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Synthetic immunology

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Medical applications of synthetic biology

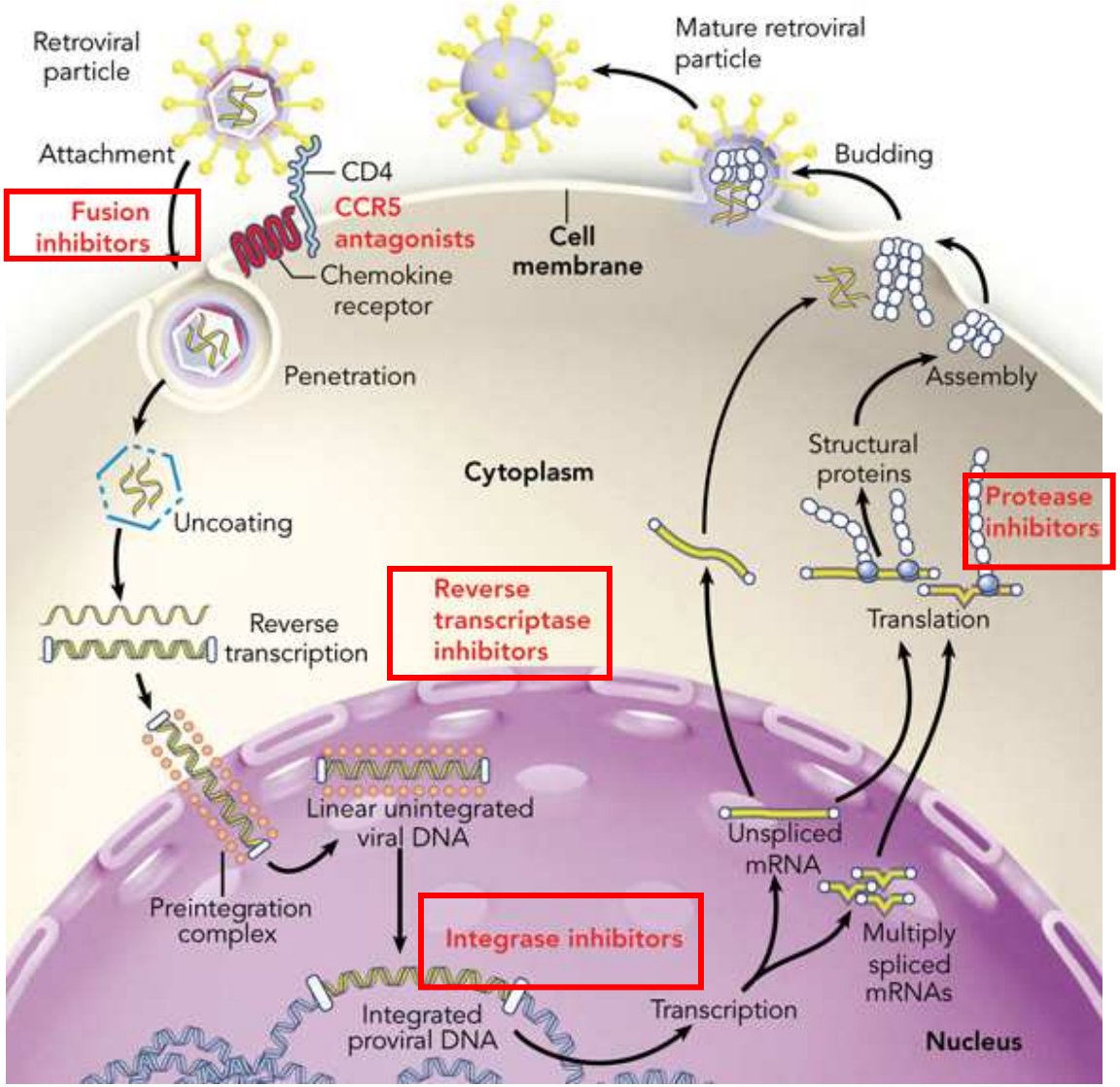
- Alternative means of drug production
- New therapeutic methods
- Engineering of response of human cells

- Immune response provides defense against danger – pathogens from the environment, endogenous threats (cancer, sterile inflammation, autoimmune diseases...)

- **Synthetic cell signaling pathway**
  - 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

Therapeutic targets of HIV life cycle



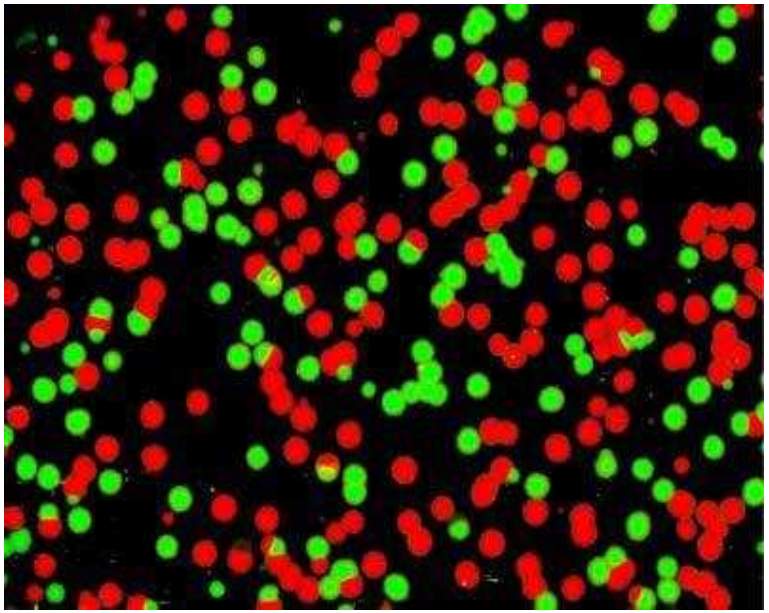
www.mja.com.au

Problems with antiviral therapy

Mutations

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

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



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

HAART – combination therapy

Price

Life-long treatment

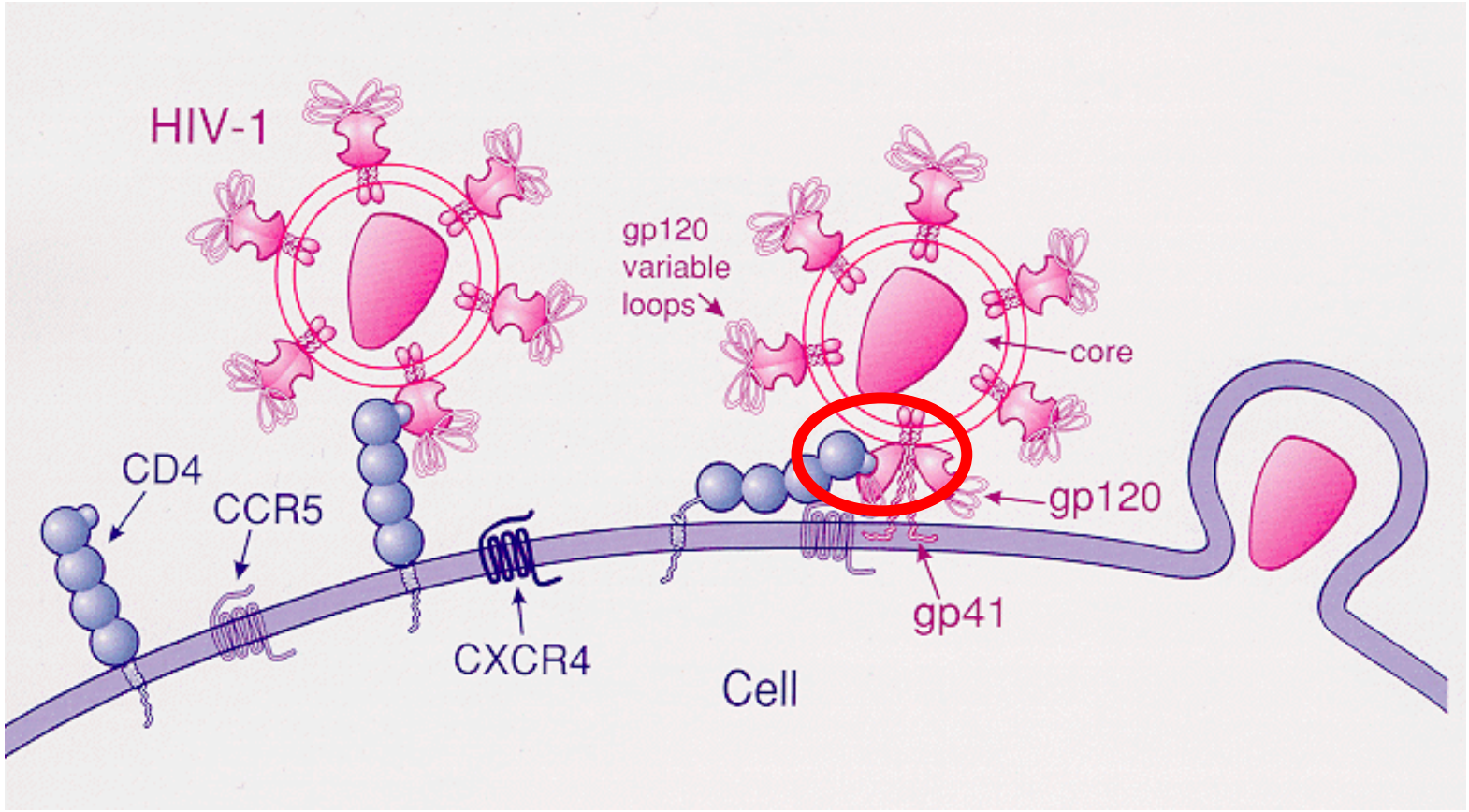
Synthetic biology approach to prepare antiviral device

