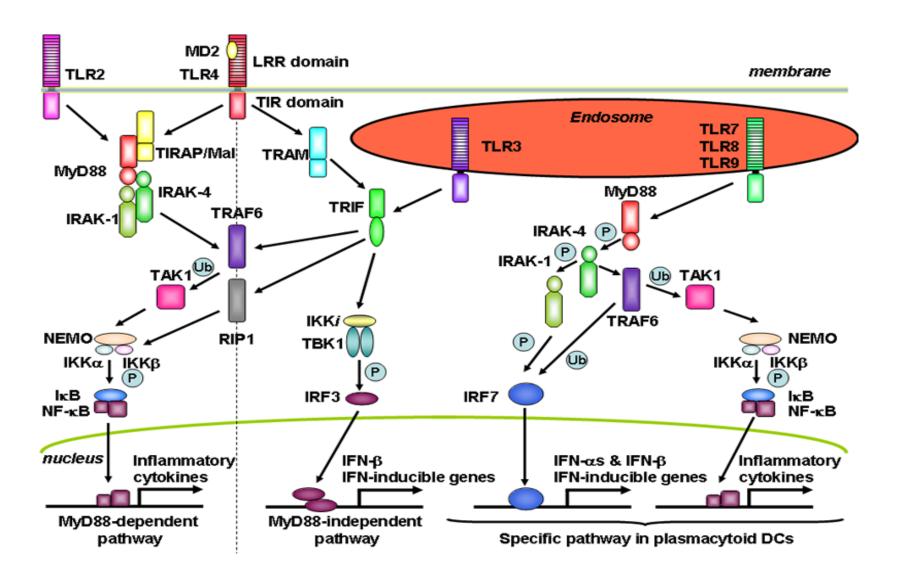
# Negative feedback loop to supress inflammation





iGEM 2006 Slovenija

## TLR signaling pathway



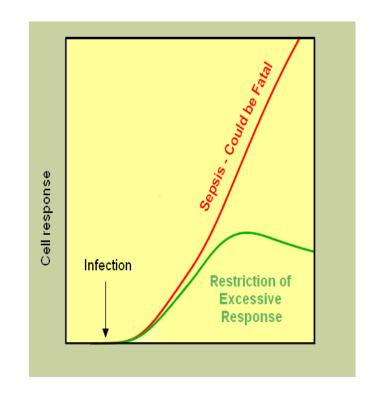
# Modification of the signaling network

#### **Basic concept:**

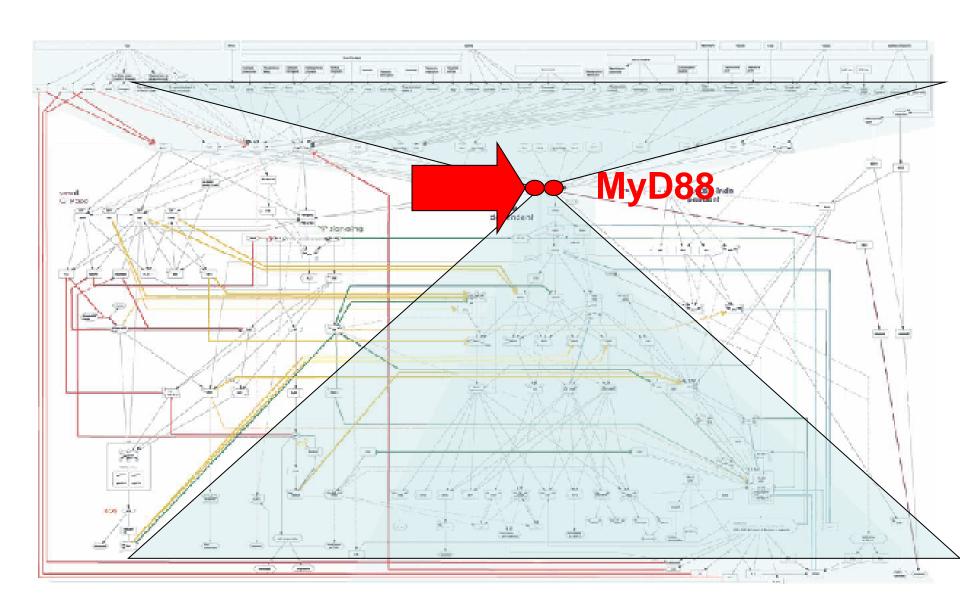
 Inhibit the excessive cellular activation but don't completely inactivate cellular responsiveness (MyD88 KO are extremely sensitive to infections)

