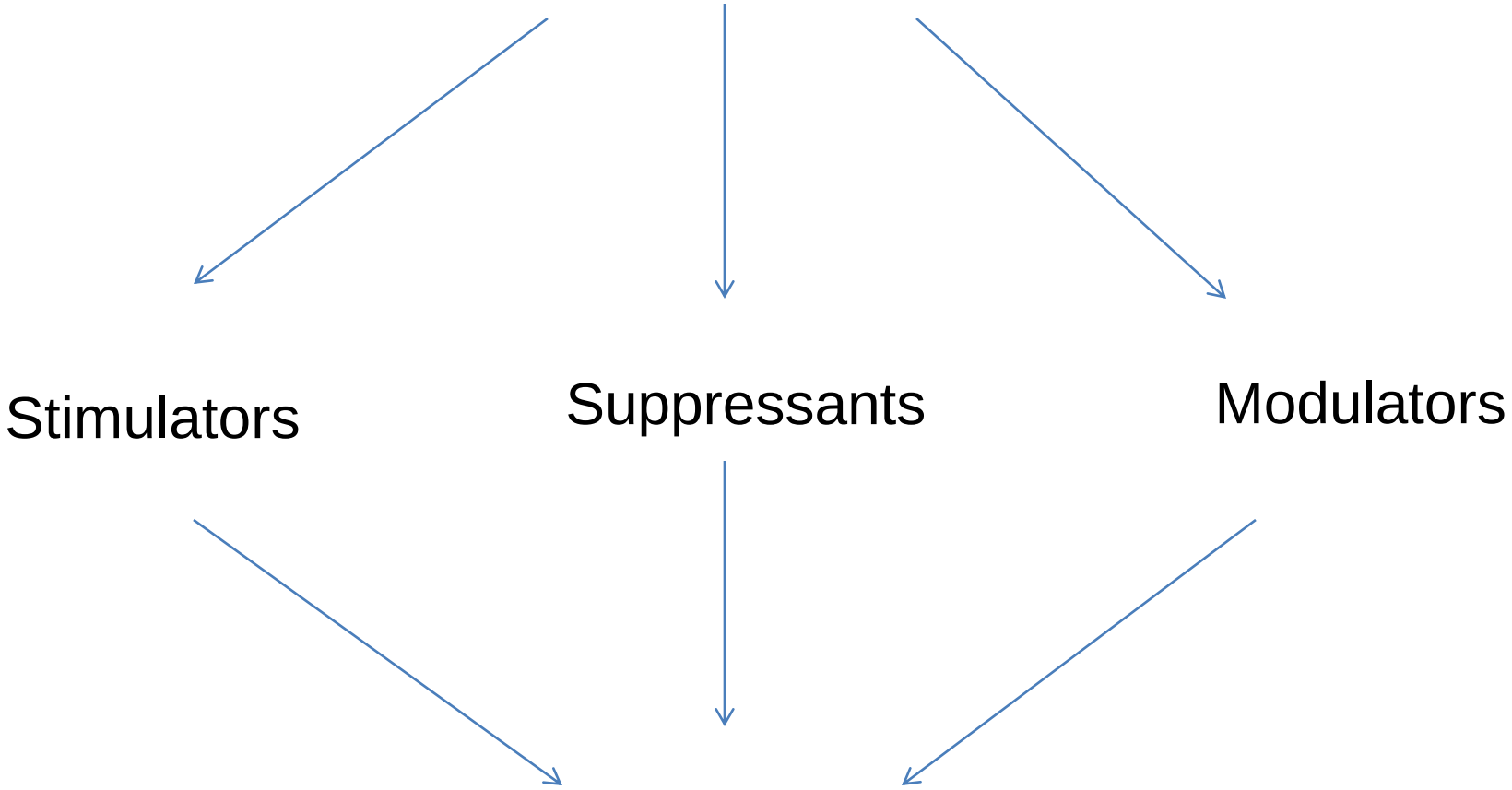
КАК ДА СЪЗДАДЕМ ВАКСИНА СРЕЩУ SARS-CoV-2

HOW TO CREATE A VACCINE AGAINST SARS-CoV-2

доц. д-р Андрей Чорбанов

Laboratory “Experimental Immunology”
Department of Immunology
Institute of Microbiology “Stefan Angelov”
Bulgarian Academy of Sciences

New drugs development



MODELS FOR TESTS

VACCINES

Live vaccines

Attenuated

Killed vaccines

Cellular

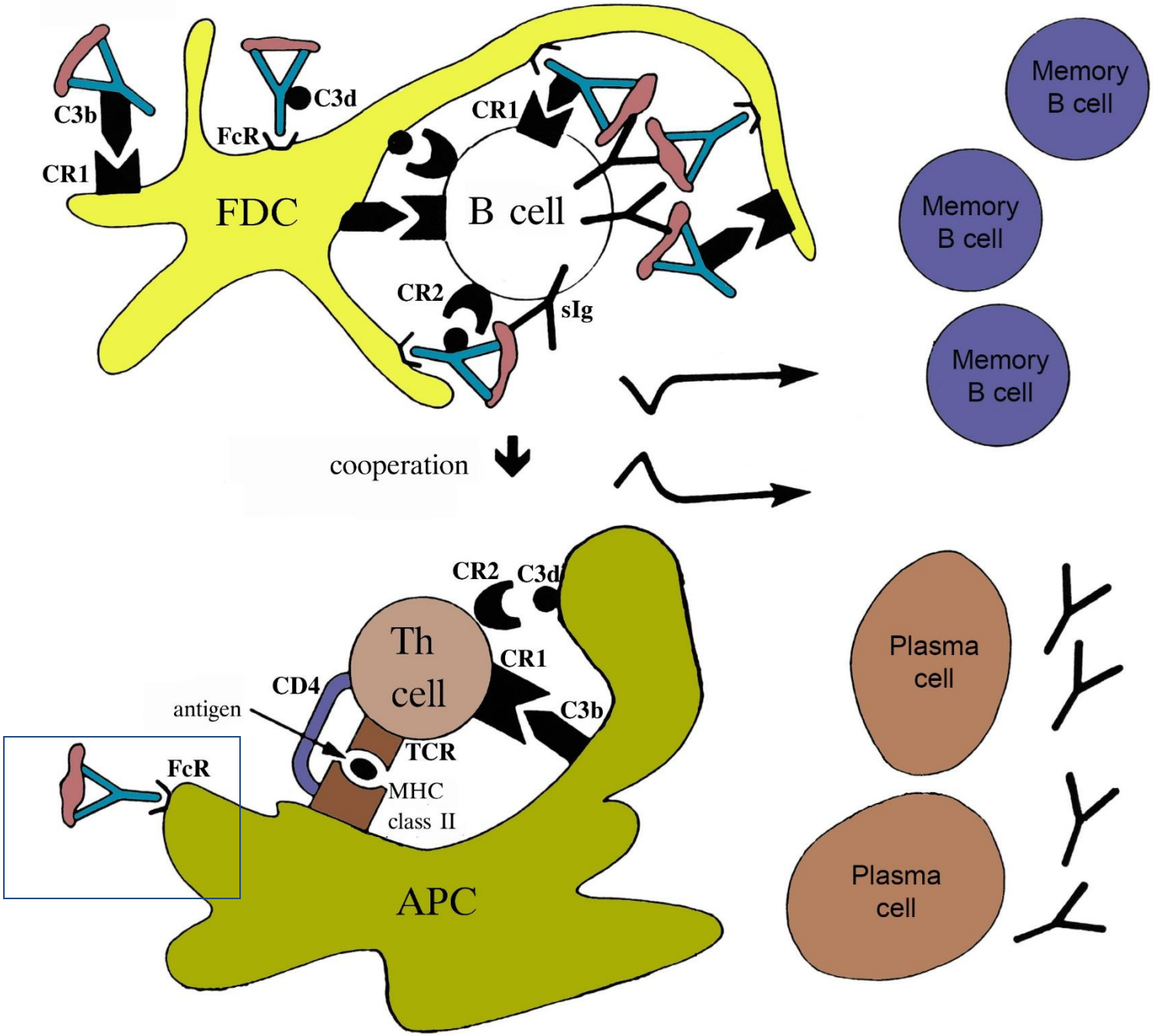
Subunit

DNA

Synthetic

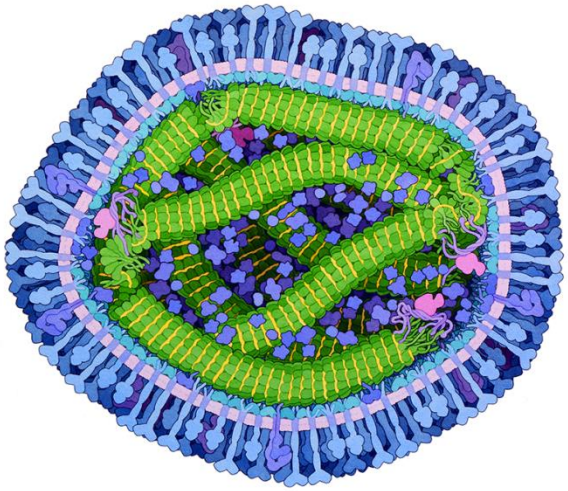
SUBUNIT VACCINES

Advantages	Disadvantages
No risks	Pure immunogenicity
Small size	Humoral answer only
Standard	Adjuvant inclusion