- Should be **INSENSITIVE TO MUTATIONS** ⇨
  BASE THE RESPONSE ON VIRAL FUNCTION !

- Tested viral functions:
  - cell attachment
  - viral protease

  also other virus-specific functions and different viruses (SARS, WNV...)

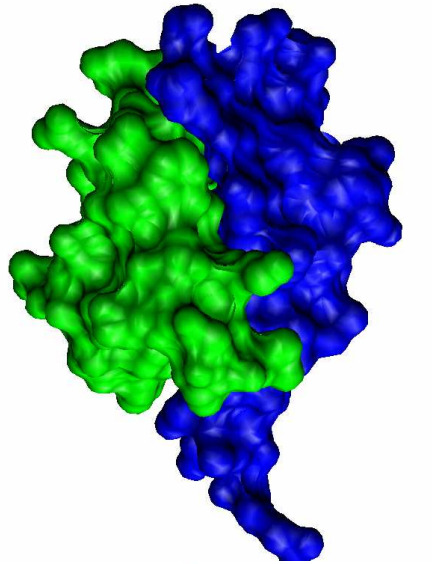
Viral attachment induces receptor heterodimerization



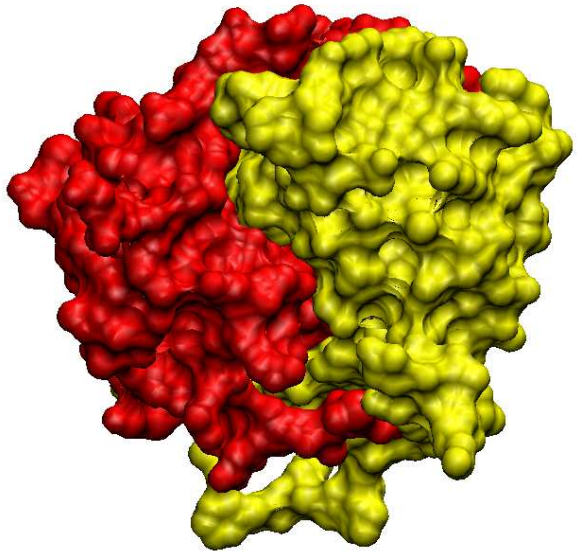
Heterodimerization of cellular transmembrane receptors at viral entry could be used to detect viral attack.

Detection of heterodimerization based on reconstitution of split proteins

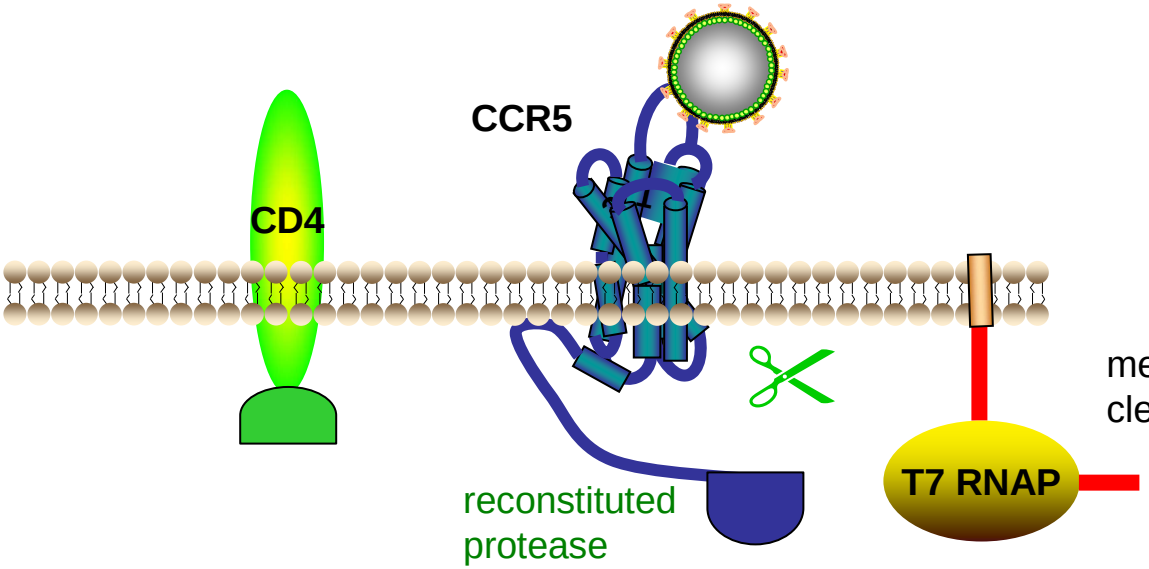
- Split ubiquitin system
  - cleavage C-terminal to ubiquitin with endogenous ubiquitin-specific protease