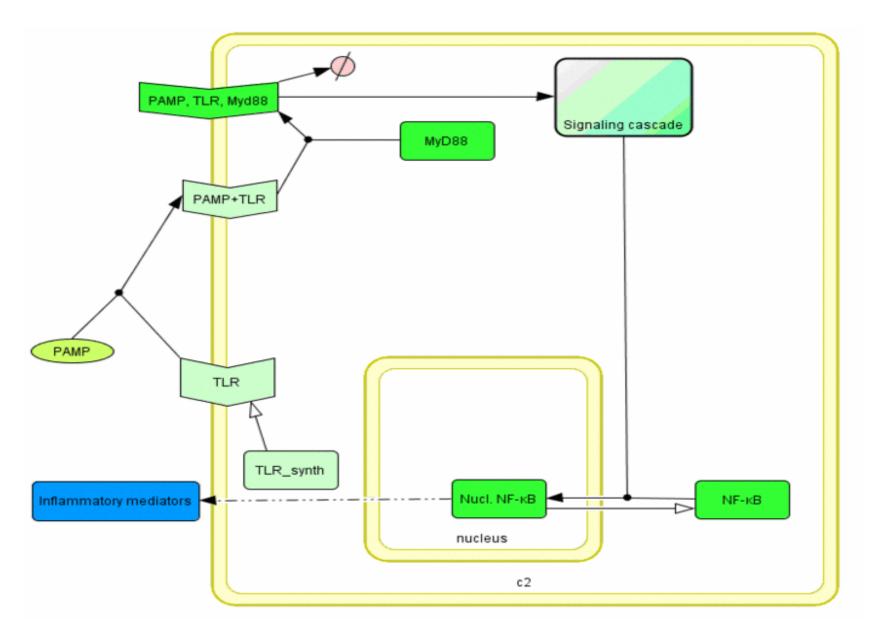
#### Realization:

 Insert into mammalian cells a feedback device with inhibitor (dnMyD88) that could repress the signaling of TLR pathway for a limited period of time



