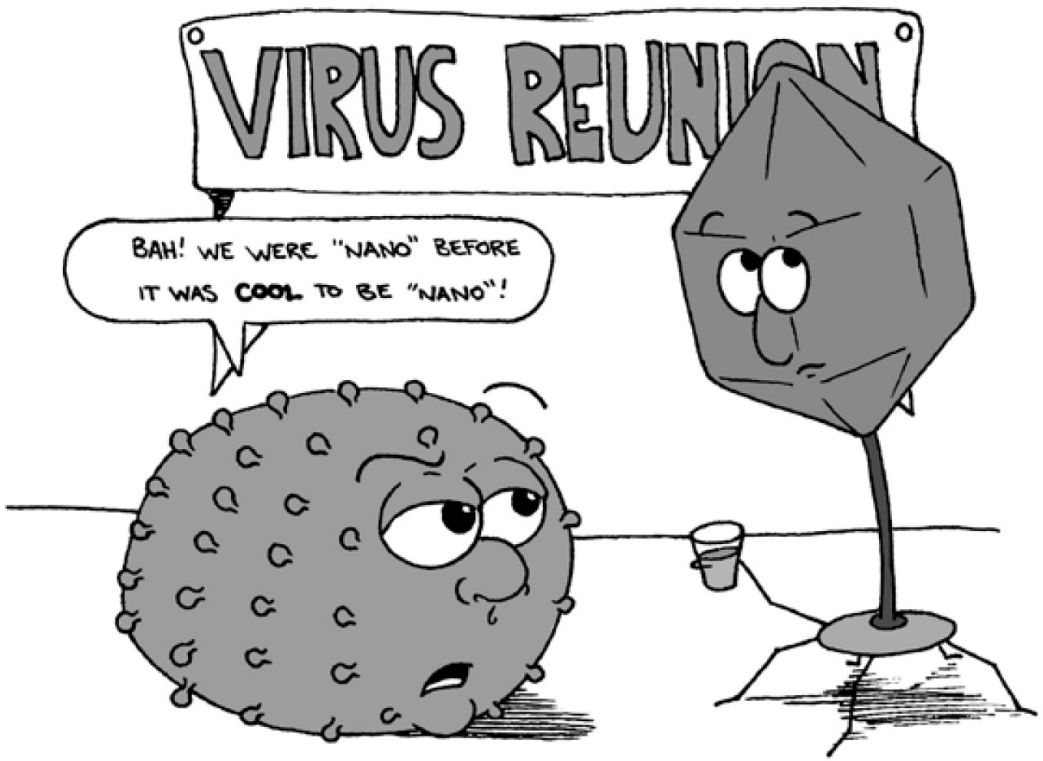
Origins, structure, and impact of viruses on the evolution of cellular life



Pavel Plevka

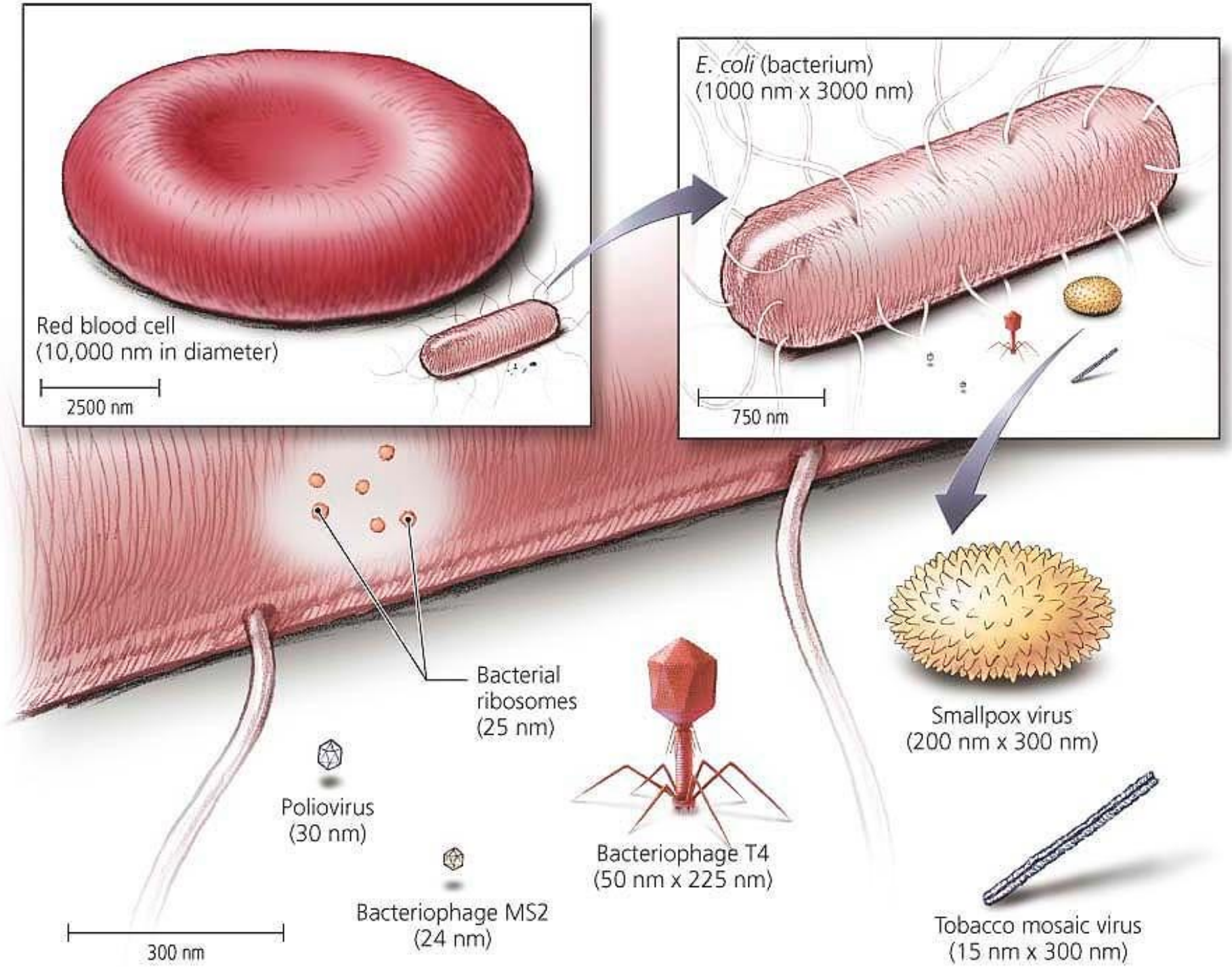
19.9. 2022

What is a virus?