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



Detection of viral attachment to cell receptors

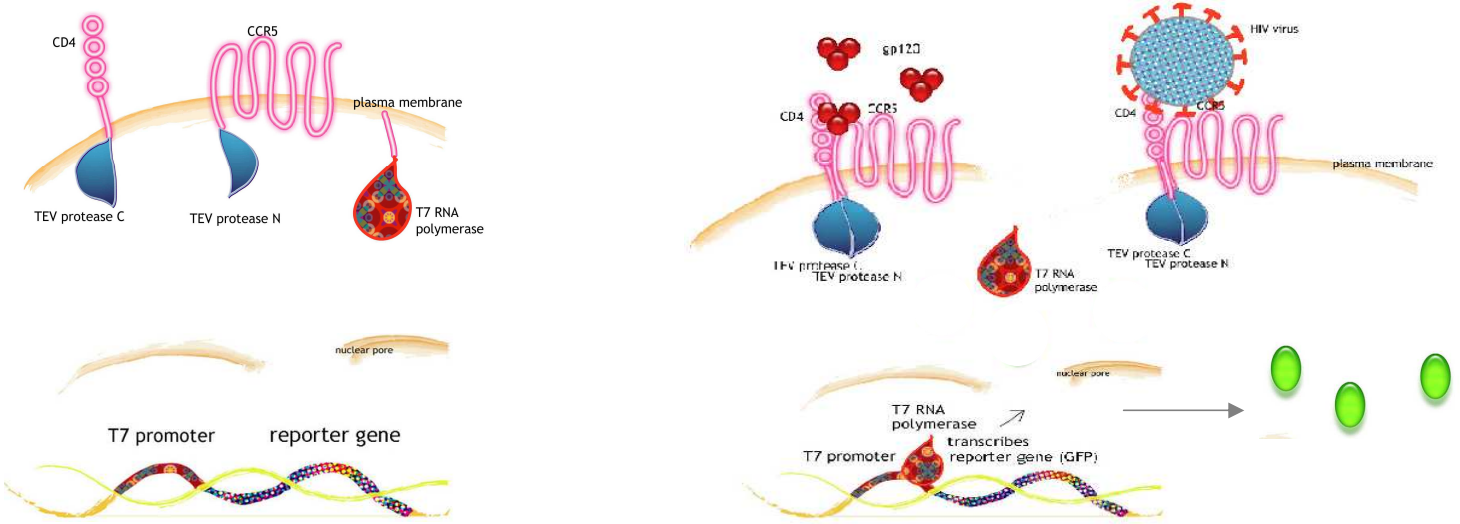


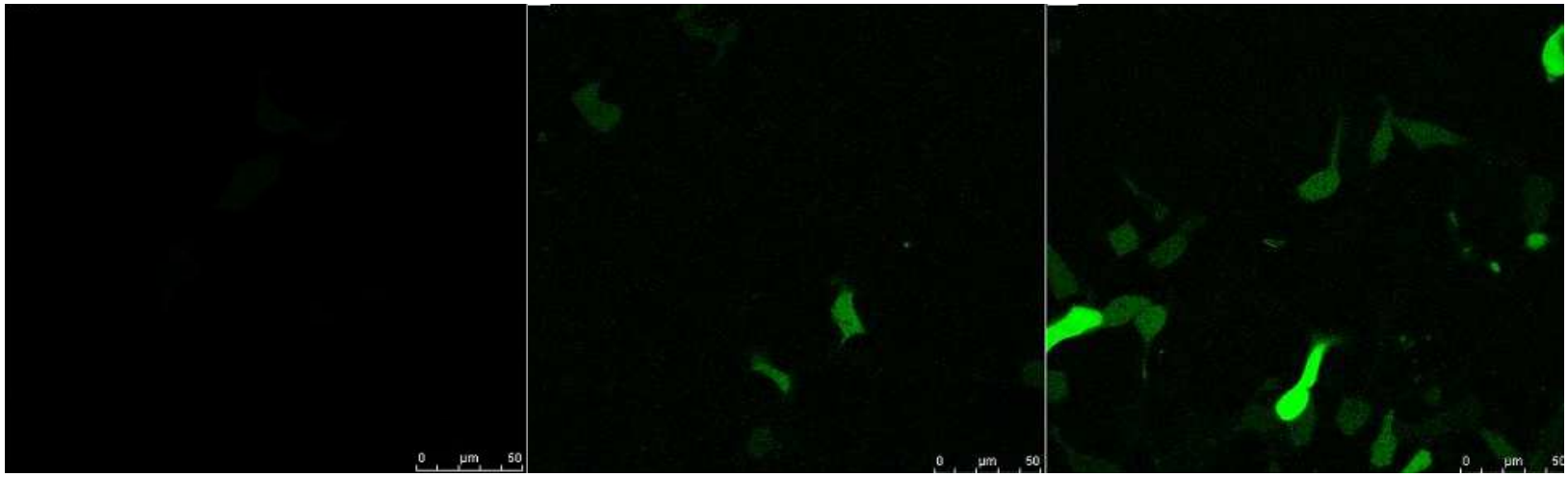
fusion of HIV coreceptors with
split TEV protease segments

translocation into the nucleus

transcription of antiviral defense genes (e.g. Caspase, IFN, ApoBec...)

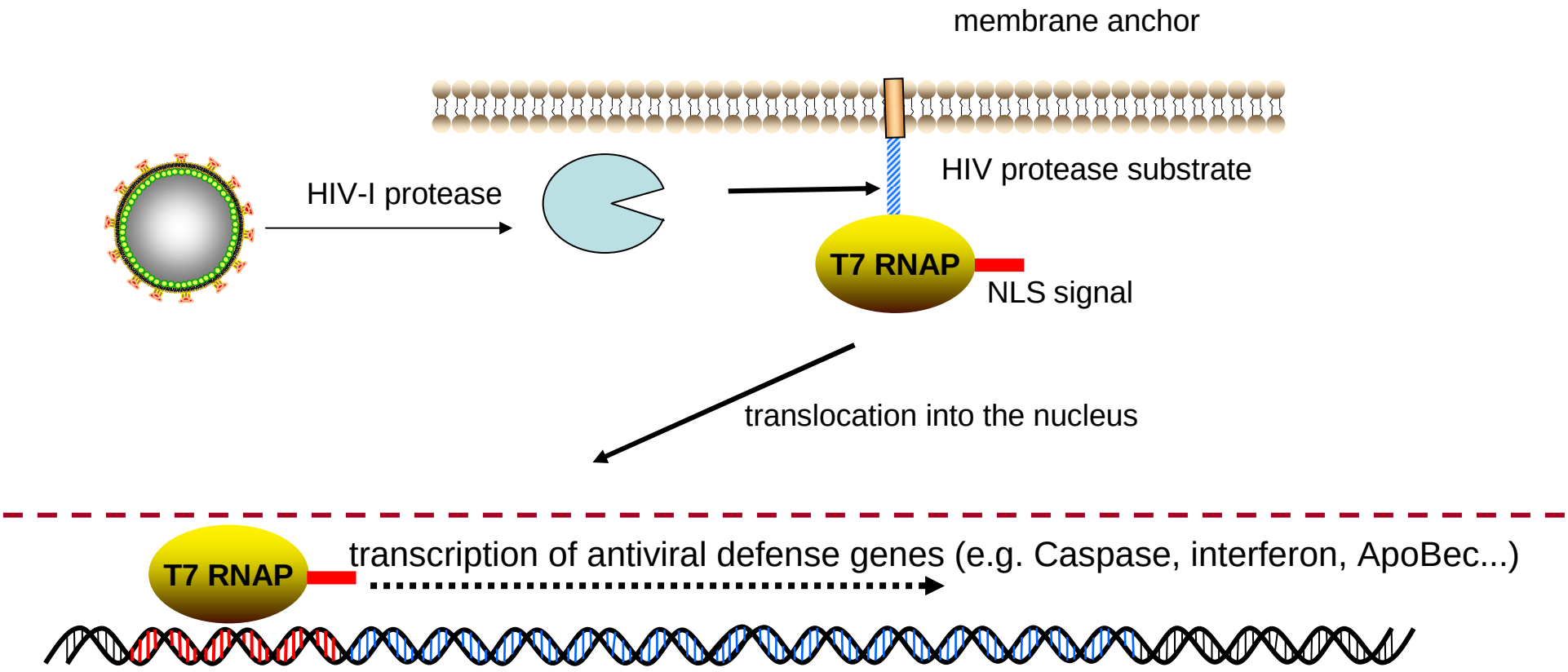
Integration of two steps:
Split TEV-T7-based cell activation



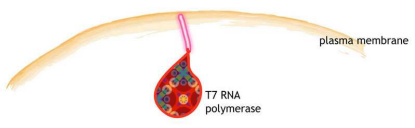


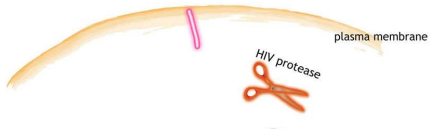
Control **+ gp120** **+ Pseudovirus**

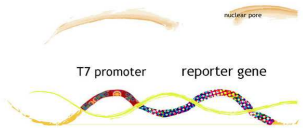
Detection based on the HIV protease activity