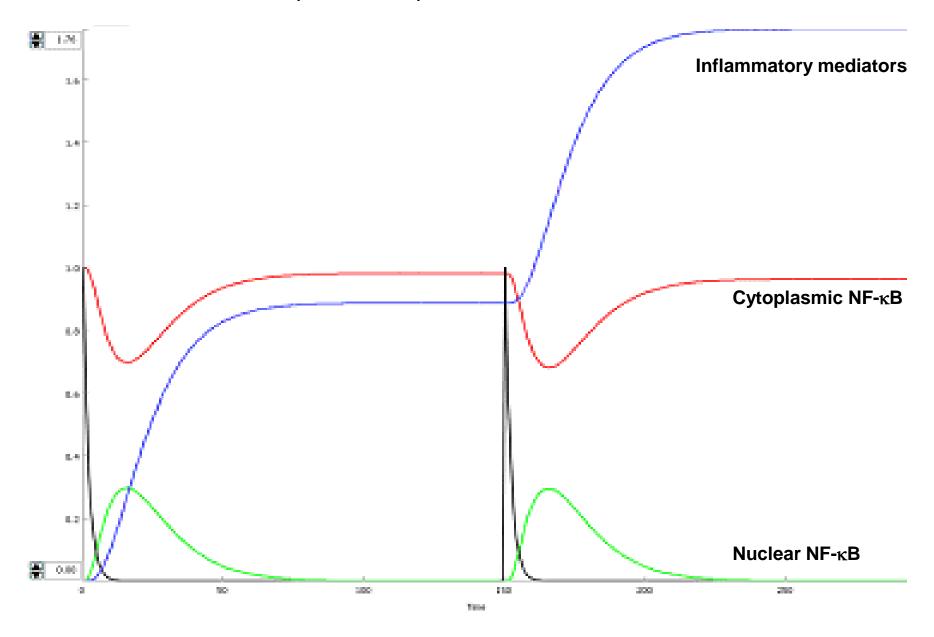
## MyD88 as the central adapter

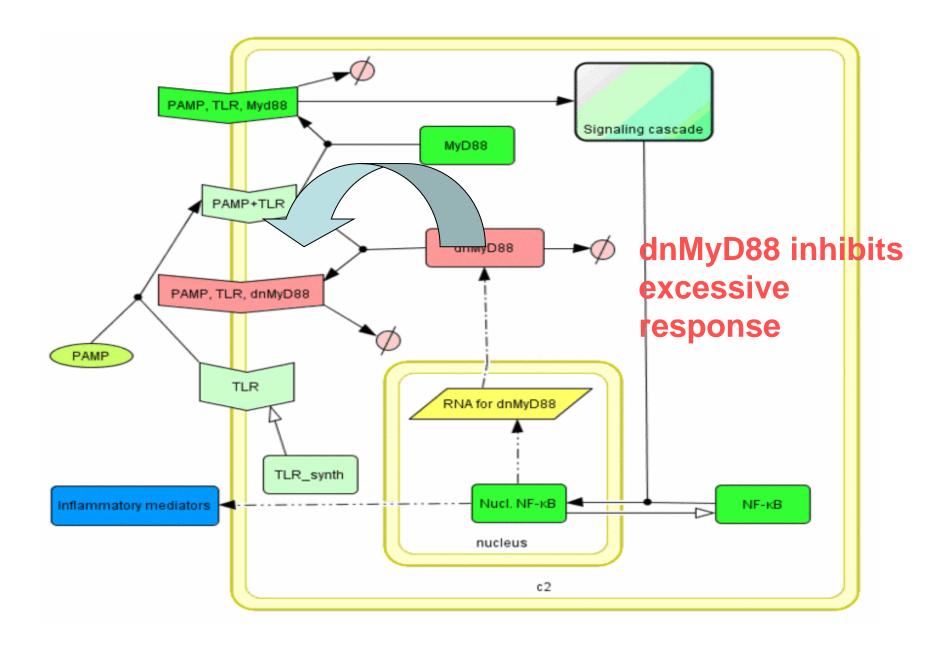




Simplified model of TLR signaling

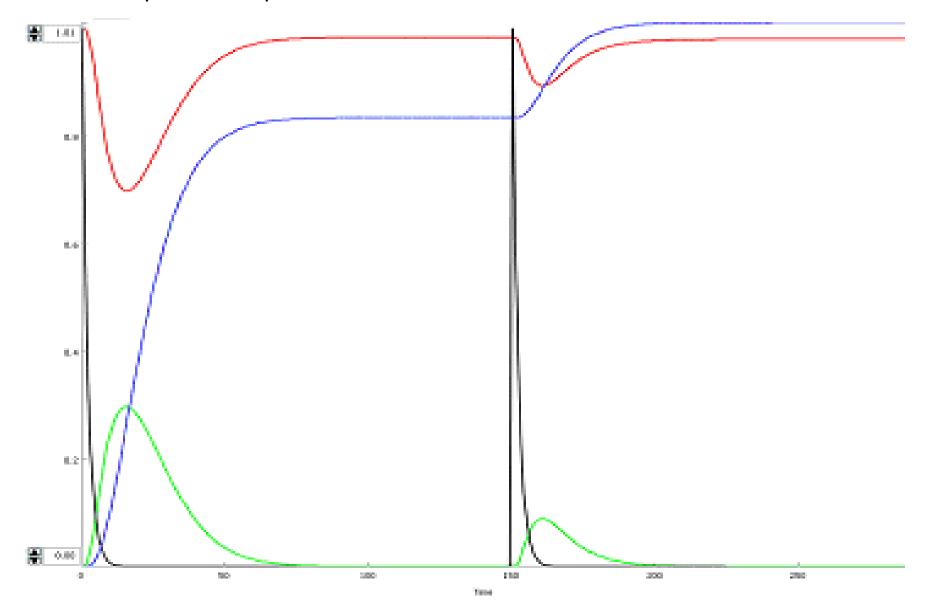
#### Normal cellular response to repeated stimulus



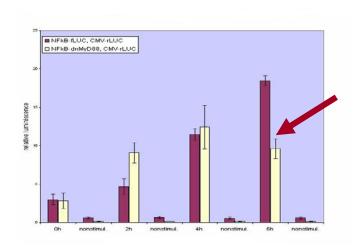


Insertion of a negative feedback loop

#### Response to repeated stimulus in cells with inserted feedback device



## Results



#### **Potential applications:**

-prevent excessive response to infection (bacterial sepsis, viremia...)

-inhibition of chronic inflammation (arthritis, lupus...)



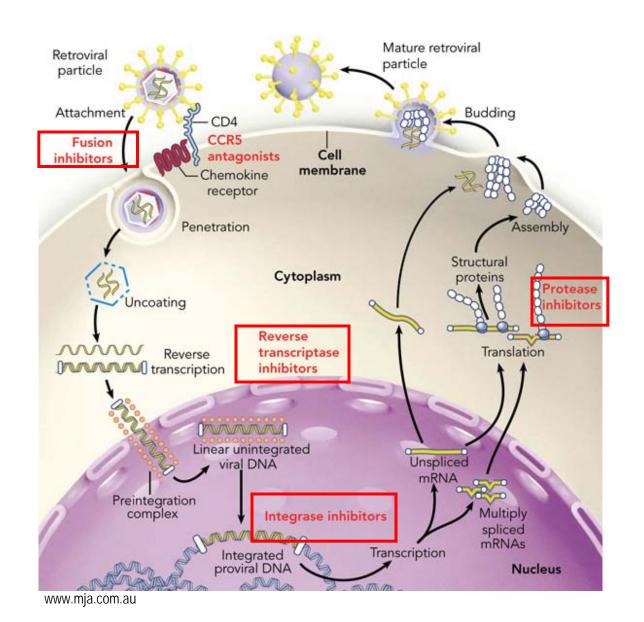
Finalist of iGEM2006: Imperial College, Slovenia (Grand Prize winner), Princeton.