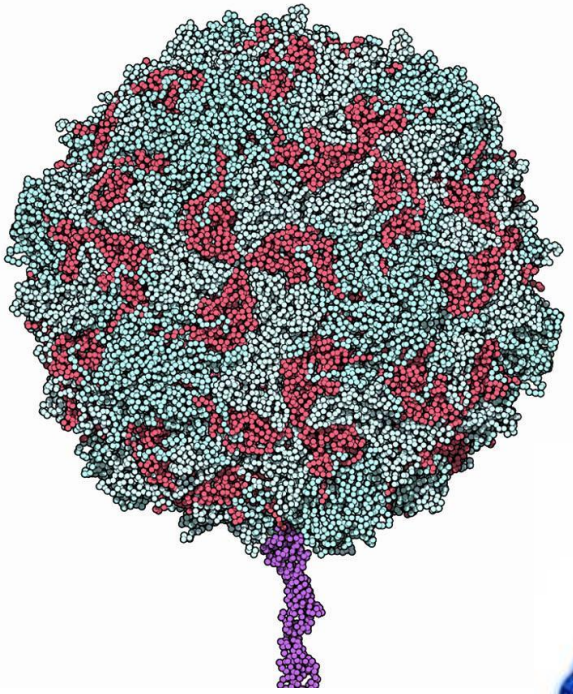

C3b
CR1
FcR
C3d
FDC
CR1
B cell
CR2
sIg
Memory B cell
Memory B cell
Memory B cell
cooperation
CR2
C3d
Th cell
CR1
CD4
antigen
C3b
TCR
FcR
MHC class II
APC
Plasma cell
Plasma cell

The perfect viruses … for vaccine design

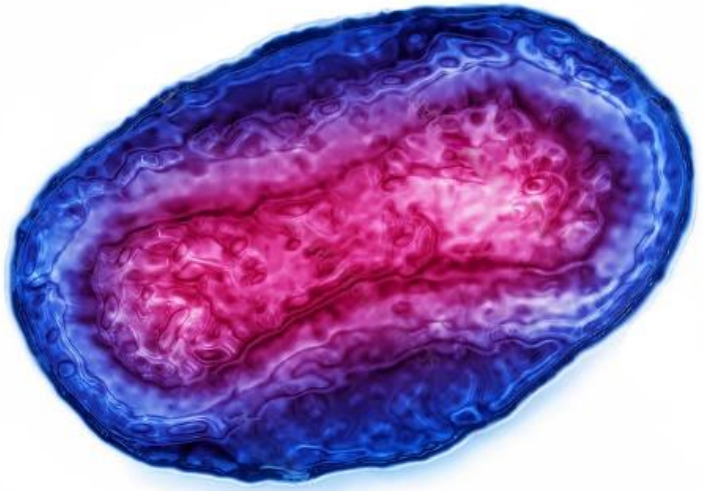
Measles



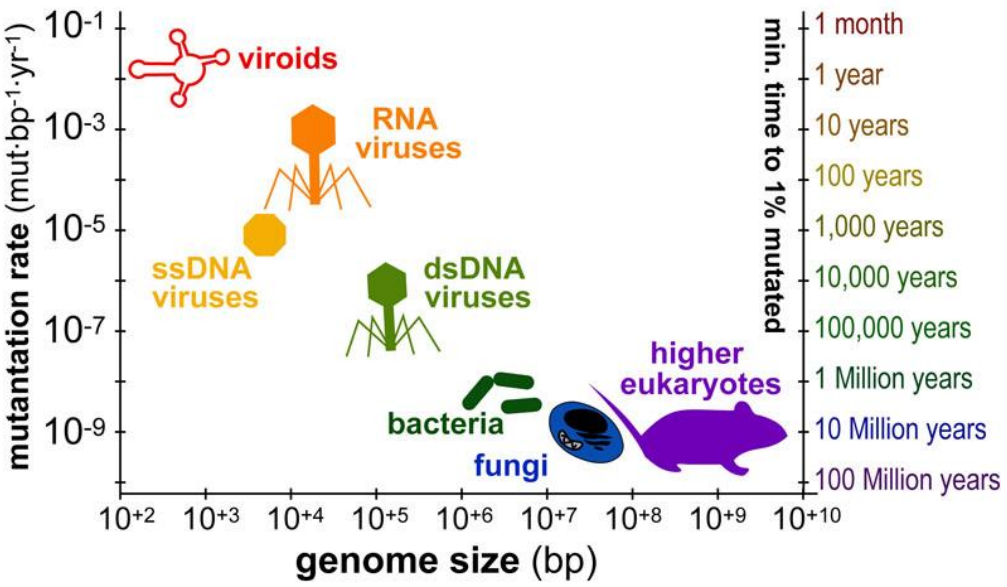
Polio

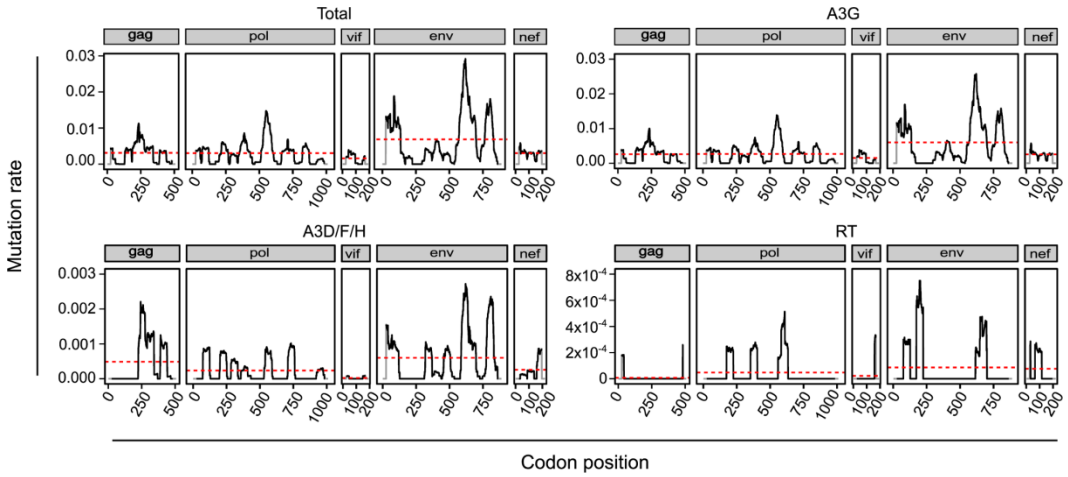


Pox



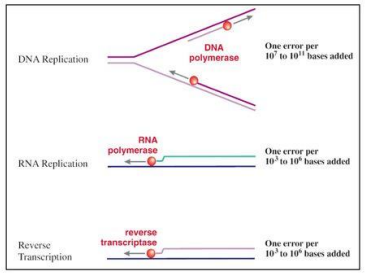
Mutation rate





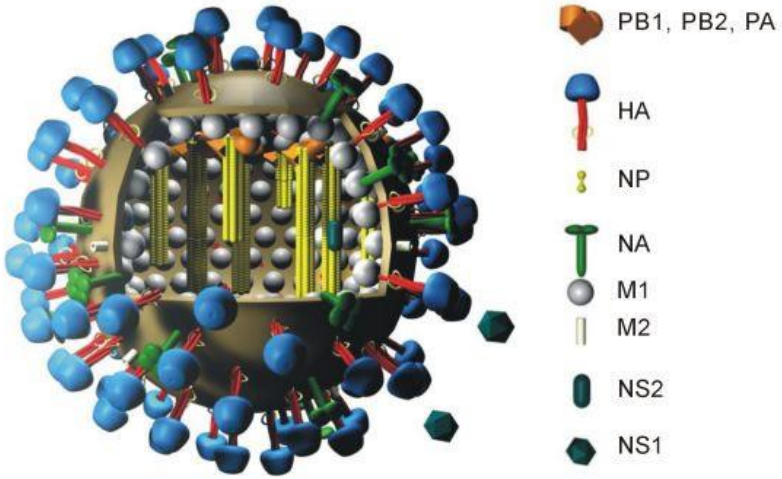
Evolution requires mutation: **High mutation rates (genome replication is inaccurate)**

- Mutations occur when nucleic acids are copied (i.e. genome replication).
- Error rate of human DNA polymerase is approximately 10^{-9} (3 mutations per replication of the human genome).
- Error correction machinery lowers this to 10^{-11}
- Virus RNA and DNA polymerases are much more error prone.
  - –RNA dependent RNA pol: $10^{-3} – 10^{-6}$
  - –DNA polymerases: $10^{-6} – 10^{-7}$



Vaccines:

Influenza Virus Structure:



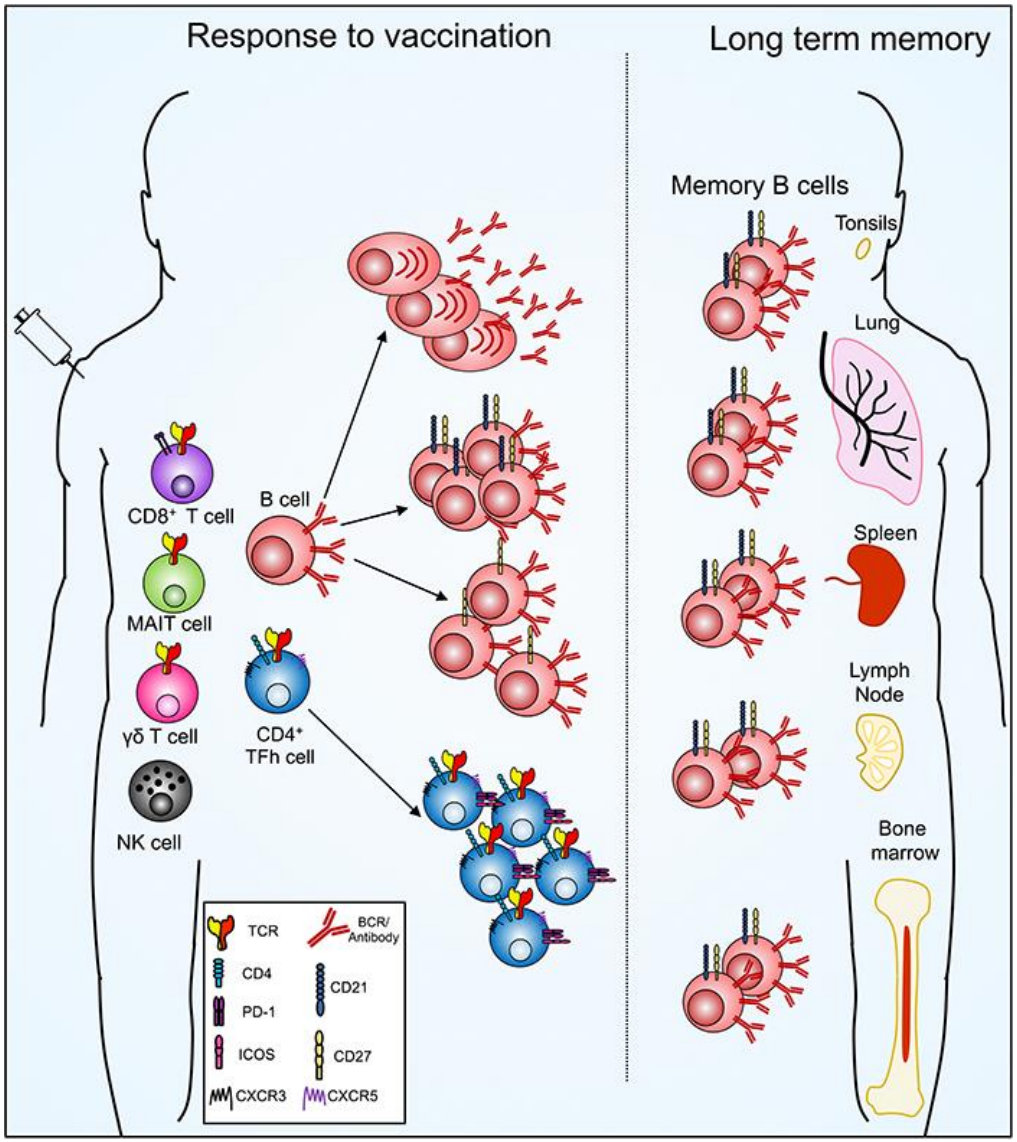
Structure of an influenza A virus. Image copyright by Dr. Markus Eickmann, Institute for Virology, Marburg, Germany.

1. **Conventional vaccines:** Induce mainly a humoral response

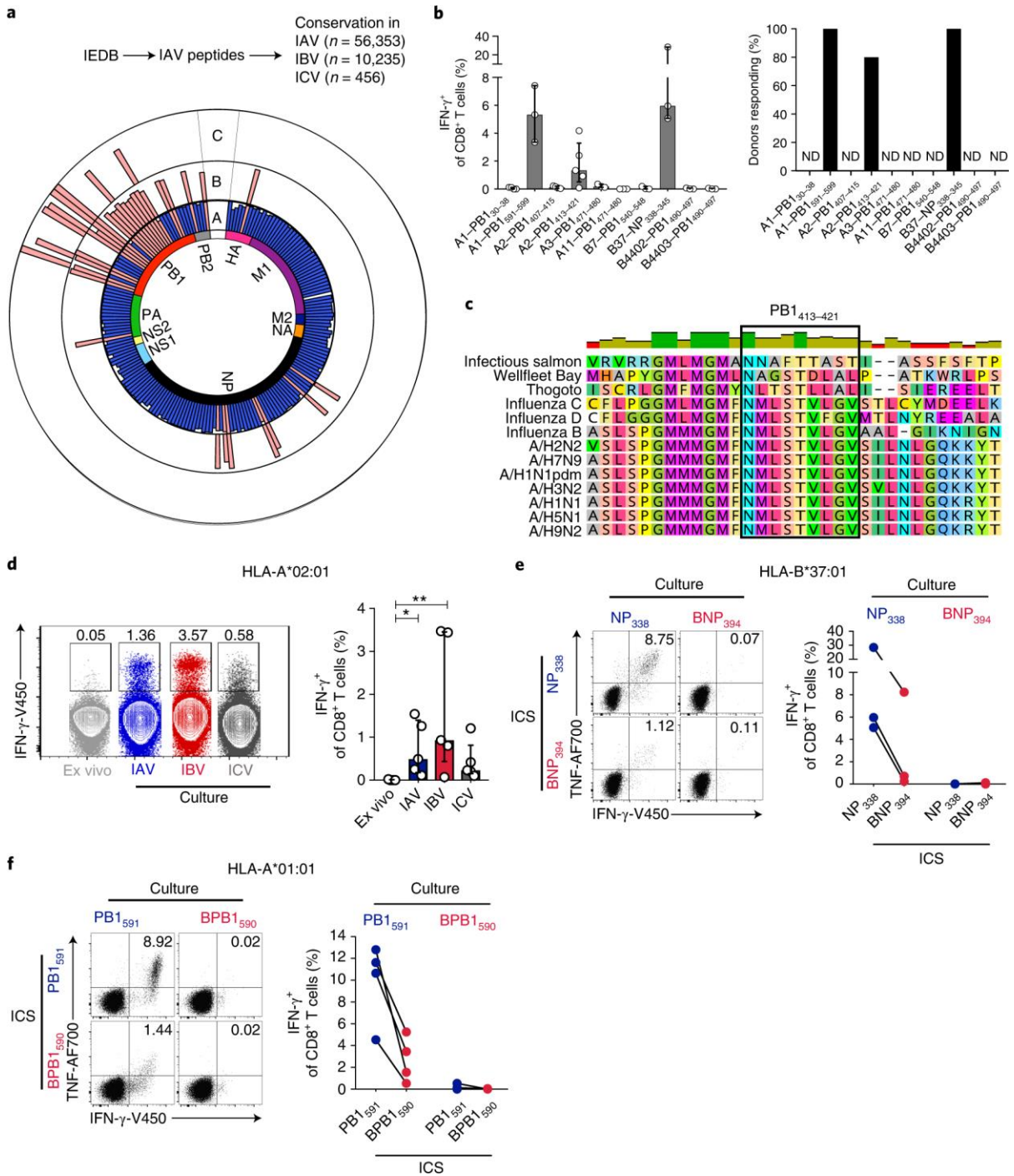
2. **DNA vaccines:**

• allow the processing, modification and presentation of the antigen to the host's immune system in a manner similar to that which would occur during a natural infection

•can induce long-lasting immune responses and is a highly efficient method for inducing cytotoxic lymphocytes (CTLs)



Koutsakos, M. et al. *Sci Transl Med* 2018 Feb 14; **10**(428). pii: eaan8405. doi: 10.1126/scitranslmed.aan8405.



Influenza A, B and C viruses (IAV, IBV and ICV, respectively) circulate globally and infect humans, with IAV and IBV causing the most severe disease.

$CD8^+$ T cells confer cross-protection against IAV strains, however the responses of $CD8^+$ T cells to IBV and ICV are understudied. The breadth of $CD8^+$ T cell cross-recognition and provide evidence of $CD8^+$ T cell cross-reactivity across IAV, IBV and ICV were investigated.

We identified The immunodominant $CD8^+$ T cell epitopes from IBVs that were protective in mice and found memory $CD8^+$ T cells directed against universal and influenza-virus-type-specific epitopes in the blood and lungs of healthy humans were identified.

Lung-derived $CD8^+$ T cells displayed tissue-resident memory phenotypes. Notably, $CD38^+Ki67^+CD8^+$ effector T cells directed against novel epitopes were readily detected in IAV- or IBV-infected pediatric and adult subjects.