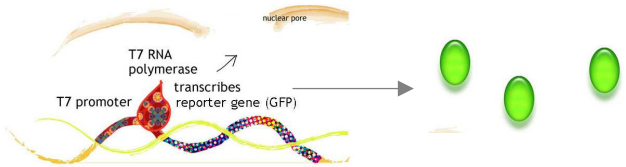


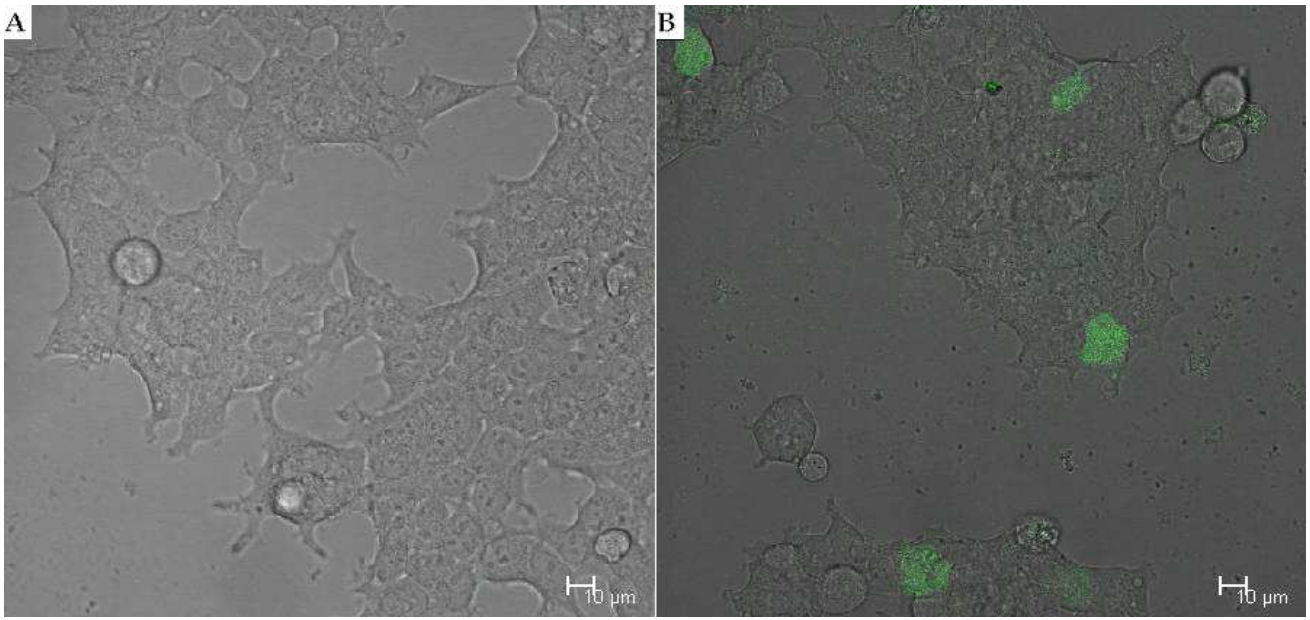
HIV protease - based cell activation





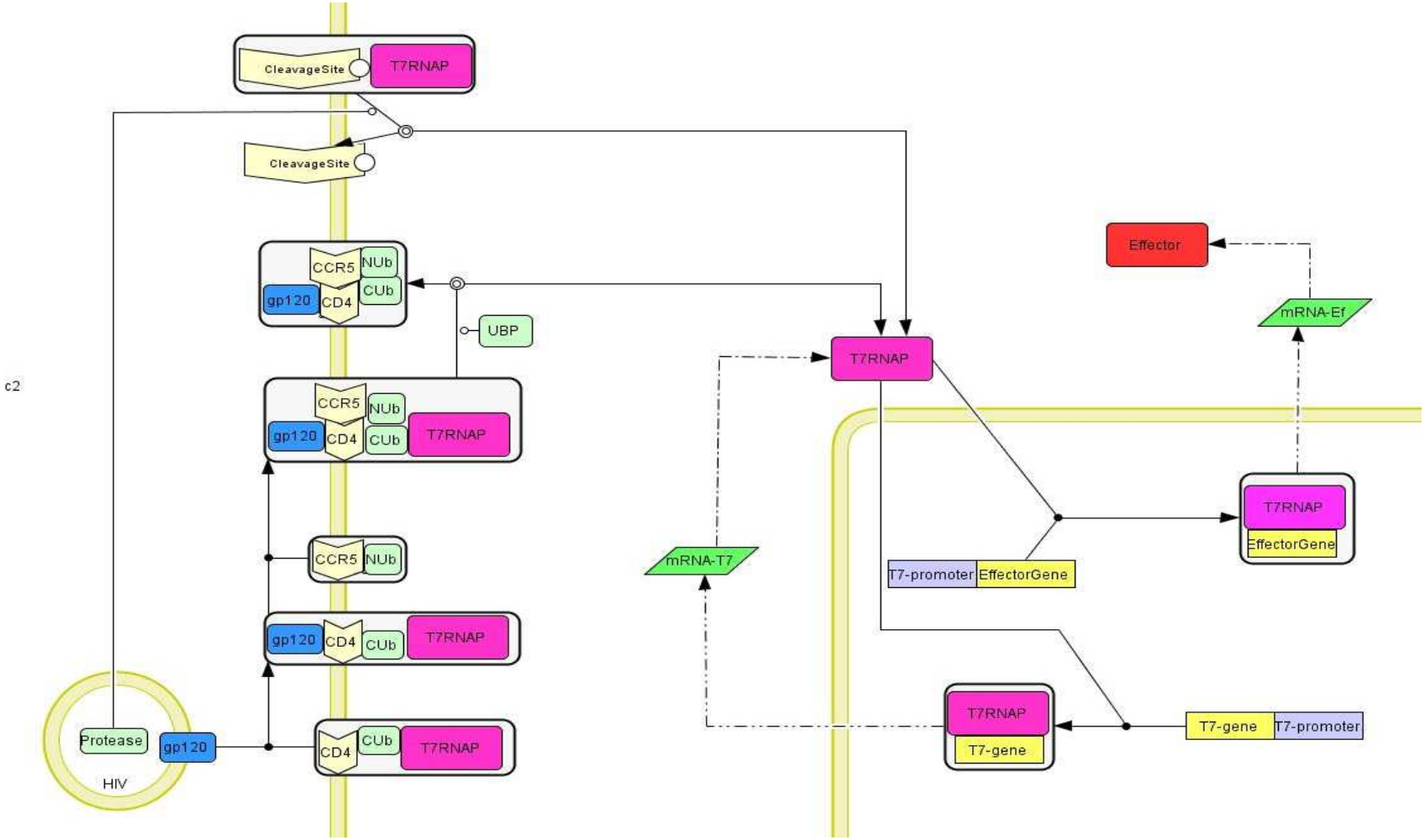




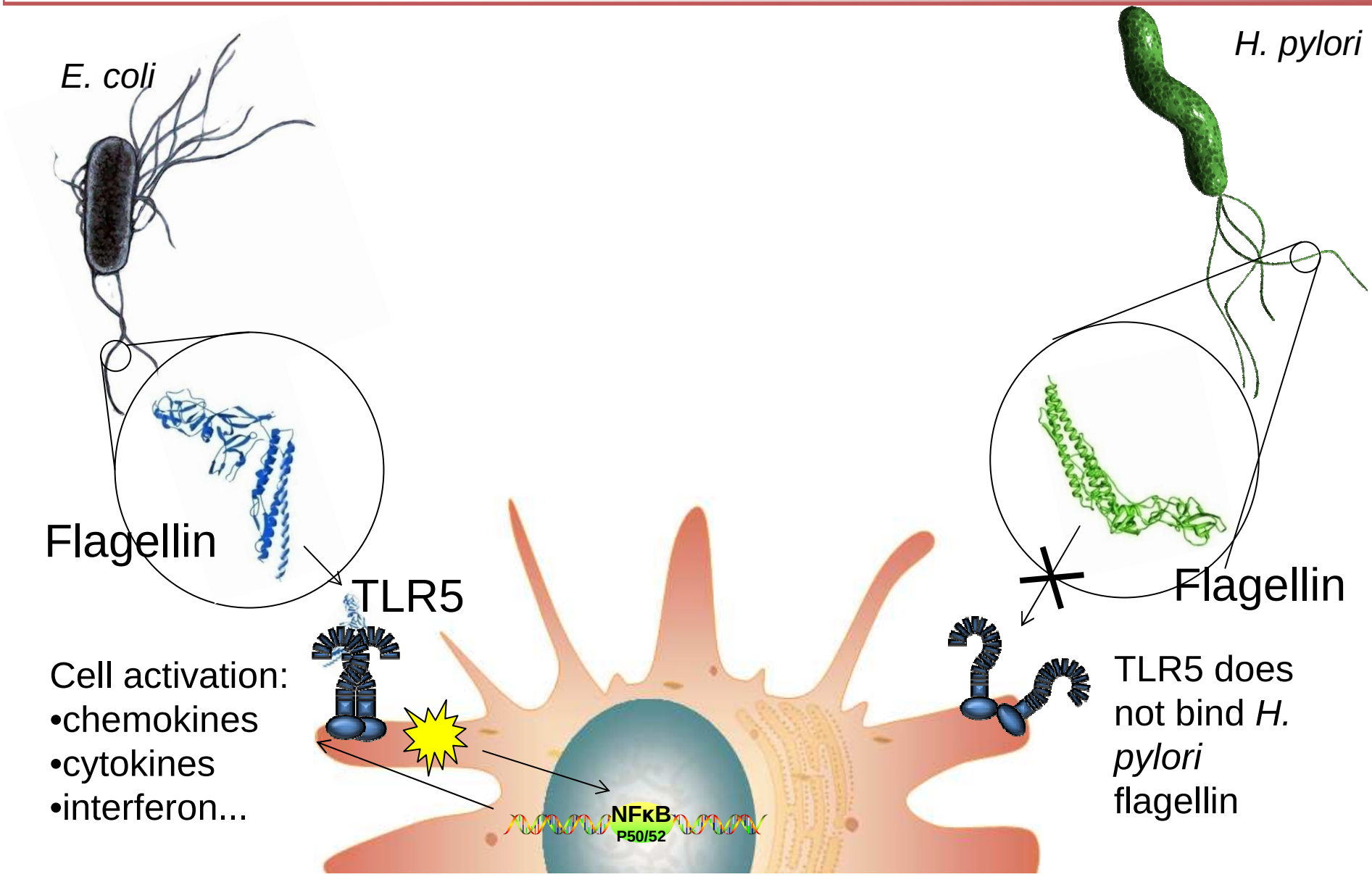


Control **+ HIV protease**

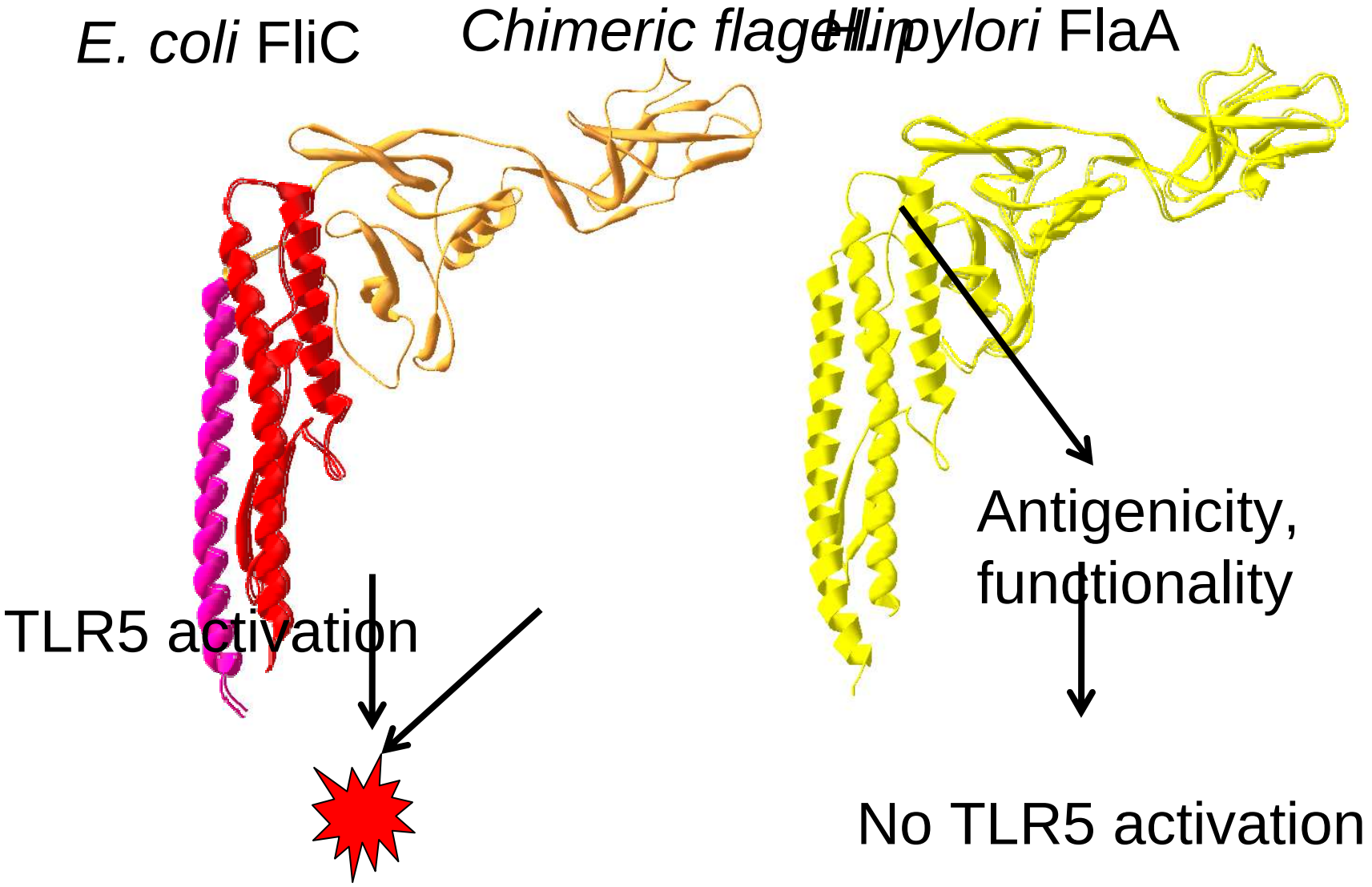