## Antiviral defense based on viral functions

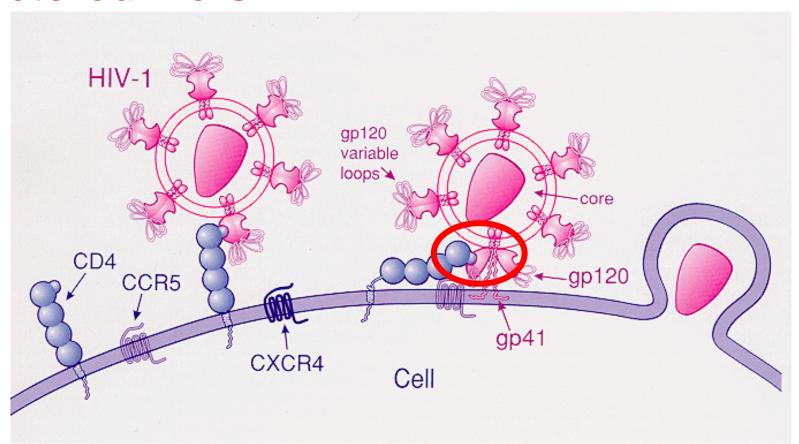


iGEM team Slovenia 2007

### Therapeutic targets of HIV life cycle

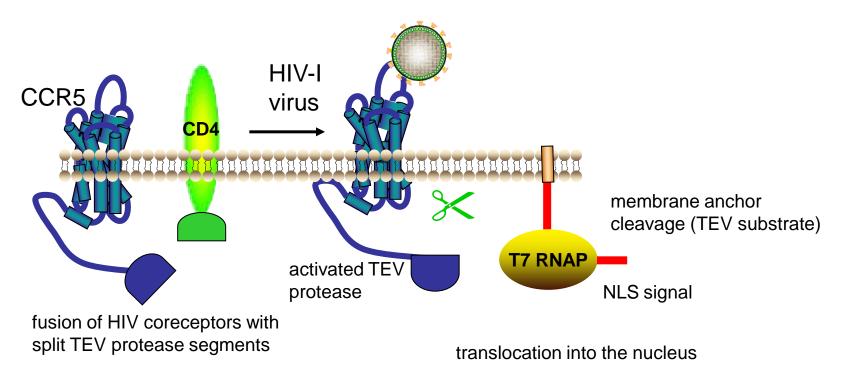


# FUNCTION-based viral detection 1: Viral attachment causes receptor heterodimers



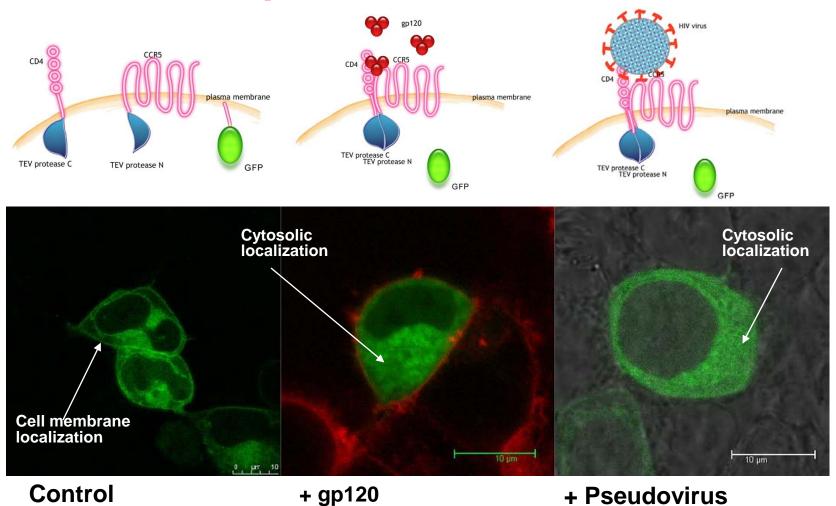
Heterodimerization of cellular transmembrane receptors at viral entry could be used to detect viral attack.

### Split TEV protease - based viral detector

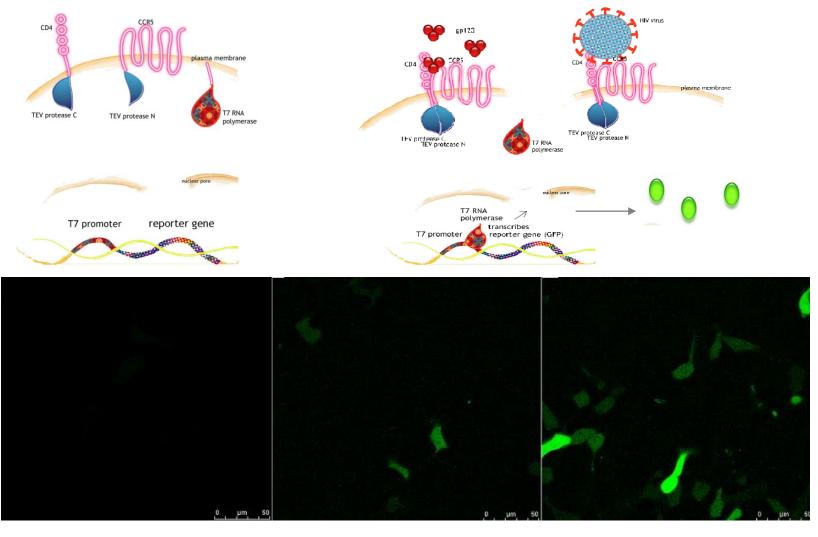


transcription of antiviral defense genes (e.g. Caspase-3, interferon, ApoBec...)

