National
Institute of Chemistry
Slovenia

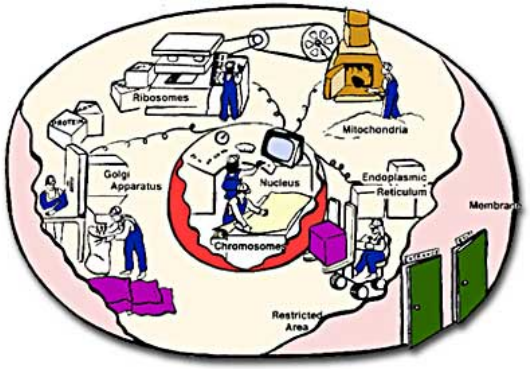
Synthetic Biology achievements and future prospects

Roman Jerala

National institute of chemistry
University of Ljubljana
Slovenia

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

Different approaches of synthetic biology

- Building on the existing chasis (modification of functional biological systems)

- Bottom up – (re)construction of complete genomes
  - currently making genome copies
  - genome transplantation, rebooting cells

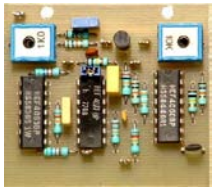
  - Creation of new minimal self-replicating systems

Modularity and hierachical organization

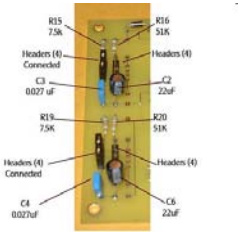
Computer networks

Computers

Modules

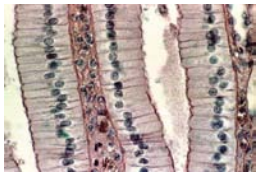


Electronic circuits

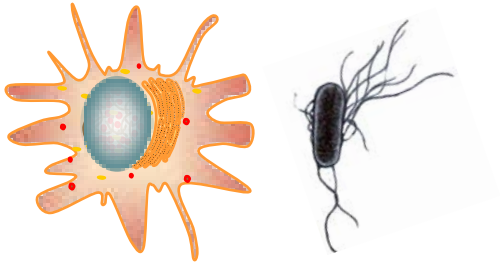


Electronic elements

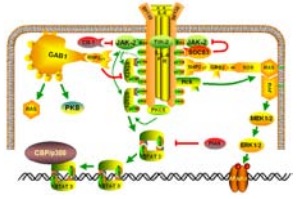
Tissues, organisms



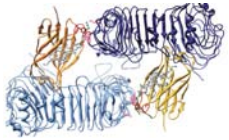
Cells



Biochemical pathways

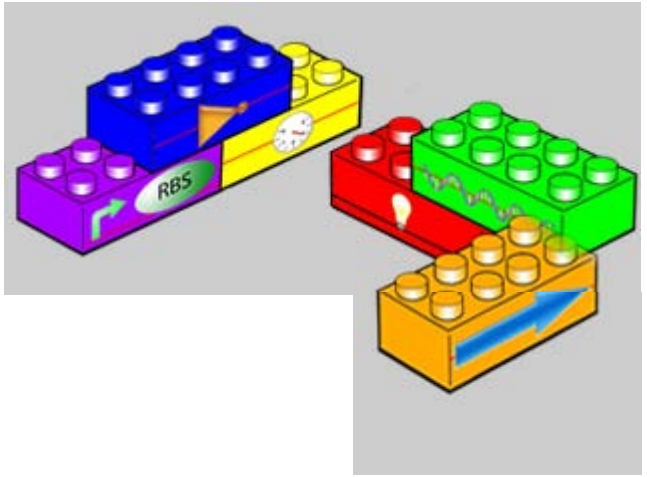


Biochemical eractions



Genes, proteins

DNA as the cellular program



BioBricks as modular building blocks:

- regulatory sequences
- protein coding sequences

Foundations for the development of Synthetic biology

- Understanding of biological processes
- Known tertiary structures of most protein folds
- Transcriptional activity of thousands of genes
- DNA sequence determination
- Gene synthesis
- ...

DNA sequence determination

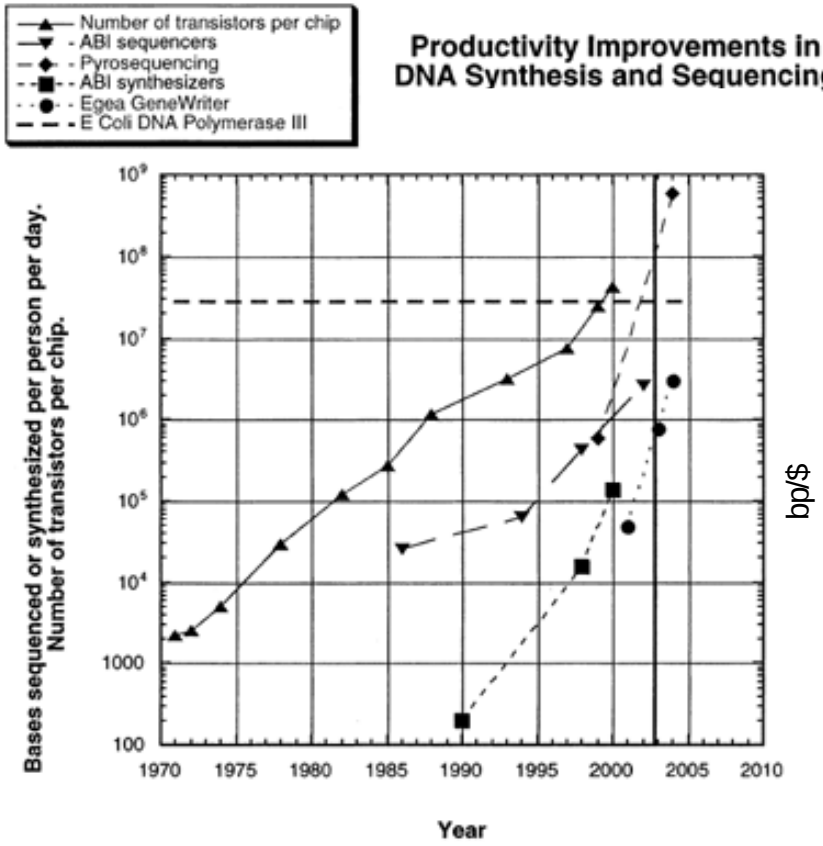
February, 2001 human genome determination was announced using first generation DNA sequencers @ 1$/bp

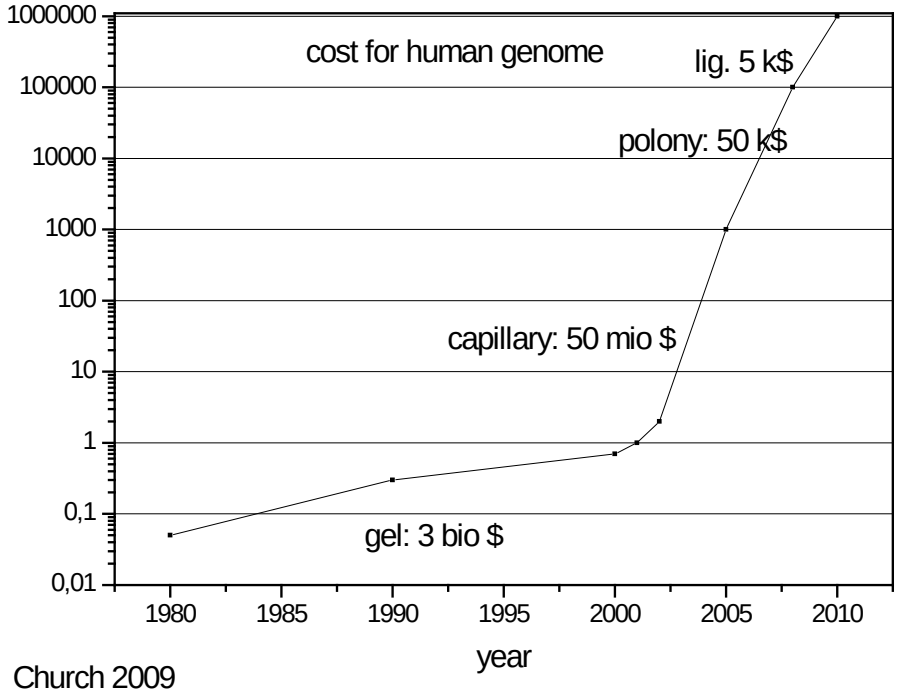
By 2011 planned determination of 1000 genomes
60x more than in previous 25 years togeather

Personal genome project – sequencing 100,000 genomes (George Church)

Pace in the last years:

10x increase/year

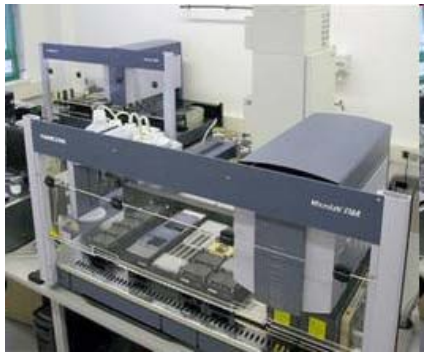




Church 2009

Rob Carlson The Pace and Proliferation of Biological Technologies

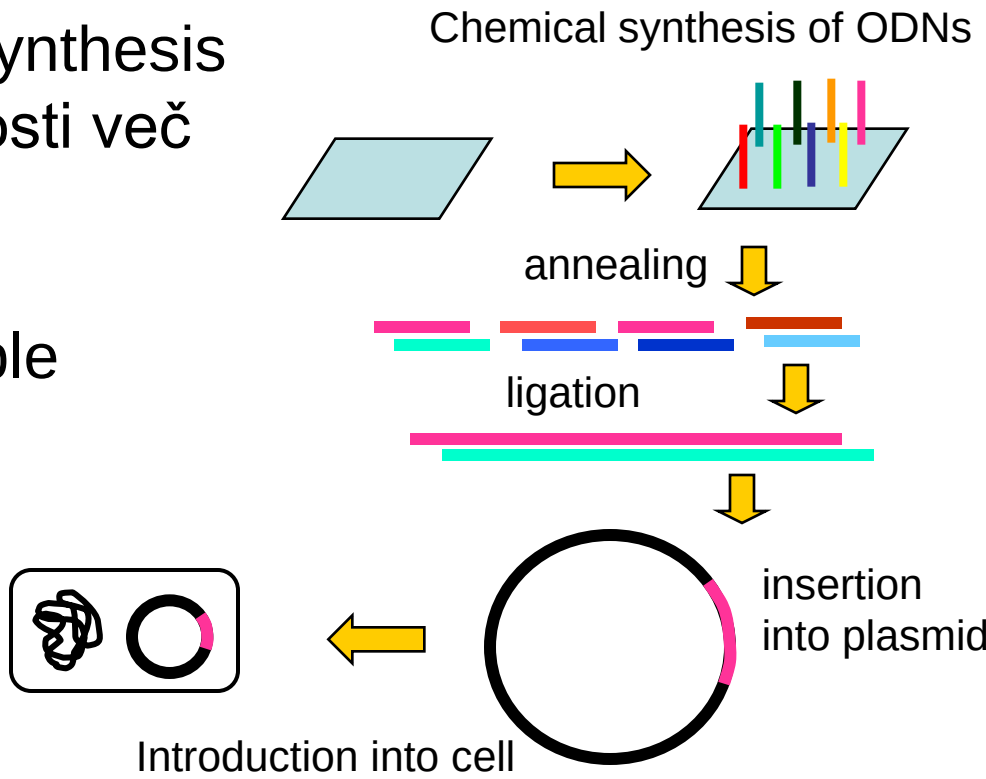
Chemical synthesis of DNA



Automatic synthesis of defined sequence

Automated parallel chemical synthesis allows to assemble DNA velikosti več 10,000 nt

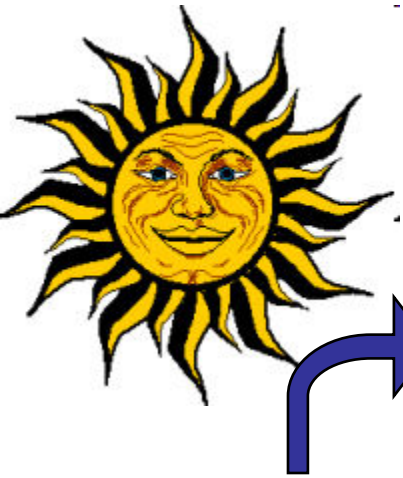
Still based on the same principle (first generation)



Potential areas of application of synthetic biology

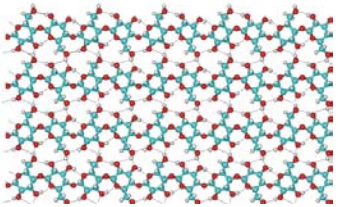
- Medicine
- Renewable sources of energy
- New materials & bionanomaterials
- Information processing
- Biosensors
- Bioremediation...

Renewable sources of energy



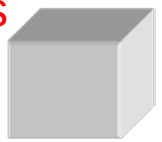
CO_2

Plant biomass

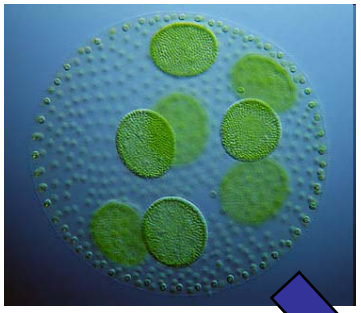


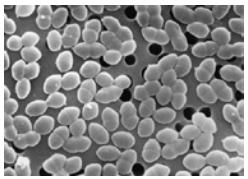
cellulose

enzymes



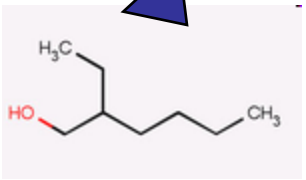
sugars

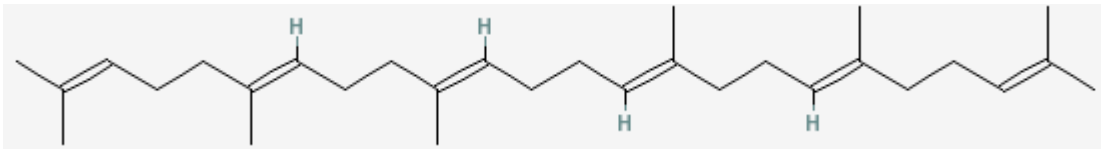




Microbial conversion

Energy Biosciences Institute





fuel

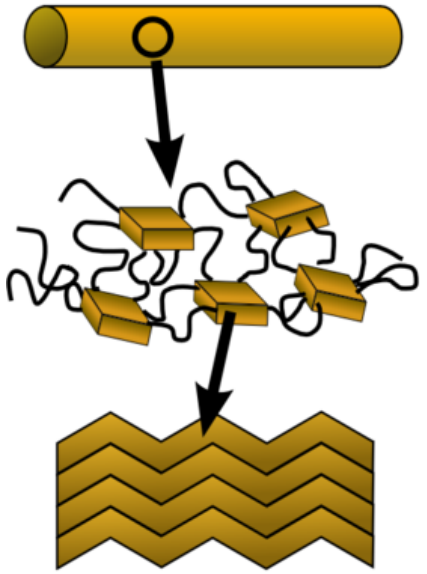
Biomimetic materials

Synthetic spider silk

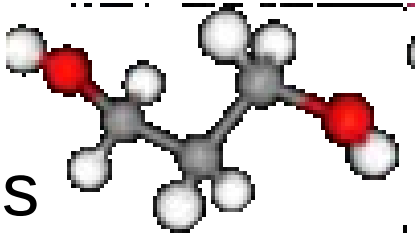
Excellent properties – stronger than Kevlar (“Biosteel”), light, not immunogenic, not allergenic (medicine), ...

Synthetic biology provides production of synthetic silk

We can add new properties –dyes, immobilized enzymes...



Microbial production of raw materials



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

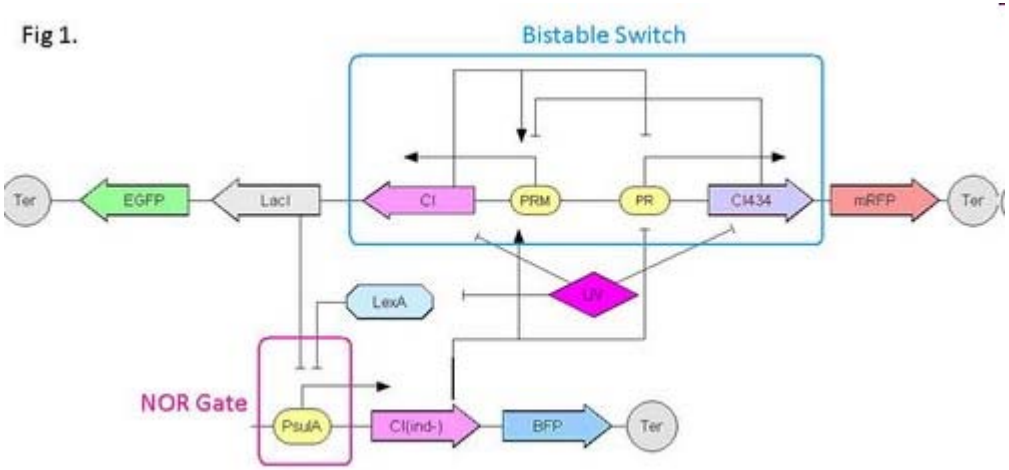
- DuPont, invested 400 M$ in 7 years
- Modification of 19 genes of *E.coli*, introduction of 8 genes from yeast and *Klebsiella*
- Production 135 g/l; 90% carbon yield, production >100,000 T/year
- First billion dollar nonmedical application of SB

Information processing

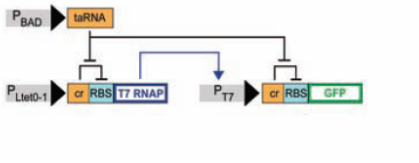
DNA contains high density of information

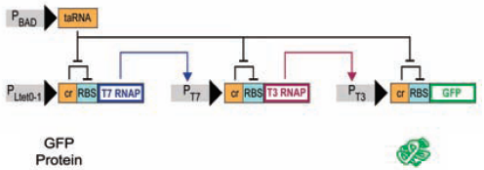
~1 bit/nm^3, much more than existing media for information storage

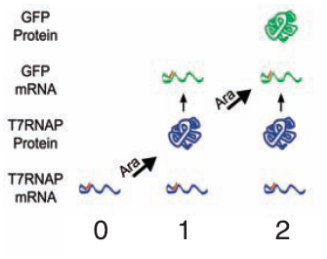
DNA processes information near thermodynamic limit: 10^{19} ops/J vs. 10^9 for computers

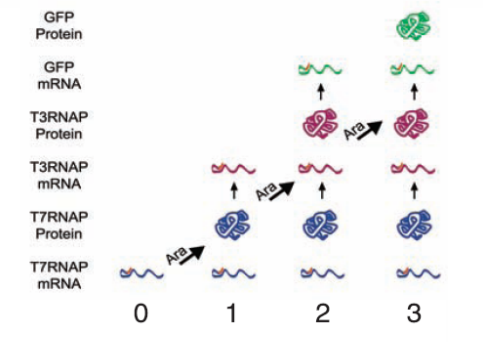


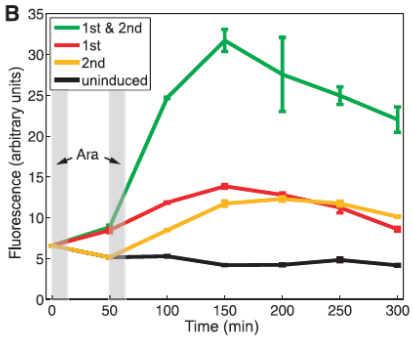
Synthetic gene networks that count

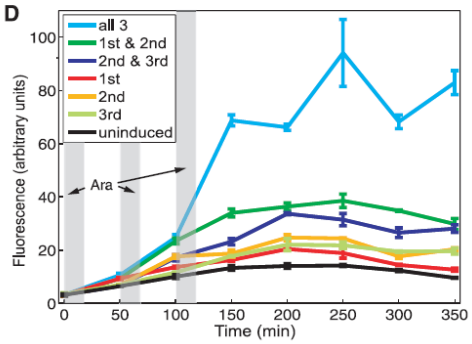


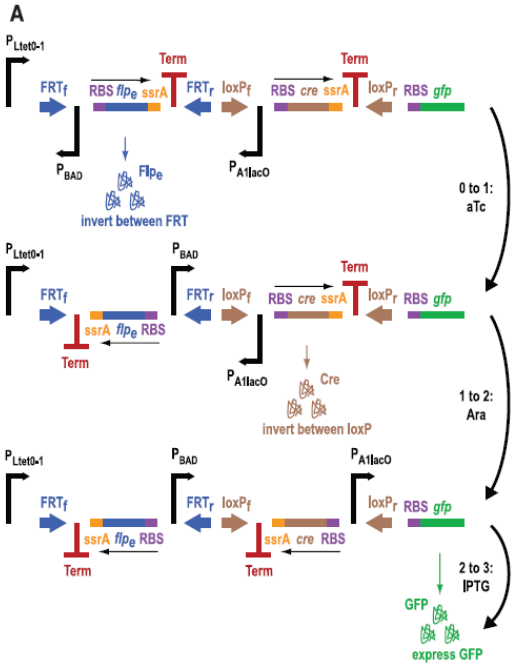


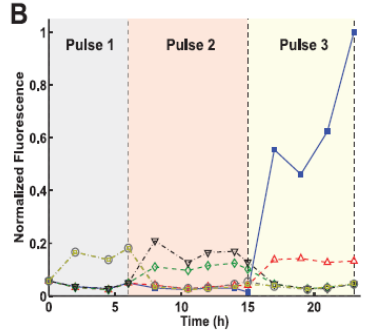


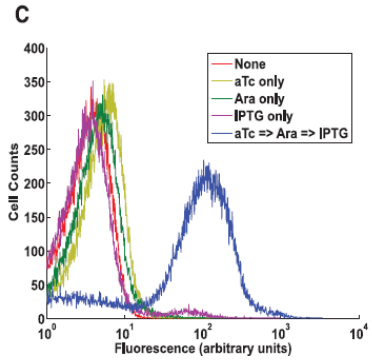








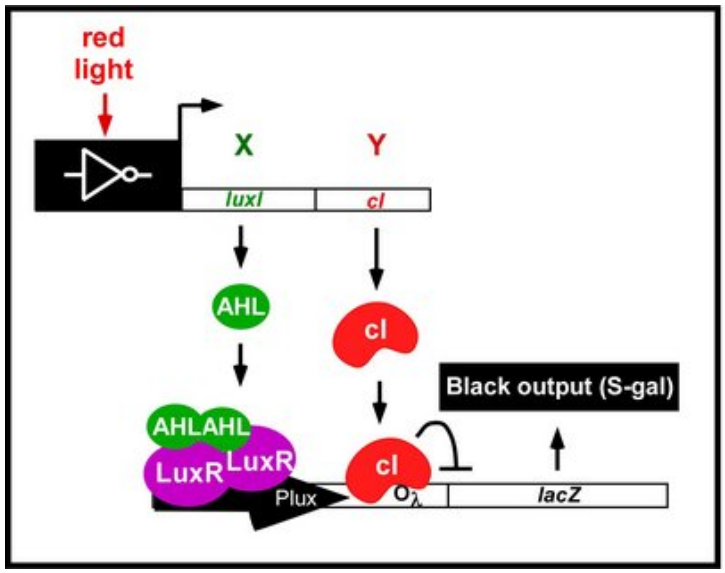


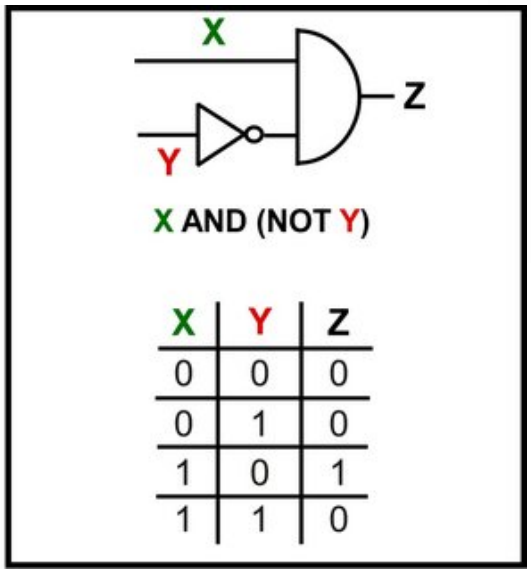


Friedland et al., Science 2009

Bio-photolitography

University of Texas
iGEM2006

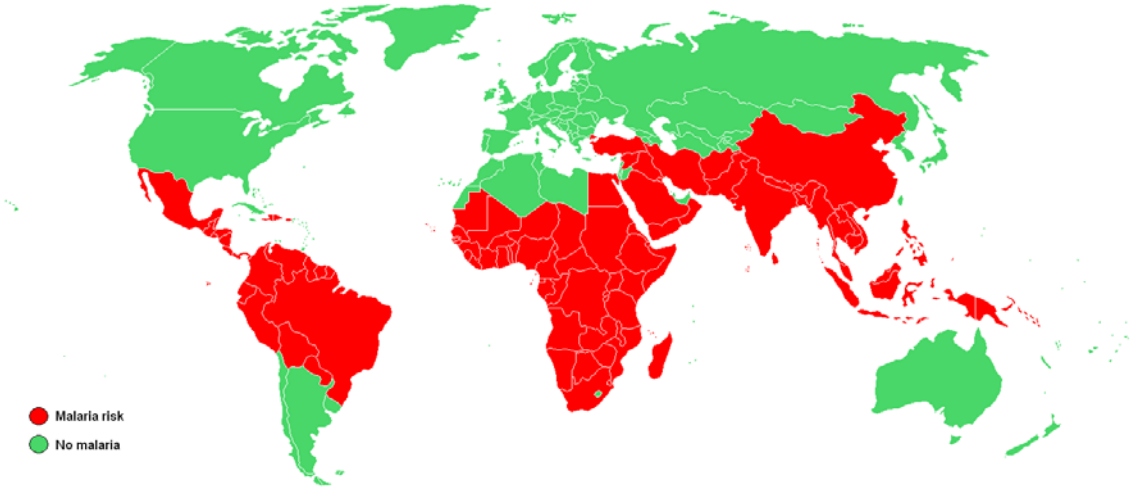




X	Y	Z
0	0	0
0	1	0
1	0	1
1	1	0

Edge detector circuit

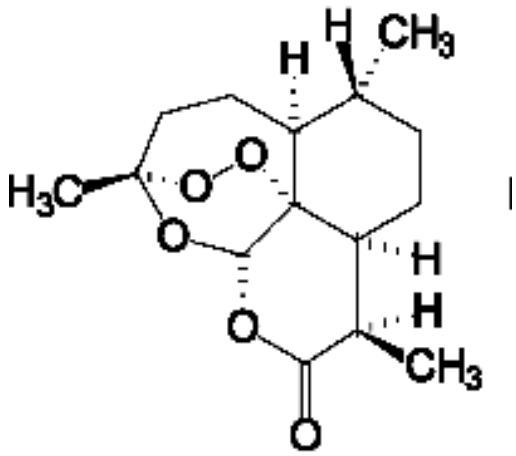
Synthesis of antimalarial drug



250 million infected
1 million deaths/year

Isolation from annual wormwood

High price
Limited production



artemisinin

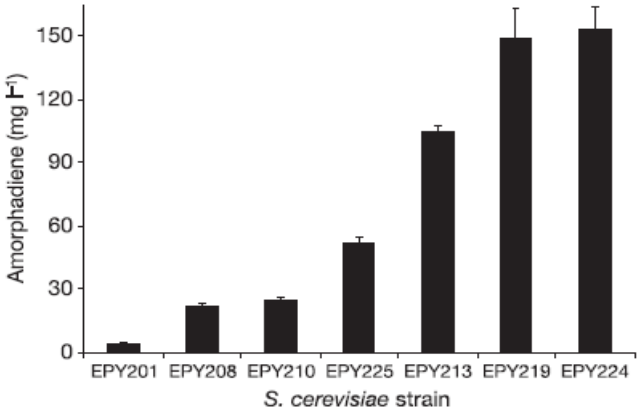
Transfer of the biosynthetic pathway into bacteria using synthetic biology