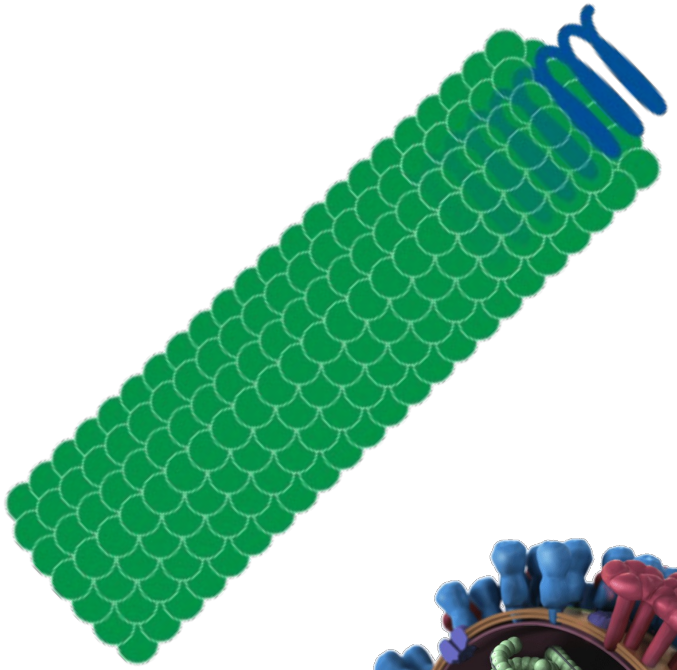
Origins of viruses

Virus structures and infection processes

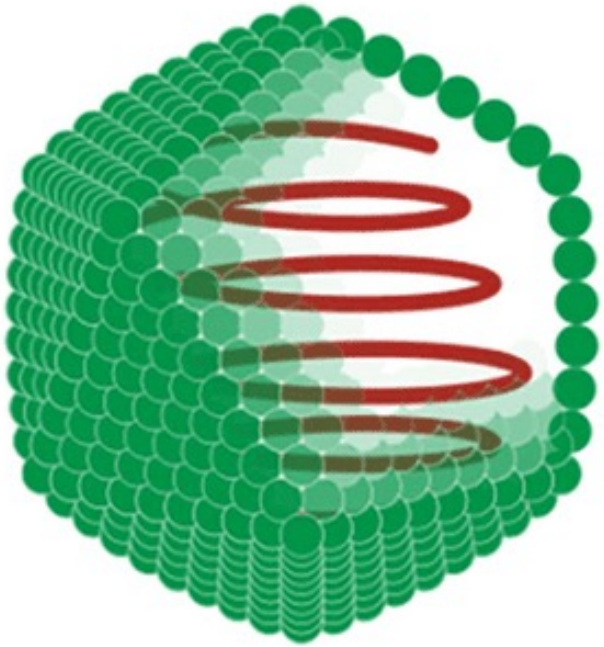
Extra


Red blood cell
(10,000 nm in diameter)
2500 nm
E. coli (bacterium)
(1000 nm x 3000 nm)
750 nm
Bacterial
ribosomes
(25 nm)
Smallpox virus
(200 nm x 300 nm)
Poliovirus
(30 nm)
Bacteriophage T4
(50 nm x 225 nm)
Bacteriophage MS2
(24 nm)
Tobacco mosaic virus
(15 nm x 300 nm)
300 nm