The study introduces a new paradigm whereby $CD8^+$ T cells confer unprecedented cross-reactivity across all influenza viruses, a key finding for the design of universal vaccines.

Koutsakos, M. et al. *Nature Immunology,* **20**,613–625 (2019)

Primate models

Nonhuman primates have been used for biomedical research for several decades. The high level of genetic homology to humans coupled with their outbred nature has made nonhuman primates invaluable preclinical models.

Restrictions:
1. Ethical
2. Price
3. Local conditions
4. Others …

Table 1 - Non-human primates used as animal models for studies on hepatitis virus infection.

HAV, Hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HDV, hepatitis D virus; HEV, hepatitis E virus.

Hepatitis virus	Non-human primates		References
	Common names	Species	
HAV	Chimpanzee	*Pan troglodytes*	10,46,51,54
	Rhesus monkey	*Macaca mulatta*	36
	African green monkey	*Cercopithecus aethiops*	46
	Owl monkey	*Aotus trivirgatus*	39,41,52
	Tamarin	*Saguinus* sp	7,9,42,47,53
	Marmoset	*Callithrix jacchus*	33,34,38
	Squirrel monkey	*Saimiri sciureus*	31,35
HBV	Chimpanzee	*Pan troglodytes*	82,91,93,94
	Baboon	*Papio* sp	77
	Tree shrew	*Tupaia* sp	83
		Macaca assamensis	84
HCV	Chimpanzee	*Pan troglodytes*	13-15,97,102, 109-112
	Rhesus monkey	*Macaca mulatta*	120,121
HDV	Chimpanzee	*Pan troglodytes*	23,123,125,129
HEV	Chimpanzee	*Pan troglodytes*	61,72
	Rhesus monkey	*Macaca mulatta*	61,65-68
	African green monkey	*Cercopithecus aethiops*	61
	Owl monkey	*Aotus trivirgatus*	60,61,72
	Tamarin	*Saguinus* sp	59,61,72
	Squirrel monkey	*Saimiri sciureus*	61

Brazilian Journal of Medical and Biological Research
On-line version **ISSN 1414-431X**

Mouse models

Of Mice and Not Men: Differences between Mouse and Human Immunology

Mice are the experimental tool of choice for the majority of immunologists and the study of their immune responses has yielded tremendous insight into the workings of the human immune system. The mouse is the most commonly used animal for modelling human disease. New approaches for generating genetically manipulated mouse models to represent human disease, as well as target the function of specific genes, has increased the importance of mice in biomedical science. However, as 65 million years of evolution might suggest, there are significant differences. Such differences should be taken into account when using mice as preclinical models of human disease. *The Journal of Immunology, 2004,* 172: 2731–2738.

Wild type (WT) mice
BALB/c
C57BL6
C3HeB
FVB/N

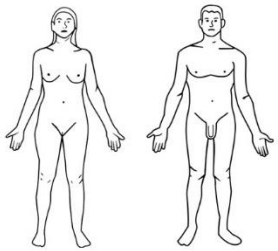
Humanized NSG Mouse model of SLE 2

NSG, or **NOD scid gamma** (NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ)

Immunodeficient NOD SCID gamma mice (NSG mice) mice tolerate normal human lymphoid cells due to a mutation affecting their recombinase system and resulting in mature T, B and NK-cell deficiency leading to absence of adaptive immune response. NSG mice are perfect recipients and upon reconstitution with human cells the humanized animals are a suitable model to investigate the efficiency of therapeutic interventions.