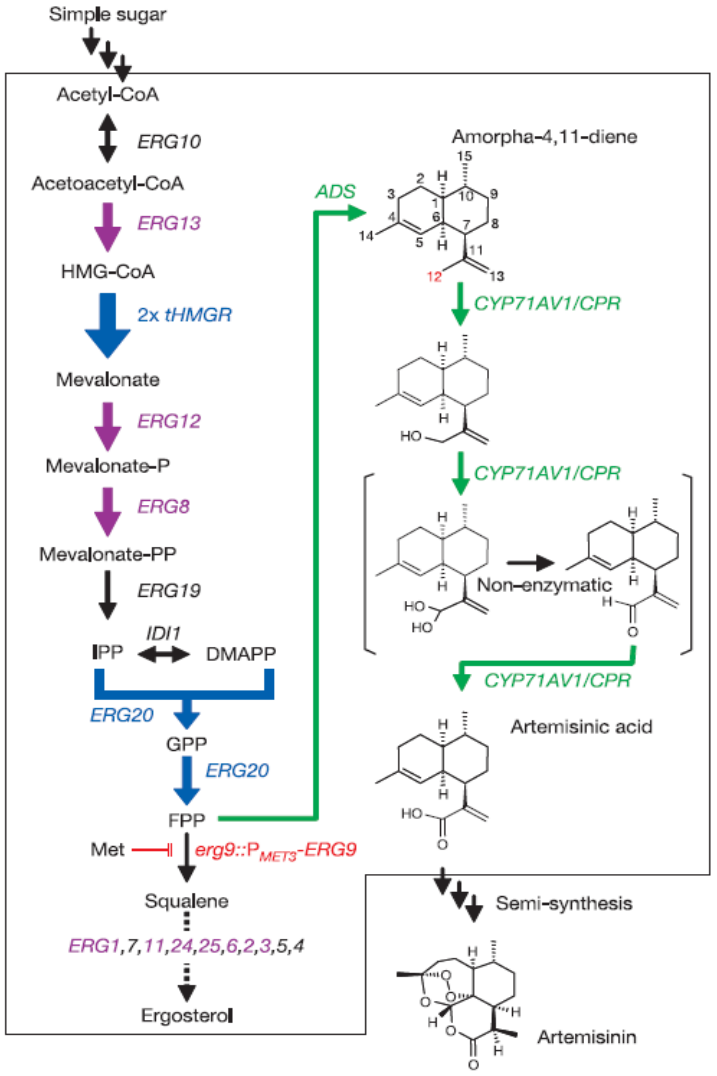
nature Vol 440|13 April 2006|doi:10.1038/nature04640

LETTERS

Production of the antimalarial drug precursor artemisinic acid in engineered yeast

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





Medical applications of synthetic biology

- Alternative means of drug production
- New therapeutics
- Engineering of response of human cells

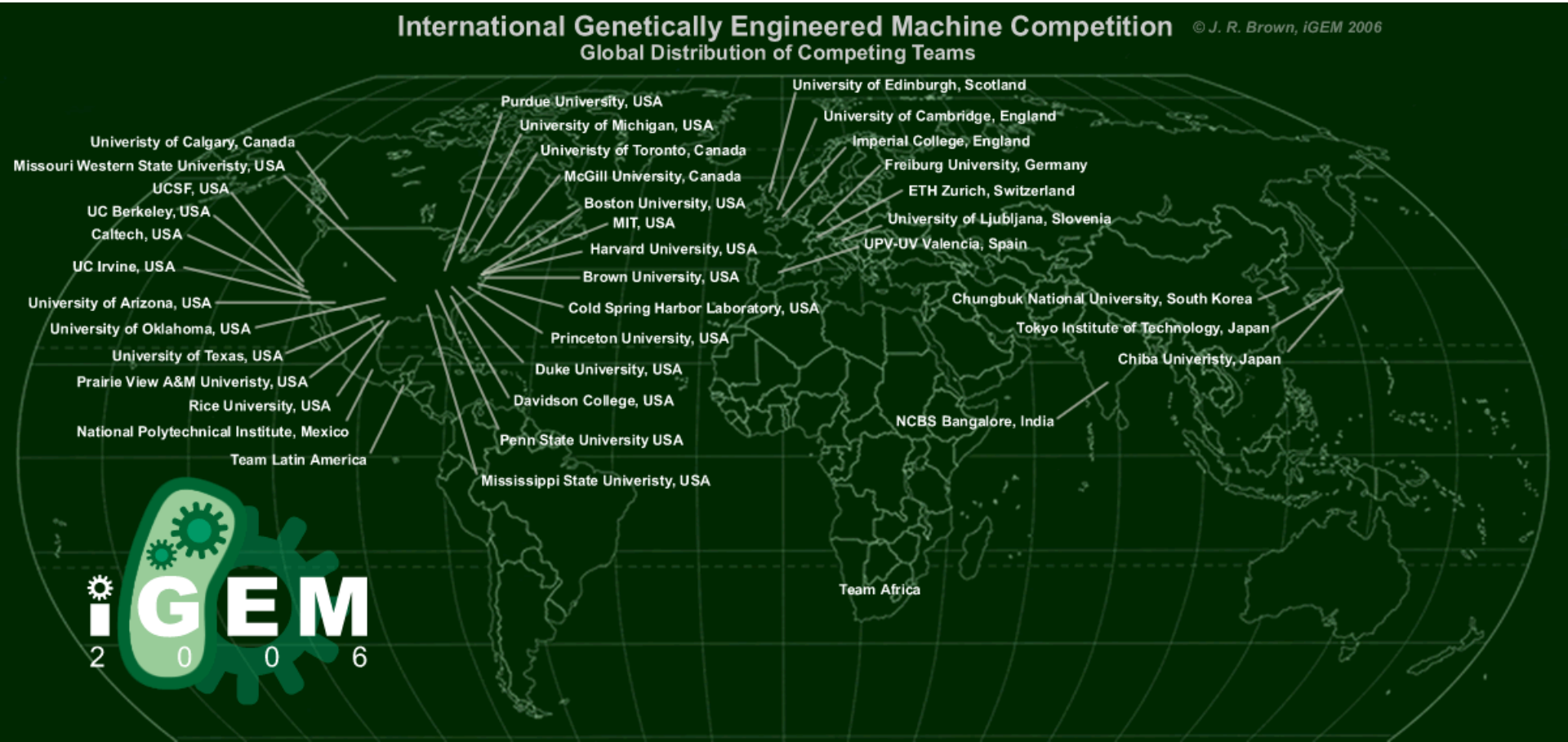
Engineering mammalian cells

- **Many similarities than differences in comparison to prokaryotic systems**
- **Disadvantages: more complex, slower (and expensive) to work with**
- **Opportunities: understanding of complex systems, potential health applications**

- **Synthetic cell signaling pathway**
  - negative feedback loop to inhibit excessive inflammatory response
  - antiviral defense based on viral function which is insensitive to viral mutations

- **Designed vaccines**
  - uncover the stealth of bacteria and make bacterial components visible to the immune system

The [International Genetically Engineered Machine competition (iGEM)] as the testing ground or sandbox for creative ideas in synthetic biology.



iGEM 2008

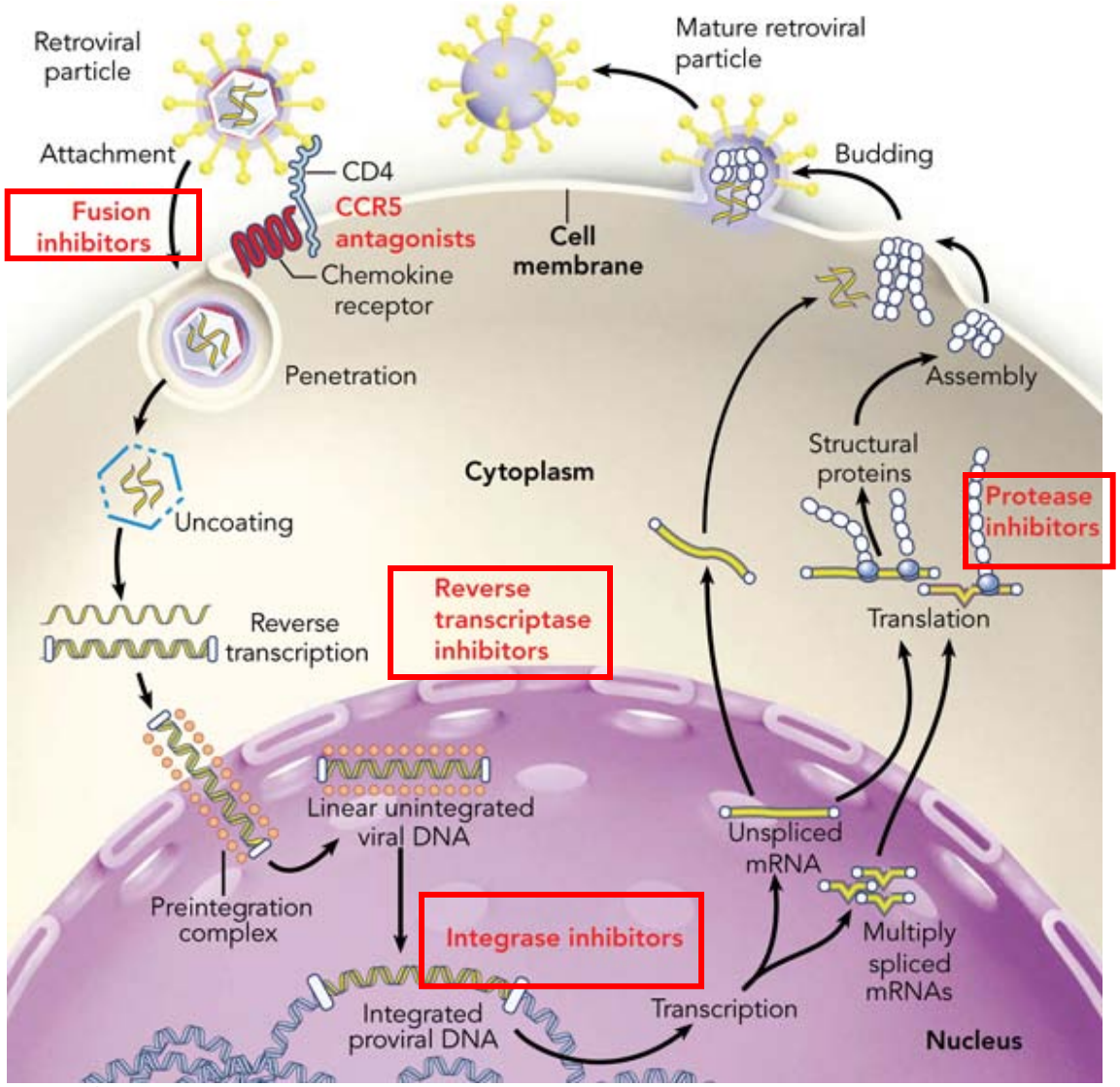
igem.org

Teams Registered for iGEM 2009 ...

These 112 teams are registered for iGEM 2009

Aberdeen_Scotland	Alberta	Amsterdam	ArtScienceBangalore
Bay_Area_RSI	BCCS-Bristol	Berkeley_Software	Berkeley_Wetlab
BIOTEC_Dresden	Bologna	British_Columbia	Brown
BrownTwo	Calgary	Cambridge	CBNU-Korea
Chiba	CityColSanFrancisco	Cornell	DTU_Denmark
Duke	Edinburgh	EPF-Lausanne	ESBS-Strasbourg
Freiburg_bioware	Freiburg_software	Gaston_Day_School	Groningen
Harvard	Heidelberg	HKU-HKBU	HKUST
IBB_Pune	IGIB-Delhi	IIT_Bombay_India	IIT_Madras
Illinois	Illinois-Tools	Imperial College London	Indiana
IPN-UNAM-Mexico	IPOC1-Colombia	IPOC2-Colombia	Johns_Hopkins
Johns_Hopkins-BAG	KULeuven	KU_Seoul	Kyoto
LCG-UNAM-Mexico	Lethbridge	McGill	METU-Gene
Michigan	Minnesota	Missouri_Miners	MIT
MoWestern_Davidson	NCTU_Formosa	Nevada	Newcastle
NTU-Singapore	NYMU-Taipei	Osaka	Paris
PKU_Beijing	Purdue	Queens	Rice
SDU-Denmark	Sheffield	SJTU-BioX-Shanghai	Slovenia
Southampton	Stanford	SupBiotech-Paris	Sweden
Tianjin	Todai-Tokyo	Tokyo-Nokogen	Tokyo_Tech
TorontoMaRSDiscovery	Tsinghua	TUDelft	TzuChiU_Formosa
UAB-Barcelona	UChicago	UCL_London	UCSF
UC_Davis	ULB-Brussels	UNC_Chapel_Hill	UNICAMP-Brazil

Therapeutic targets of HIV life cycle



www.mja.com.au

Problems with antiviral therapy

Mutations!

10 – 20% of HIV-infected individuals in USA and Europe carry drug-resistant HIV strains.

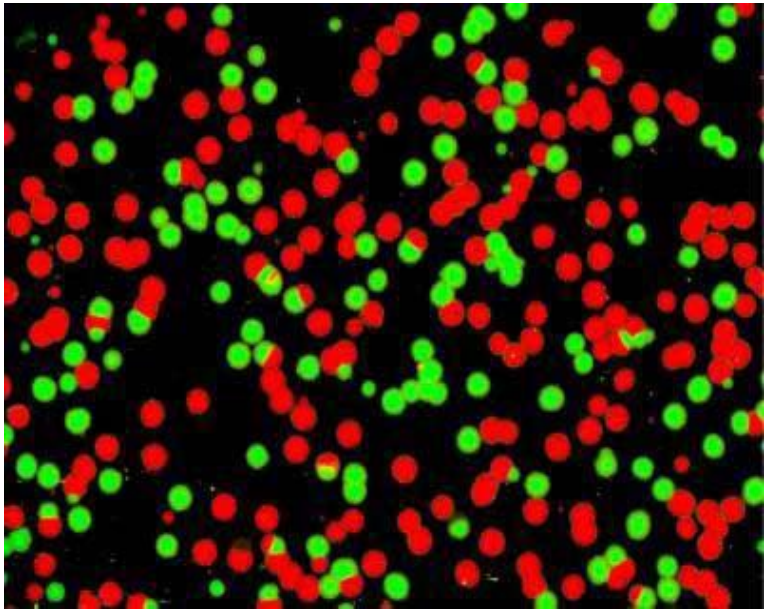
HAART – combination therapy

Price

Life-long treatment

(gene therapy a realistic option)

http://www.newscientist.com/article/dn10893.html

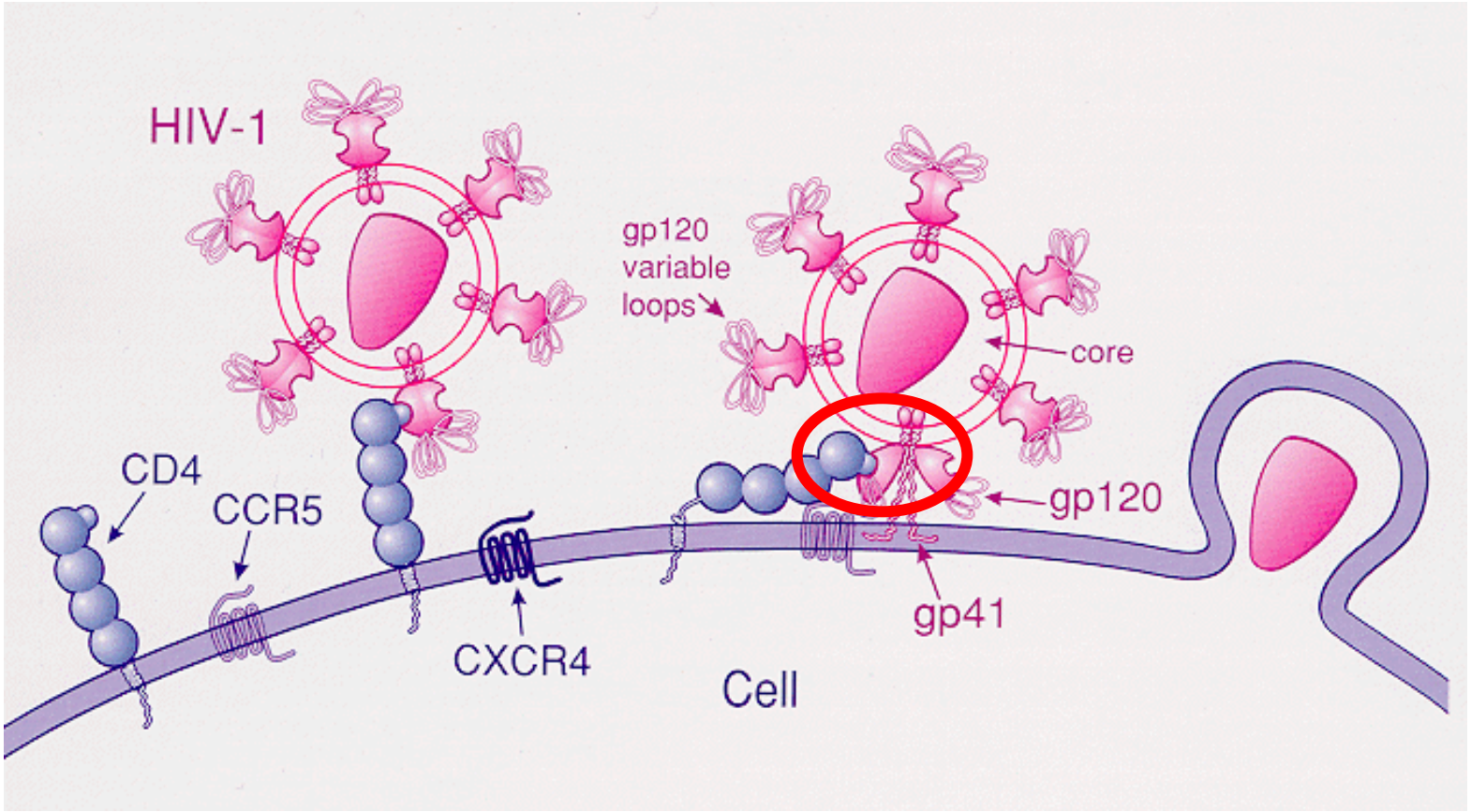


Drug sensitive (red) and drug resistant (green) HIV strains from a patient with AIDS.

Requirements for the effective synthetic antiviral device

- Should be **INSENSITIVE TO MUTATIONS** ⇨ **BASE ON VIRAL FUNCTIONS** !
- Should be **VERSATILE** - allow activation of different effectors
- **RESPONSE SHOULD BE AMPLIFIED** – triggerring of the defense by a minimal number of viral particles
- Should minimally disrupt the function of noninfected cells

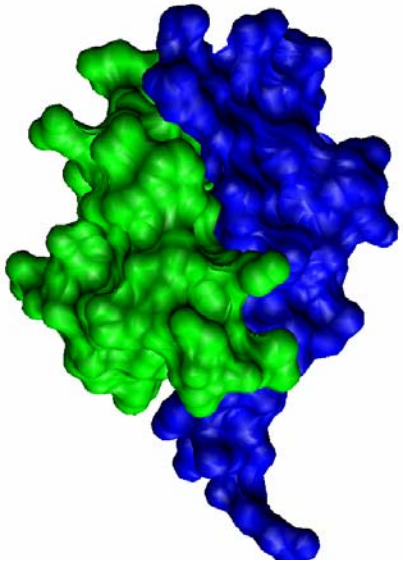
FUNCTION 1:
Viral attachment causes receptor heterodimers



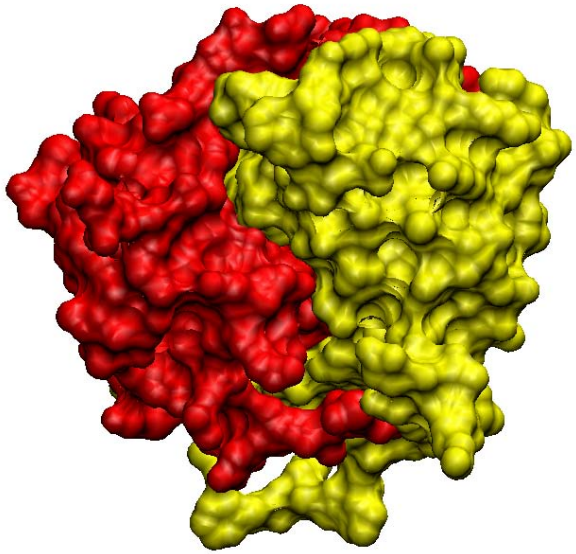
Heterodimerization of cellular transmembrane receptors at viral entry could be used to detect viral attack.