## HIV causes release of the GFP reporter from the membrane



## Integration of two steps: Split TEV-T7-based cell activation



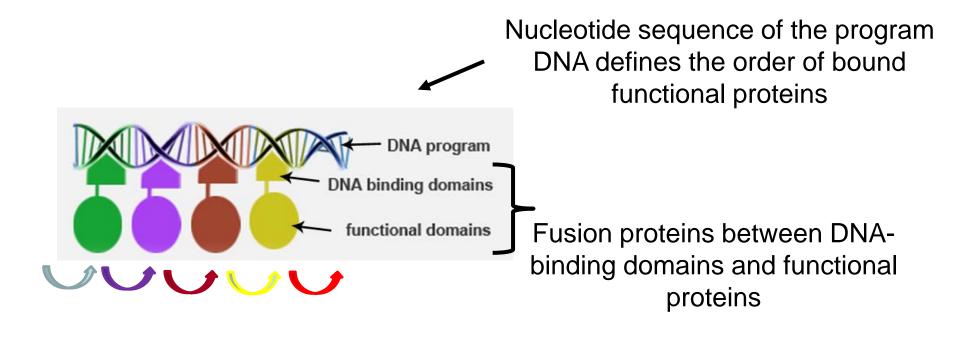
Control + gp120 + Pseudovirus

## DNA as the carrier of information

Polypeptide Assemby of chain functional domains amino acid functional domain DNA **tRNA** binding domain **RNA** DNA 9 bp sequence 3 nt sequence

### **Project iGEM2010**

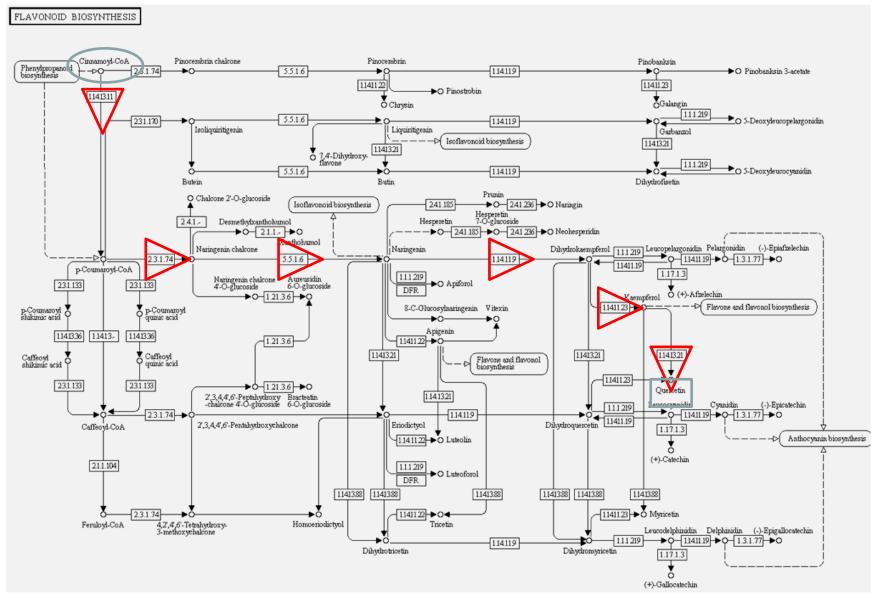
#### Assembly of functional proteins along the DNA



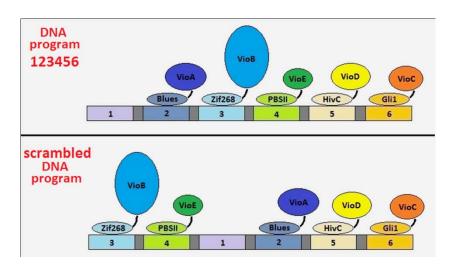
Assembly of functional protein domains fulfills complex functions