Schematic representation of the anti-HIV defense device



Helicobacter pylori - master of disguise

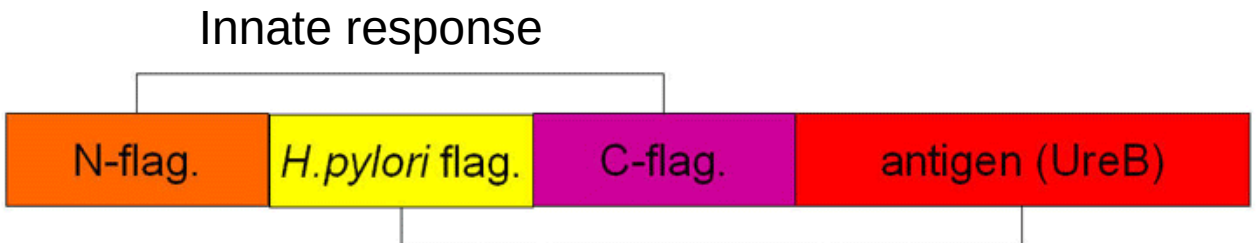


Chimeric flagellin



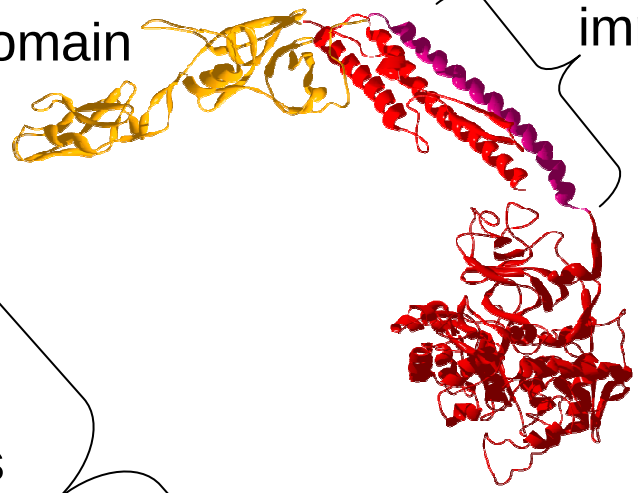
Chimeric flagellin

Track 1



H. pylori variable flagellin domain

activation of innate immunity (TLR5)