Detection of heterodimer formation based on reconstitution of split proteins

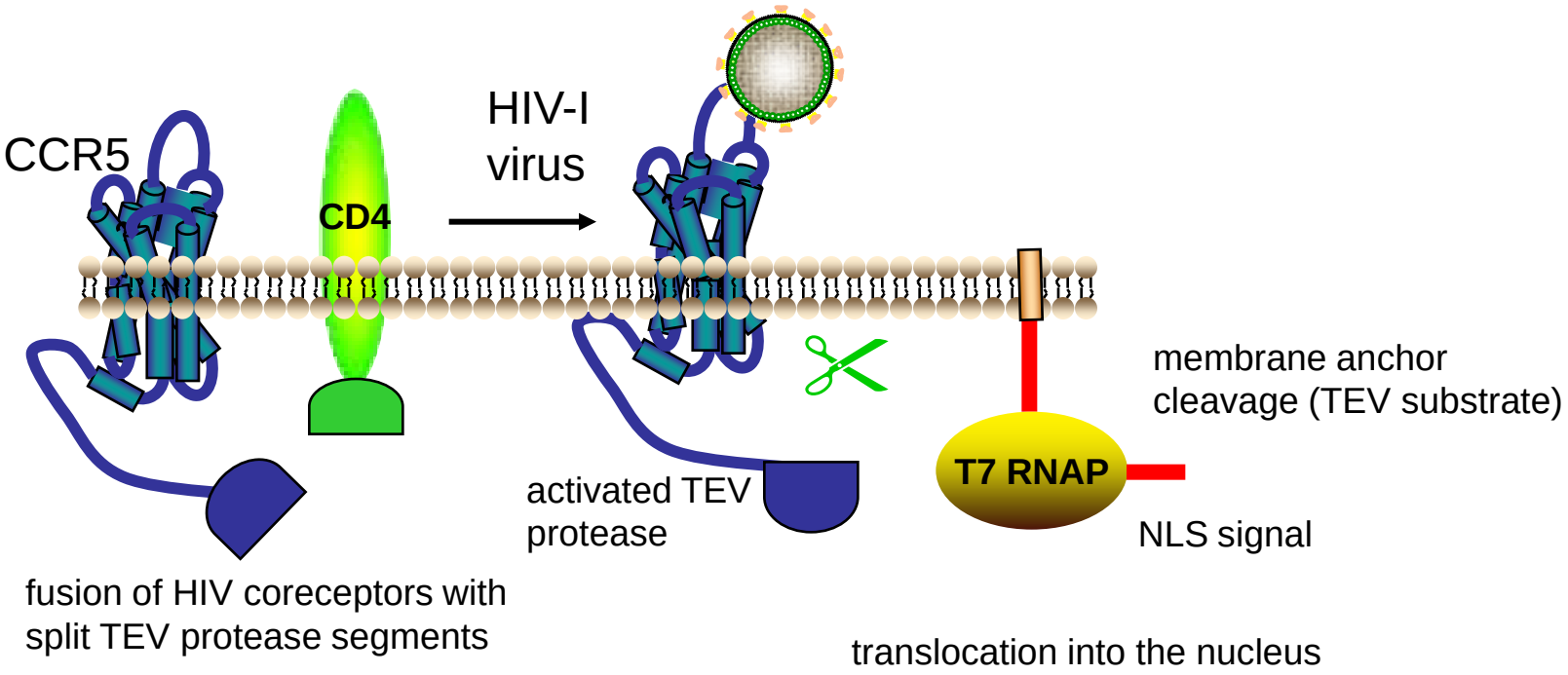
- Split ubiquitin system
  - cleavage C-terminal to ubiquitin with endogenous ubiquitin-specific protease



- Split tobacco etch virus (TEV) protease system
  - cleaves specific recognition site



Split TEV protease - based viral detector



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

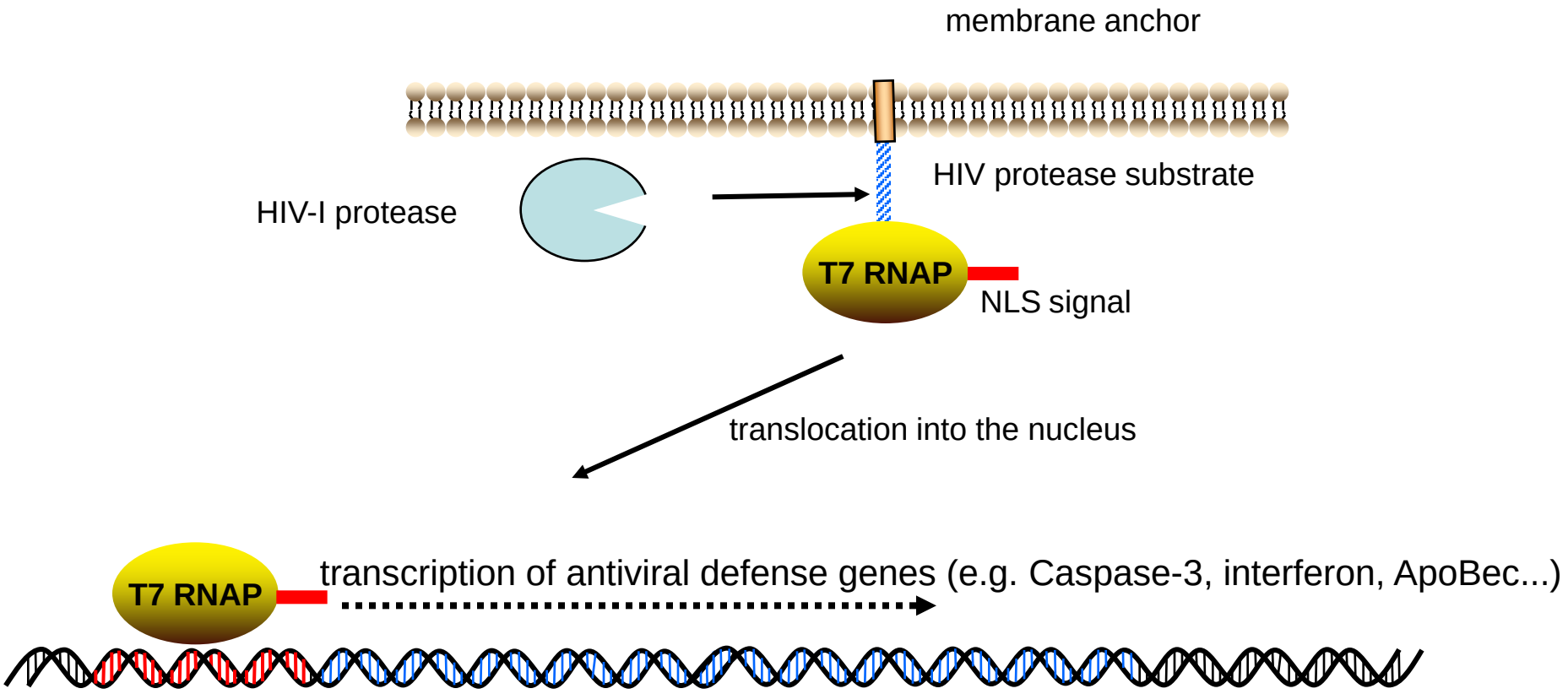
FUNCTION 2: HIV protease activity

- **HIV protease has a specific recognition site:**

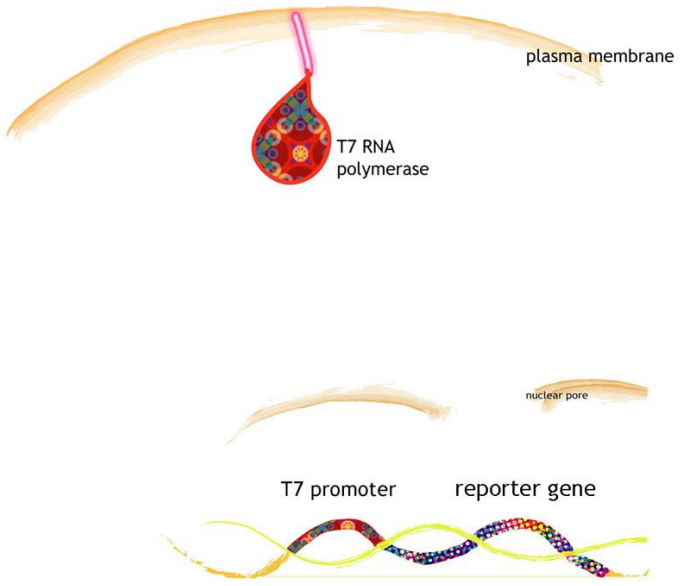
SQNY↓PIVQ

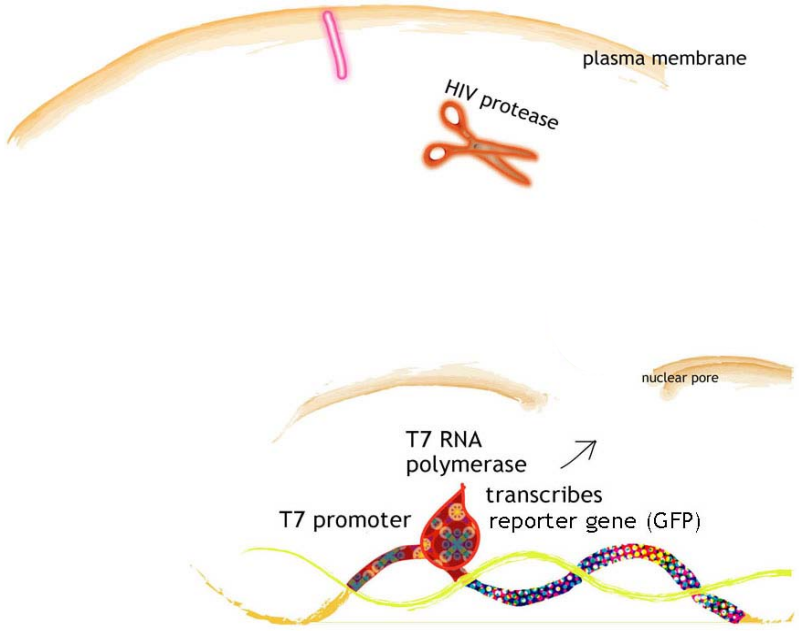
- **HIV protease activity can detect the late stage of HIV infection.**

Viral detection based on HIV protease activity



Localization-based switch of T7 RNA polymerase activation





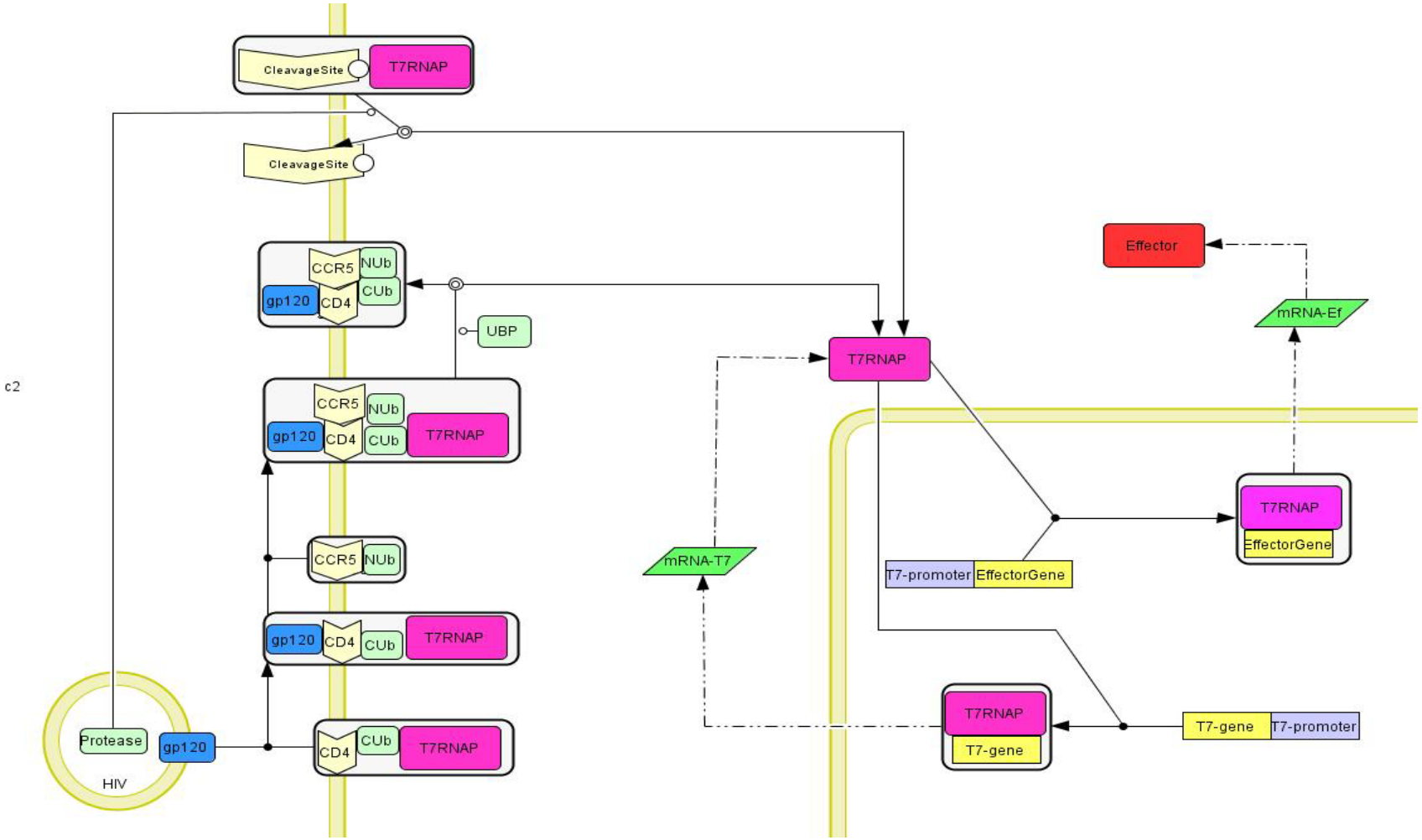
Inactive state:

T7 RNA polymerase is anchored to the membrane and is separated from DNA

Active state:

T7 RNA polymerase is released from the membrane and directed to the nucleus (NLS) – transcription of antiviral effectors

Model of the anti-HIV defense device



Experimental results

Implementation of two types of devices each based on a different viral function:

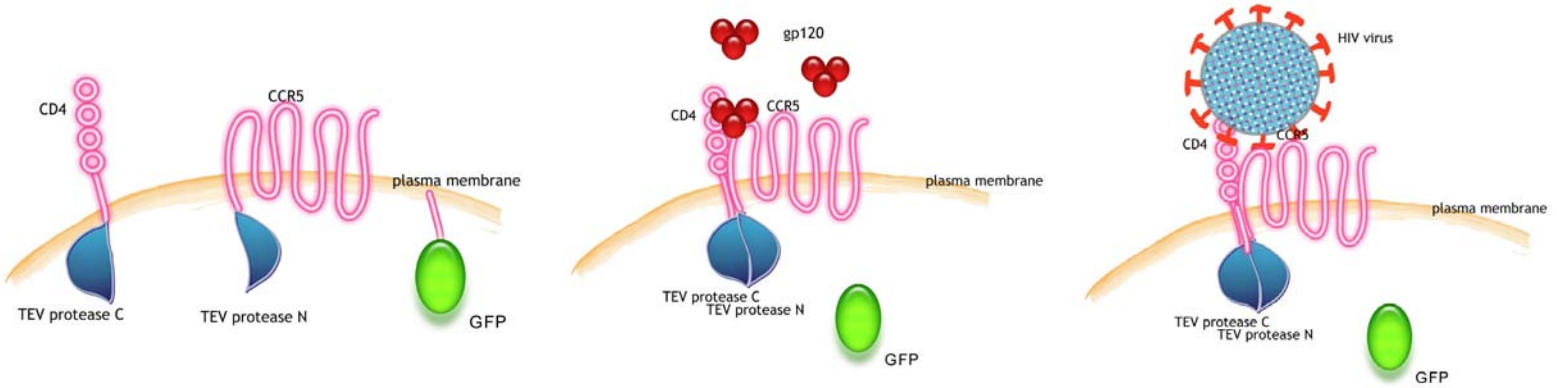
- **Viral attachment**

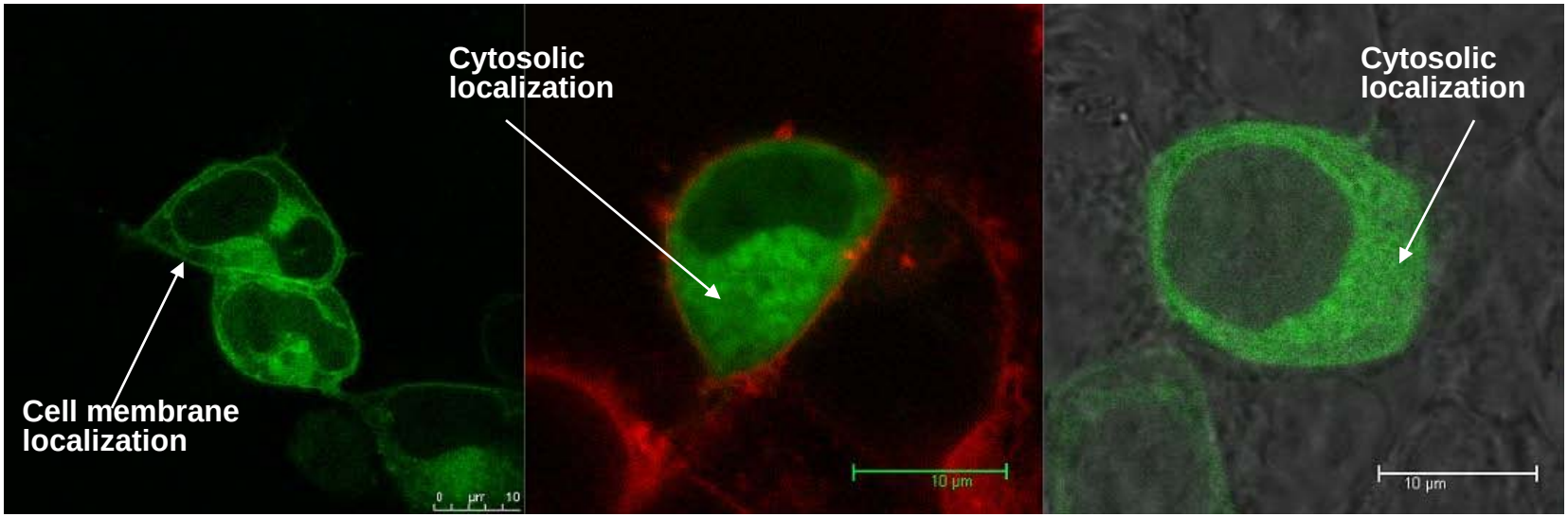
  ⇨ receptor heterodimerization

  ⇨ reconstitution of split protein (split ubiquitin and split TEV protease system)

- **HIV protease activity**

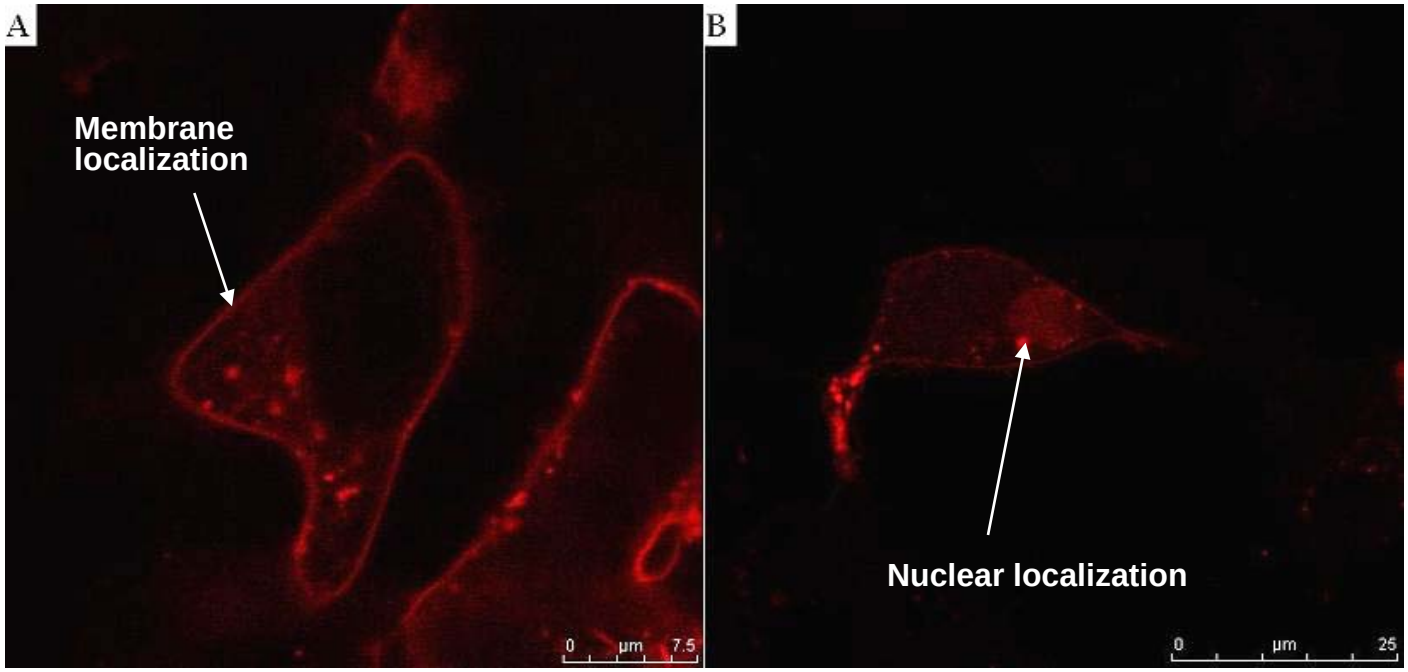
HIV causes release of the GFP reporter from the membrane





Control **+ gp120** **+ Pseudovirus**

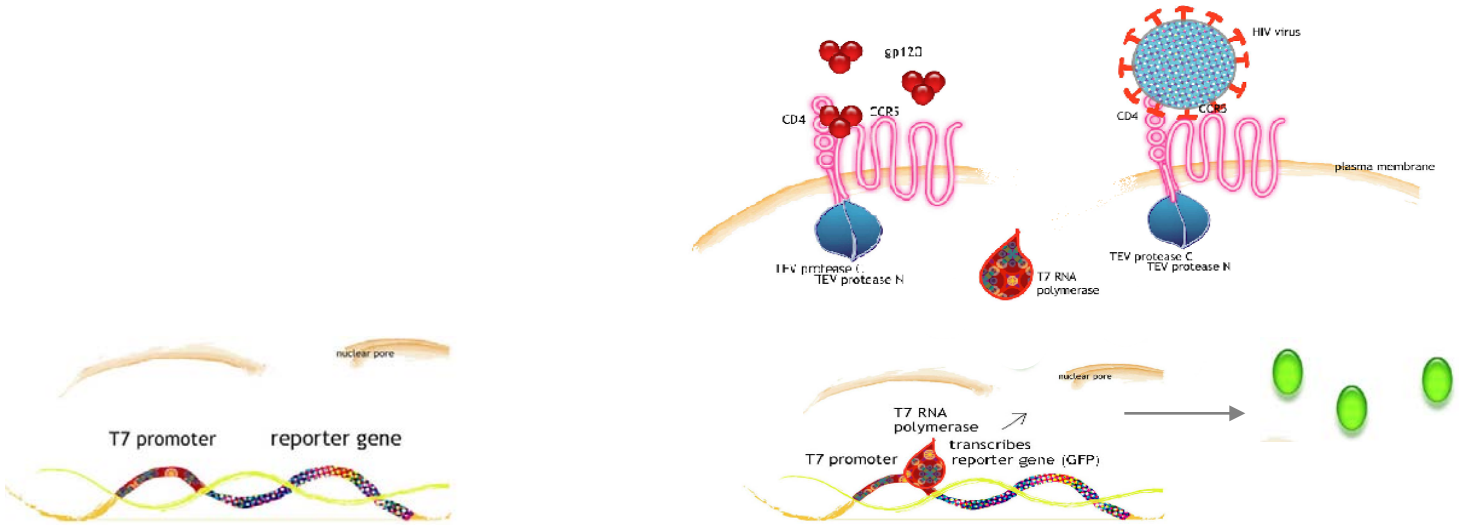
HIV protease causes translocation of fluorescent reporter into the nucleus