СЪЗДАВАНЕ НА ХУМАНИЗИРАН МОДЕЛ

SPF (Specific pathogen free) animal house



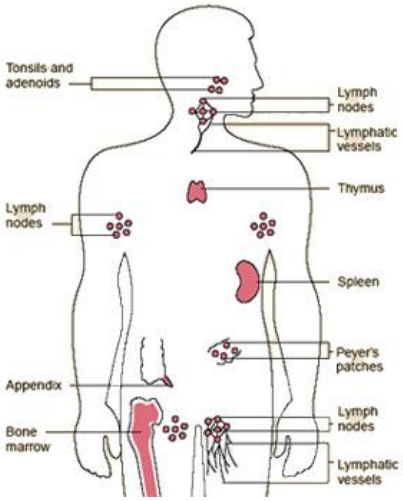
Healthy donors

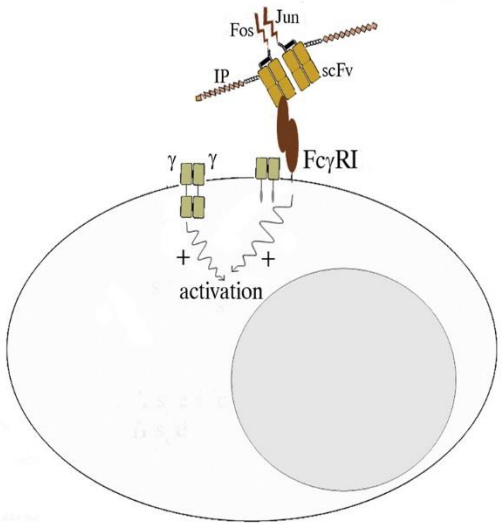
Tonsils

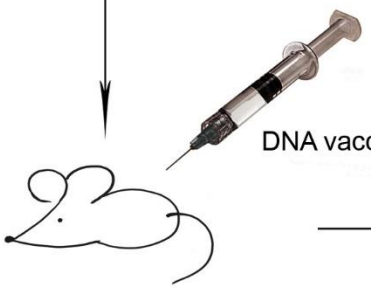
Human tonsil cells isolation

Cell transfer

$5x10^7$ cells







DNA vaccine

NSG mice

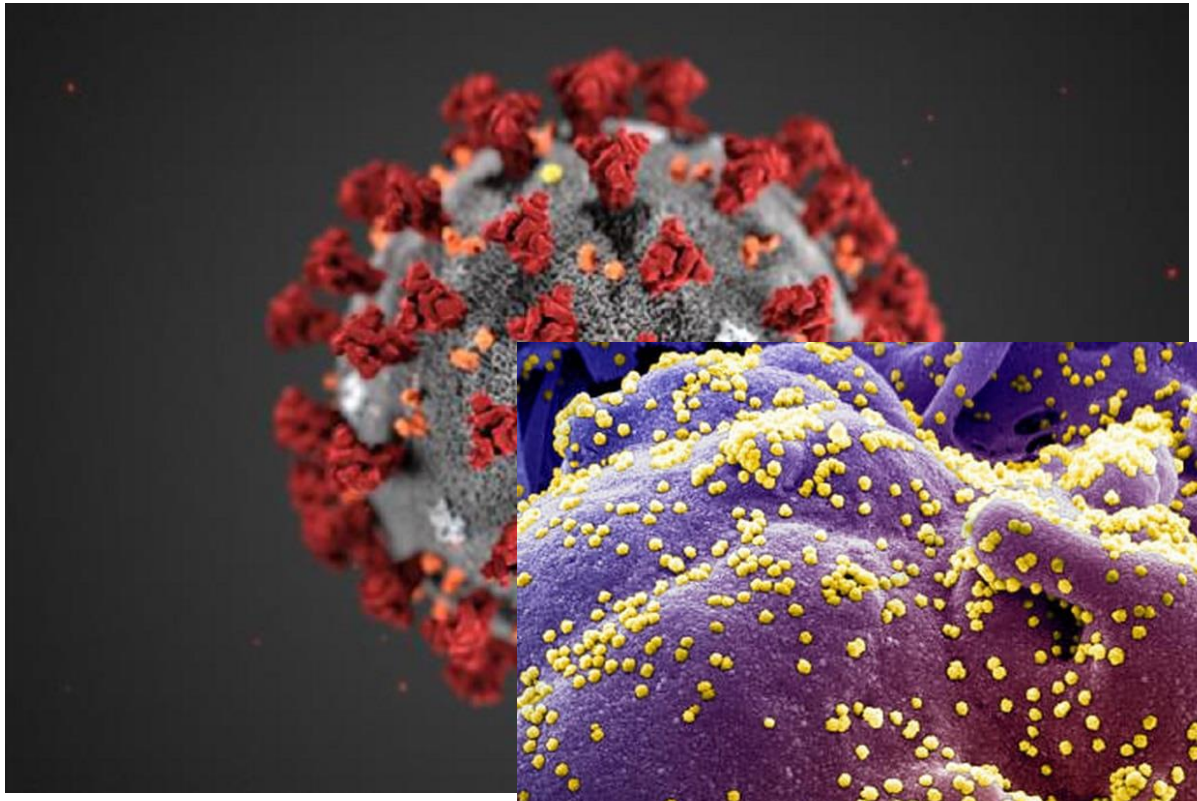
10 weeks

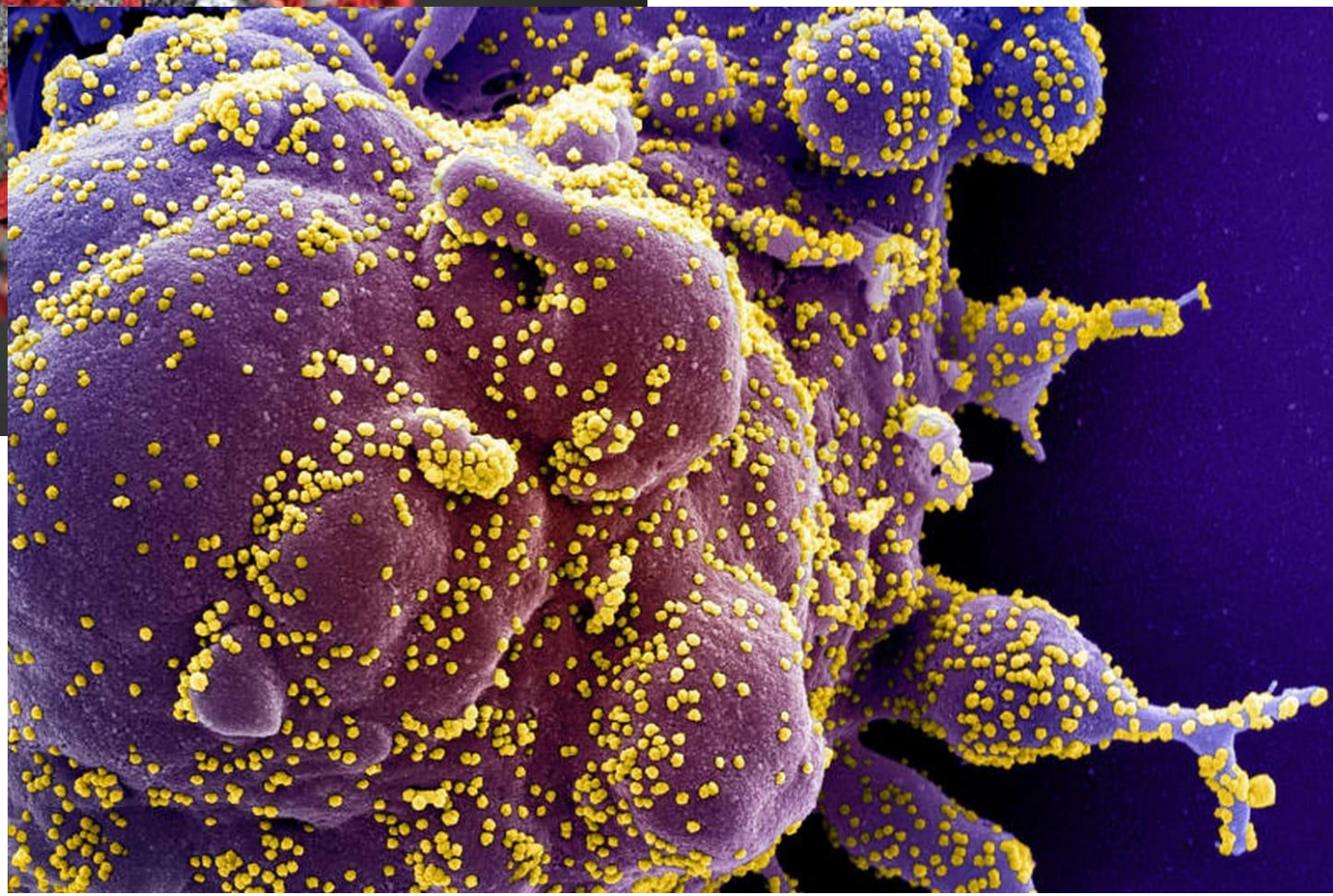
NSG mice

Anti-influenza antibodies

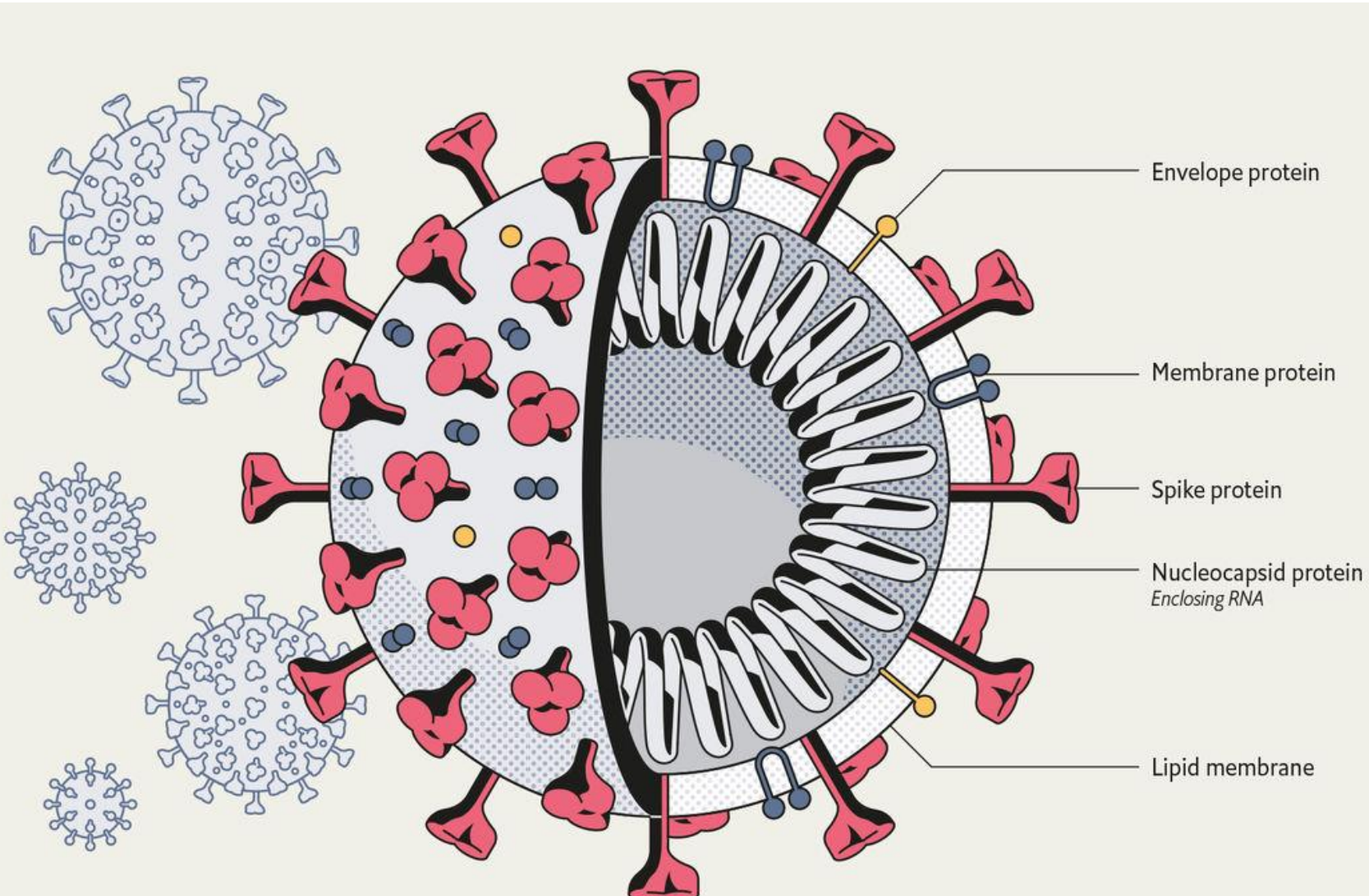
Anti-influenza cytotoxic T cells

SARS-CoV-2

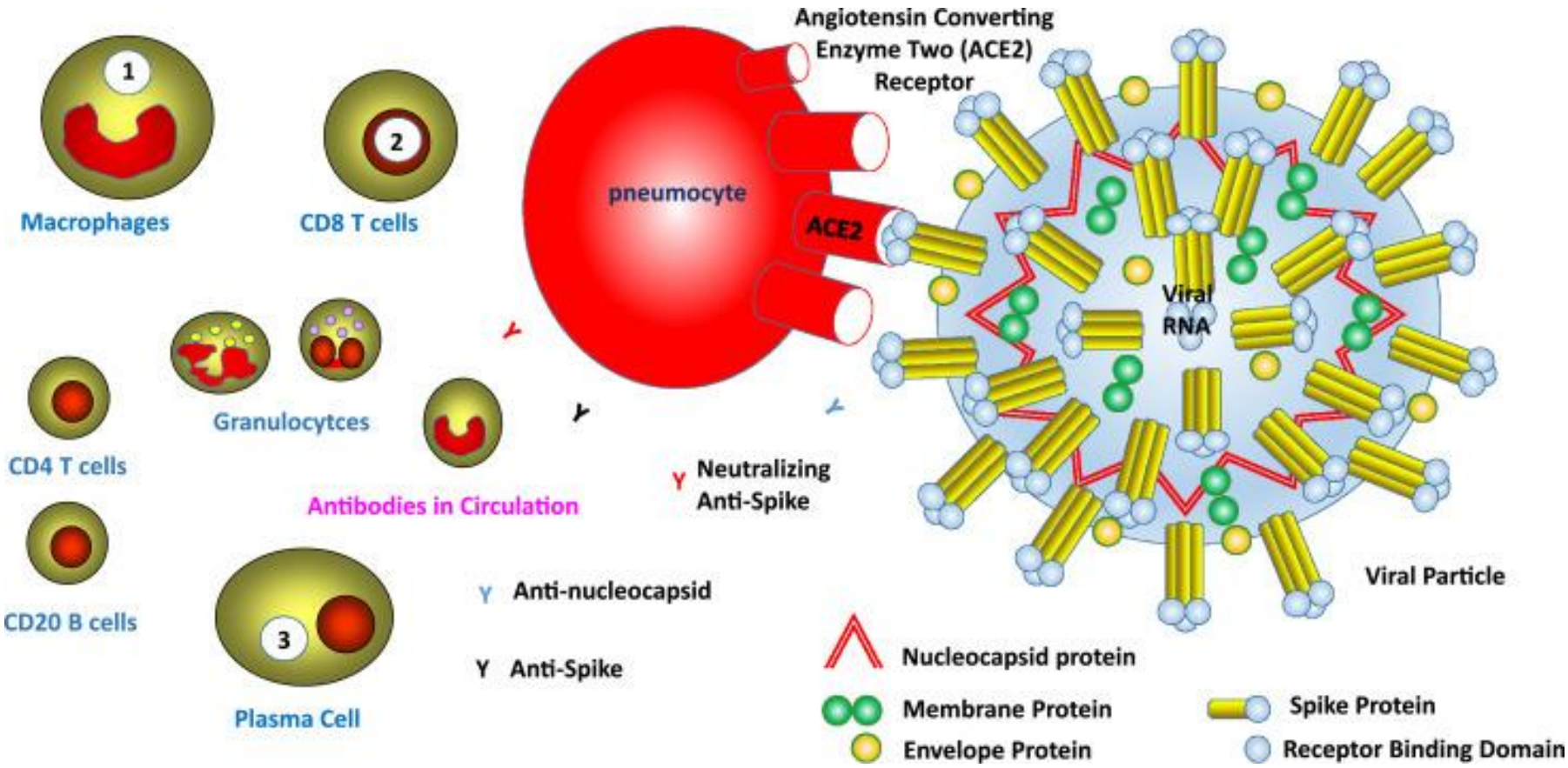




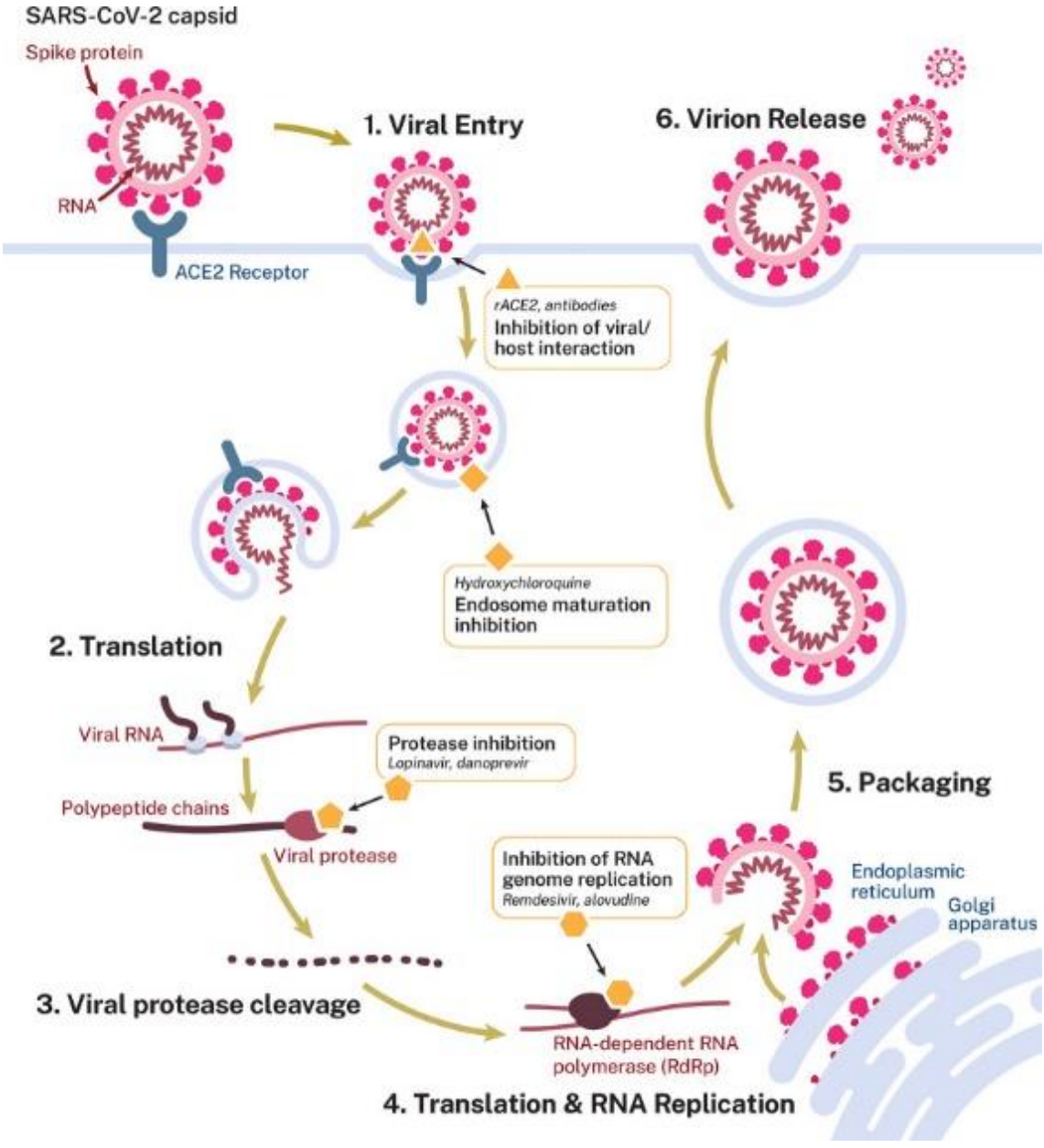
SARS-CoV-2 structure



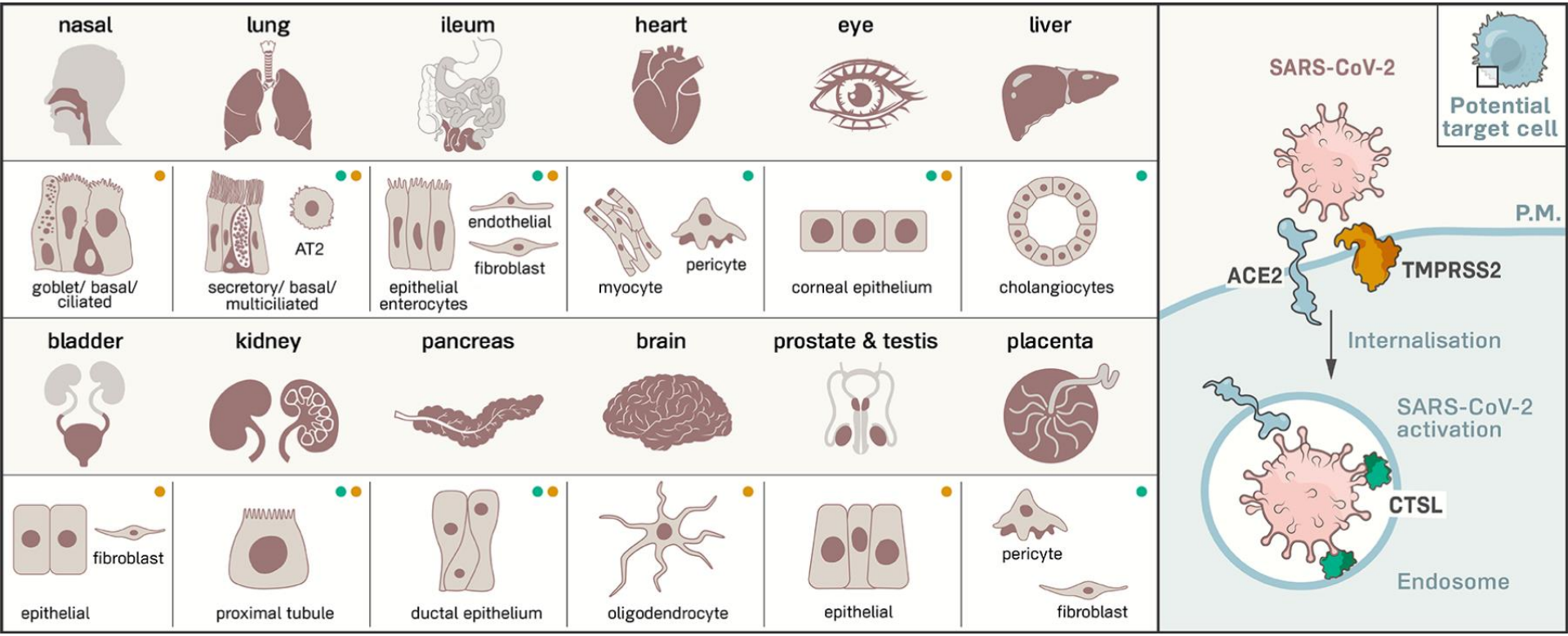
Virus penetration into the host cells