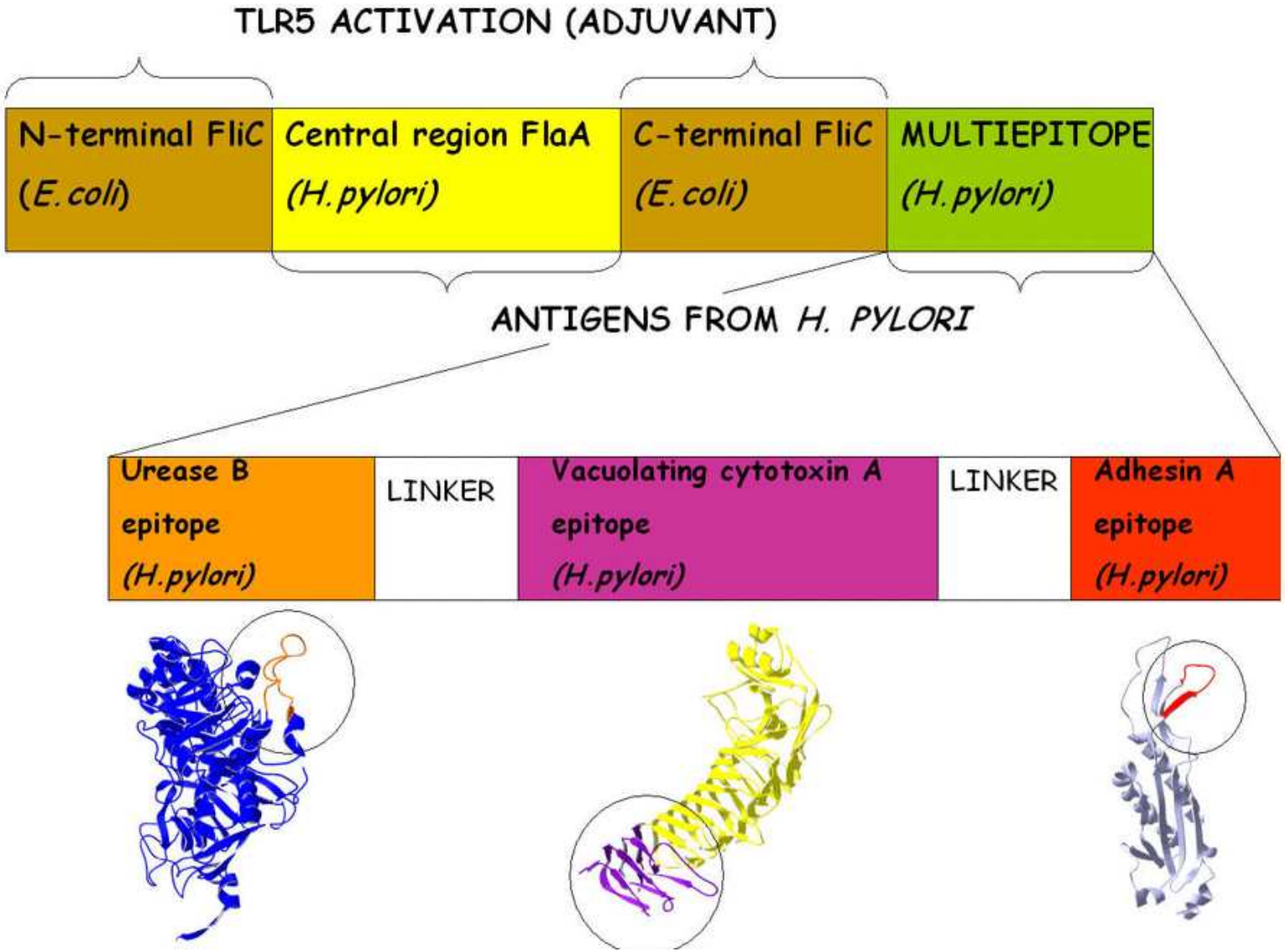


protein antigens (activation of adaptive immunity)

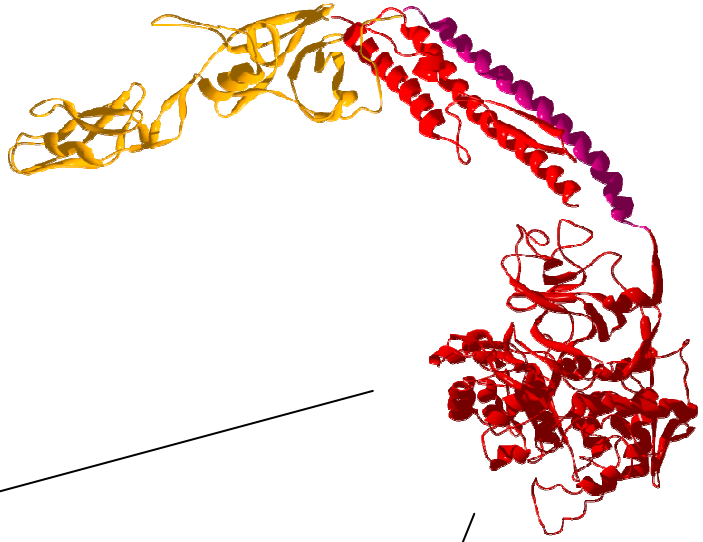
H. pylori antigen (e.g. UreB)