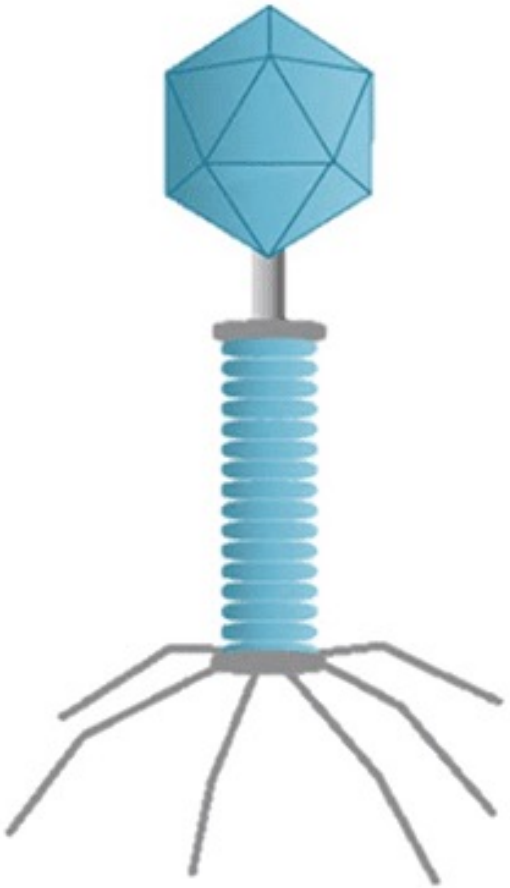
Helical

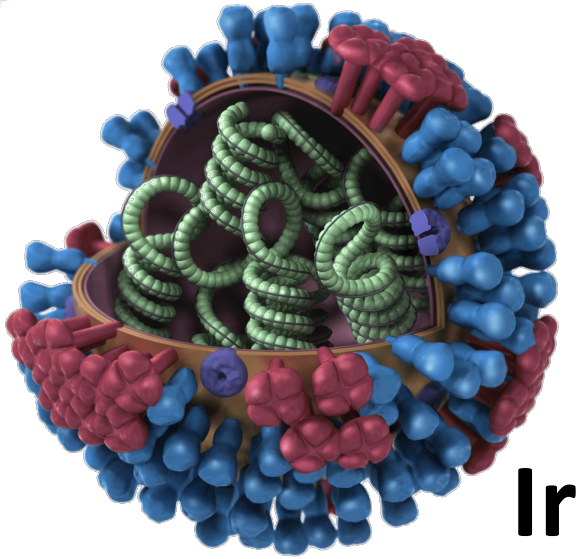


Icosahedral



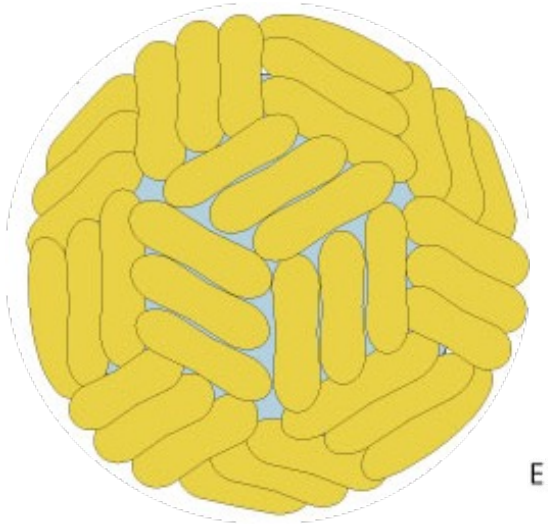
Complex

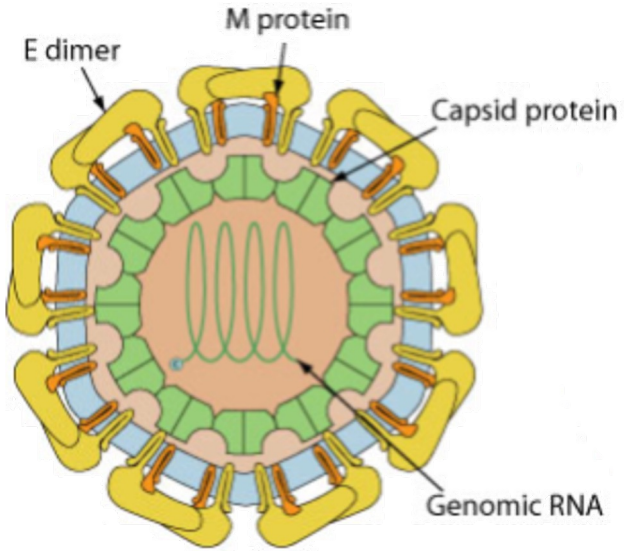




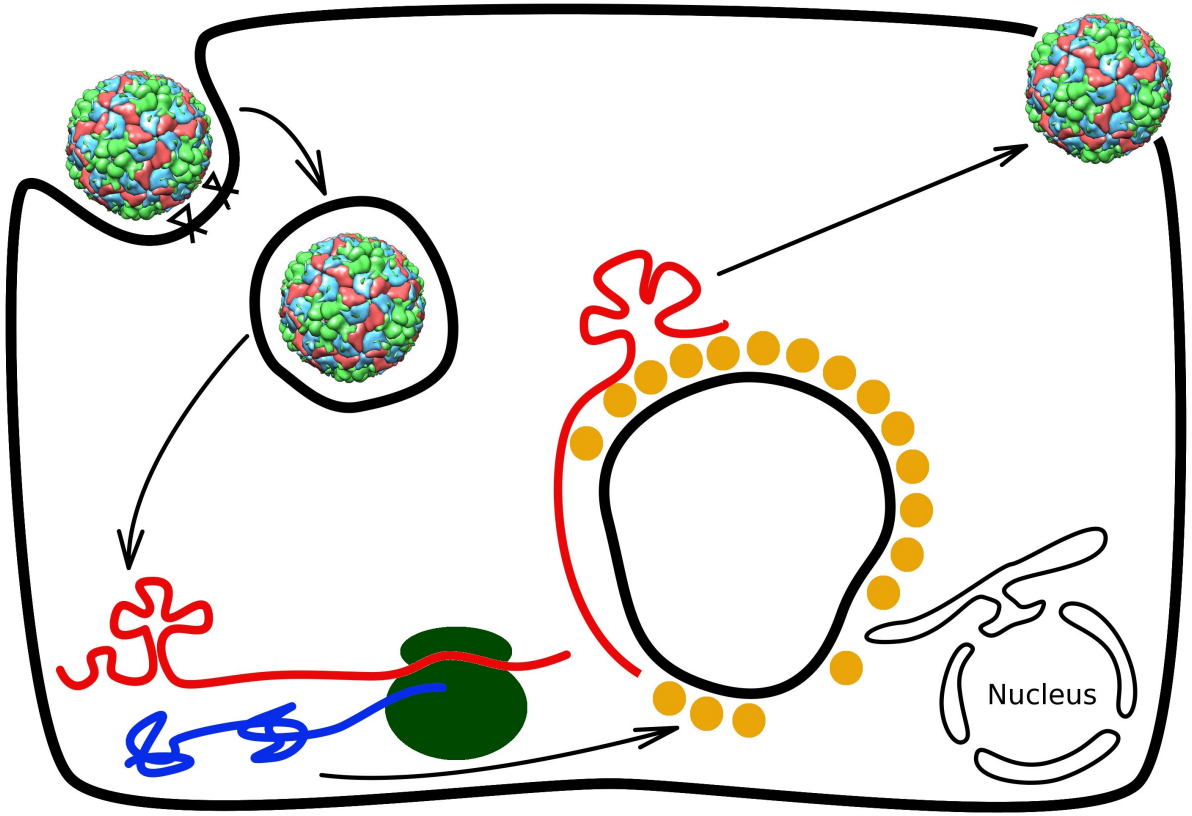
Irregular

Virion

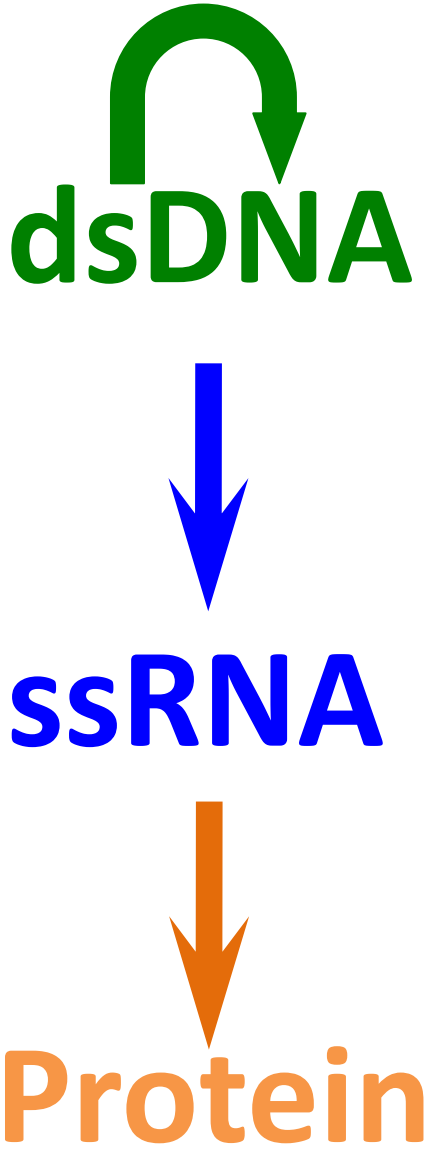




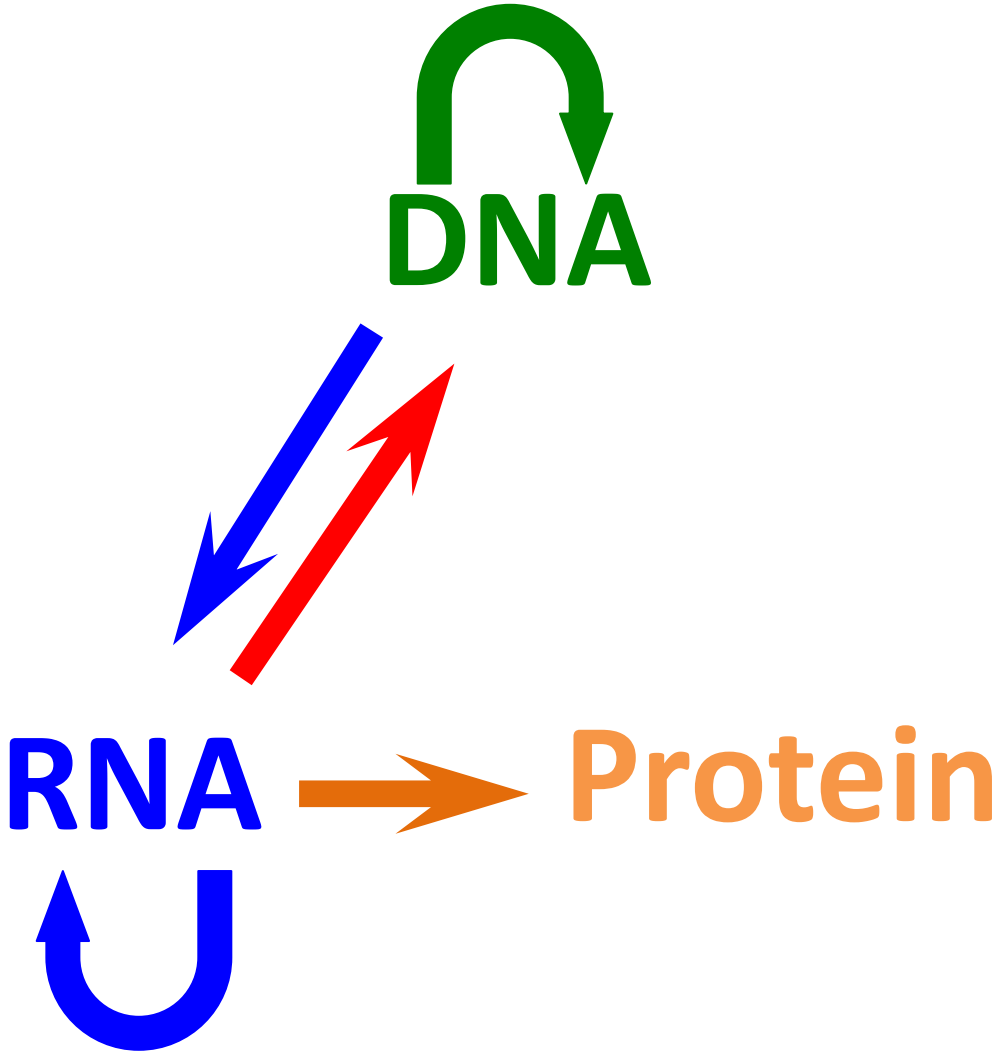
Infected cell

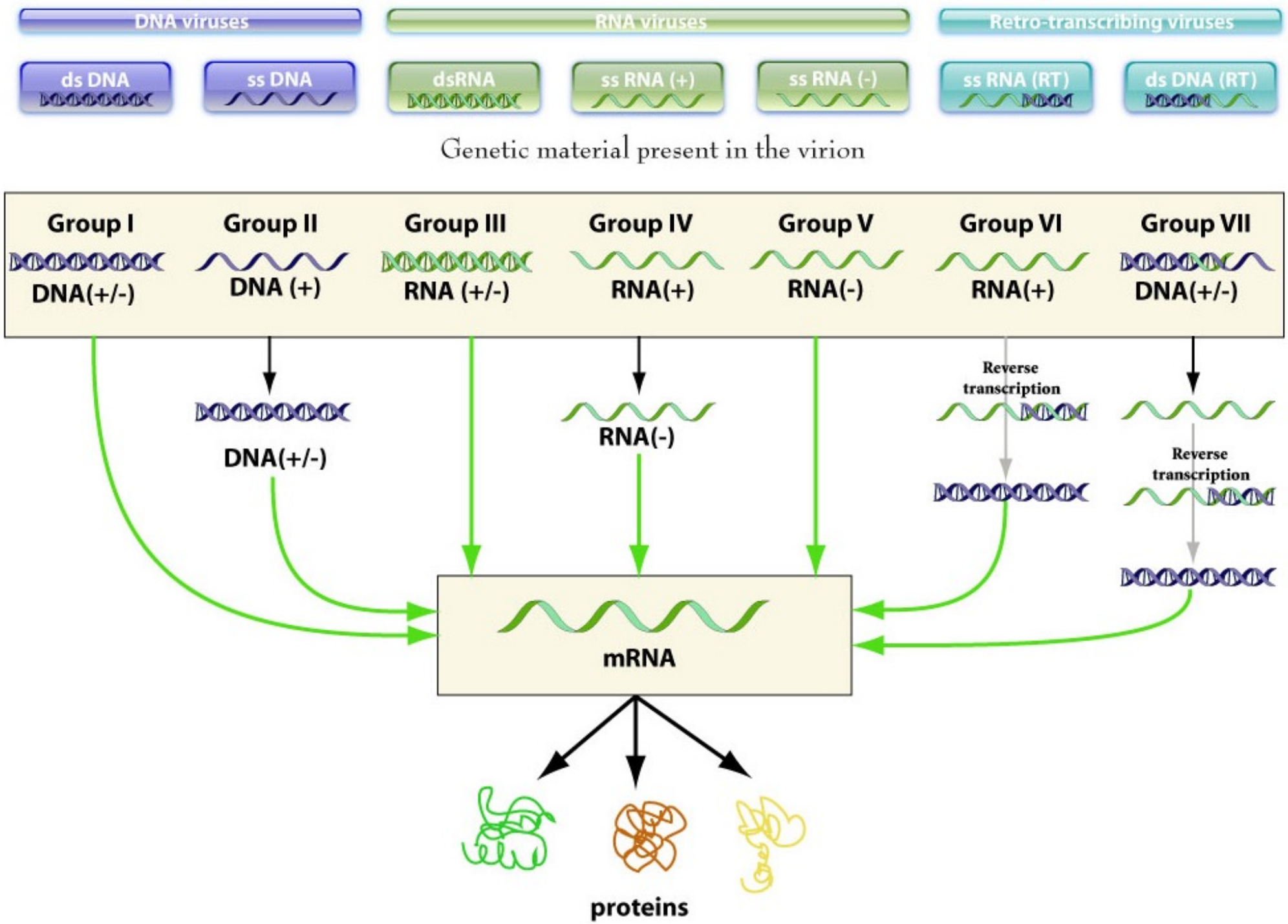


Cellular organisms