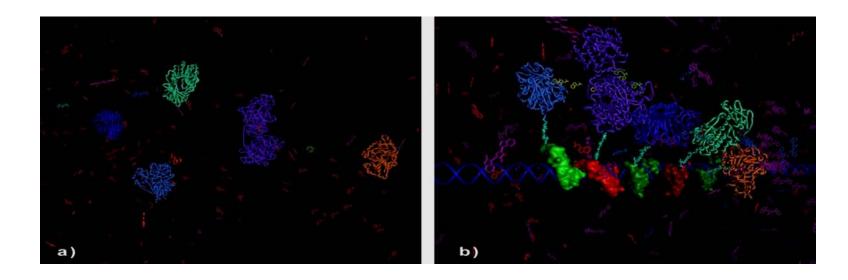


## Complex biosynthetic pathways



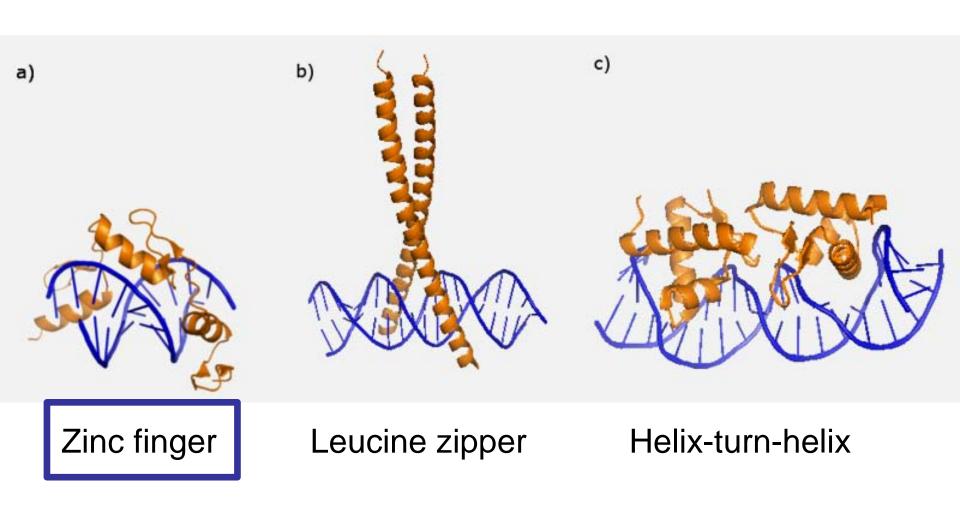
## Biosynthetic pathway based on DNA program







## **DNA** binding domains



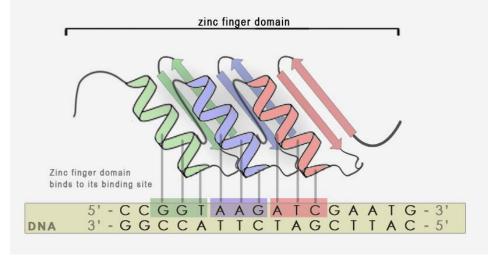


### Selection of DNA binding domains

- Zinc fingers
- Modular design (multiples of 3)
- >700 characterized zinc fingers readily available in ZiFDB
- 262.144 possible binding sites for nonameres
- Longer recognition sequence → more specific binding

 Reversing the problem - we can select well characterized zinc fingers and use its cognate DNA motif for the DNA

program



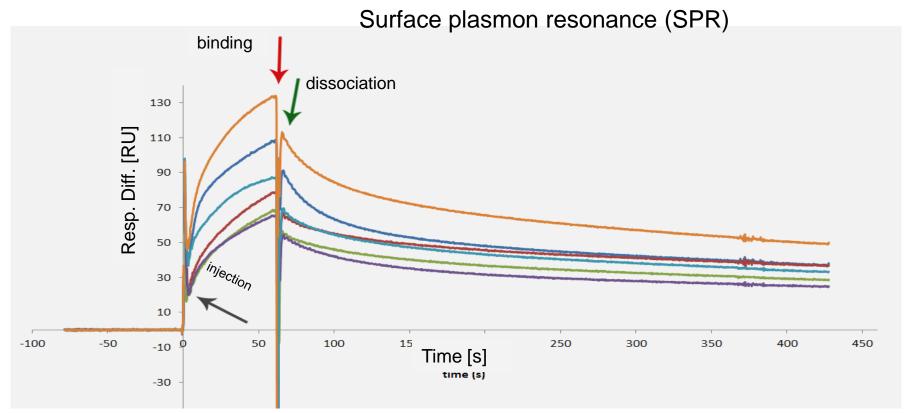


#### **Characterization of ZNFs**

Selected zinc fingers:

Gli1, HIVC, Zif268, PBSII, Jazz, Blues

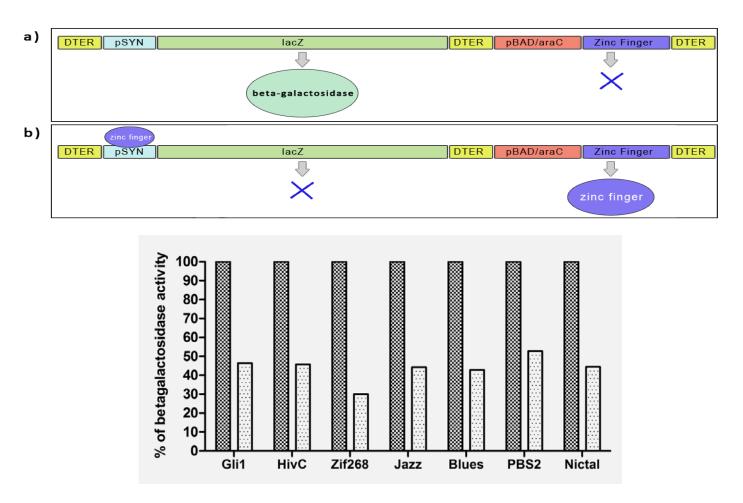
→ cloned → expressed → purified → characterized