Multiepitope

Track 1



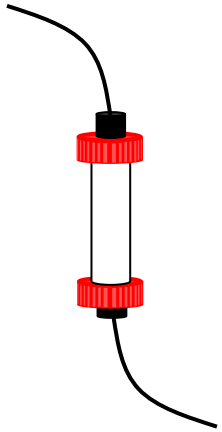
Implementation

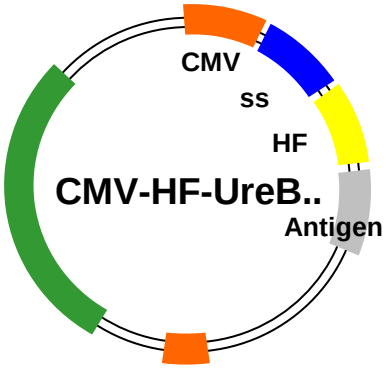


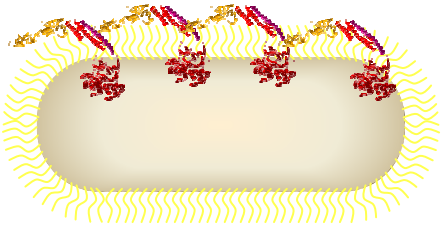
protein vaccine

DNA vaccine

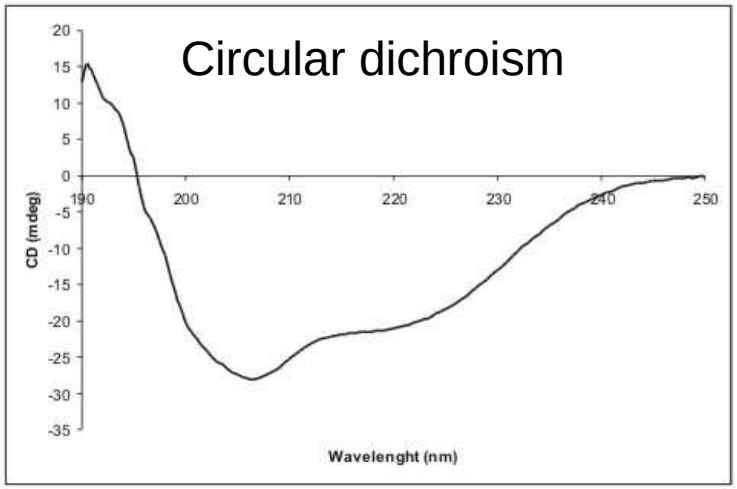
engineered bacteria



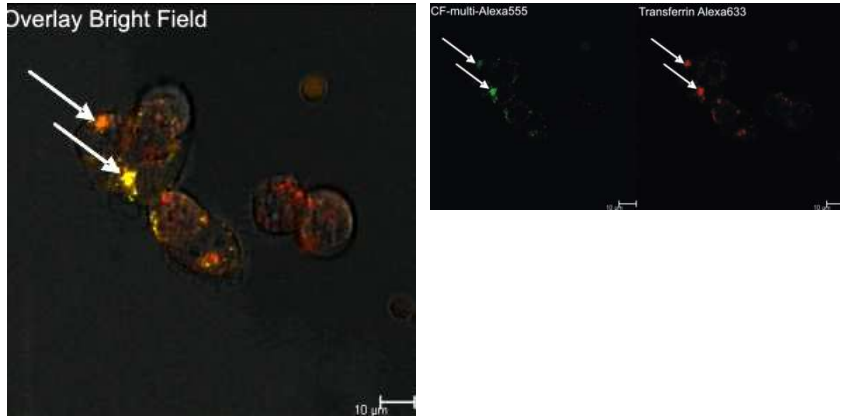




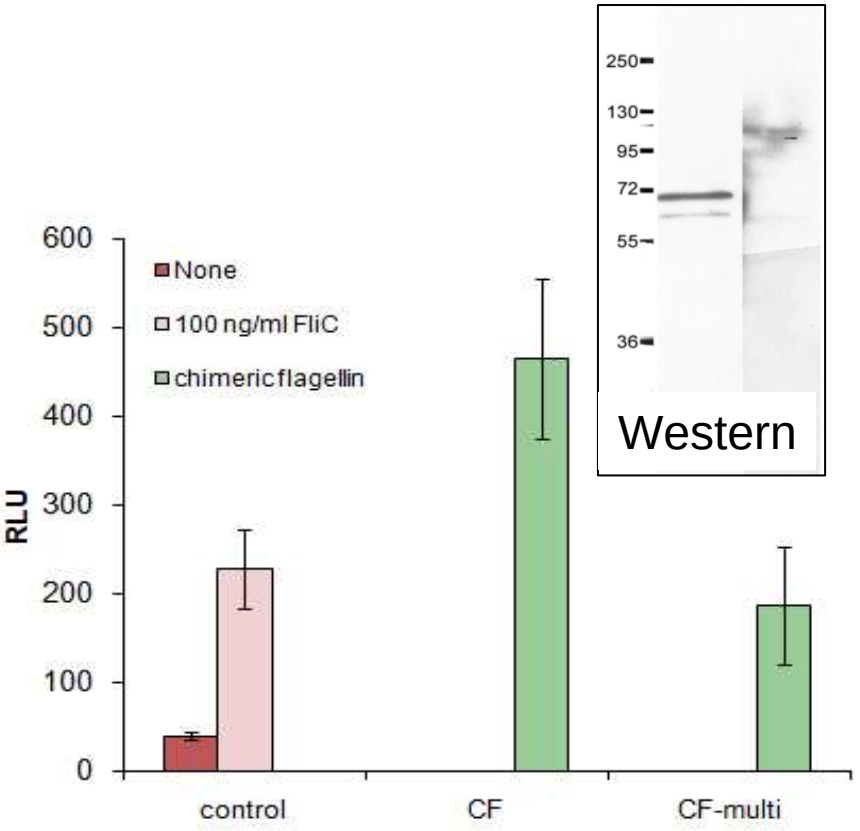
Protein vaccine



Isolated chimeric flagellin is correctly folded.

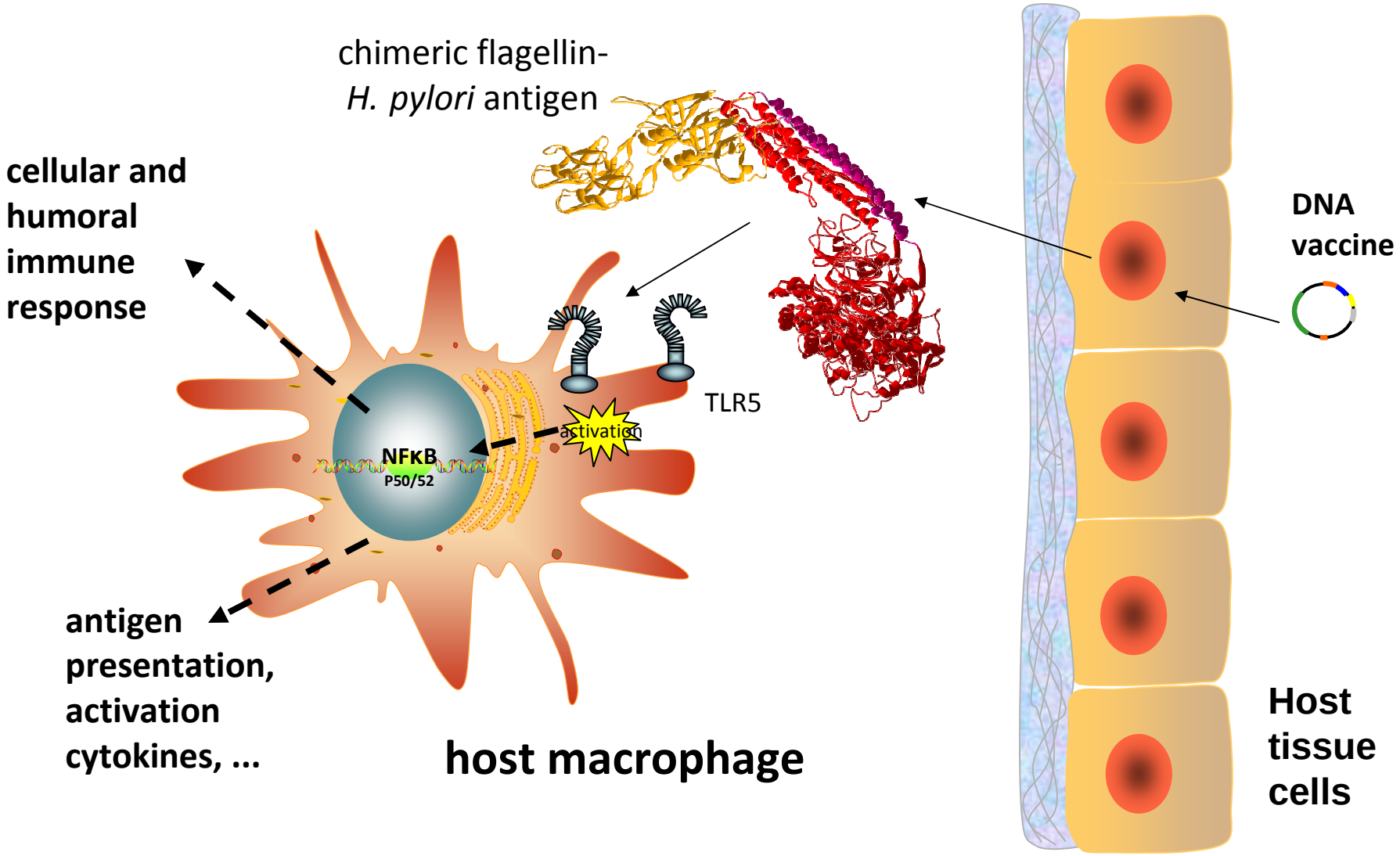


Cells internalize fluorescently labeled vaccine

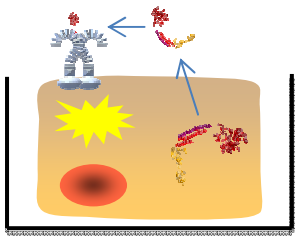


Isolated recombinant chimeric flagellin activates cells through TLR5.