Viruses




DNA viruses
RNA viruses
Retro-transcribing viruses
ds DNA
ss DNA
dsRNA
ss RNA (+)
ss RNA (-)
ss RNA (RT)
ds DNA (RT)
Genetic material present in the virion
Group I
DNA(+/-)
Group II
DNA (+)
Group III
RNA (+/-)
Group IV
RNA(+)
Group V
RNA(-)
Group VI
RNA(+)
Group VII
DNA(+/-)
DNA(+/-)
RNA(-)
Reverse transcription
Reverse transcription
mRNA
proteins

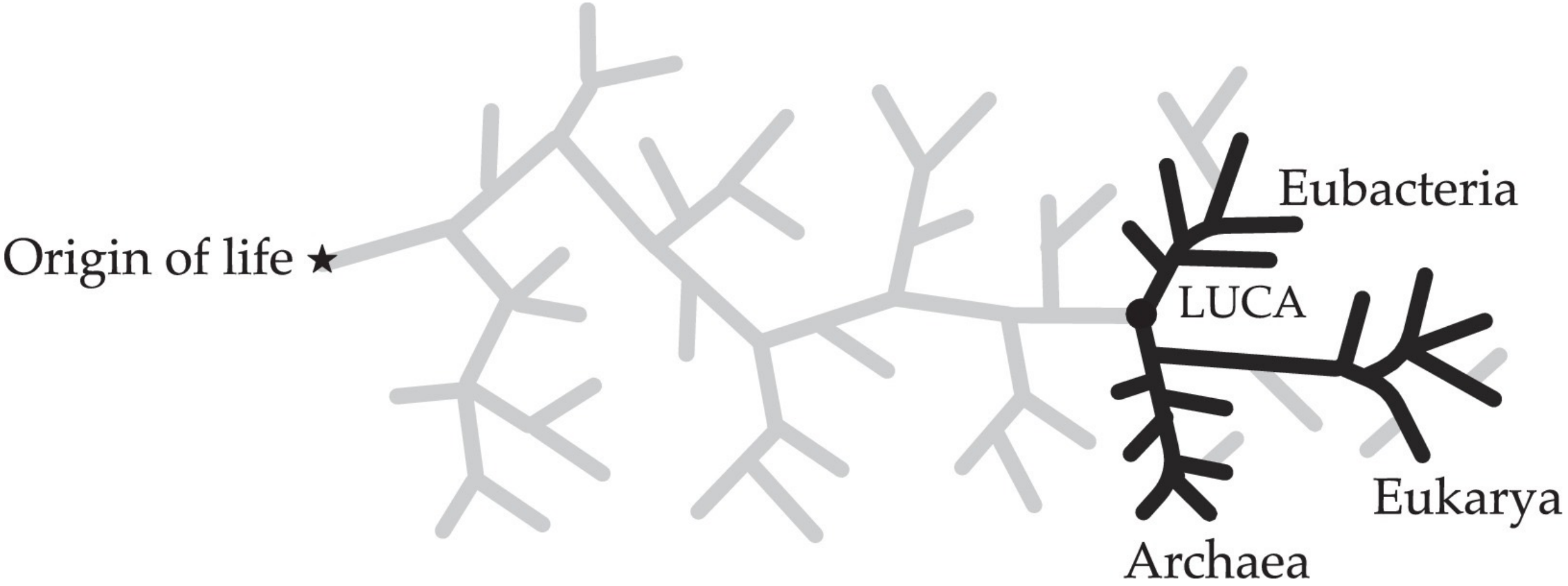
What is a virus?