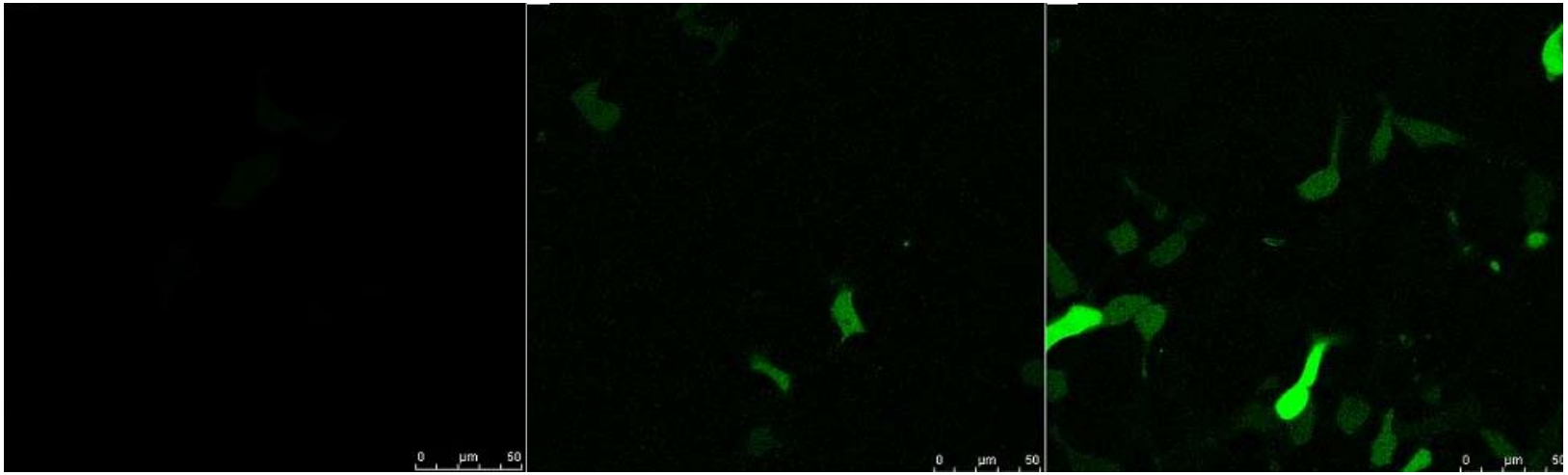


control

+ HIV protease

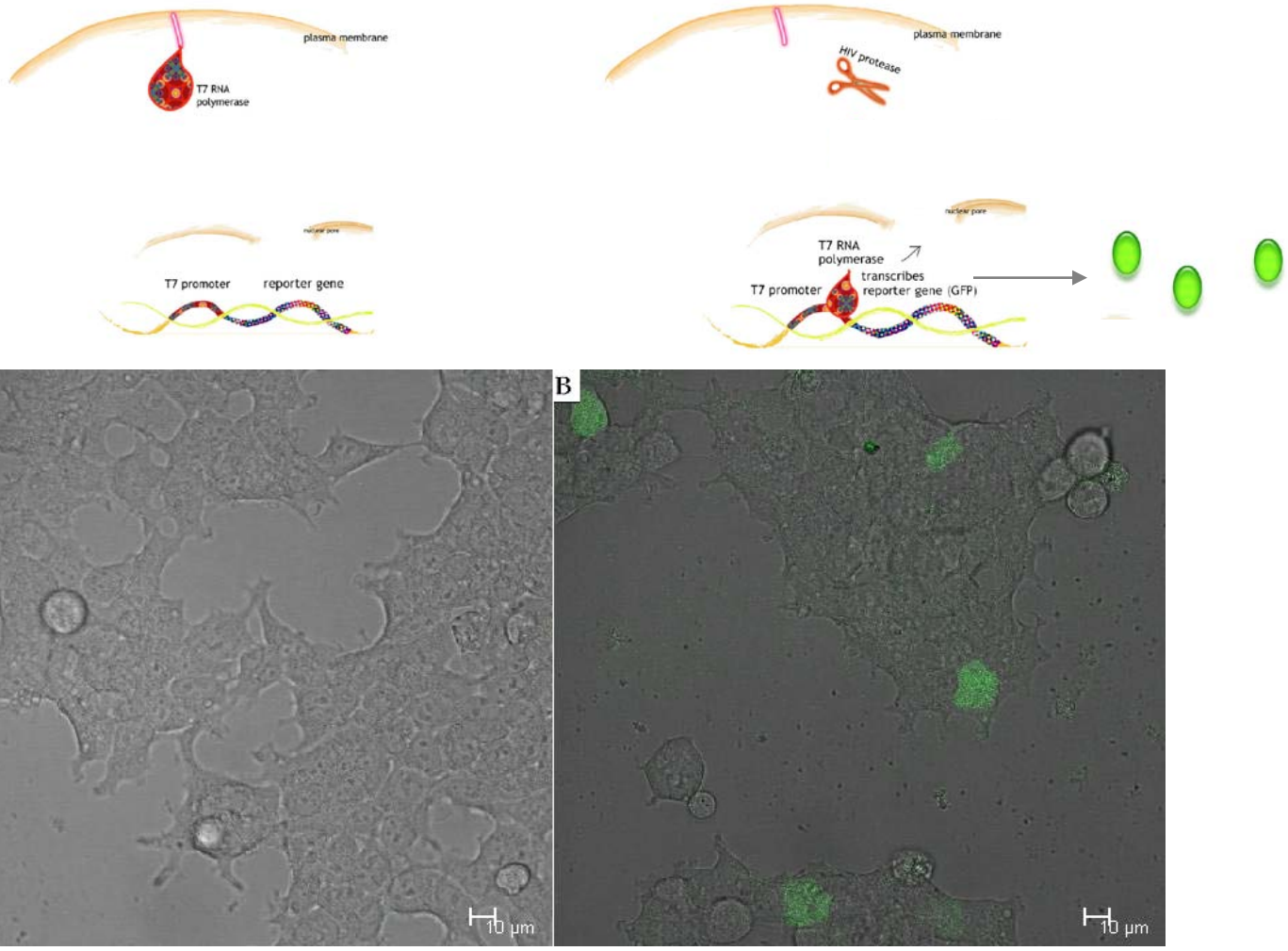
Integration of two steps: Split TEV-T7-based cell activation





Control **+ gp120** **+ Pseudovirus**

HIV protease - based cell activation



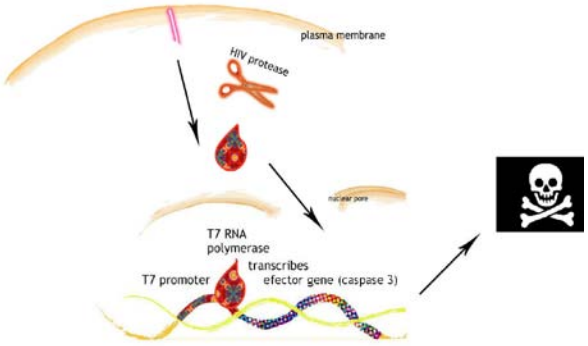
Control **+ HIV protease**

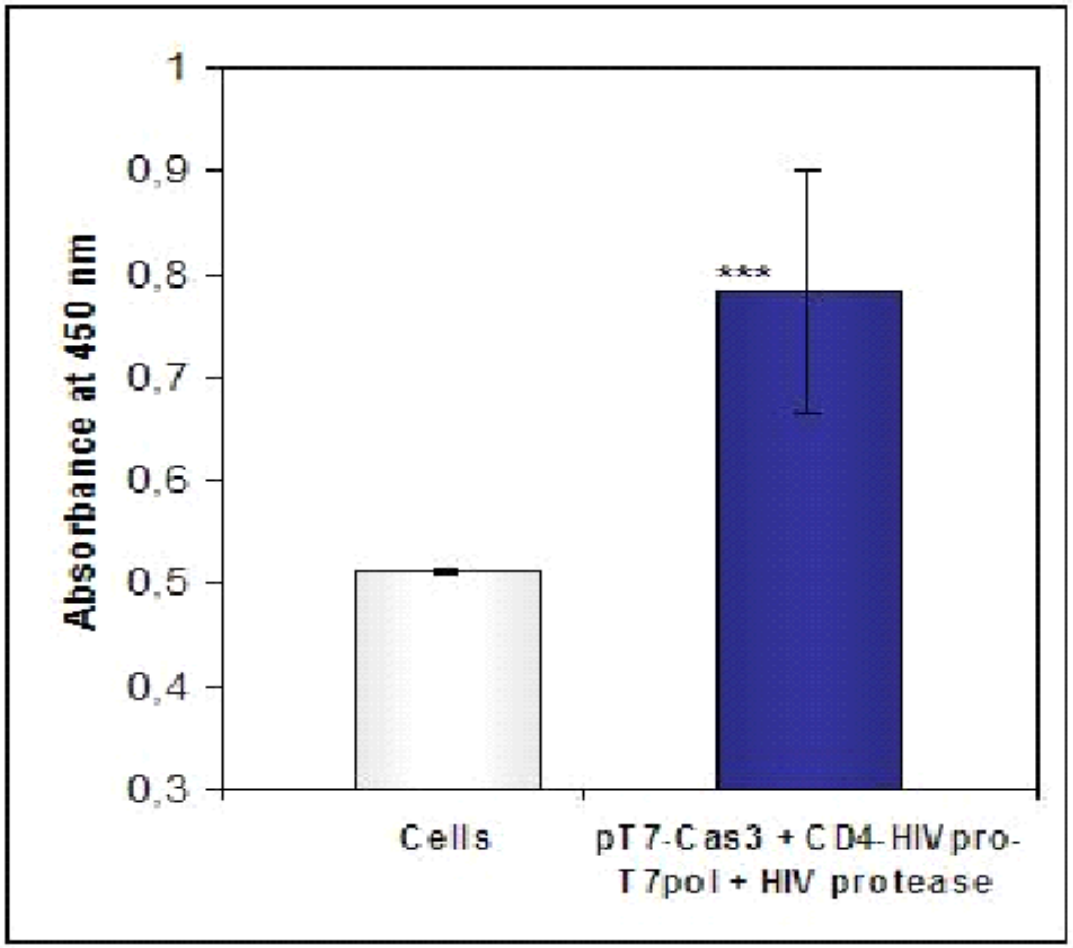
Effectors for antiviral defense

-Kill infected cells (e.g. activate apoptosis)

-Provide protection to other cells (e.g. IFNα, inhibitors of viral fusion...)

Apoptotic caspase-3 expression triggered by HIV protease

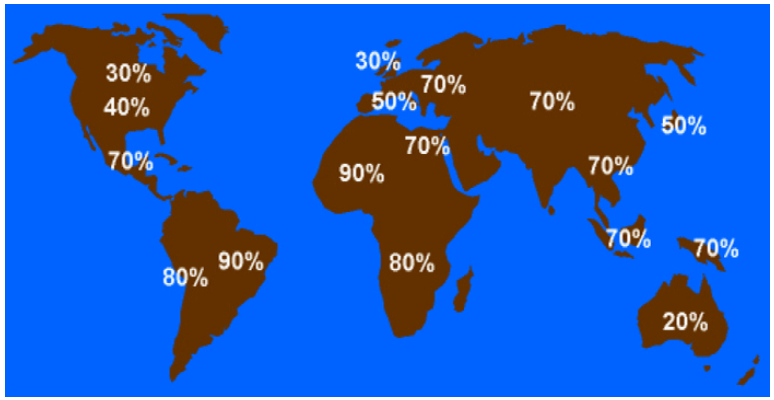


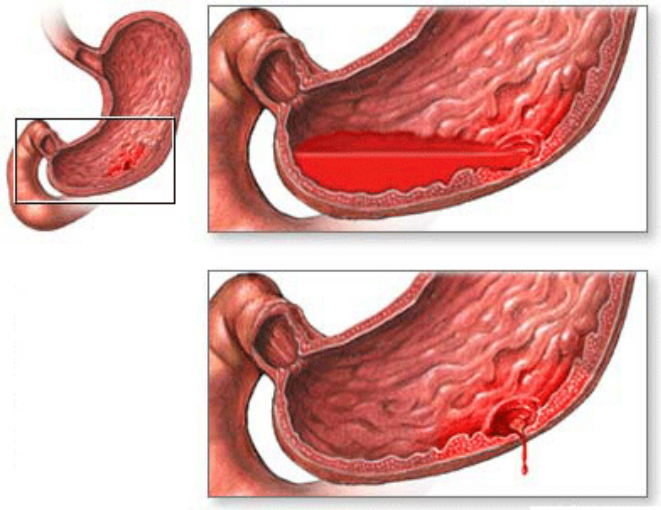


Similar **“function-based mutation-independent”** principle could be used:

- utilizing other HIV–specific processes (e.g. integration, reverse transcription..)
- against other viruses with specific protease or cellular receptors (e.g. HCV, SARS, WNV...).

Helicobacter pylori



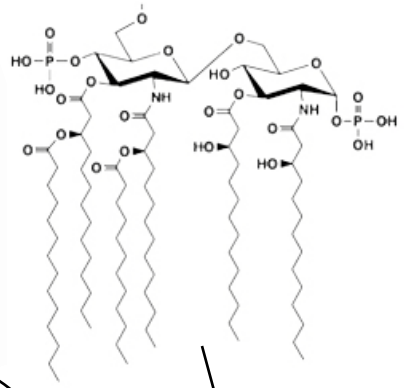


- About 50 % of world population infected
- Main cause of peptic ulcers and gastric cancer
- 7 million get sick every year
- 8th leading cause of death by 2010
- Antibiotic treatment is effective, BUT:
  - costs
  - antibiotic resistance
  - reinfection

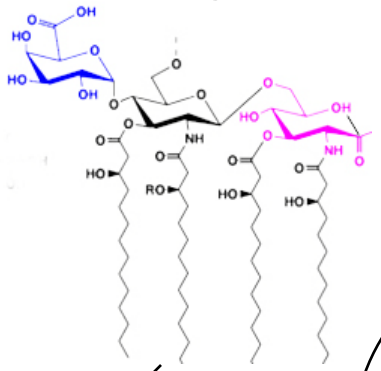
Master of disguise

E. coli

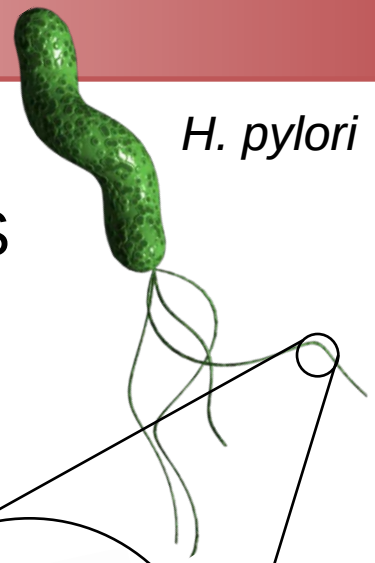
Hexaacylated LPS

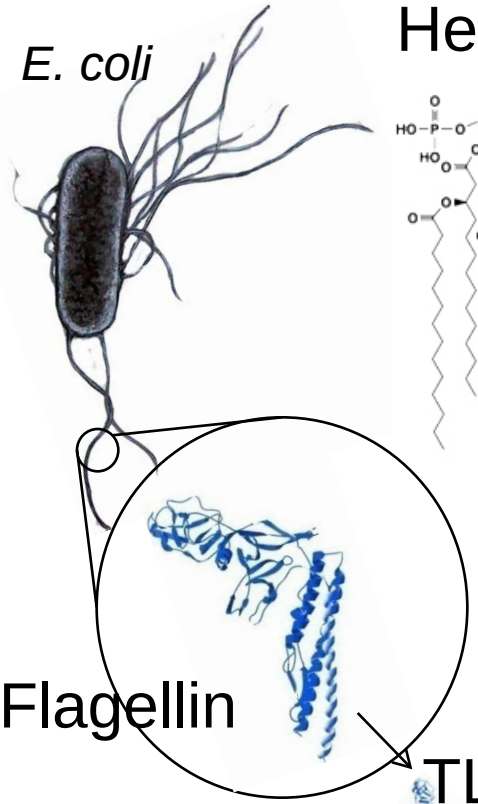


Tetraacylated LPS



H. pylori

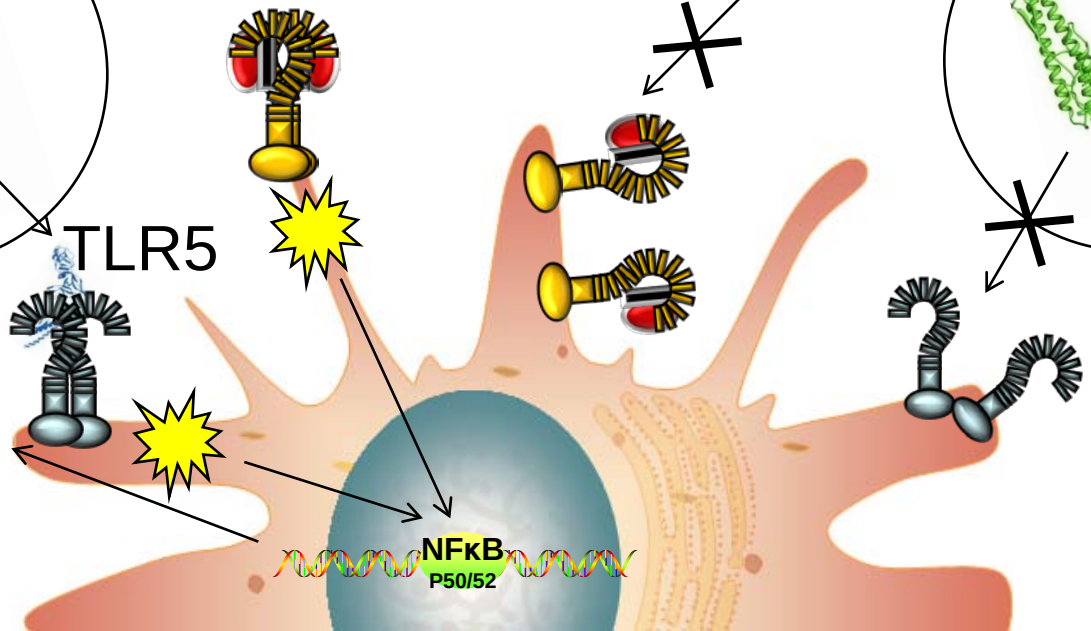


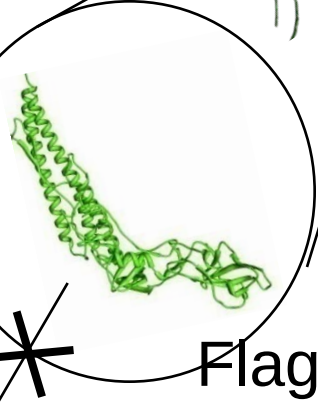


Flagellin

TLR4

TLR5





Flagellin

Cell activation:
- chemokines
- cytokines
- interferon...

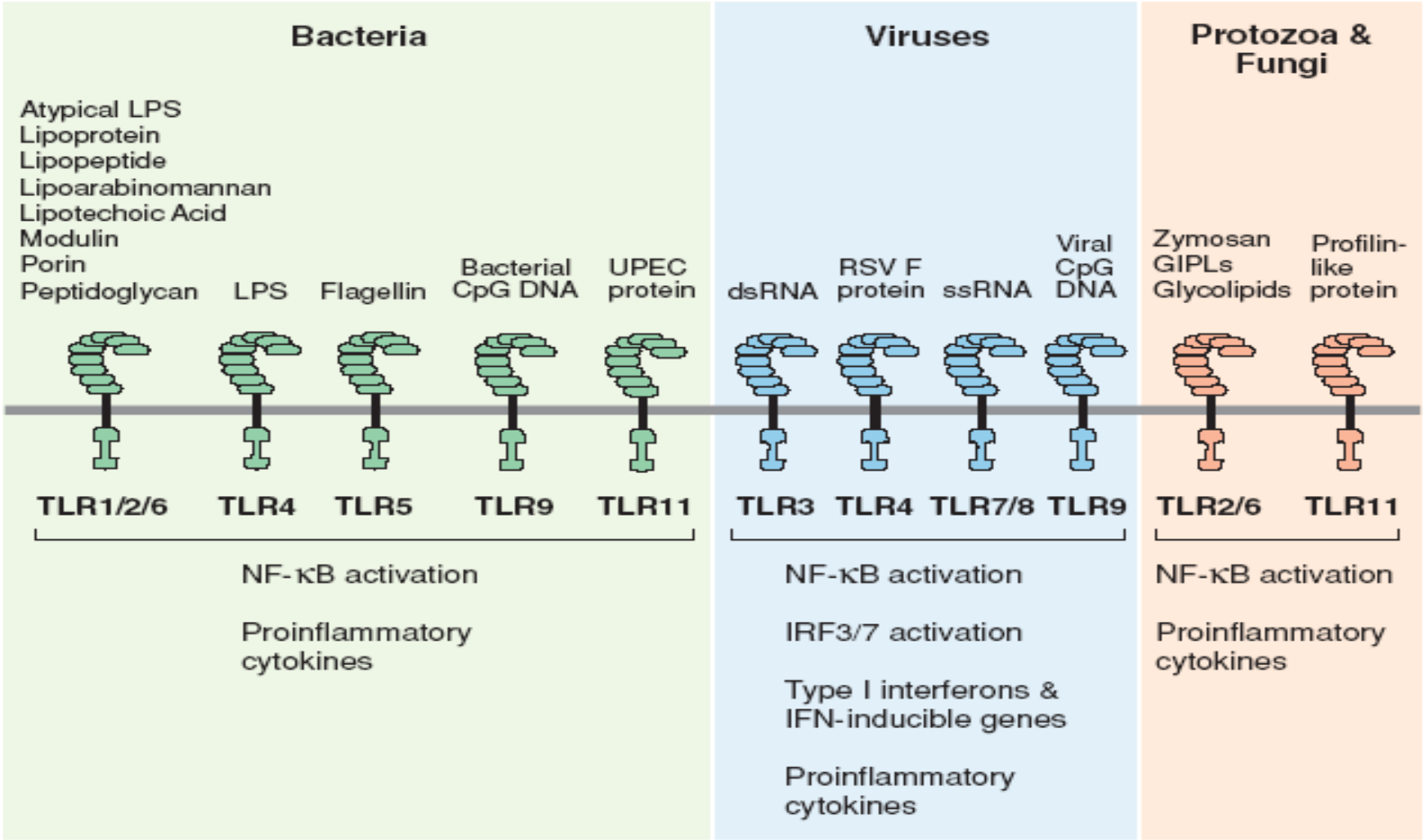
TLR5 does not bind *H. pylori* flagellin

TLR receptors sense the presence of bacteria

- main sensors of innate immunity response

- Toll-like receptor molecules:
- a family of membrane receptors
- recognize different molecules distinctive for patogens