All tested ZNFs bind to their target DNA



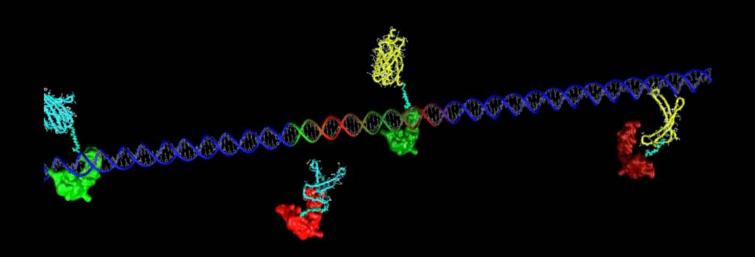
### Experimental testing of seven artificial repressors



ZNFs act as artificial repressors

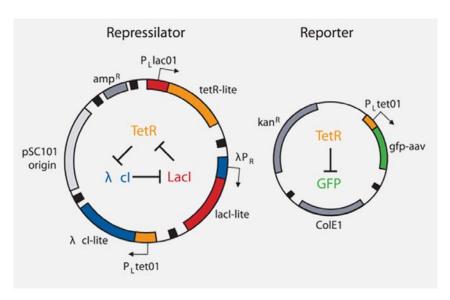


### **Assembly of multiple DNA binding domains**

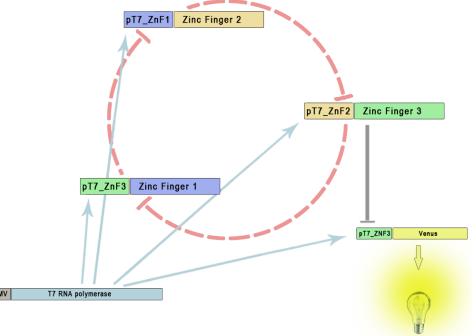


## Application of artificial ZNF repressors: Biological oscillators

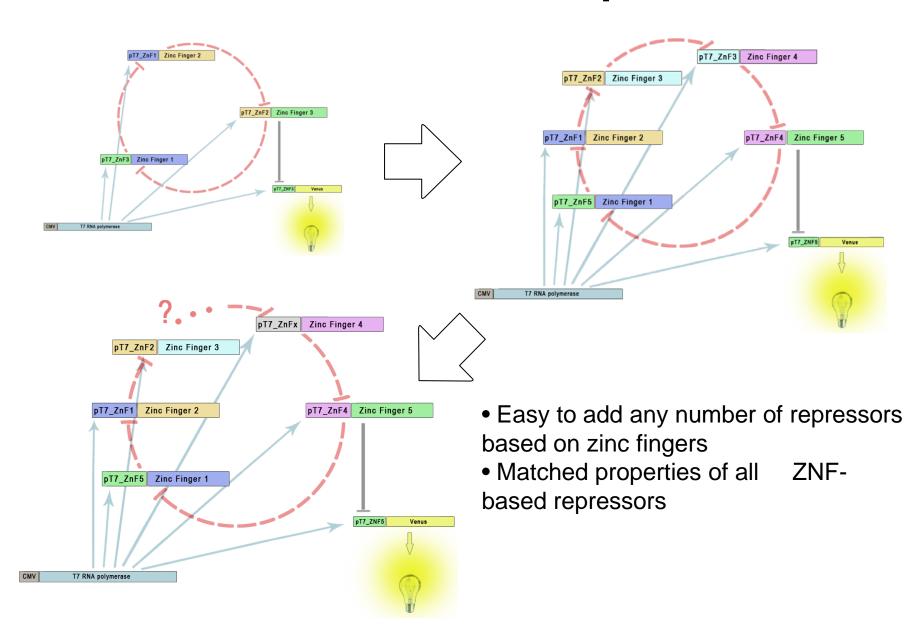
- Cyclic topology with 3 bacterial repressors
- Limited selection of available natural repressors
- Use zinc fingers as artificial repressors new features?
- Deterministic and stochastic simulations



(Elowitz et al., 2000, Nature)



### **Extended ZNF-based Repressilator**



### Repressilator

Three ZNFs (period of oscillation: 2,7 h)

Four ZNFs (no oscillation)