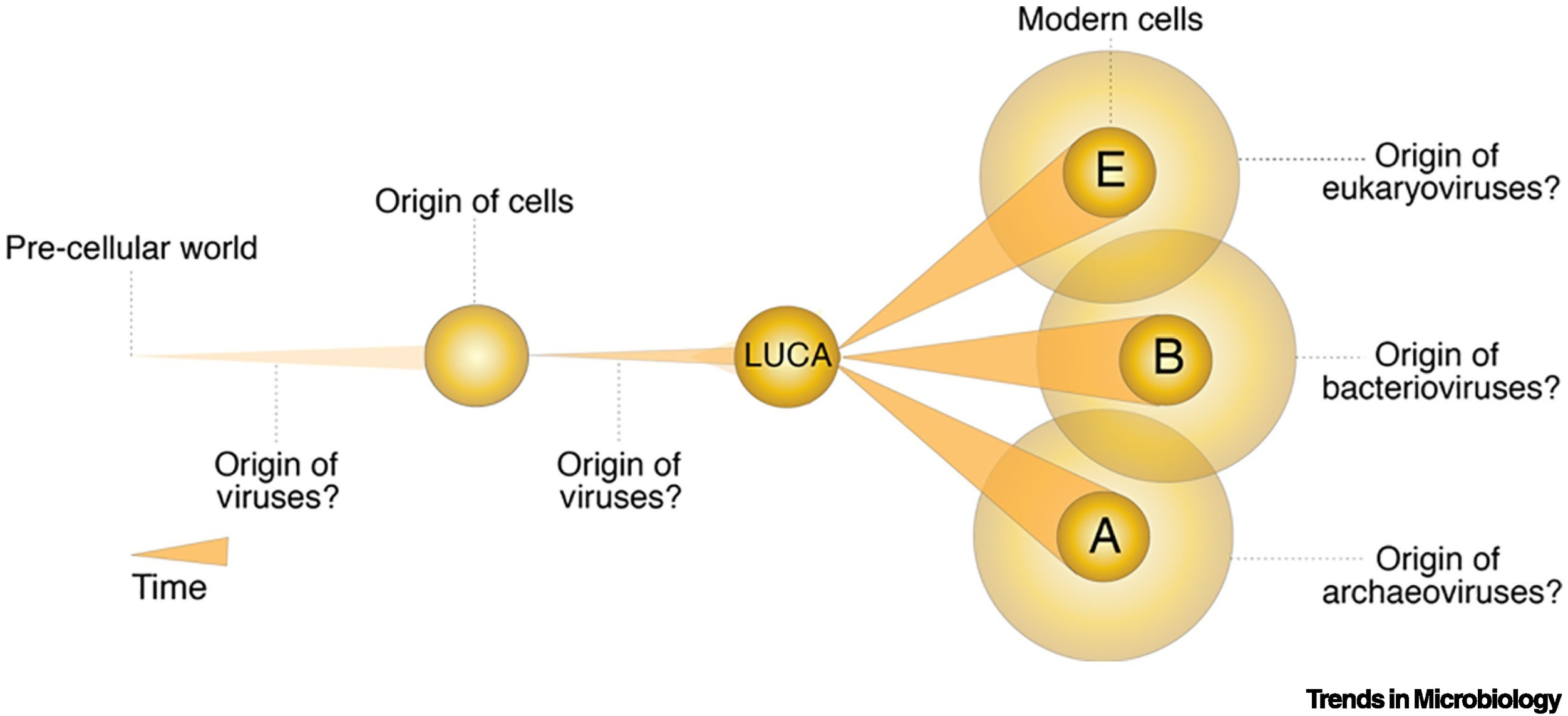
Origins of viruses

Virus structures and infection processes

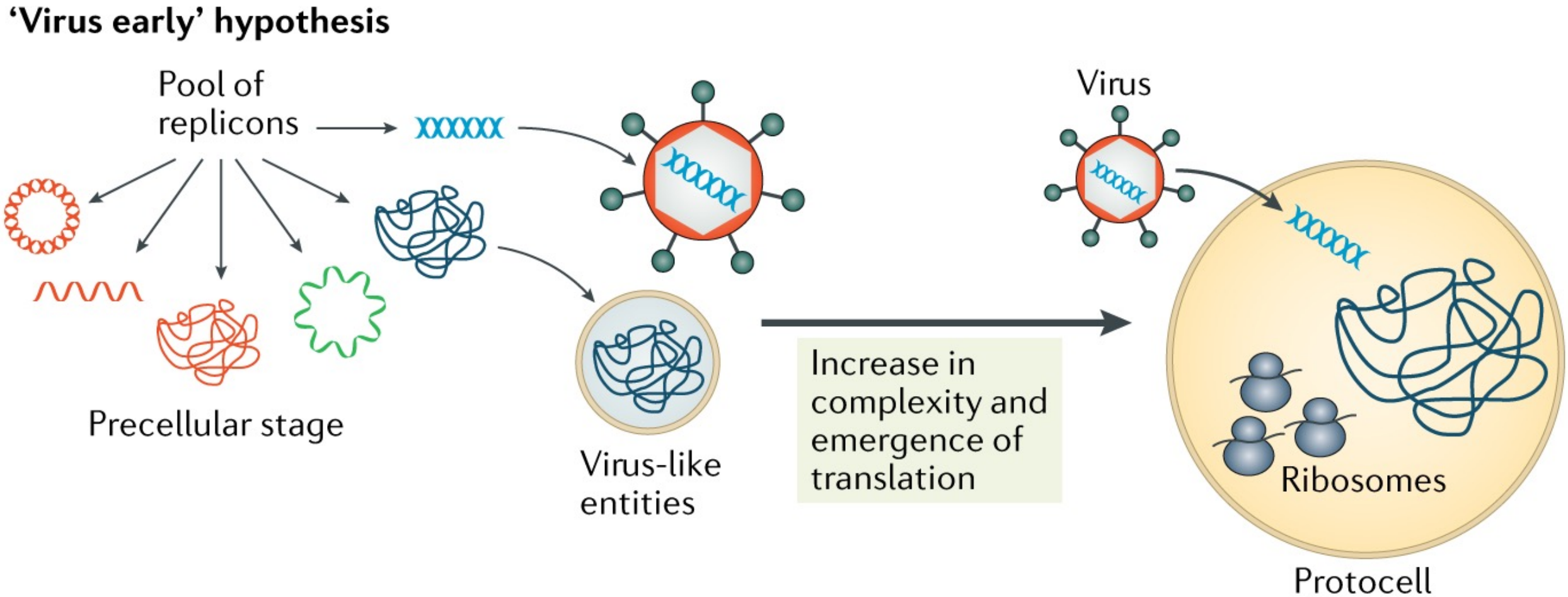
Extra

Last Universal Common Ancestor (LUCA)

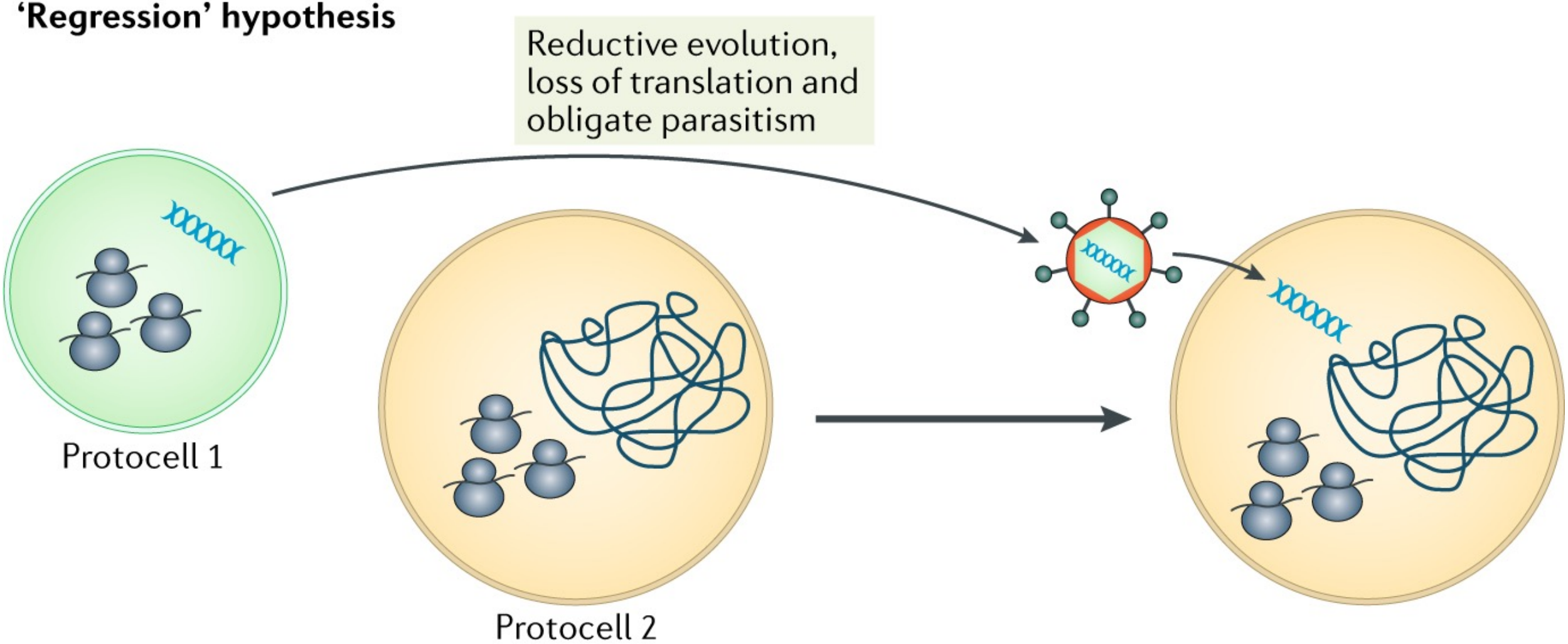




Investigating the Concept and Origin of Viruses, Nasir et al., Trends in Microbiology, 2020



Origin of viruses: primordial replicators recruiting capsids from hosts, Krupovic et al., Nature Reviews Microbiology, 2019.



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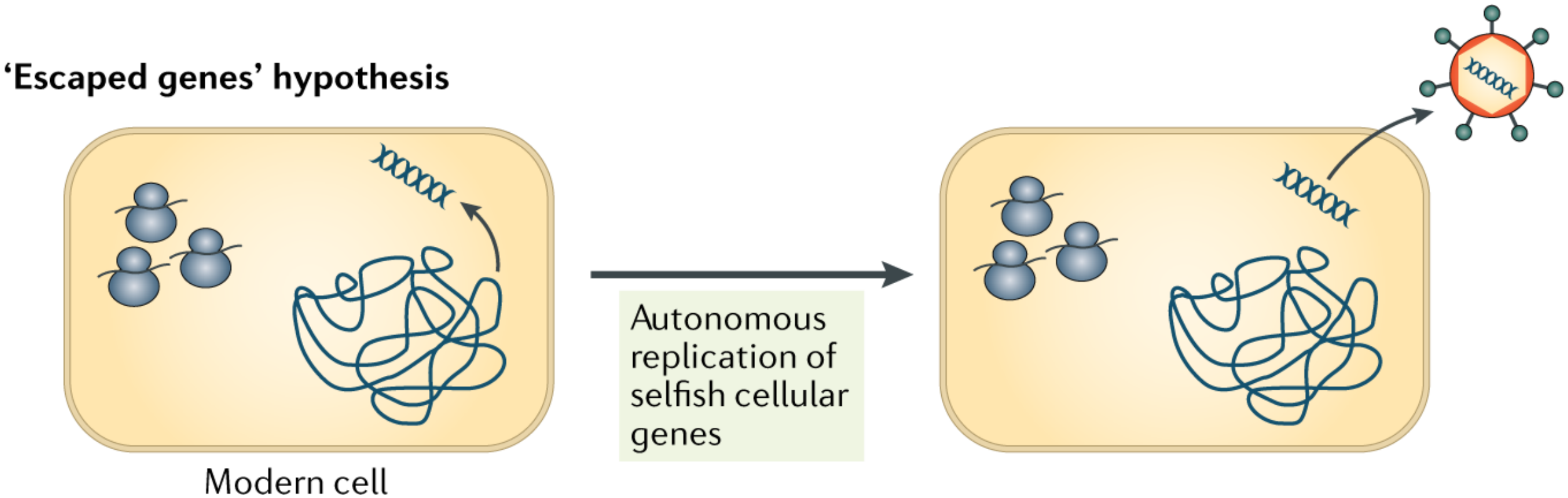