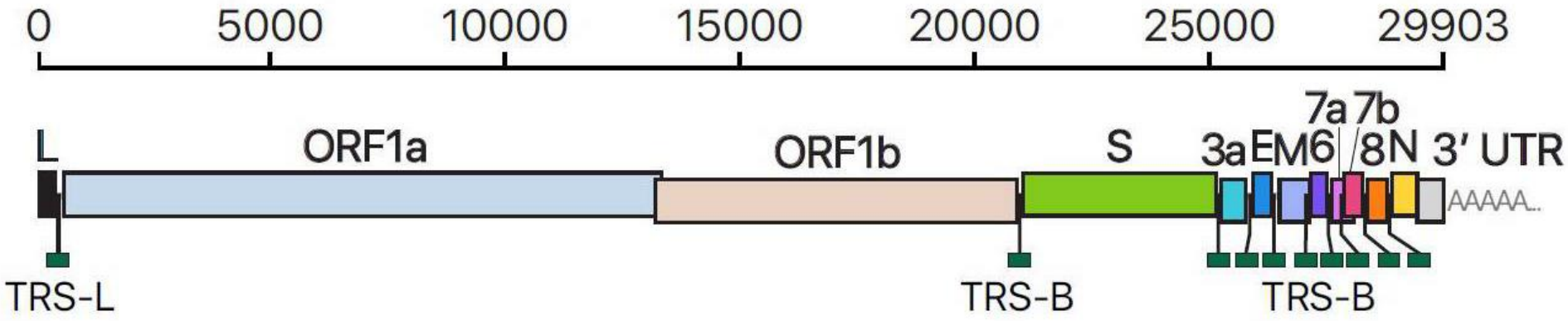
Virus penetration into the host cells

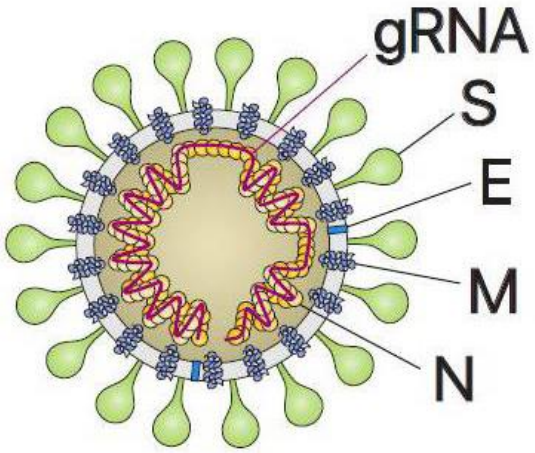


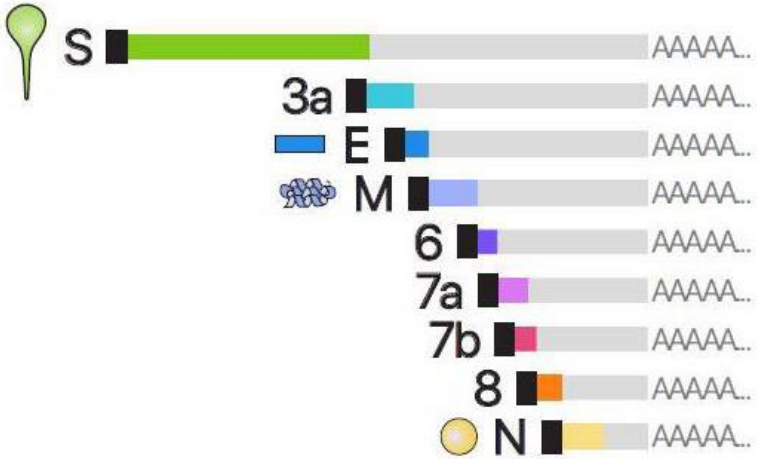
Potential SARS-CoV-2 targets



Prediction of SARS-CoV-2 epitopes

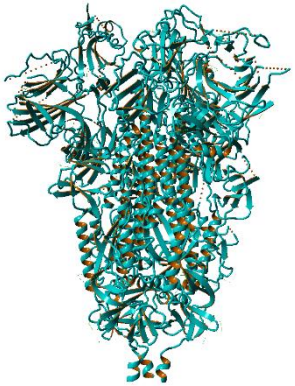




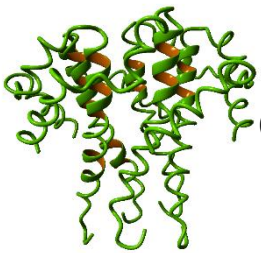


N-protein
9 epitopes

M-protein
15 epitopes



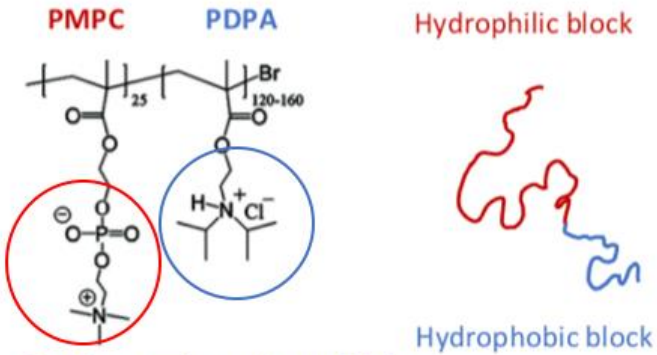
S-protein
34
epitopess



E-protein
17
epitopes

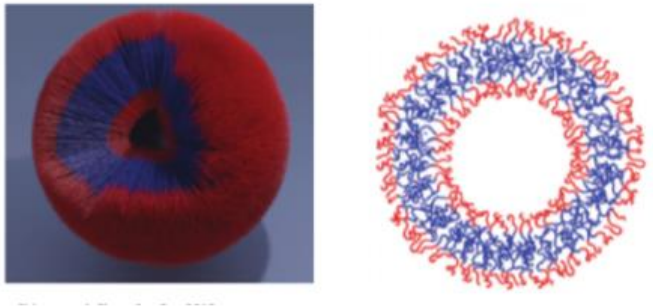
Self-assembly process PMPC-PDPA POs

Block copolymer



Self-assembly
at pH ≥6-6,3

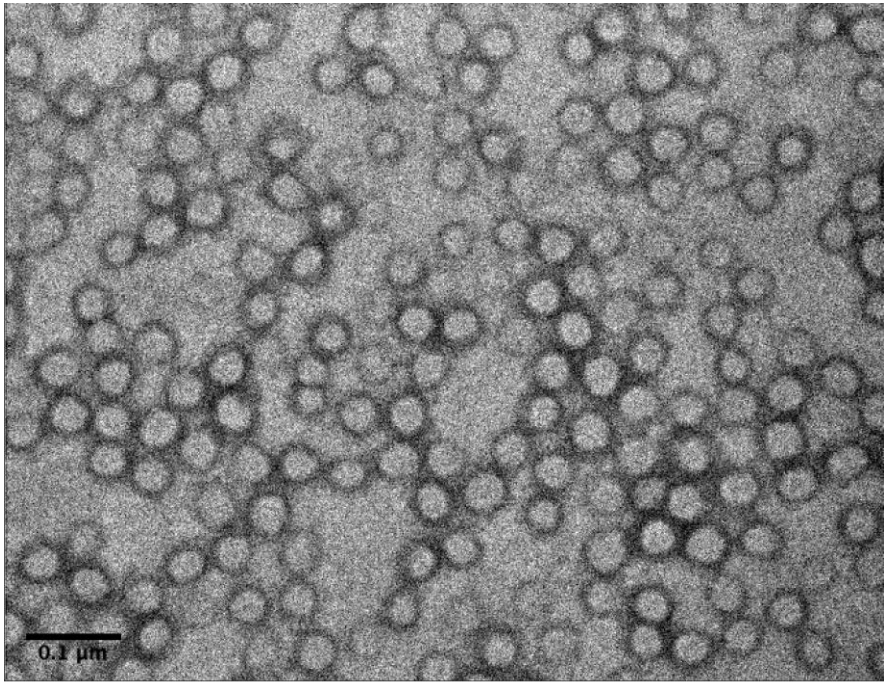
Polymersomes

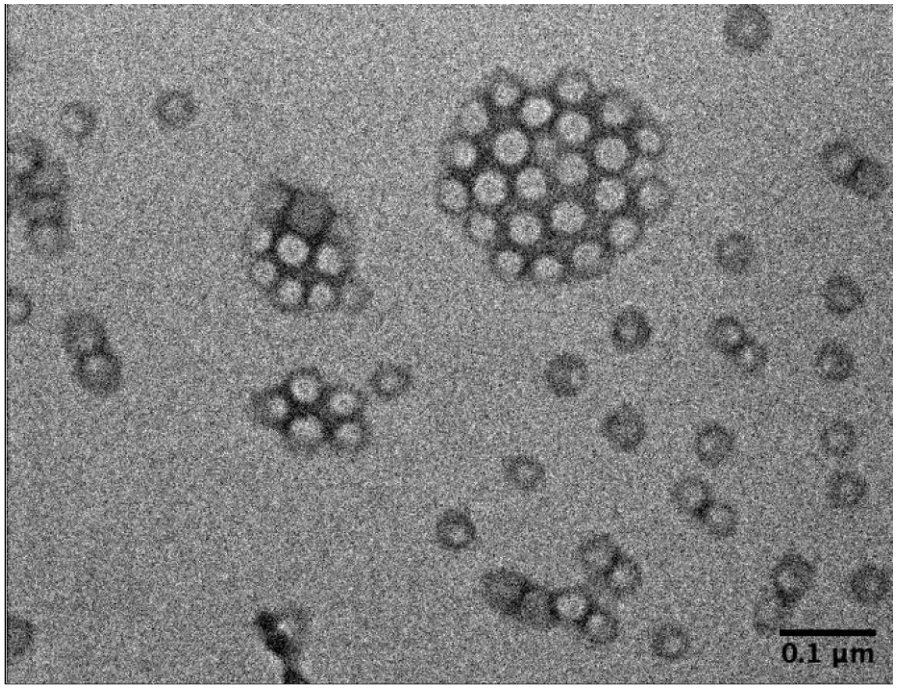


Rideau et al, Chem Soc Rev 2018