TLRs and their agonists

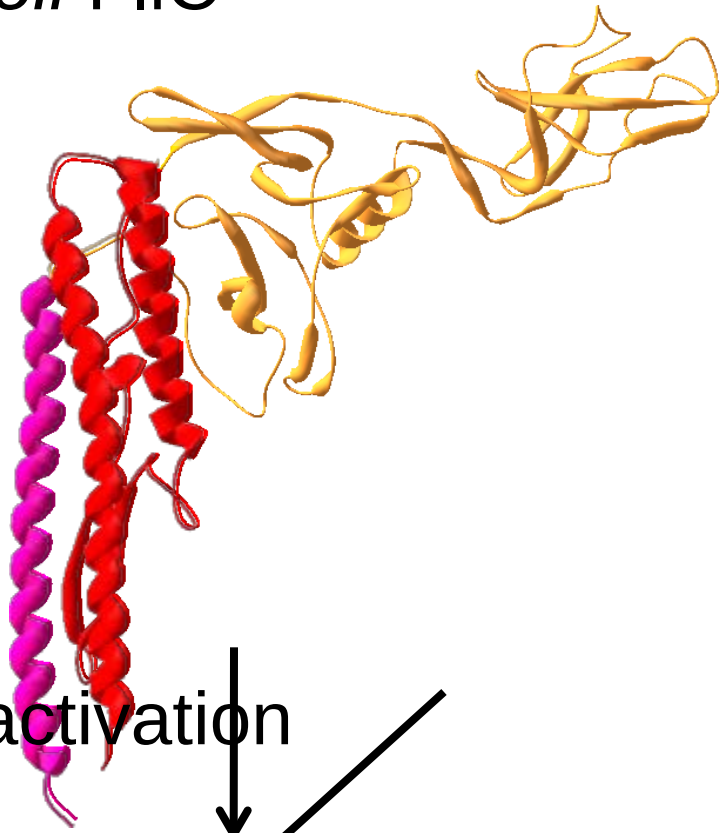


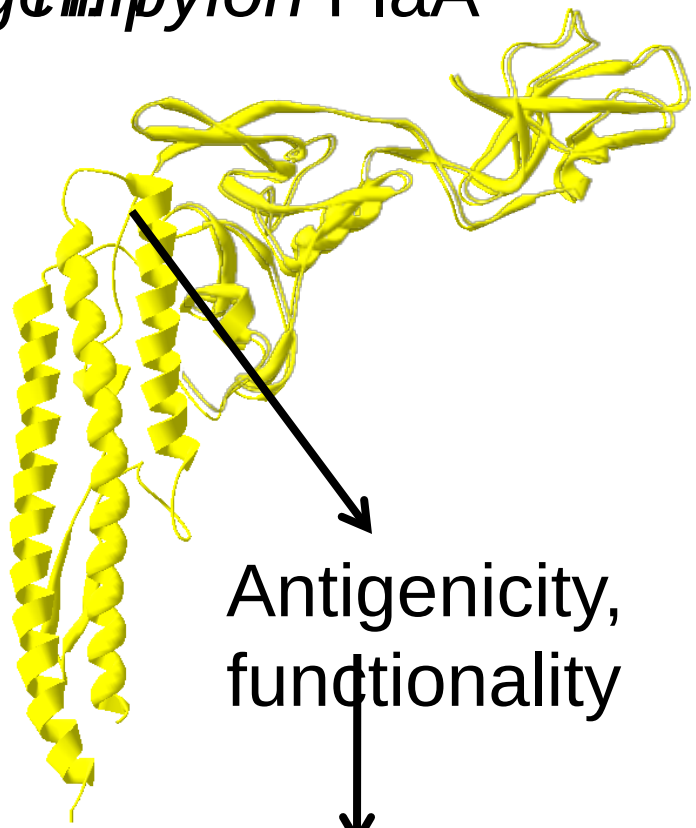
Chimeric flagellin

E. coli FliC

Chimeric flagellin

H. pylori FlaA





TLR5 activation

Antigenicity, functionality

No TLR5 activation

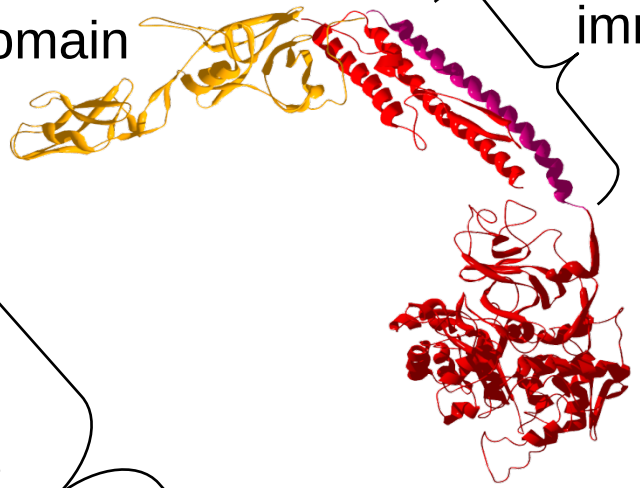
Chimeric flagellin

Innate response

Adaptive response against *H. pylori*

H. pylori variable flagellin domain

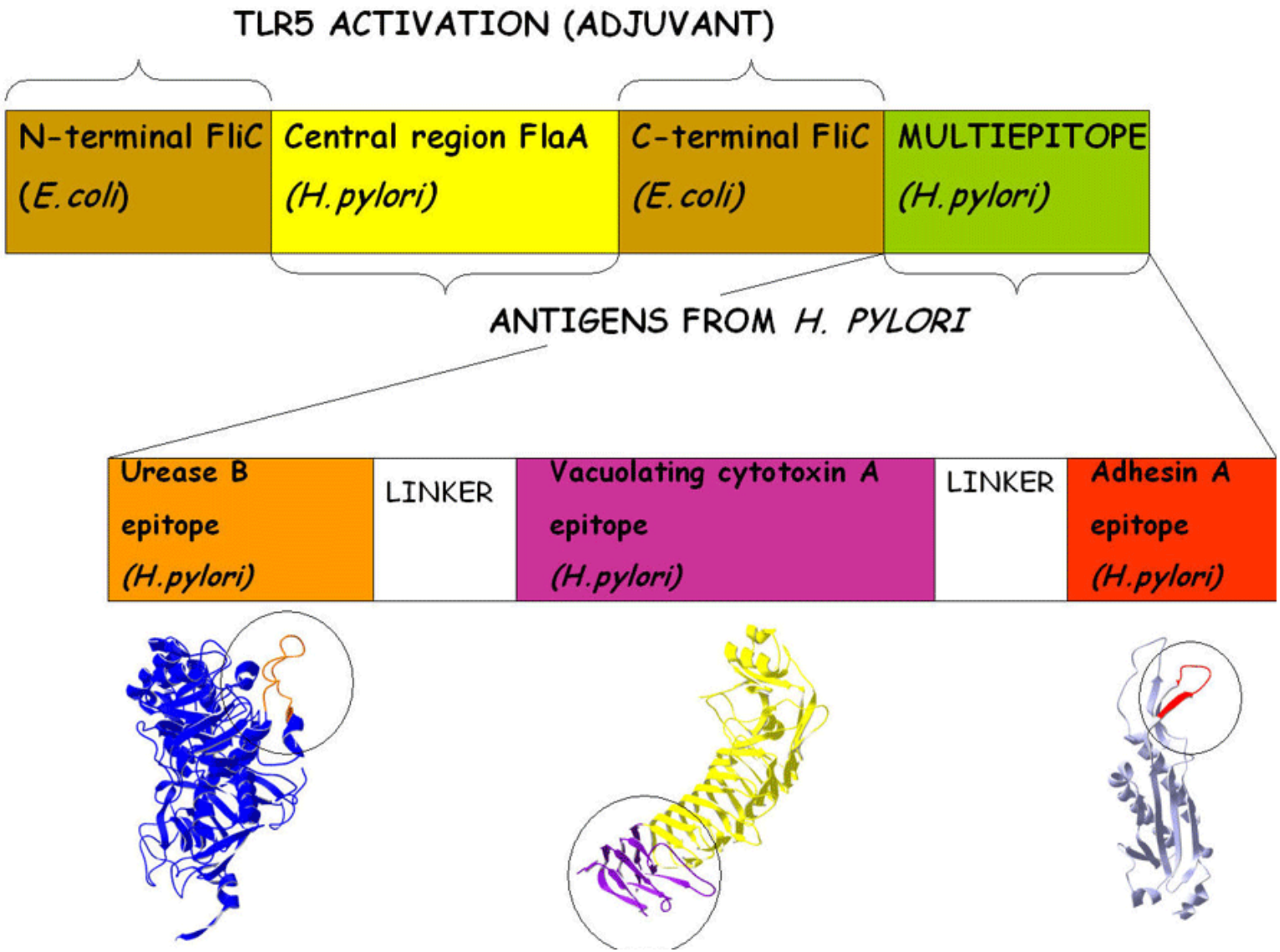
activation of innate immunity (TLR5)



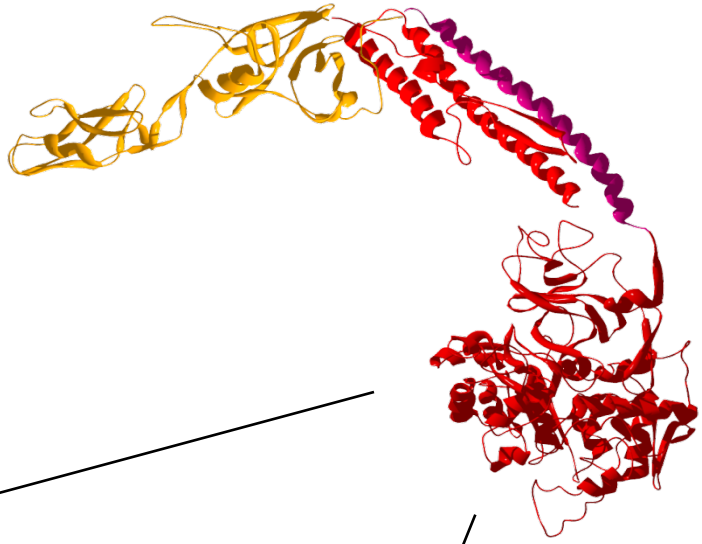
protein antigens (activation of adaptive immunity)

H. pylori antigen (e.g. UreB)

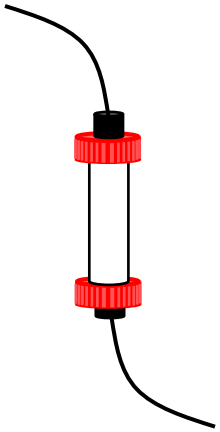
Multiepitope



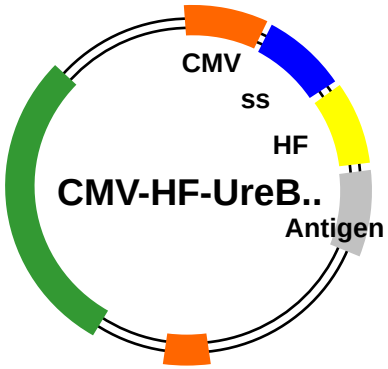
Implementation



protein vaccine



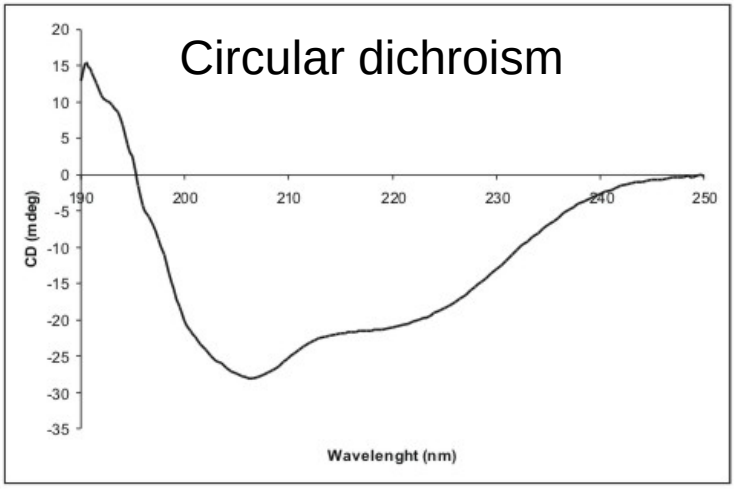
DNA vaccine



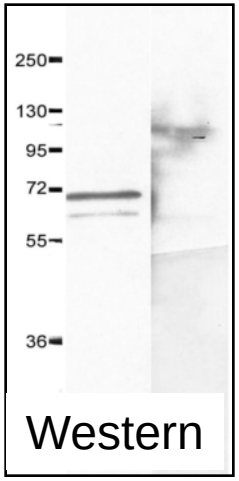
engineered bacteria

Implementation 1
Protein vaccine

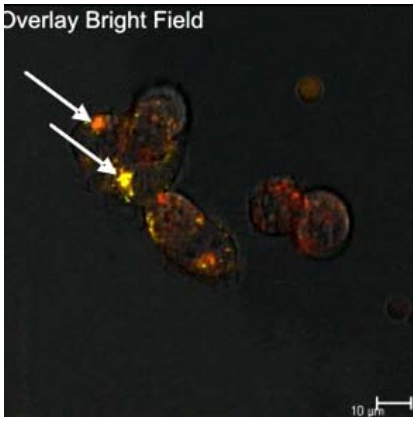
Track 1a

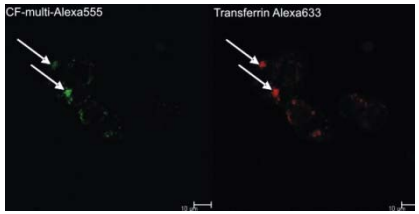


Isolated chimeric flagellin is correctly folded.



Isolated recombinant chimeric flagellin activates cells through TLR5.

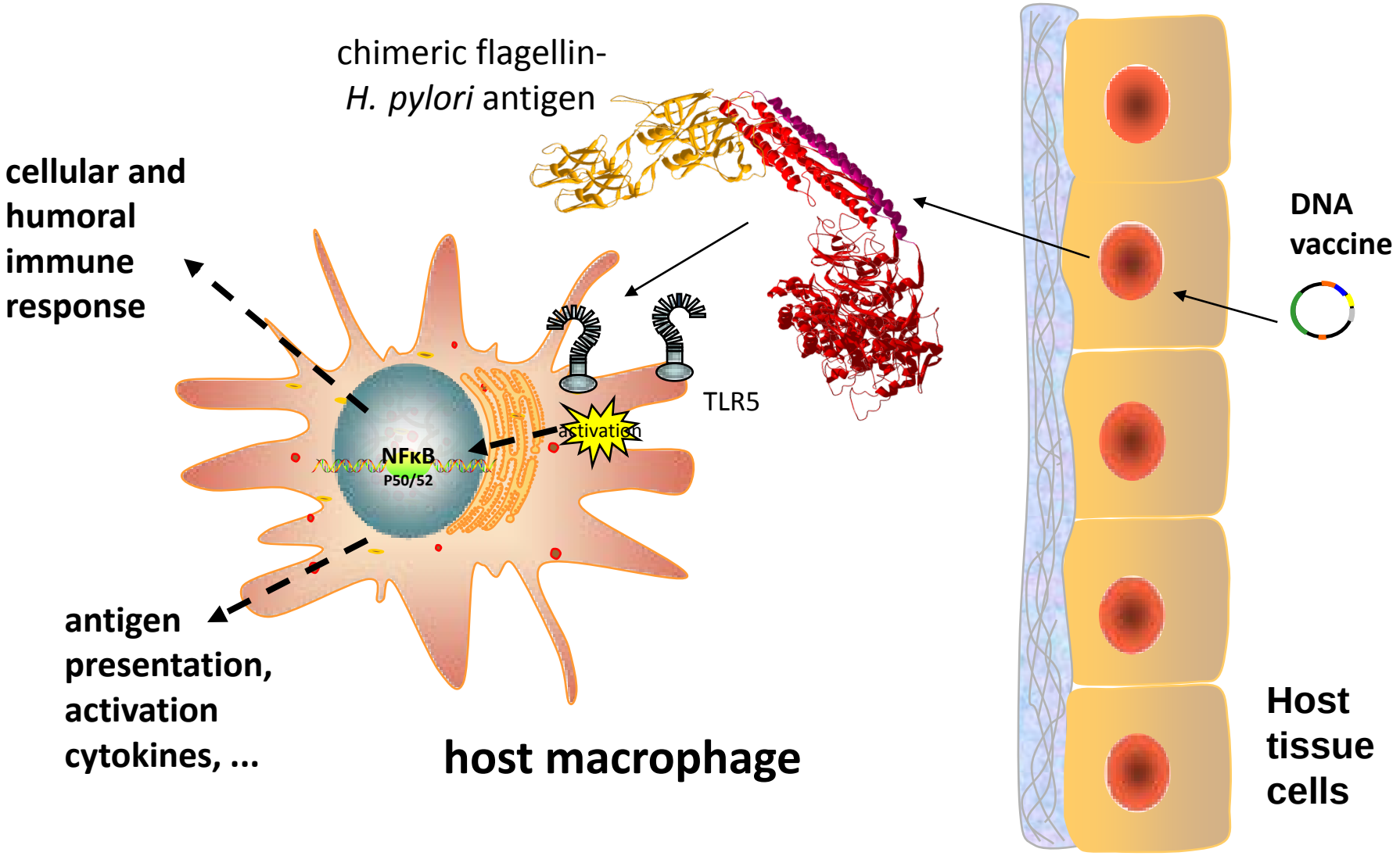




Cells internalize fluorescently labeled vaccine

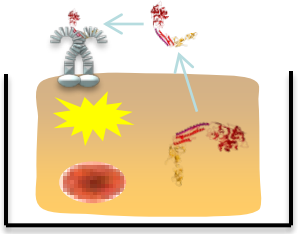
Implementation 2
DNA vaccine

Track 1b

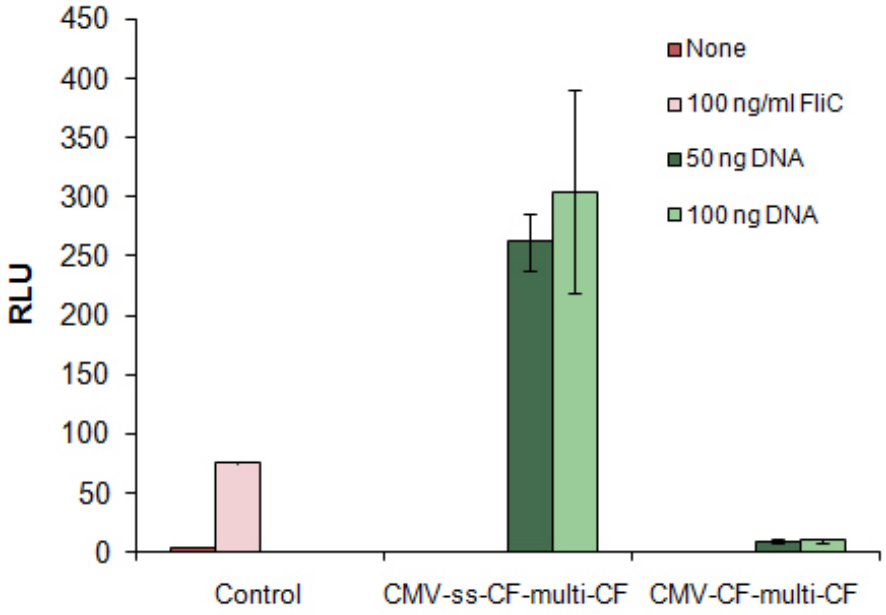


Implementation 2
DNA vaccine

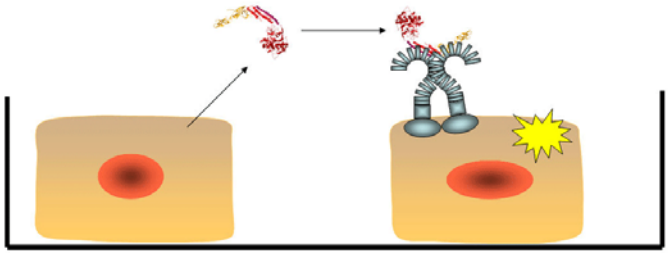
Track 1b

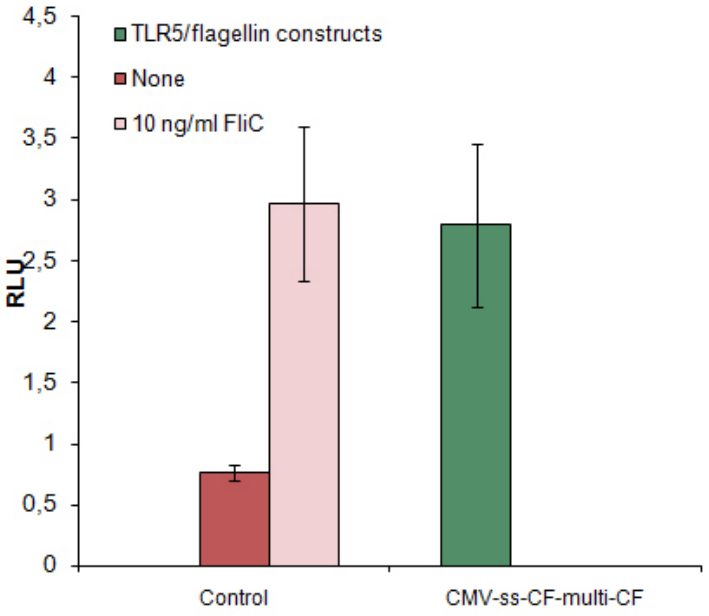


Cell, cotransfected with TLR5 and DNA vaccine



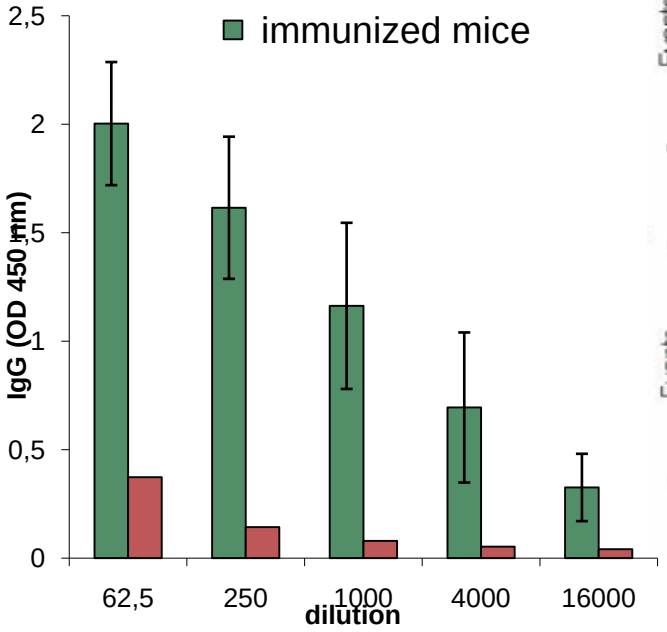
Chimeric flagellin needs to be secreted in order to activate cells with TLR5.



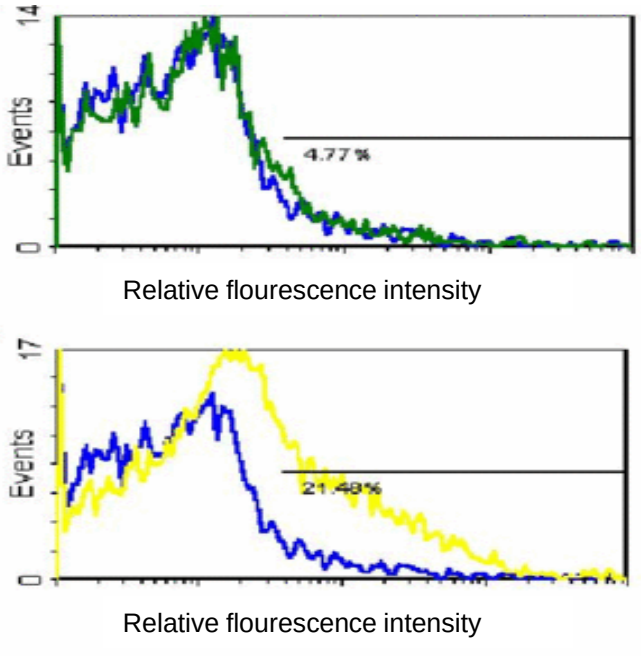


Transactivation of TLR5 by secreted chimeric flagellin.

in vivo efficiency of the vaccine



Strong immunoreactivity in the sera of immunized mice (CF-MULTI).