Origin of viruses: primordial replicators recruiting capsids from hosts, Krupovic et al., Nature Reviews Microbiology, 2019.

What is a virus?

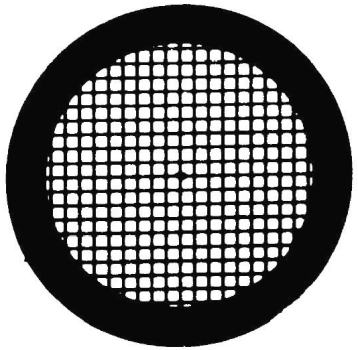
Origins of viruses

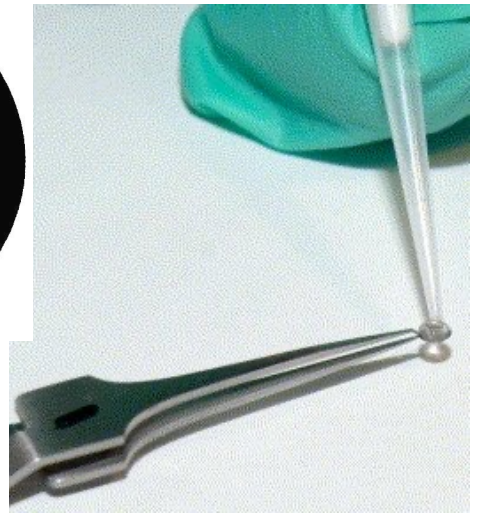
Virus structures and infection processes