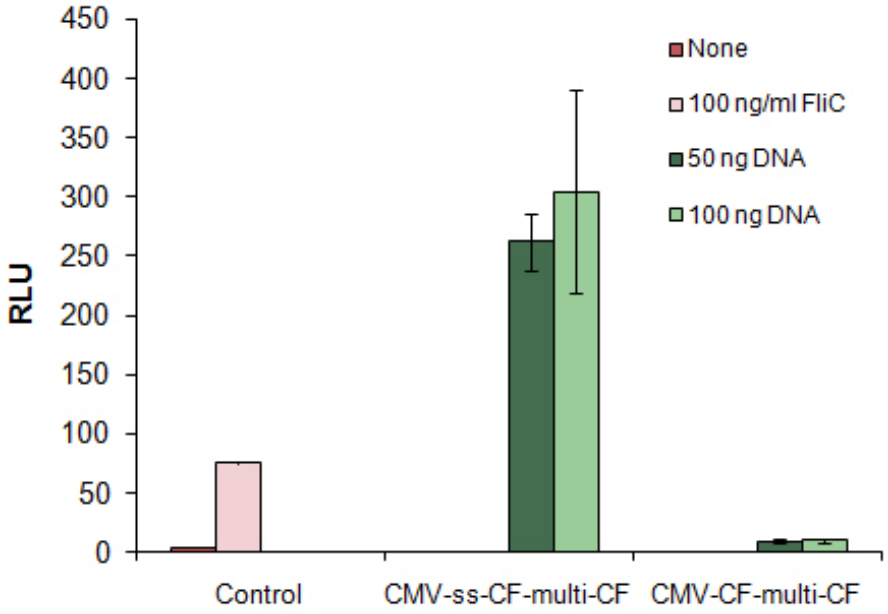
DNA vaccine


chimeric flagellin-
H. pylori antigen
cellular and humoral immune response
TLR5
activation
NFκB
P50/52
antigen presentation, activation cytokines, ...
host macrophage
DNA vaccine
Host tissue cells

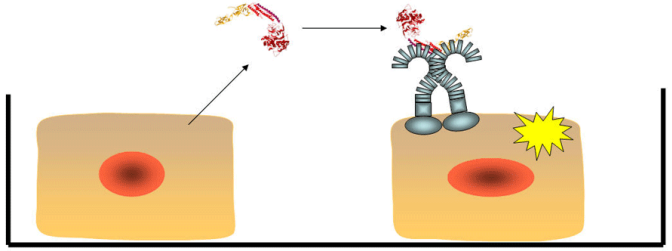
DNA vaccine

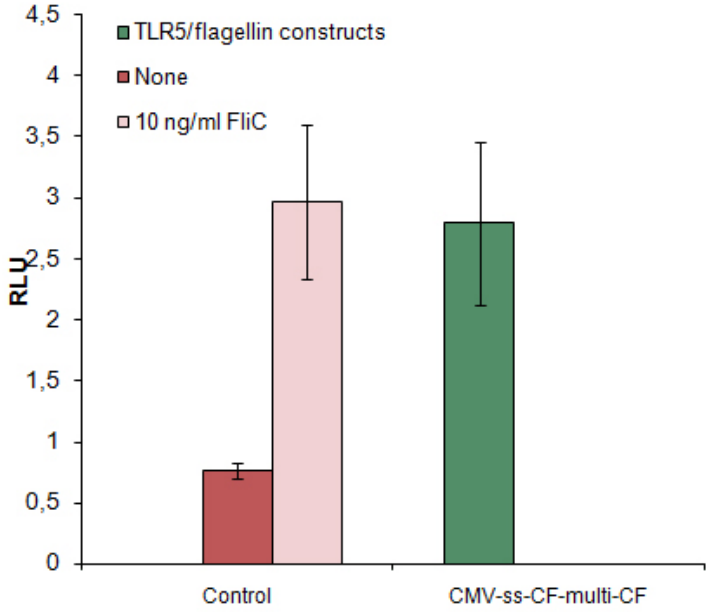


Cell, cotransfected with TLR5 and DNA vaccine



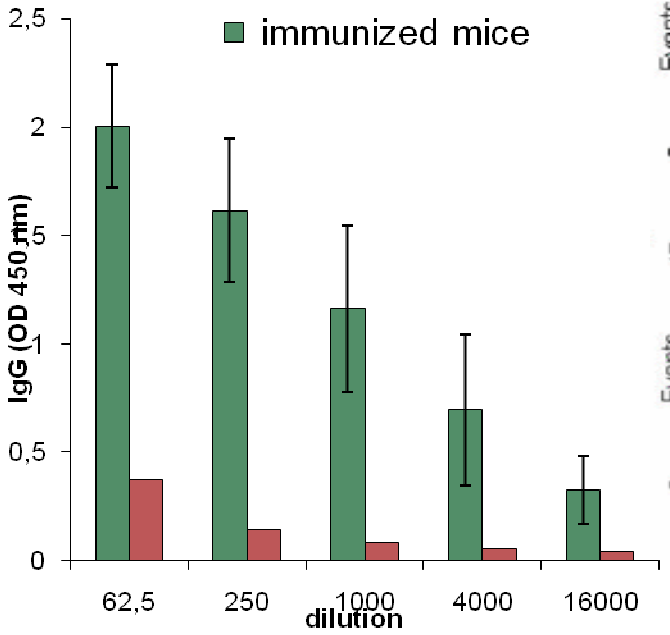
Chimeric flagellin needs to be secreted in order to activate cells with TLR5.



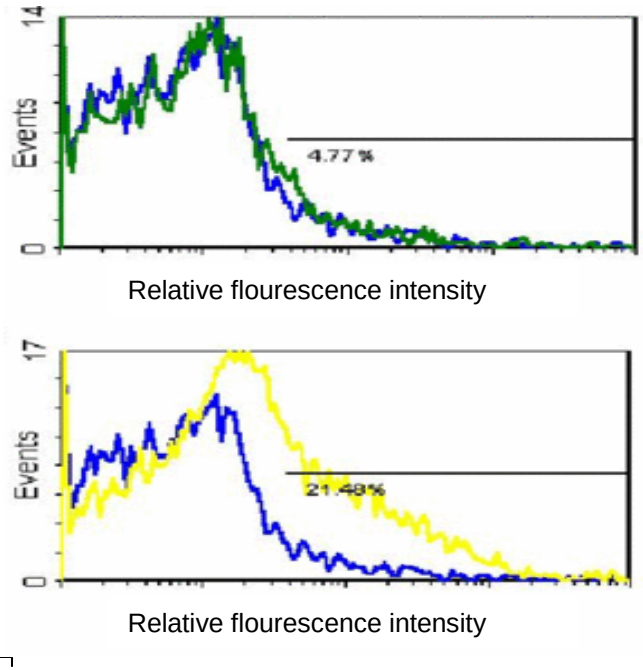


Transactivation of TLR5 by secreted chimeric flagellin.

Efficiency of the vaccine



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

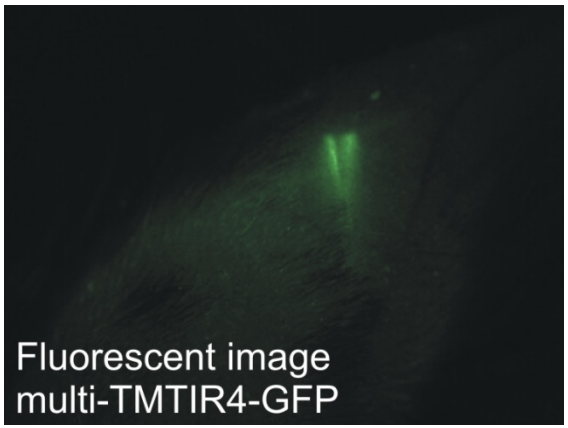


Immune serum against designed epitope (MULTI) recognizes live *H. pylori*

neg. control

Secondary Ab to *H. pylori*

CF-sera to *H. pylori*



Electroporated DNA vaccine is expressed in mouse leg

The future of Synthetic immunology

Combination of understanding of the molecular mechanisms and tools of synthetic biology opens exciting therapeutic potentials

Antiviral genetic device based on viral function can be applicable against several different viruses

Designed vaccines that activate both innate and adaptive immune response have great potentials against infection, as well as cancer and other diseases

Further potentials and developments of synthetic immunology include engineering of defined cell populations, tissues, targeted delivery etc.

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)