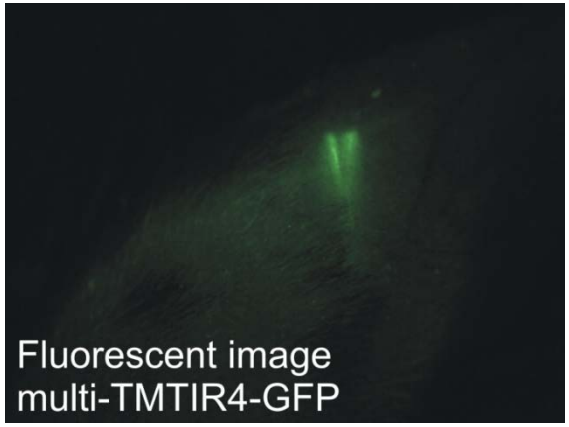


Immune serum against designed epitope (MULTI) recognizes live *H. pylori*

neg. control

Secondary Ab to *H. pylori*

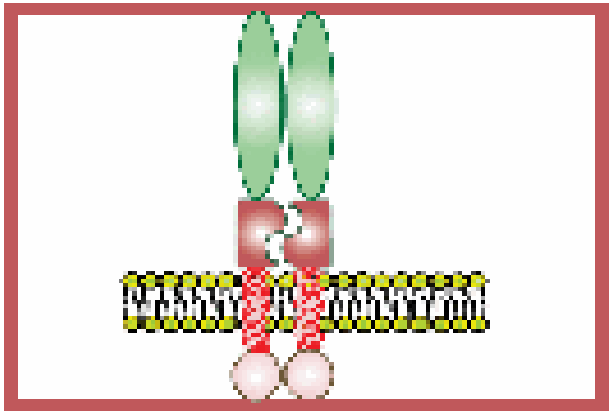
CF-sera to *H. pylori*



Electroporated DNA vaccine is expressed in mouse leg

Colocalization of antigen and TLR activation

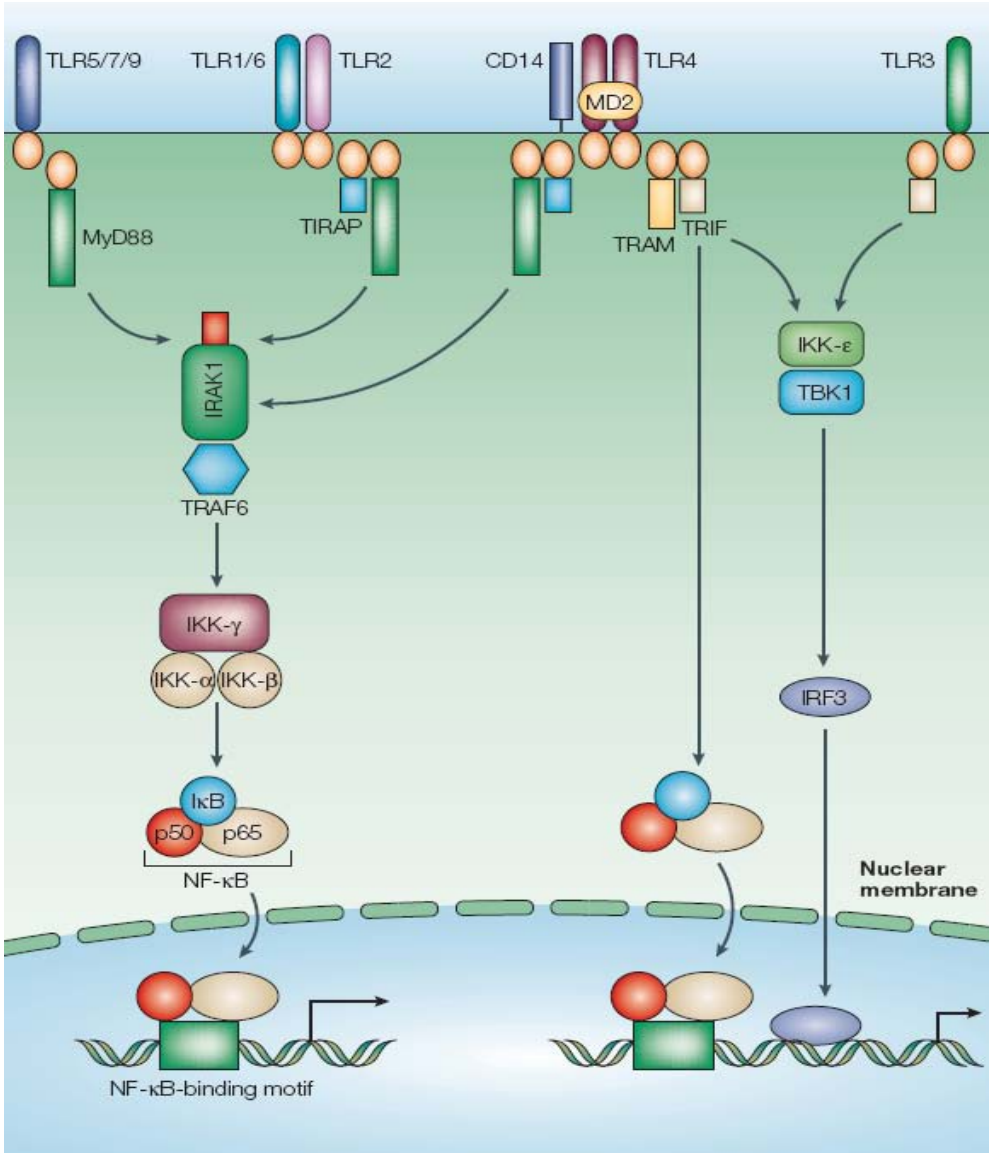
- Activate TLRs by dimerization, colocalization with antigen



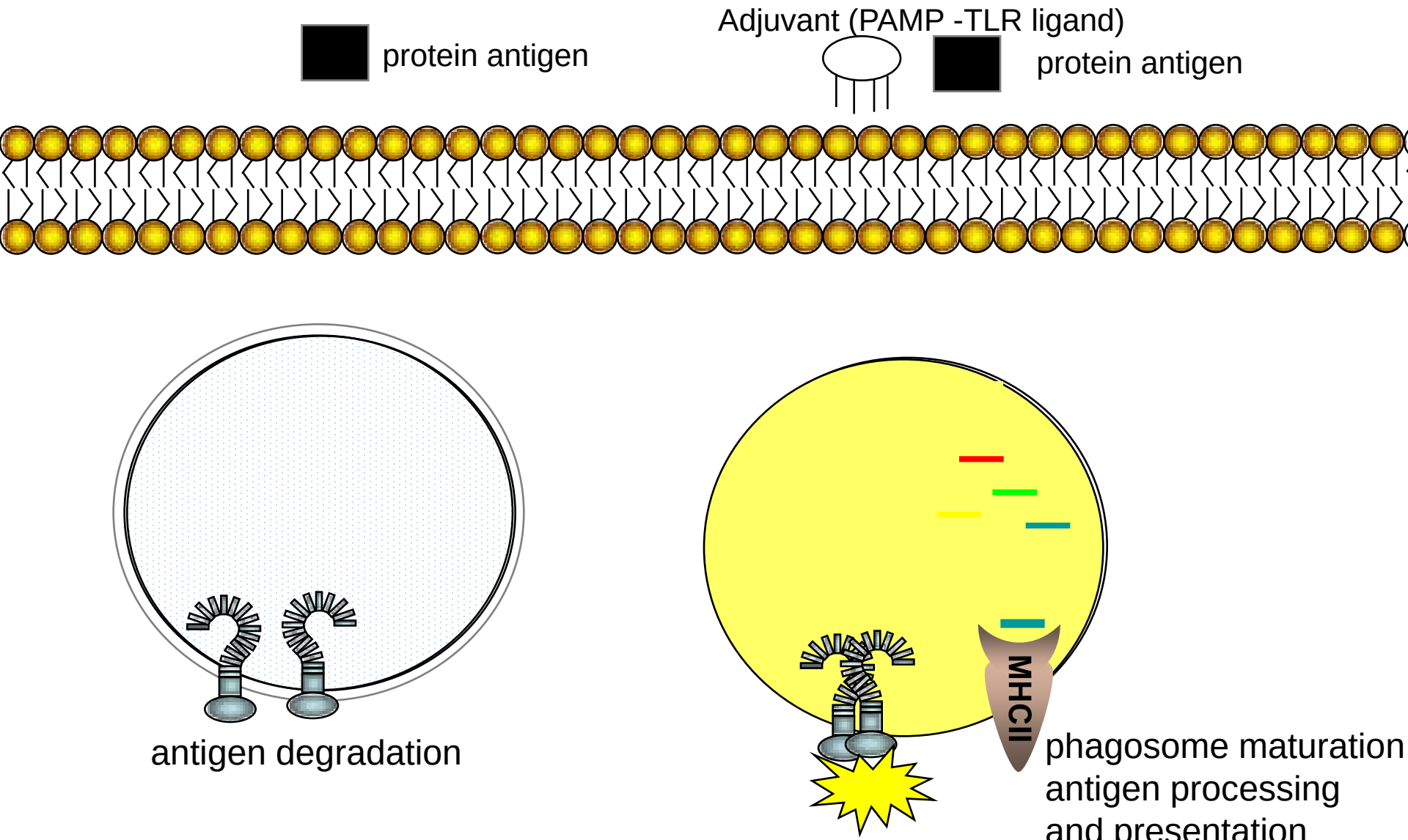
Track 2

TLR signaling

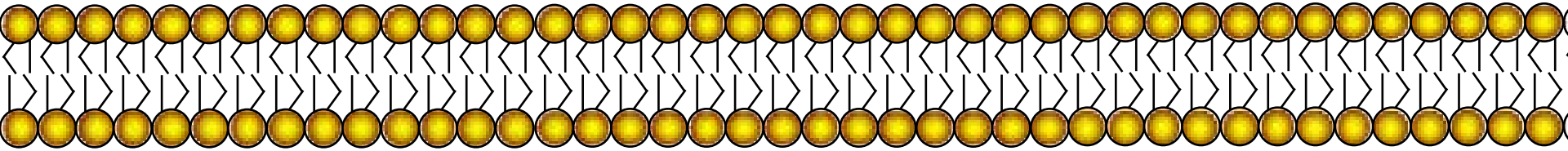
Track 2



TLR signaling activates antigen processing

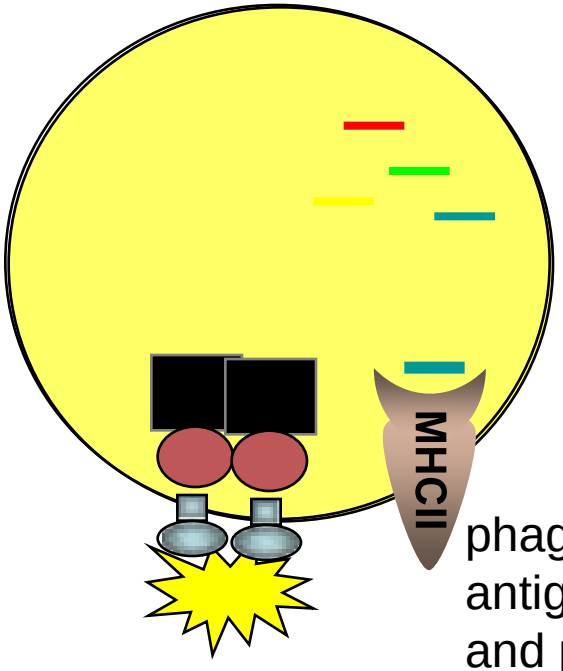


Constitutively active antigen-TLR fusion



Antigen and TLR signal are colocalized

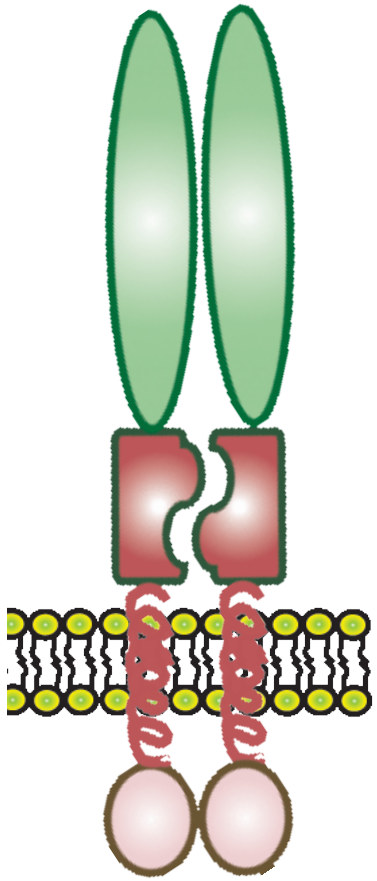
No additional adjuvant is required



phagosome maturation
antigen processing
and presentation

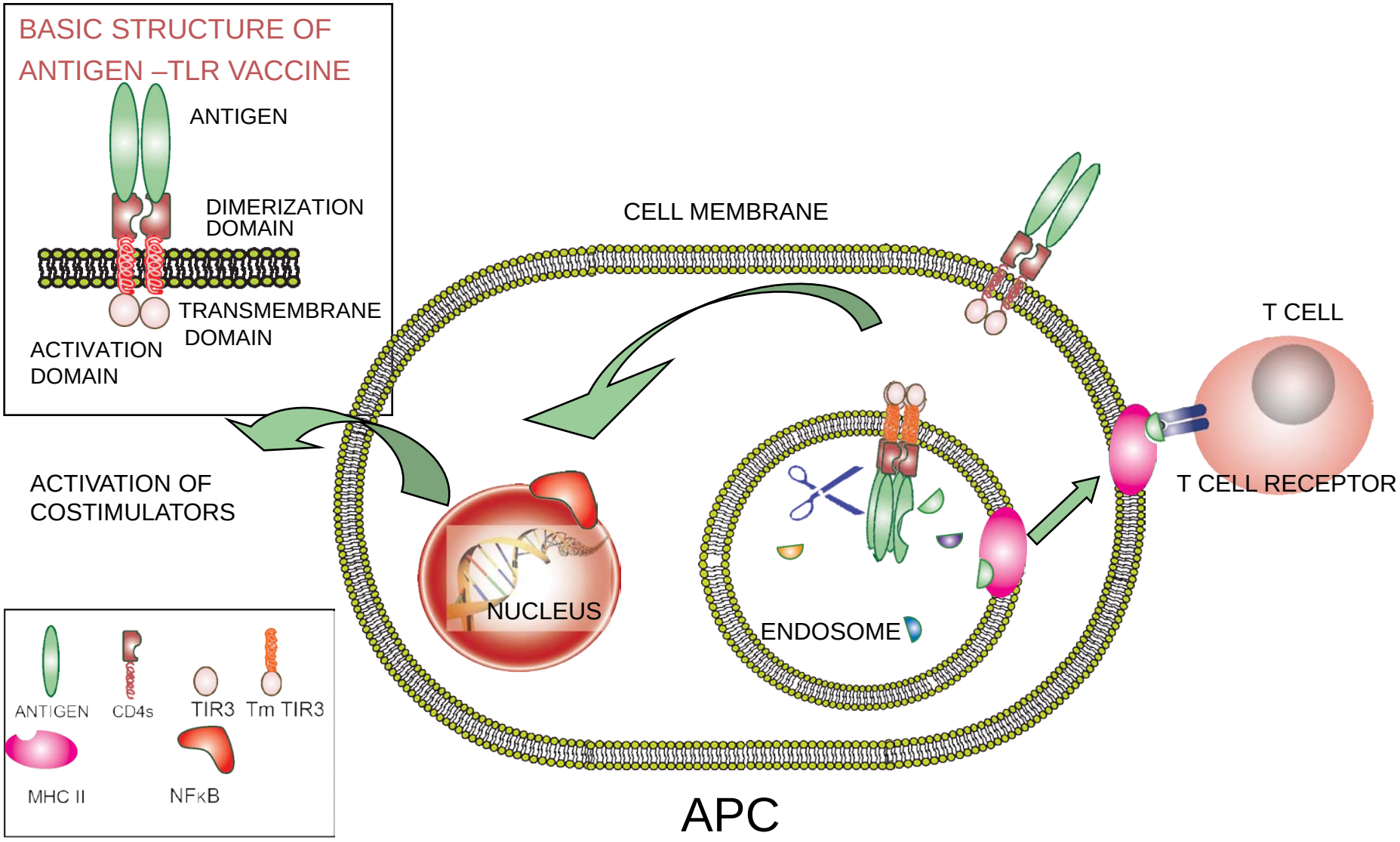
Modular composition of vaccine in track 2

- Antigens → Urease B Multiepitope
- Dimerization domain → CD4e
- TM domain → CD4sh TLR3 TM
- Active domain → TIR3 TIR4
- Peptide tags → His_6 tag HA tag

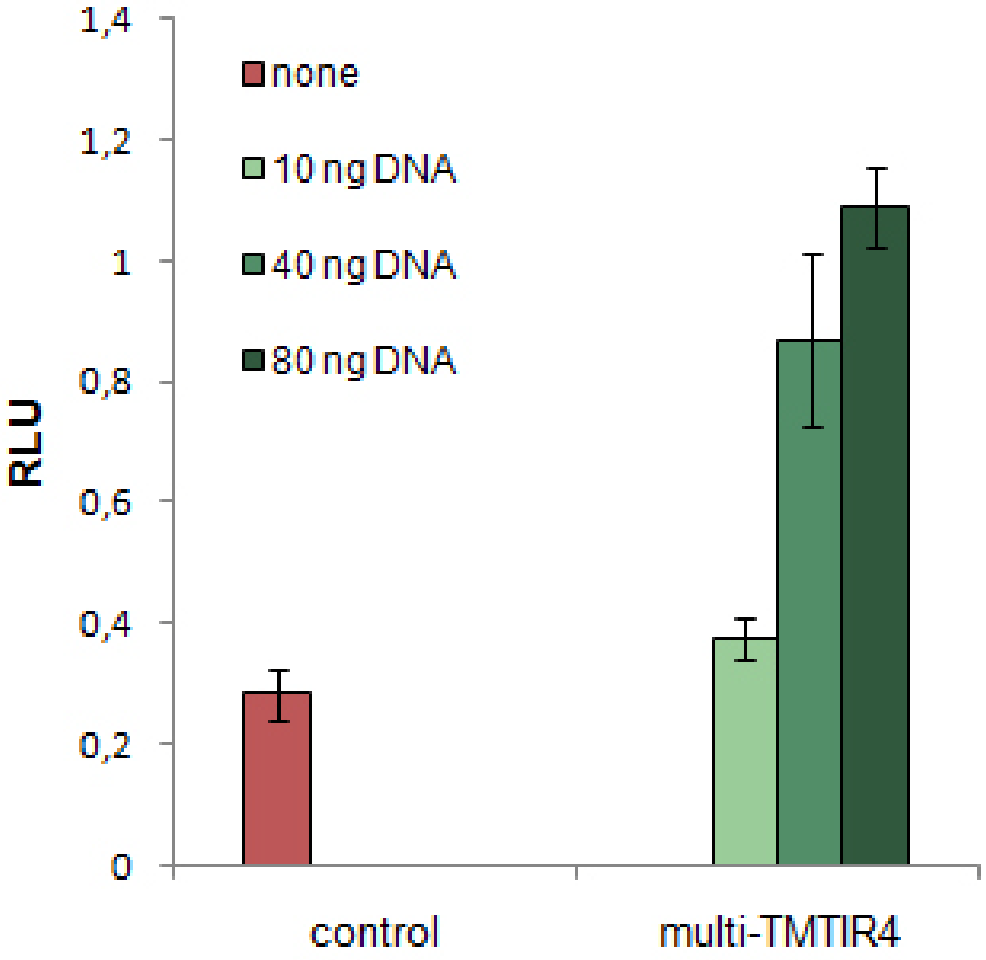


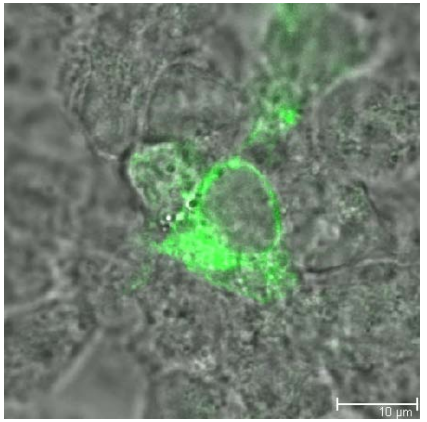
The function of TLR-fusion vaccine

Track 2



TLR4 activation

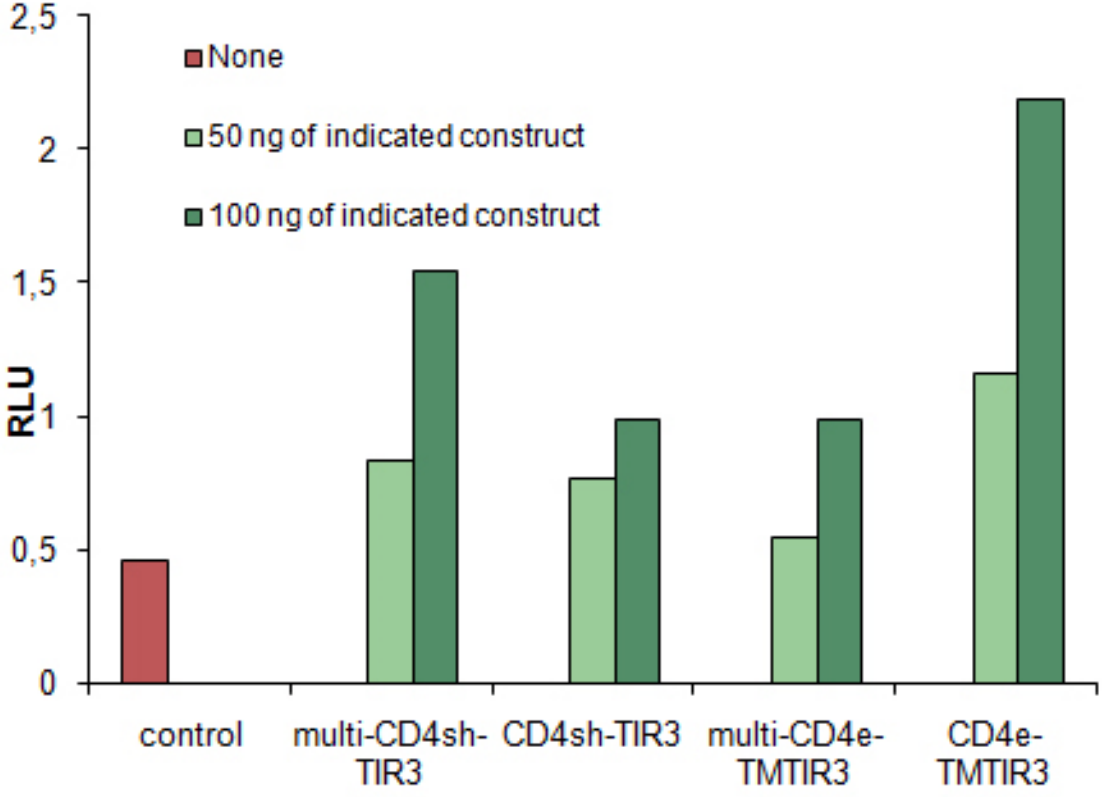


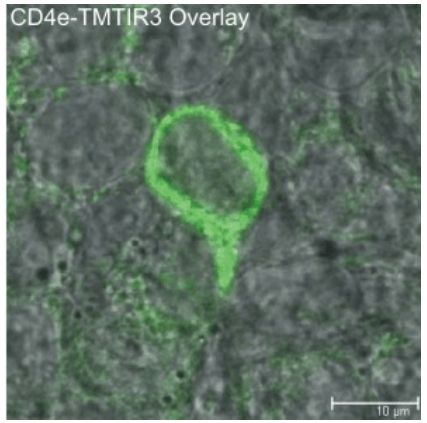


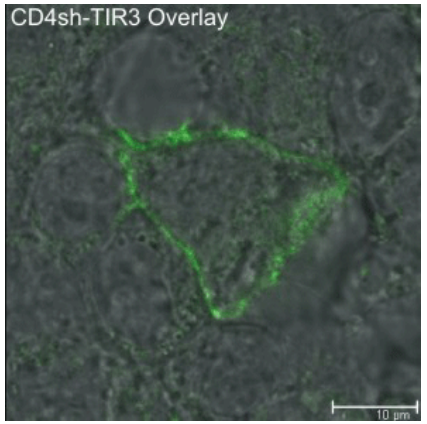
Localized at cell membrane and in cellular vesicles.