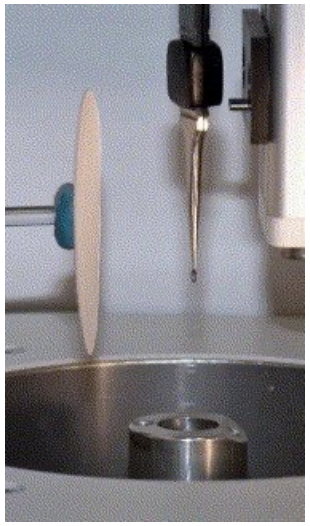
Extra

1. Virus purification

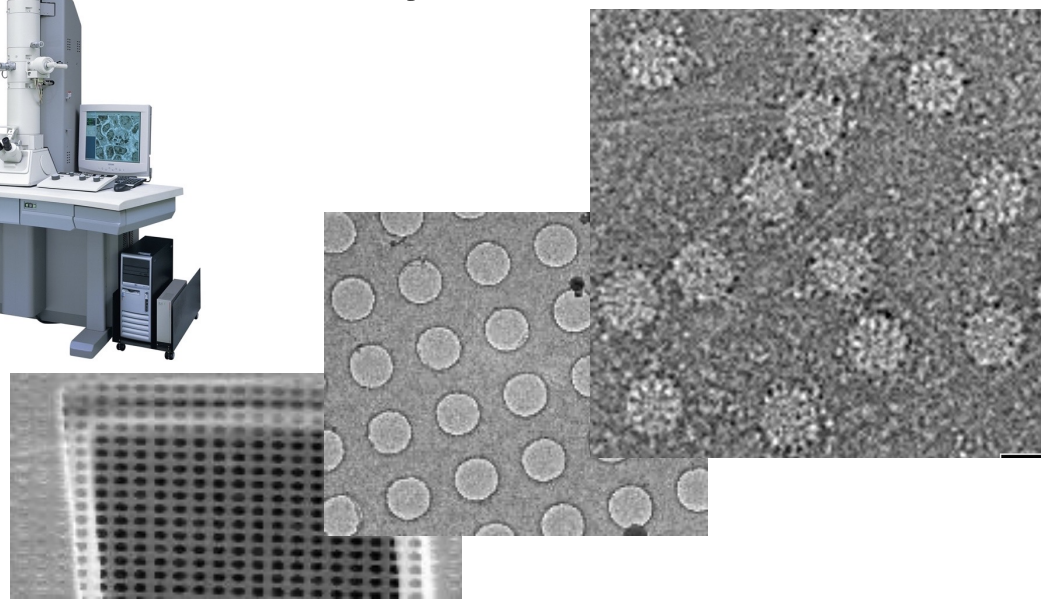
2. Grid preparation



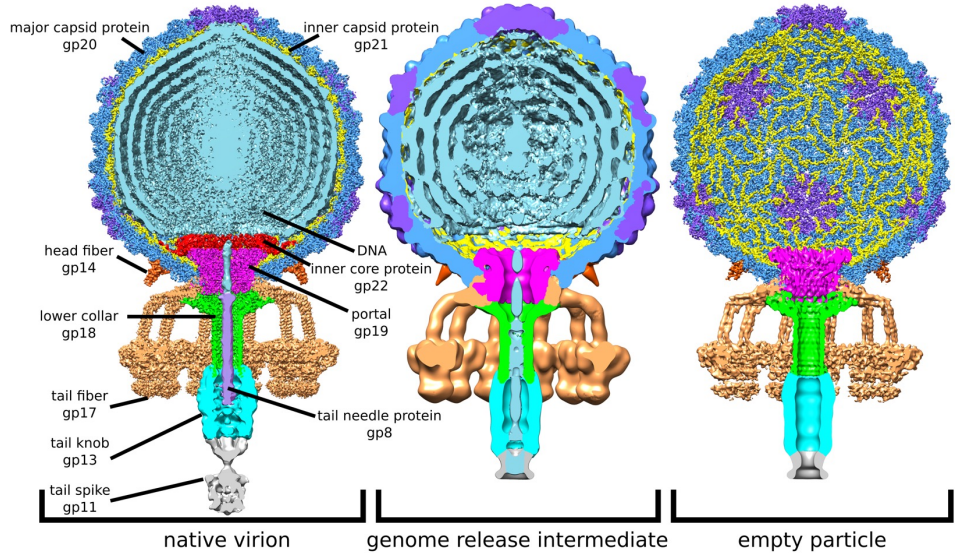


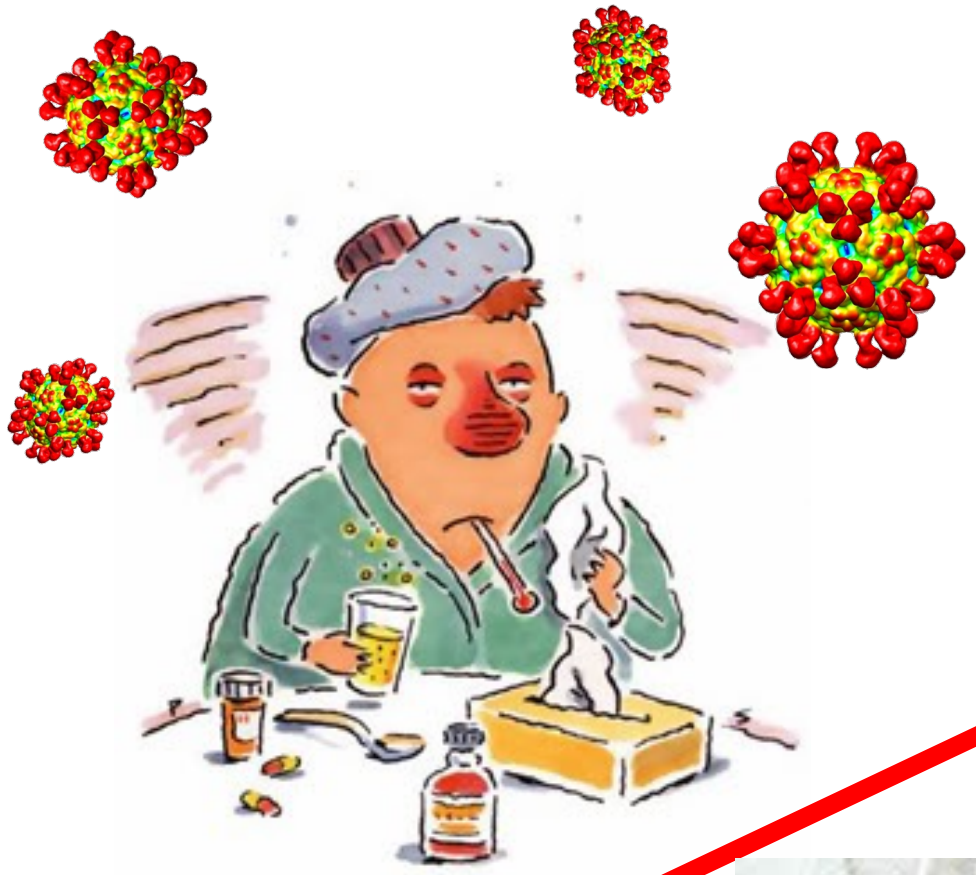


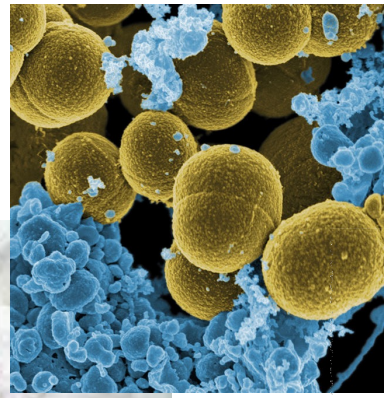
3. cryo-EM

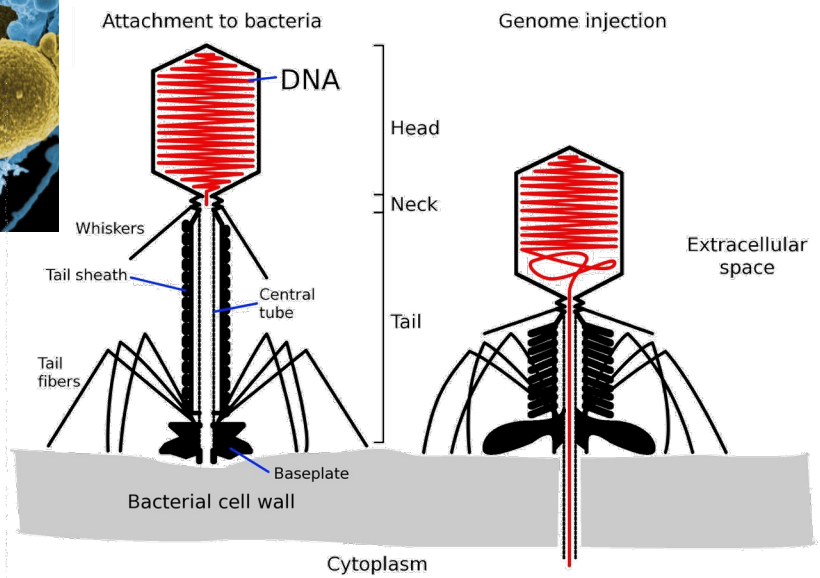


4. Reconstruction

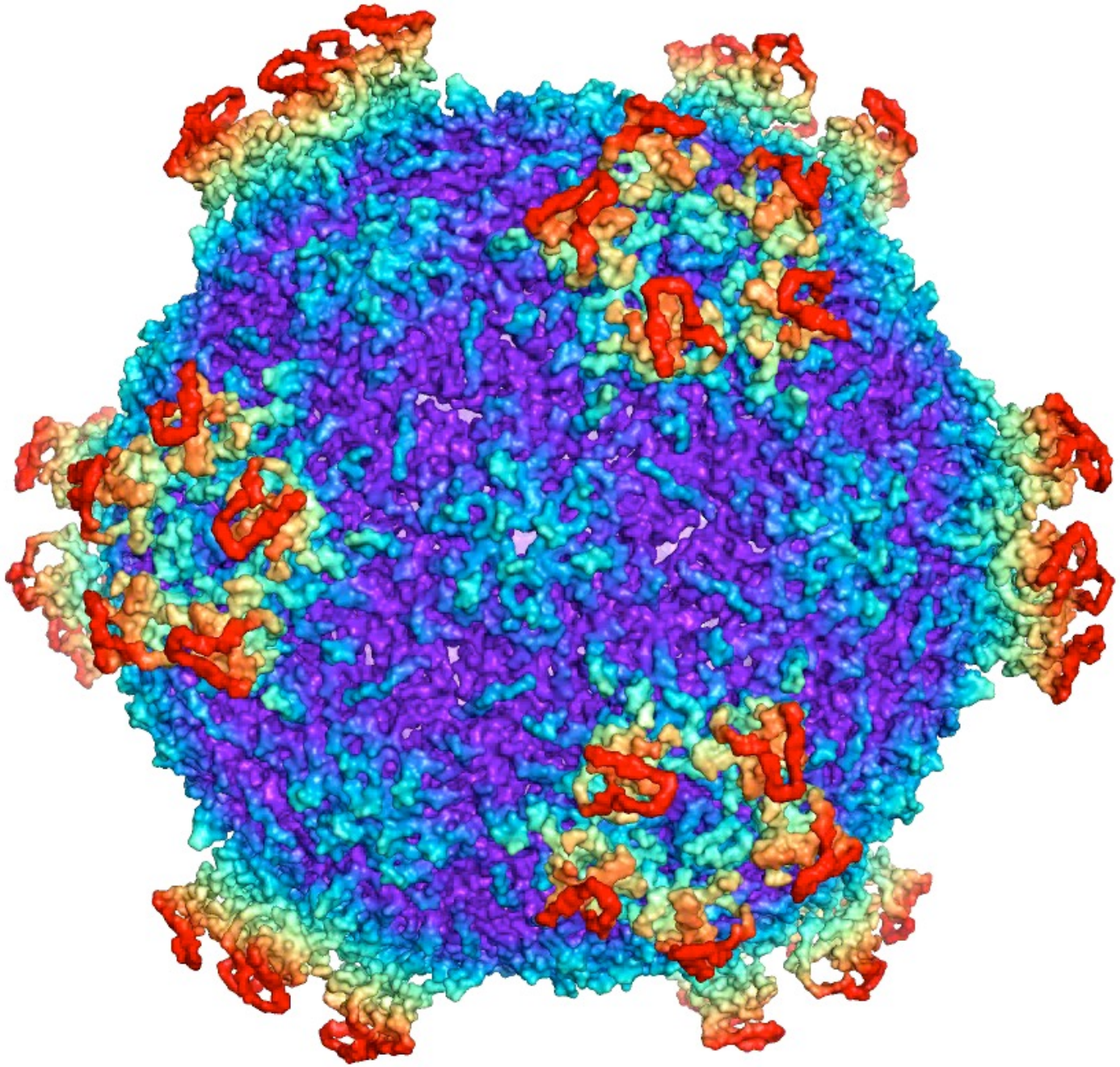


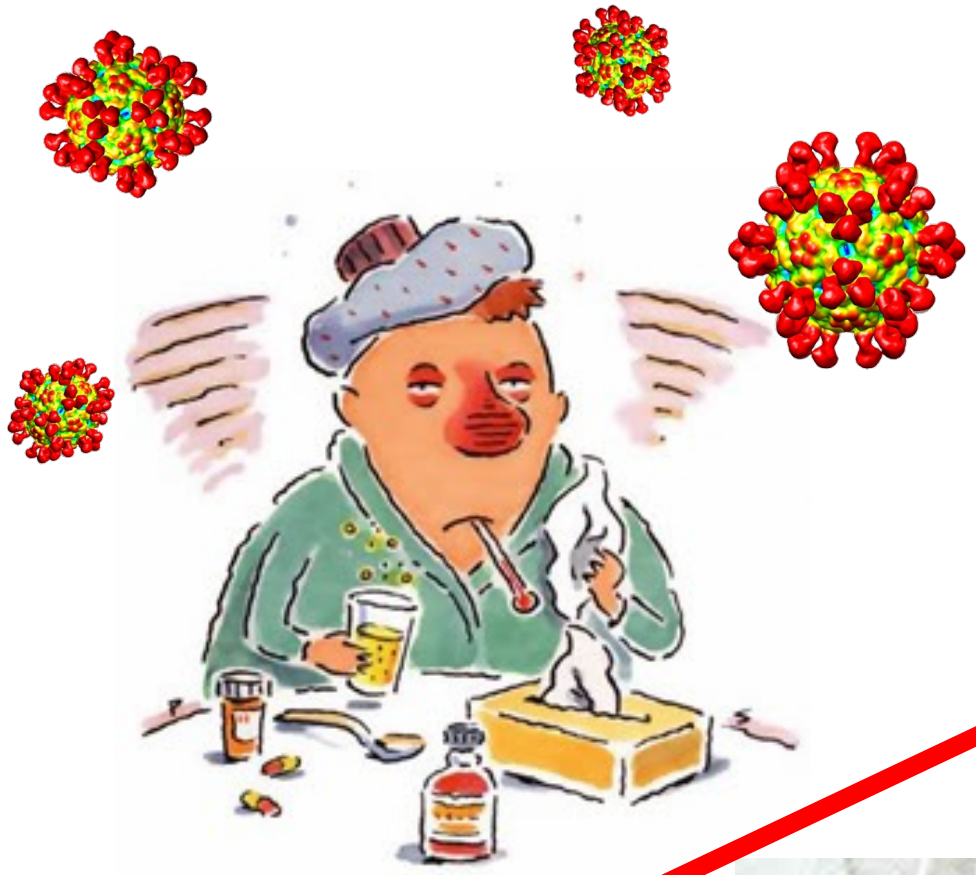


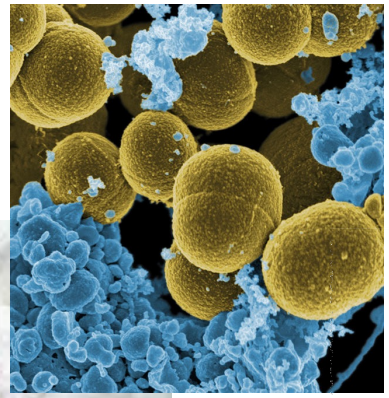