50.000

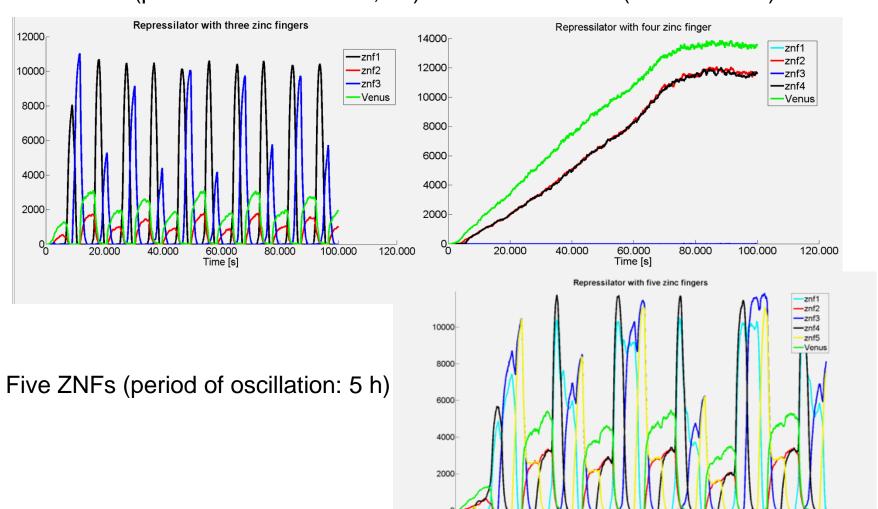
Time [s]

60.000

70.000

80.000

40.000



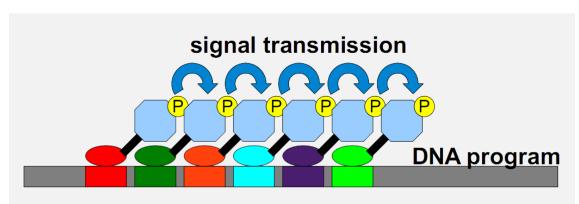


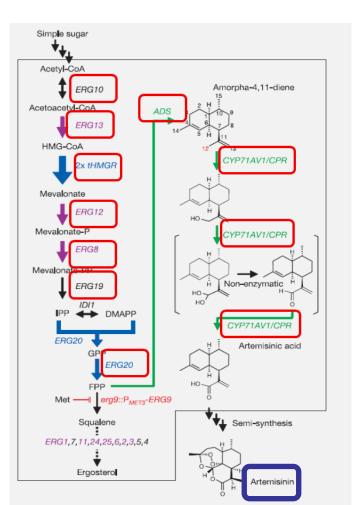
### **Perspectives**

- Complex biosynthetic pathways z involving many steps/reactions (e.g. drugs, biofuel...)
- Mutienzyme catalytic reactions in vitro

#### More than just biosynthesis:

- Pattern recognition
- Information processing (using protein kinases, proteases etc.)





Keasling et al., 2003, Nature Biotechnology

## Capabilities of synthetic biology

#### **Current:**

Synthesis and assembly of complete biosynthetic and signaling pathways (tens of genes)

### **Cutting edge:**

Assembly of a **copy** of complete bacterial genomes based on synthetic DNA (up to million nucleotides)

Jump start the living bacteria based on synthetic genome

## Perspectives and limitations

Assembly of artificial information processing logic on the existing chassis.

Assembly of completely new adaptive organisms based on designed components.

#### The Main limitations:

Understanding of the function of components and their interactions as the complete system

Understanding of the minimal set of genes for the self replicating organism (estimate ~300 genes)

## Slovenian teams 2006-2010











#### 2006

Monika Ciglič, BF Ota Fekonja, BF Jernej Kovač, FKKT Alja Oblak, BF Jelka Pohar, BF Matei Skočai, BF Rok Tkavc, BF

#### 2007

Marko Bitenc, BF Peter Cimermančič, FKKT Rok Gaber, BF Saša Jereb, FKKT Katja Kolar, FKKT Anja Korenčič, FKKT Andrei Ondračka, FKKT

#### 2008

Eva Čeh, BF Vid Kočar, FKKT Katja Kolar, FKKT Tibor Doles, BF Ana Lasič, MF Jerneja Mori, BF Anže Smole, BF

#### 2009

Sabina Božič, FKKT Nika Debeljak, BF Urška Jelerčič; FMF Jan Lonzarić, FKK Anja Lukan, FKKT Špela Miklavič, BF Marko Verce, BF

#### 2010

Jernej Turnšek Neic Tjaša Tina IIc Tina Lebar Matej Žnidarič Mattia Petroni Jure Bordon Rok Pustoslemšek Rok Črešnovar

#### **Mentors**

Mojca Benčina, Monika Avbelj, Karolina Ivičak, Nina Pirher, Gabriela Panter, Mateja Manček Keber (KI), Marko Dolinar(FKKT), Simon Horvat (BF), Iva Hafner Bratkovič, Helena Gradišar, Ota Fekonja, Jelka Pohar, Rok Gaber, Tomaž Koprivnjak, Jerneja Mori, Irena Vovk, (KI), Gregor Anderluh, Vesna Hodnik (BF), Miha Mraz, Miha Moškon, Nikolaj Zimic (FRI), Roman Jerala (KI, FKKT)

# "See things not as they are, but as they might be"

Robert Oppenheimer

Watermark in the synthetic genome of Mycoplasma laboratorium