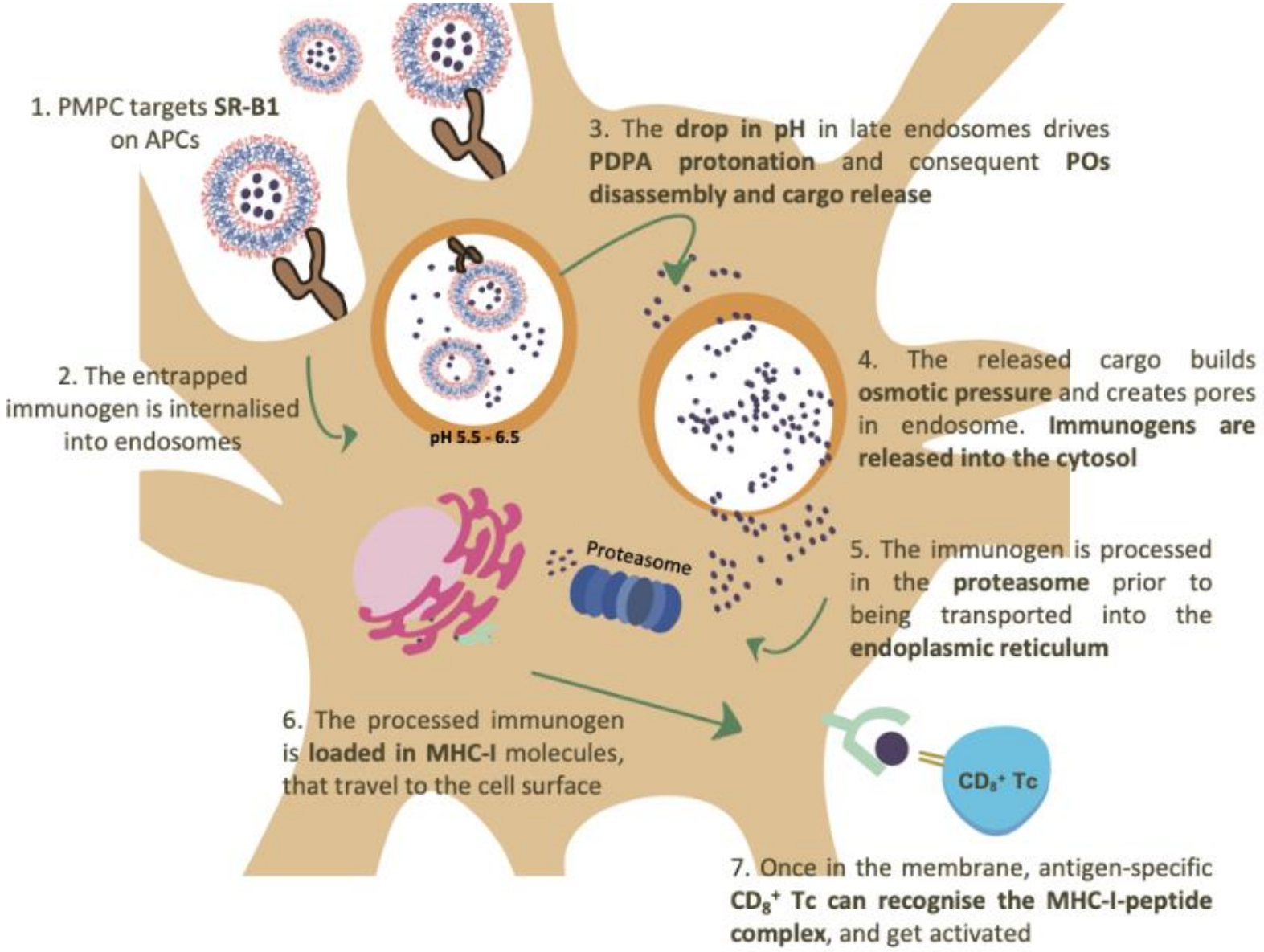
- Tertiary amine: pH sensitivity
- PC: Selective targeting in cells

Transmission Electron Microscopy images of PMPC-PDPA polymersomes (PTA staining)

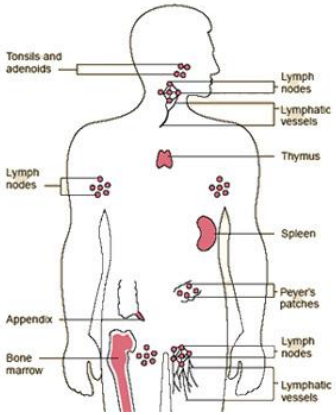




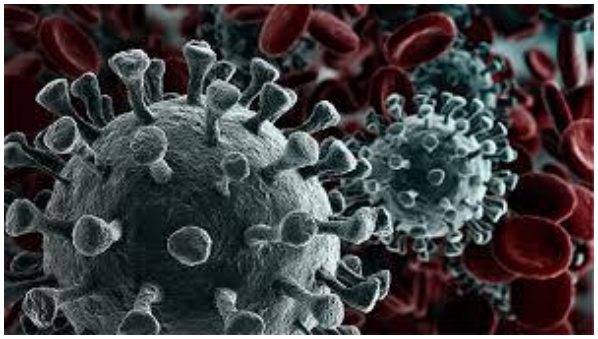
Schematic representation of PMPC-PDPA target of APC and antigen cross presentation

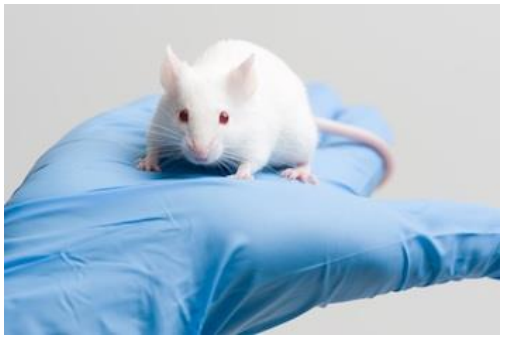


СЪЗДАВАНЕ НА ХУМАНИЗИРАН МОДЕЛ И ТЕРАПИЯ С ВИРУС-НАПОДЯВАЩИ ВАКСИНАЛНИ ЧАСТИЦИ



Human tonsil cells isolation





Cell transfer

$5x10^7$ cells

Virus-like particles vaccination

NSG mice

10 weeks

NSG mice

Anti-SARS-CoV-2 antibodies

Cytokines

Anti-SARS-CoV-2 cytotoxic T cells

Histology

THE TEAM

Bulgarian Academy of Sciences
Institute of Microbiology
Department of Immunology
Sofia, Bulgaria
Assoc. prof. Andrey Tchorbanov
Dr Nikolina Mihaylova
Dr Kalina Nikolova-Ganeva
Dr Petya Ganova
Dr Iliyan Manoylov
Silvya Bradyanova
Gabriela Boneva
Nikola Ralchev
Emiliya Stoyanova
Blagovesta Boneva
Alexandar Markovski

Department of Immunology
Eötvös Loránd University
Budapest, Hungary
Dr Jozsef Prechl

Faculty of Pharmacy,
Medical University, Sofia
Prof. Irini Doytchinova
Dr Ivan Dimitrov
Dr Mariyana Atanasova

University College London
London, GB
Prof Giuseppe Battaglia
Barbara Ibarzo Yus

http://labexpimm.eu
tchorban@microbio.bas.bg


ASTRO DONUT


1914
2014
СОФИЯ
100

БЪДЕТЕ НОРМАЛНИ, МАСКИРАЙТЕ СЕ С КАКВОТО ВИ ХАРЕСВА ...