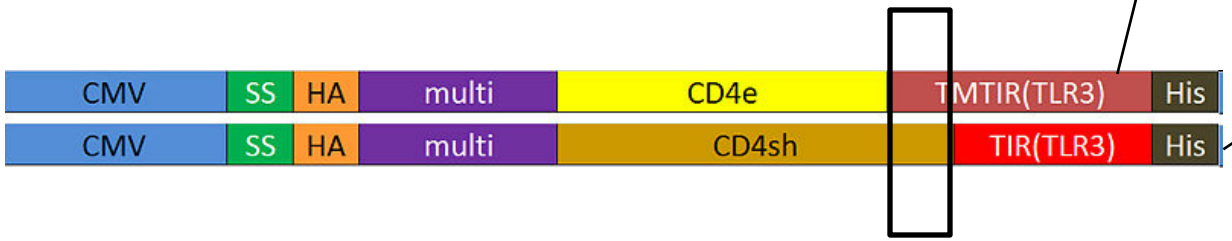
Modulating localization

Track 2







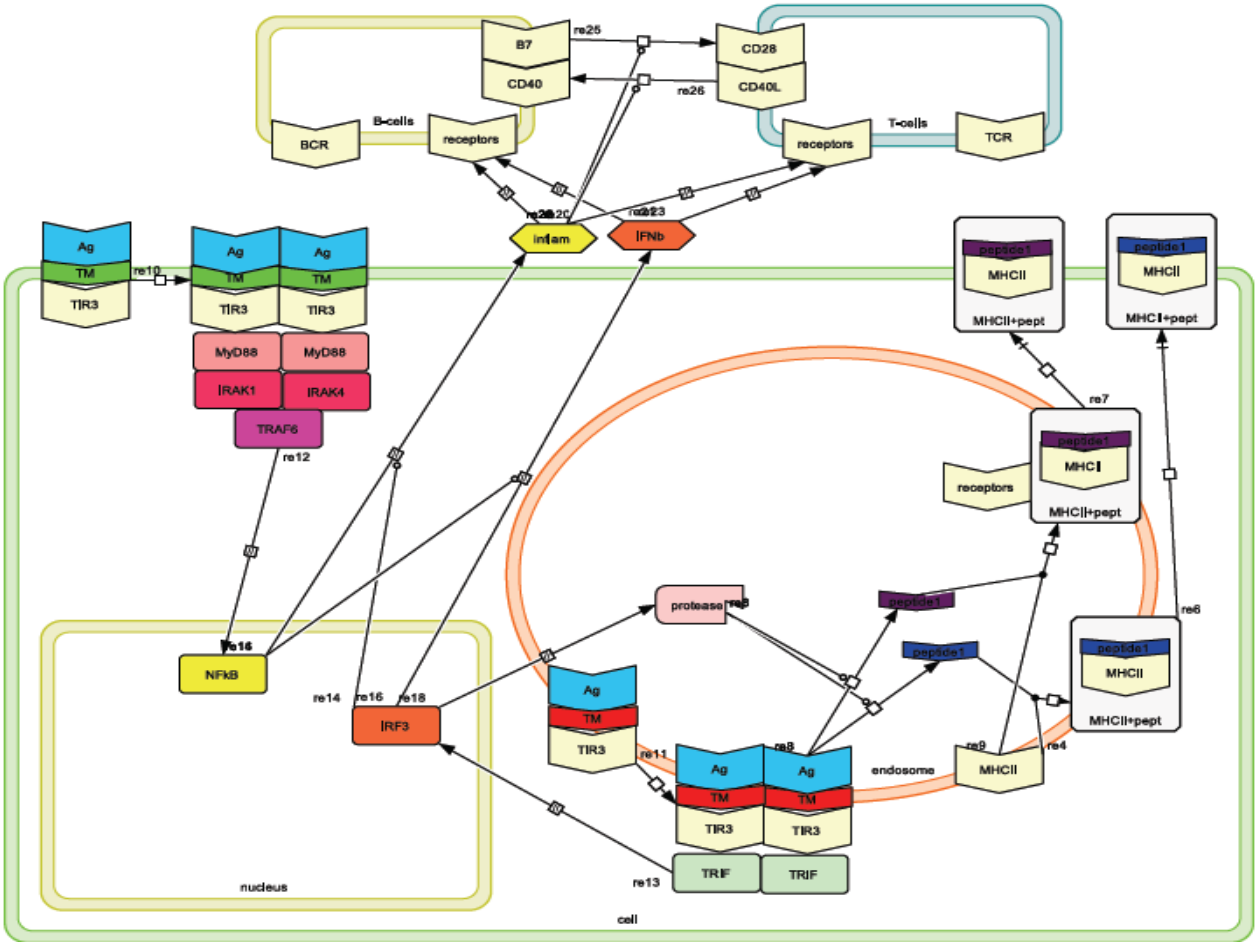


Localization

Signaling network of TLR-fusion vaccine

Track 2

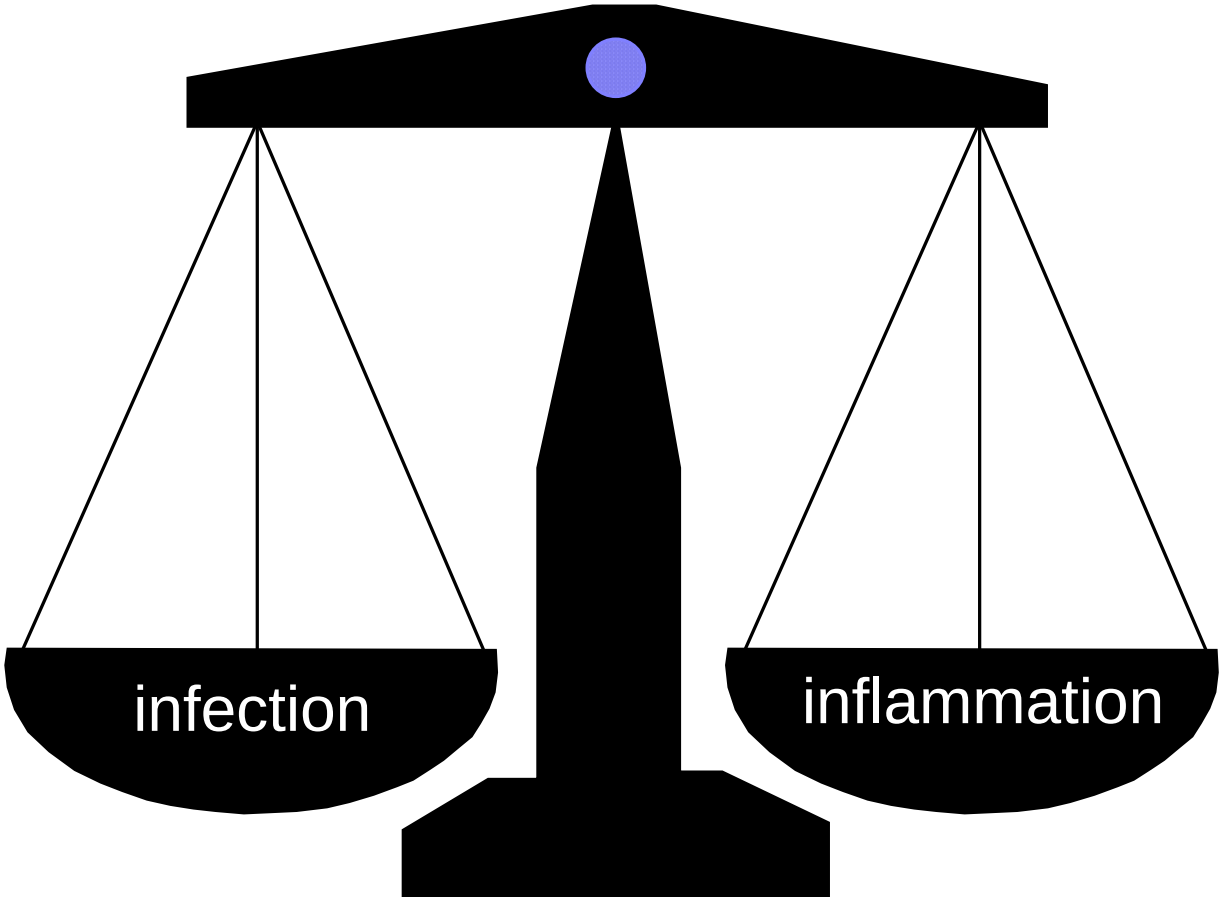
Combined activation of several TLRs **mimics stimulation by complete microbe** (+synergy between MyD88- and TRIF-dependent signaling)



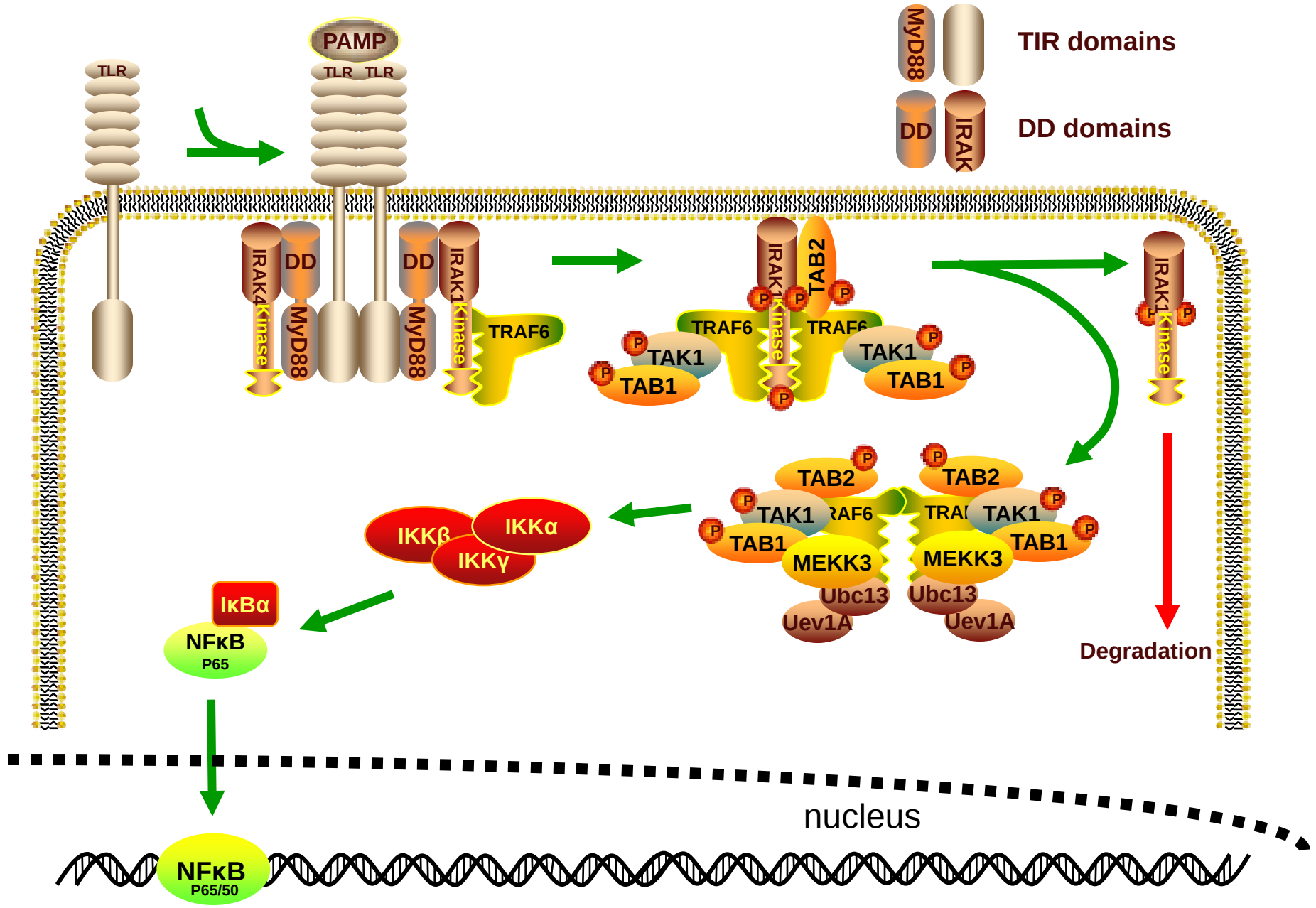
Achievements

- Synthetic biology and BioBrick principles were used to produce synthetic vaccine
- Reengineered *H. pylori* flagellin that became visible to the immune system with additional designed antigens attached
  - Tested three vaccine implementations
- Reengineered TLR signaling network to colocalize activation of innate signaling and antigen processing in a single molecule
- Demonstrated intense *in vivo* antibody response

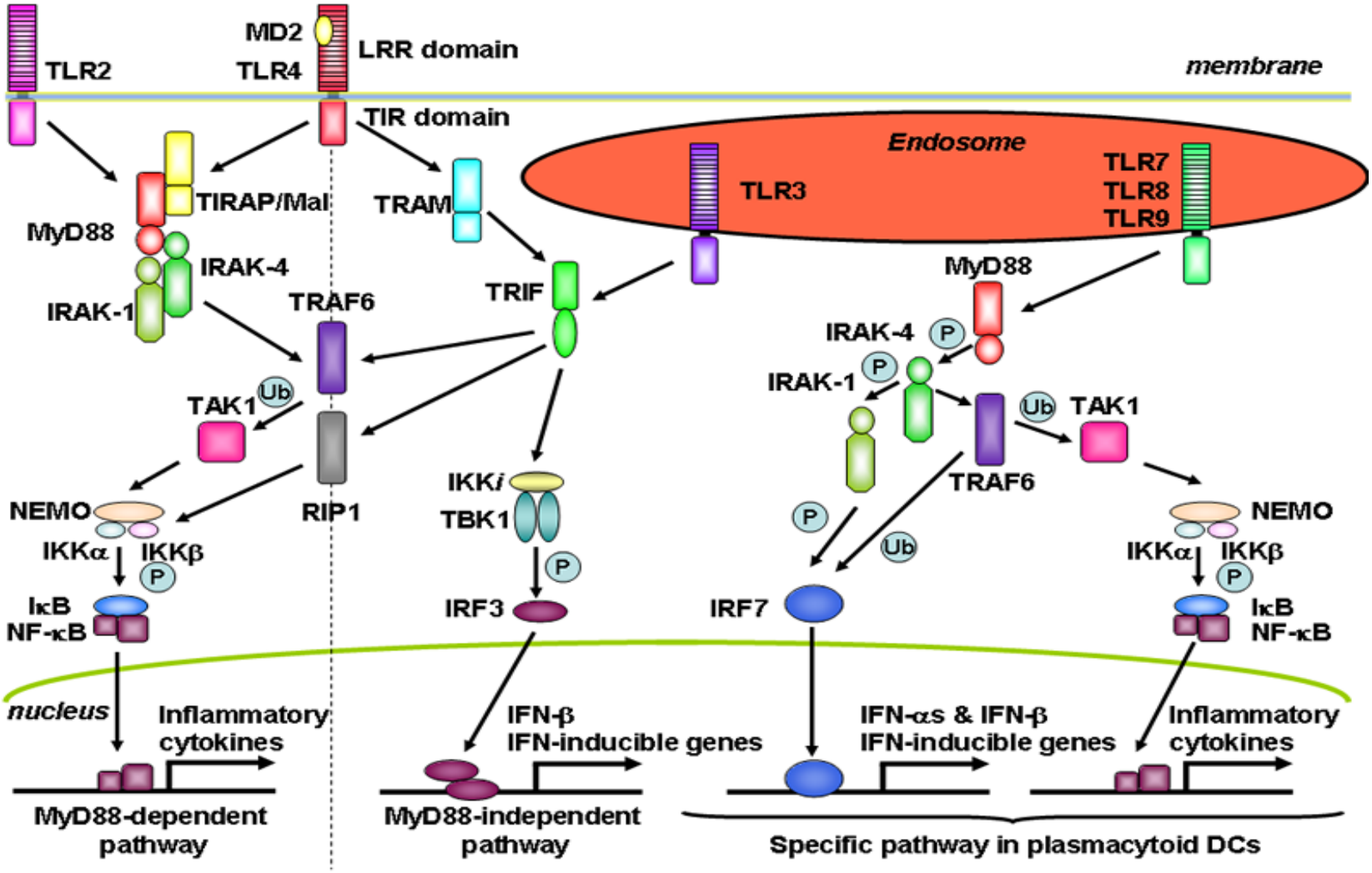
Immune response has to keep the balance between bacterial infection and inflammation



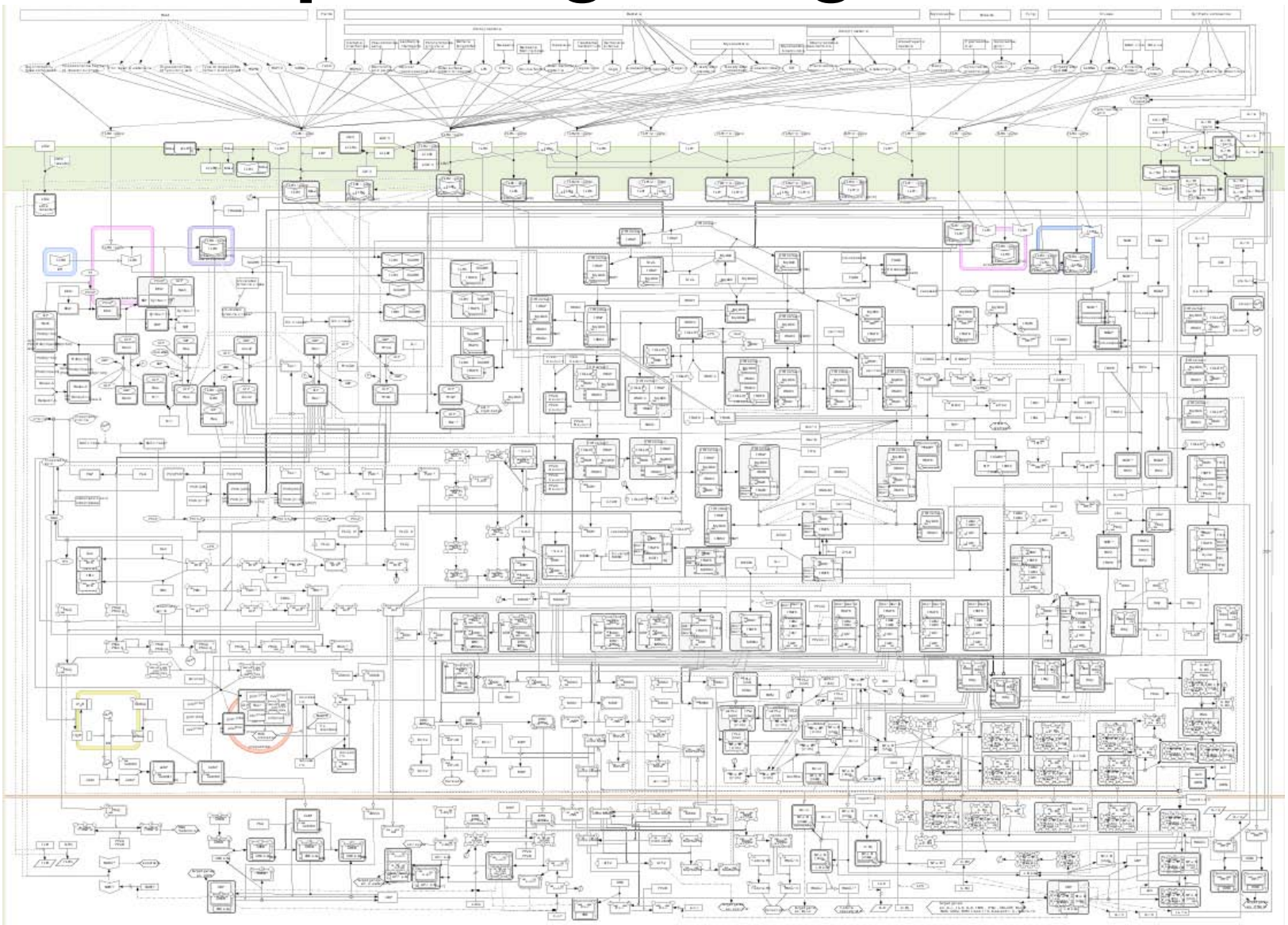
TLR Signaling pathway



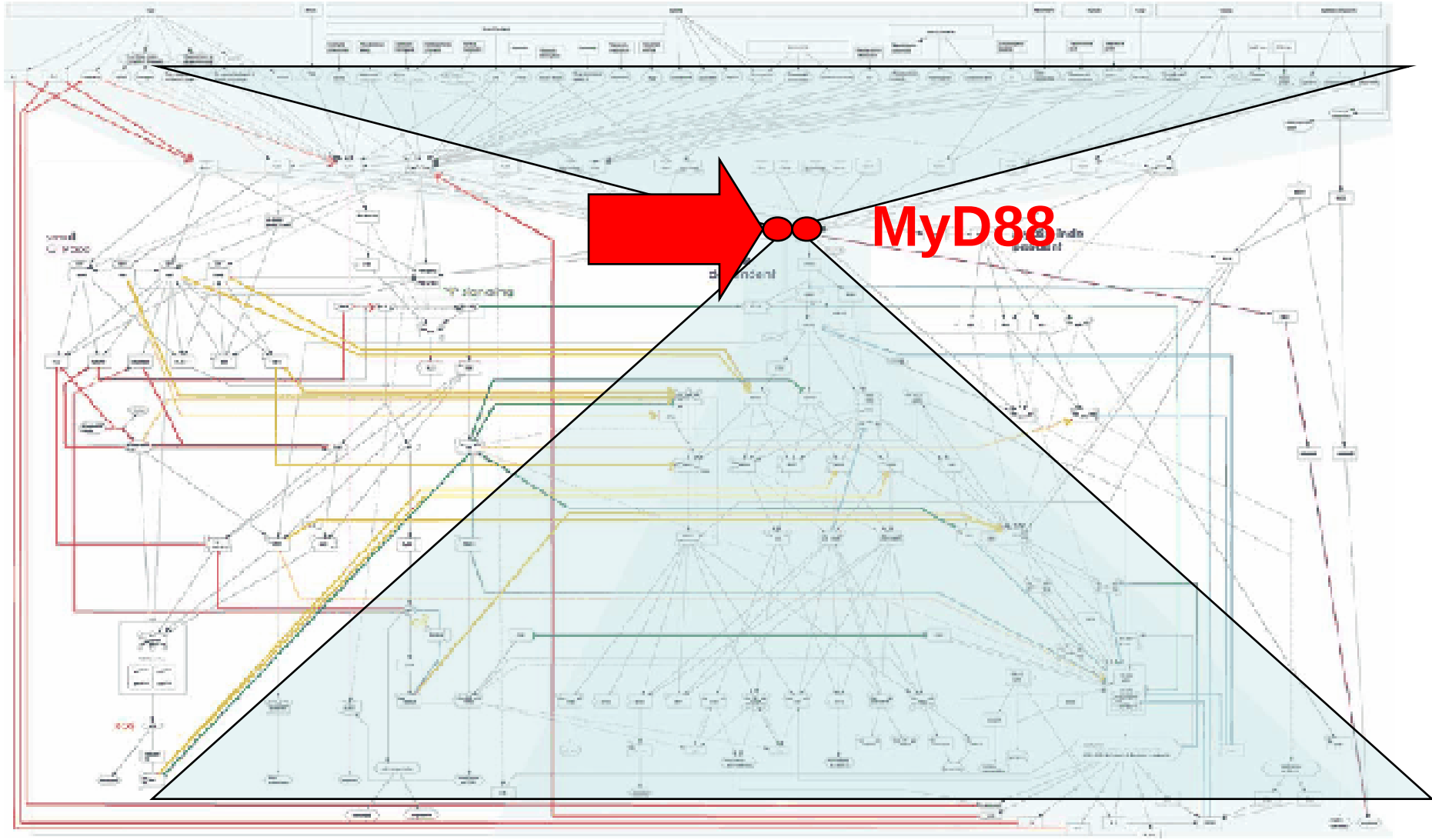
TLR signaling pathway



TLR signal pathway involves a complex signaling network



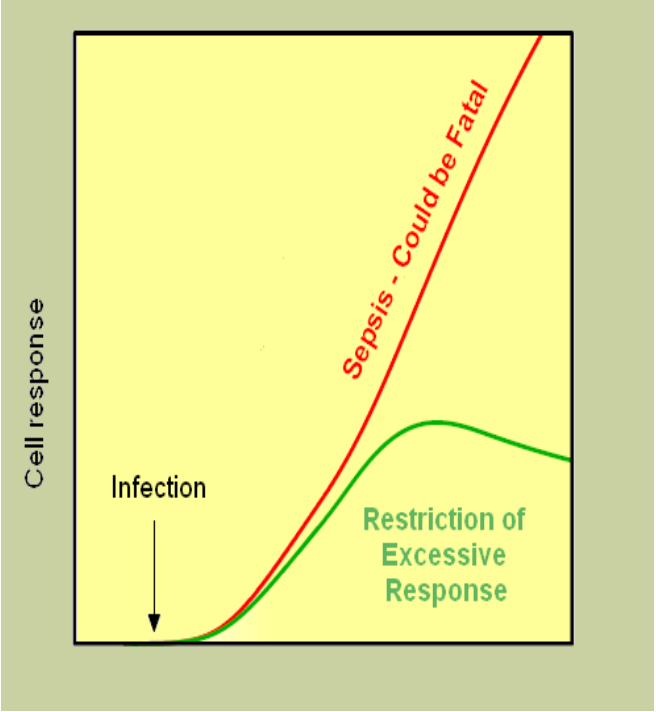
“All Paths lead through MyD88”



Modification of signaling network

Basic concept:

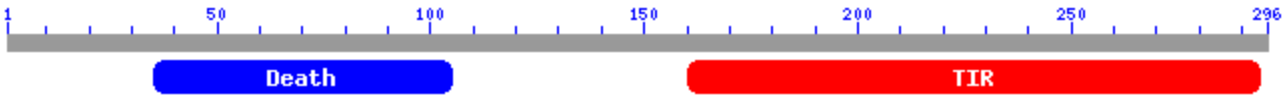
- Inhibit the excessive cellular activation but don't completely inactivate cellular responsiveness (MyD88 KO are extremely sensitive to infections)

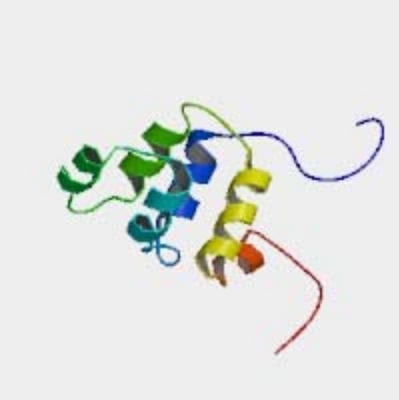


Realization:

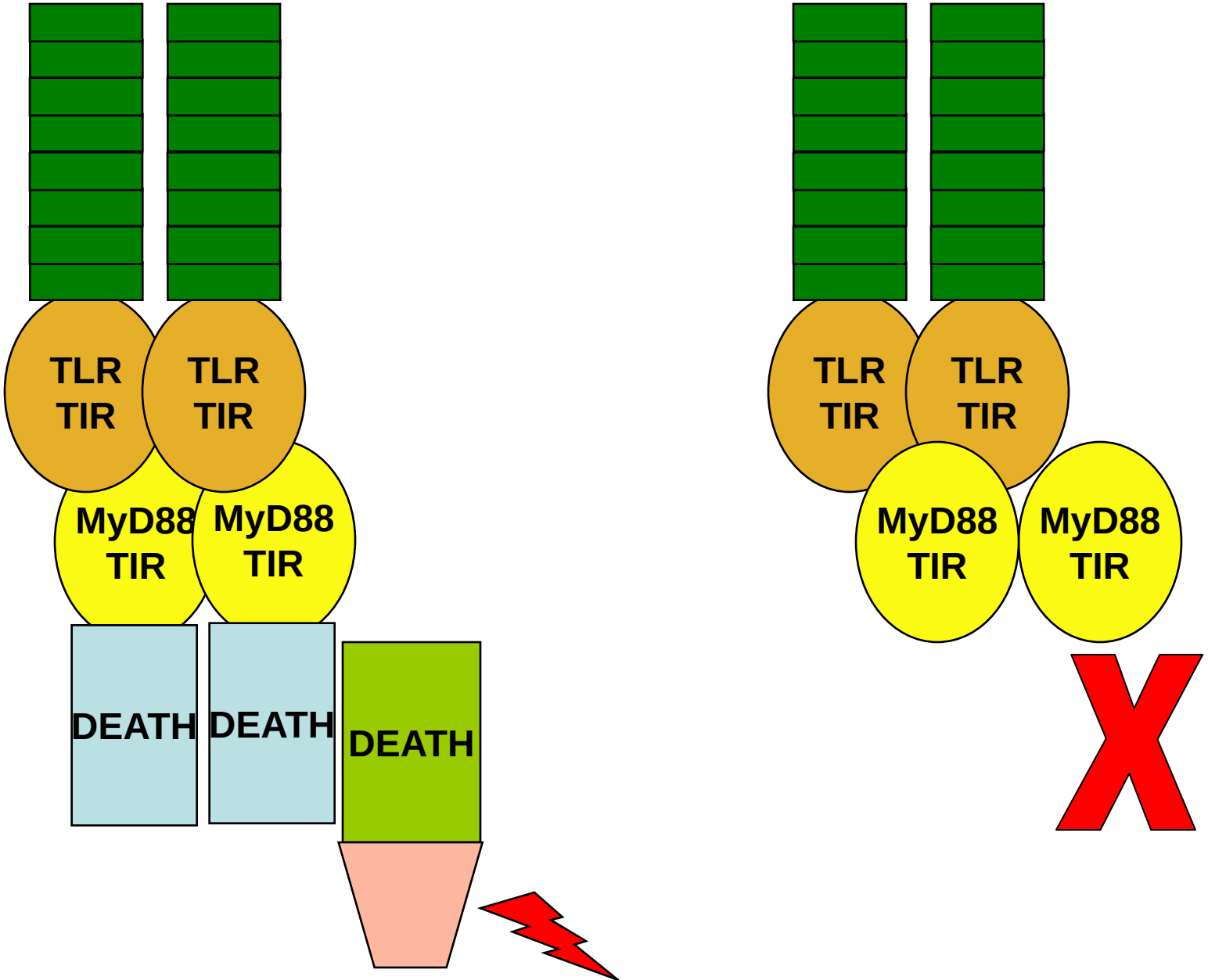
- Insert into mammalian cells a feedback device with inhibitor(dnMyD88) that would repress the signaling of TLR pathway for a limited period of time

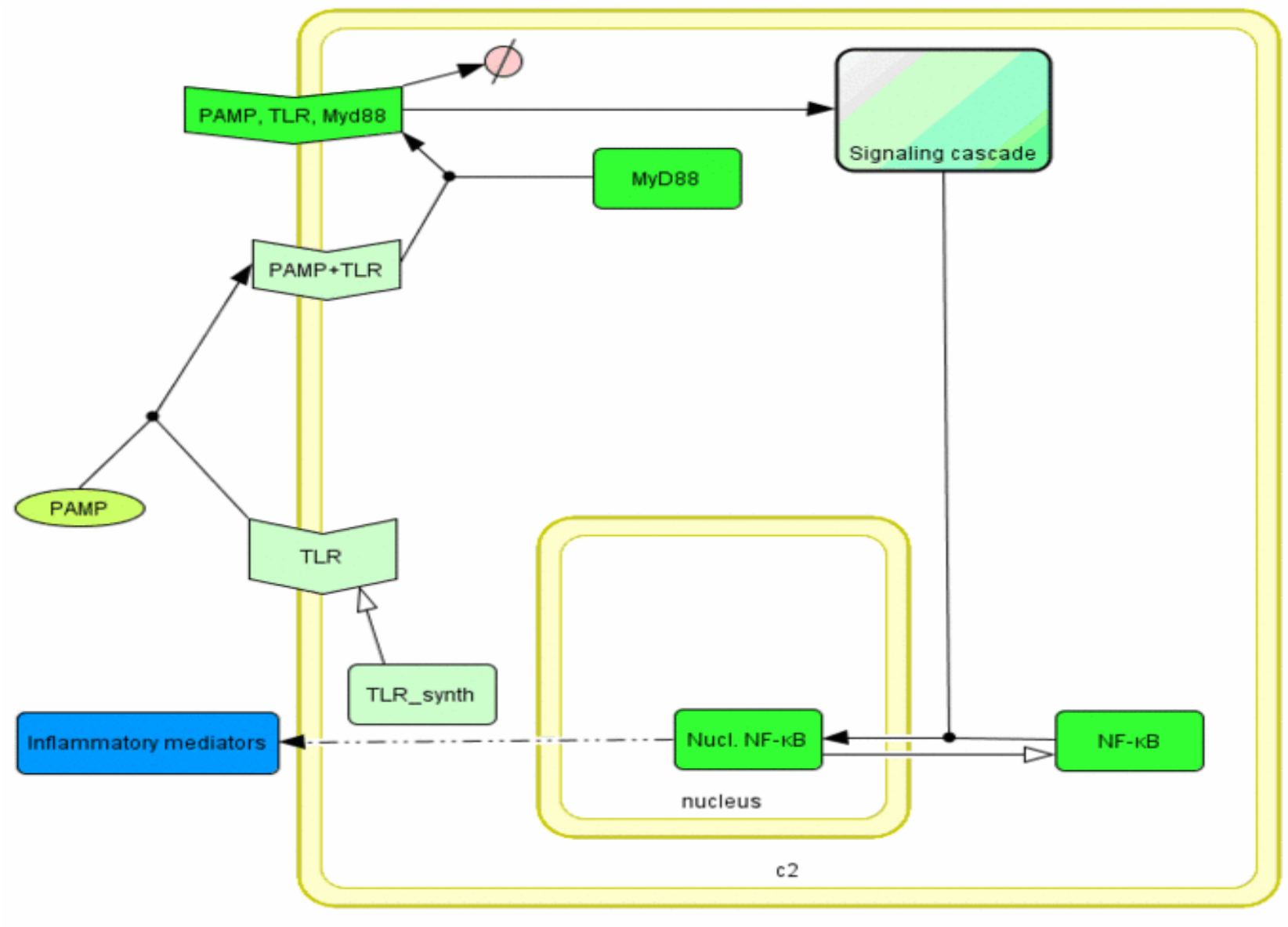
Domain structure of MyD88



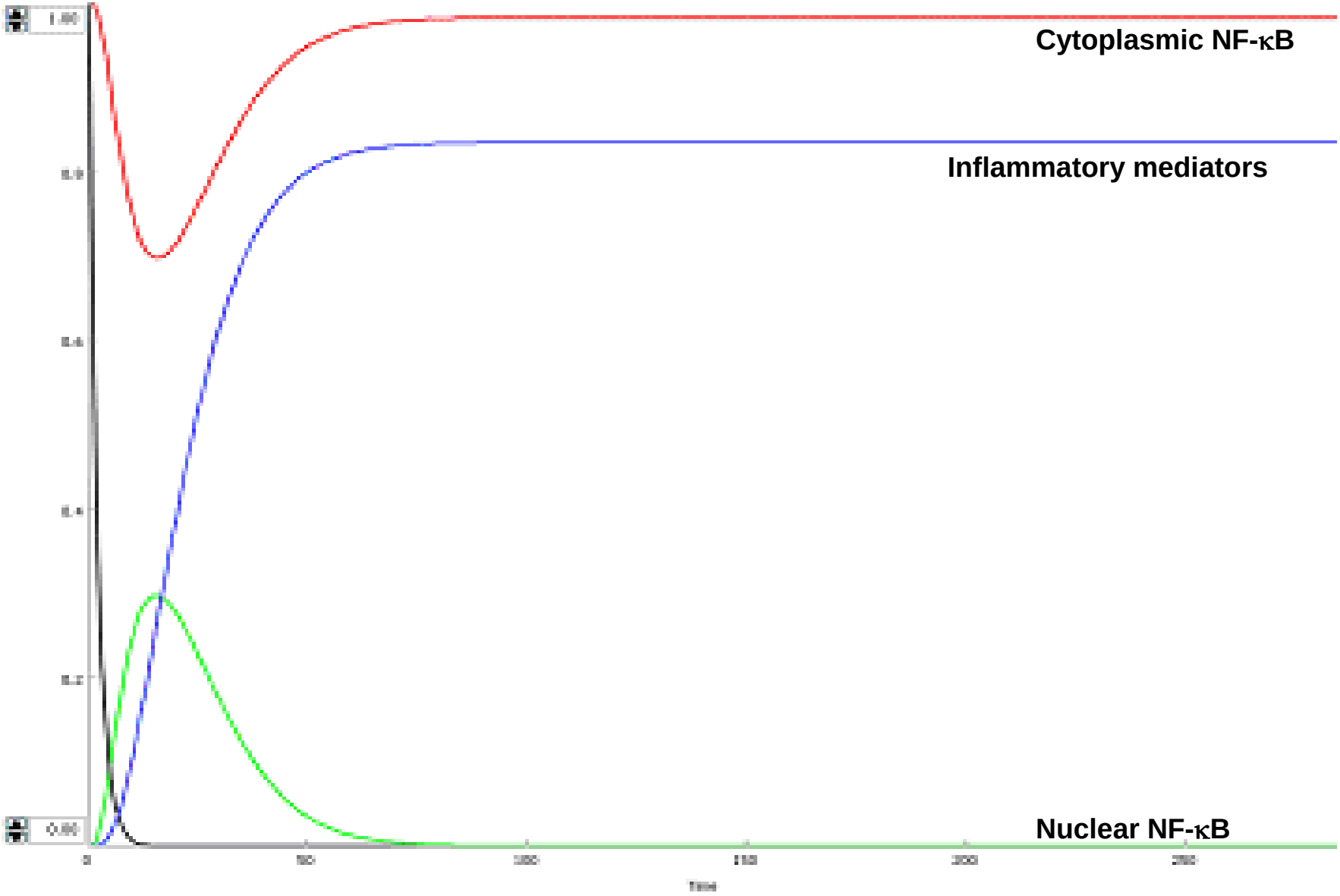


Dominant negative MyD88 inhibition

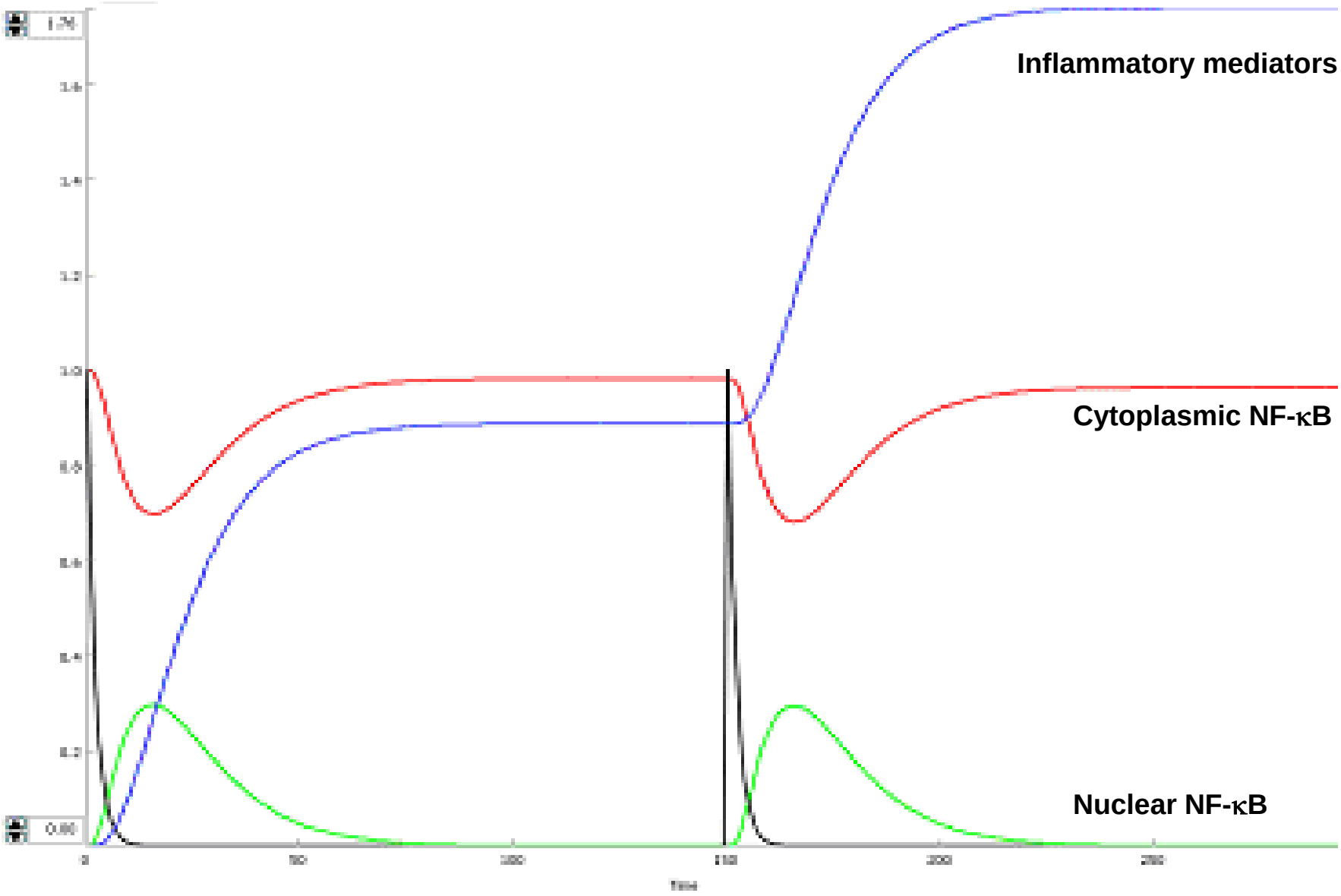


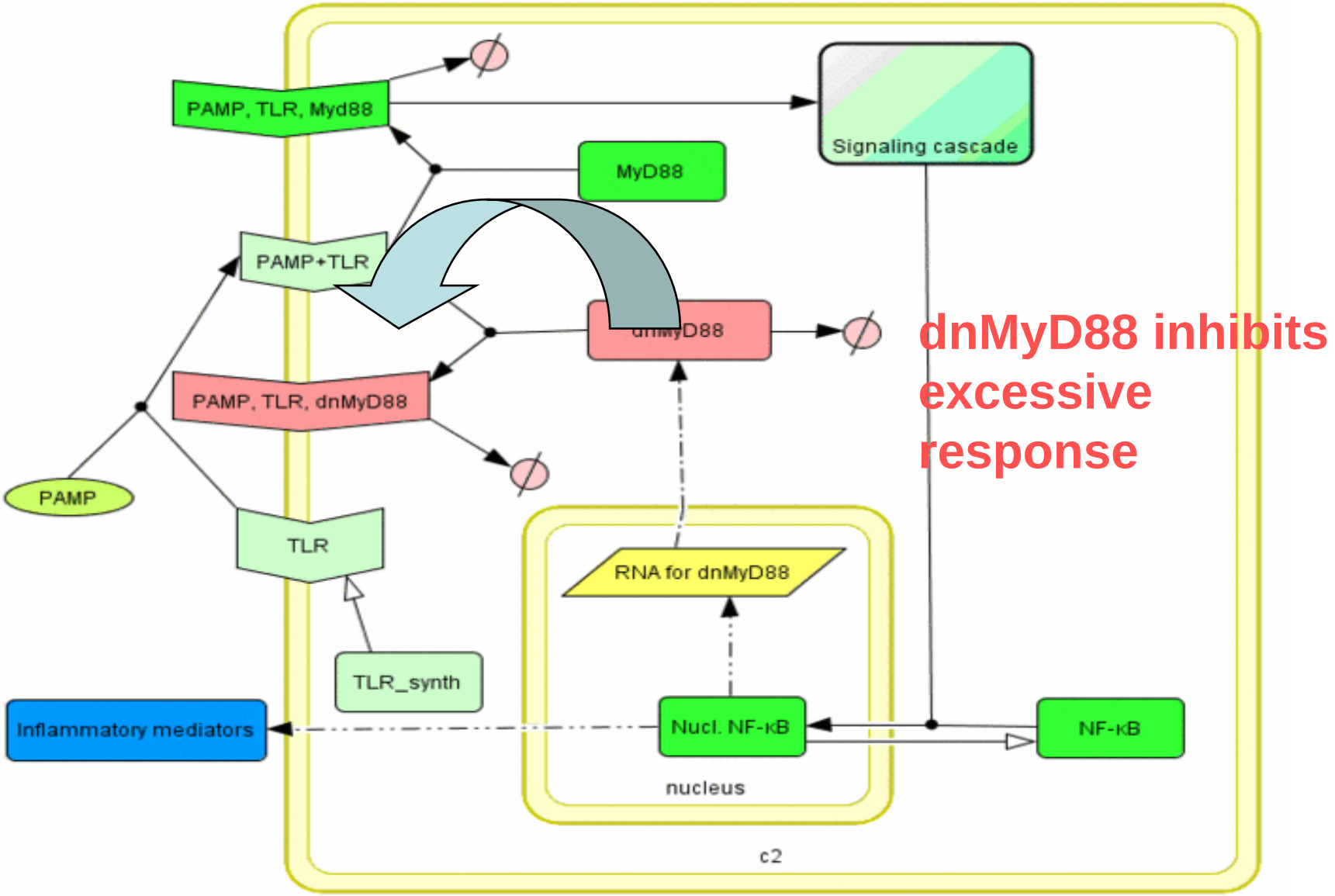


Simplified model of TLR signaling


Cytoplasmic NF-κB
Inflammatory mediators
Nuclear NF-κB

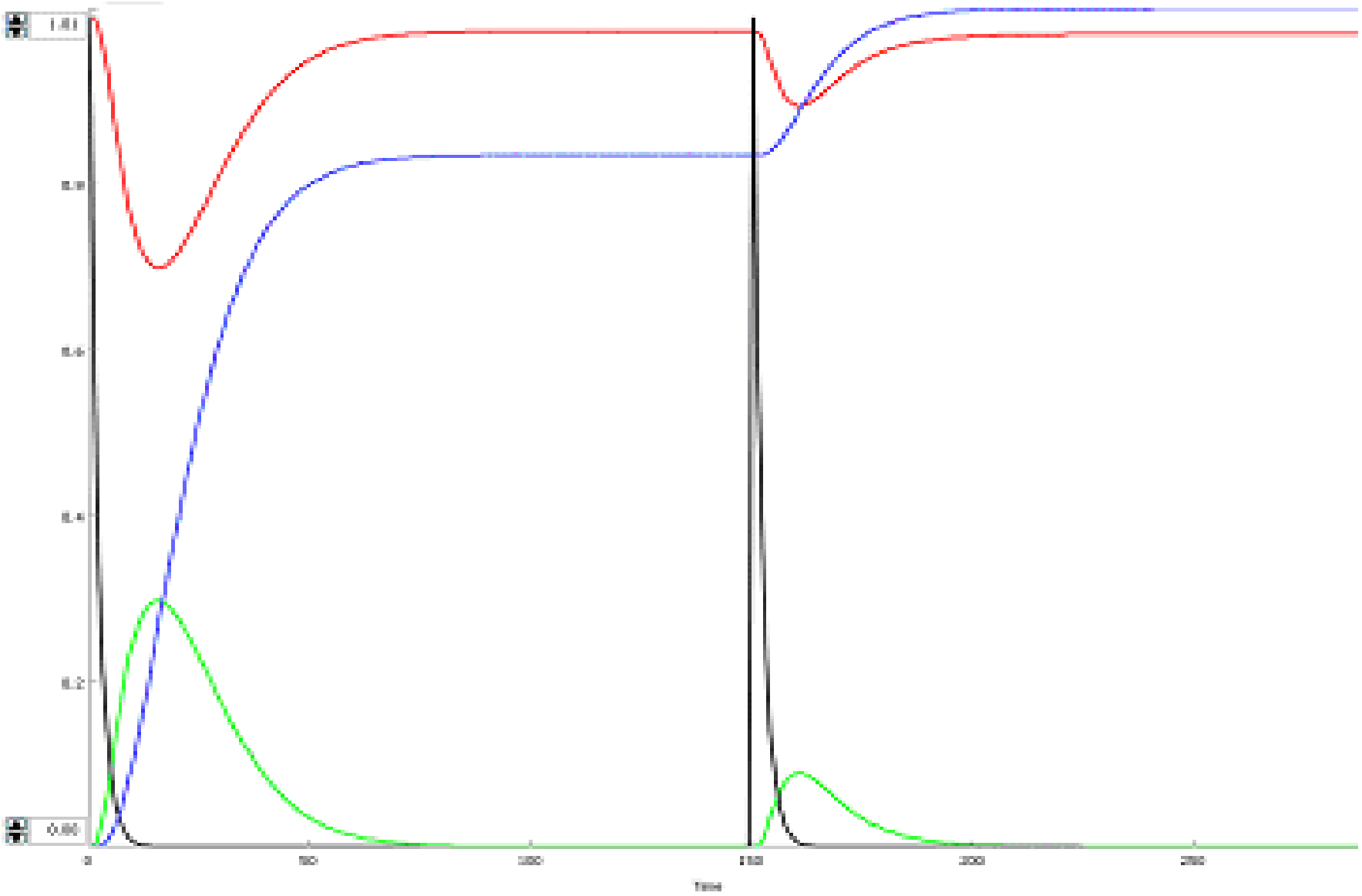
Normal cellular response to repeated stimulus



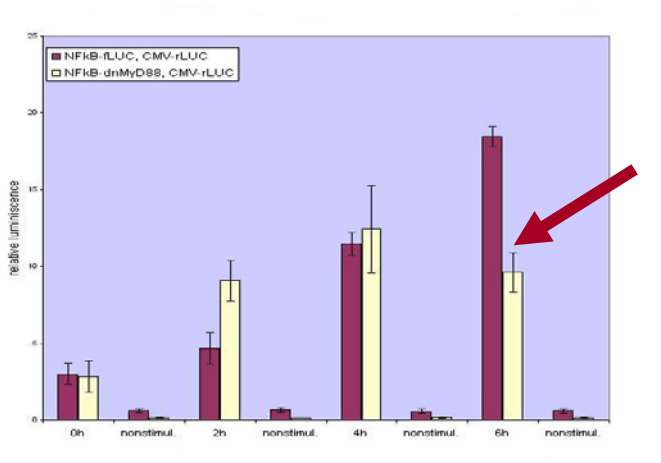


Insertion of a negative feedback loop

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...)

Slovenian team won the Grand prize in the final against the Imperial college and Princeton.

virotrap® 160 ng

A Synthetic Biology Approach Against HIV

2007

at a glance:

1925 minutes of talks
1200 participants
825 jamboree attendees
84 teams
77 presentations
24 awards
22 weeks of work
21 countries

News:

- Slovenia takes home the grand prize for iGEM 2008!
- Share any publicity that your team has received on the Publicity page.

Congratulations to the Slovenia team,
iGEM 2008 Grand Prize winners!

Current bottlenecks of SB

Tools

Chemical synthesis at genomic scale (second generation DNA synthesizers)

Modularity, standardization

Automatisation of gene manipulation (lab on a chip)

Fundamentals

Lack of understanding of cell (organism) as a system (systems biology)

Understanding of protein folding and interactions

The Future of synthetic biology

- Applications
  - cost effective renewable fuel sources
  - advanced (nano)biomaterials
  - medical applications

- Fundamental advances
  - synthesis of complete genomes of bacteria and their activation
  - Identification of a minimal genome
  - *de novo* programming of a genome

Slovenian teams 2006-2008

2006

•Monika Ciglič, BF
•Ota Fekonja, BF
•Jernej Kovač, FKKT
•Alja Oblak, BF
•Jelka Pohar, BF
•Matej Skočaj, BF
•Rok Tkavc, BF

2007

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

2008

- Eva Čeh, BF
- Vid Kočar, FKKT
- Katja Kolar, FKKT
- Ana Lasič, MF
- Jan Lonzarić, FKKT
- Jerneja Mori, BF
- Anže Smole, BF

Mentors

Mojca Benčina (KI), Monika Ciglič (KI), Karolina Ivičak (KI), Nina Pirher (KI), Gabriela Panter (KI), Mateja Manček Keber (KI), Marko Dolinar(FKKT), Simon Horvat (BF), Roman Jerala (KI, FKKT)