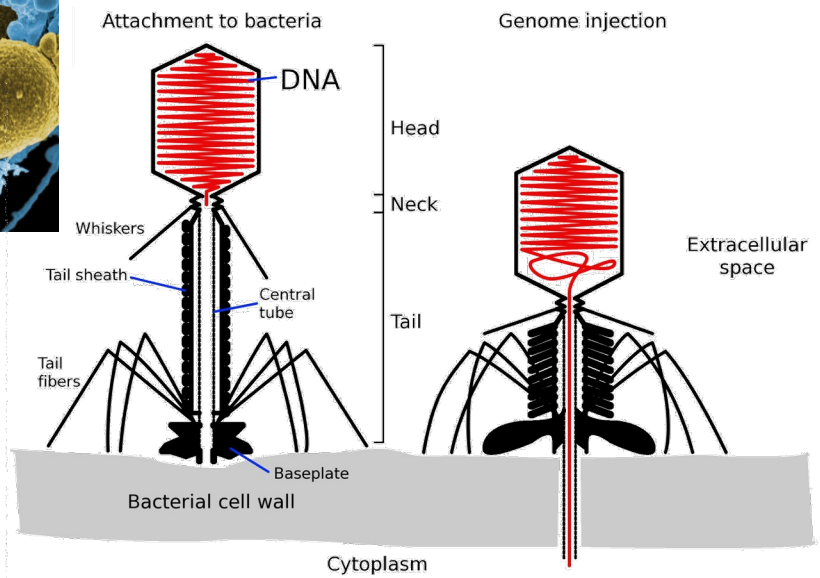

Attachment to bacteria
Genome injection
DNA
Head
Neck
Whiskers
Tail sheath
Central tube
Tail
Tail fibers
Baseplate
Extracellular space
Bacterial cell wall
Cytoplasm

Slow bee paralysis virus








Attachment to bacteria
Genome injection
DNA
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Central tube
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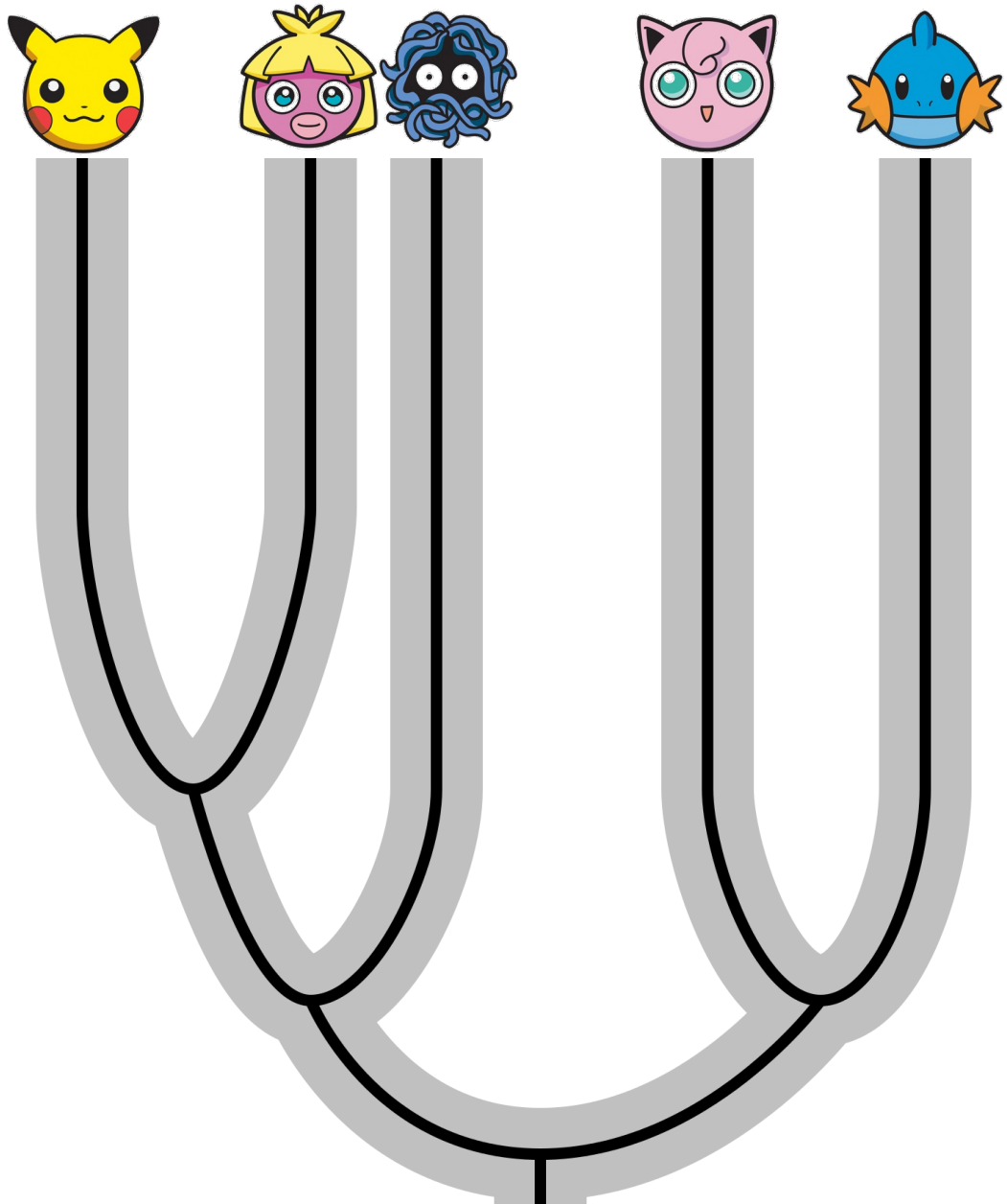
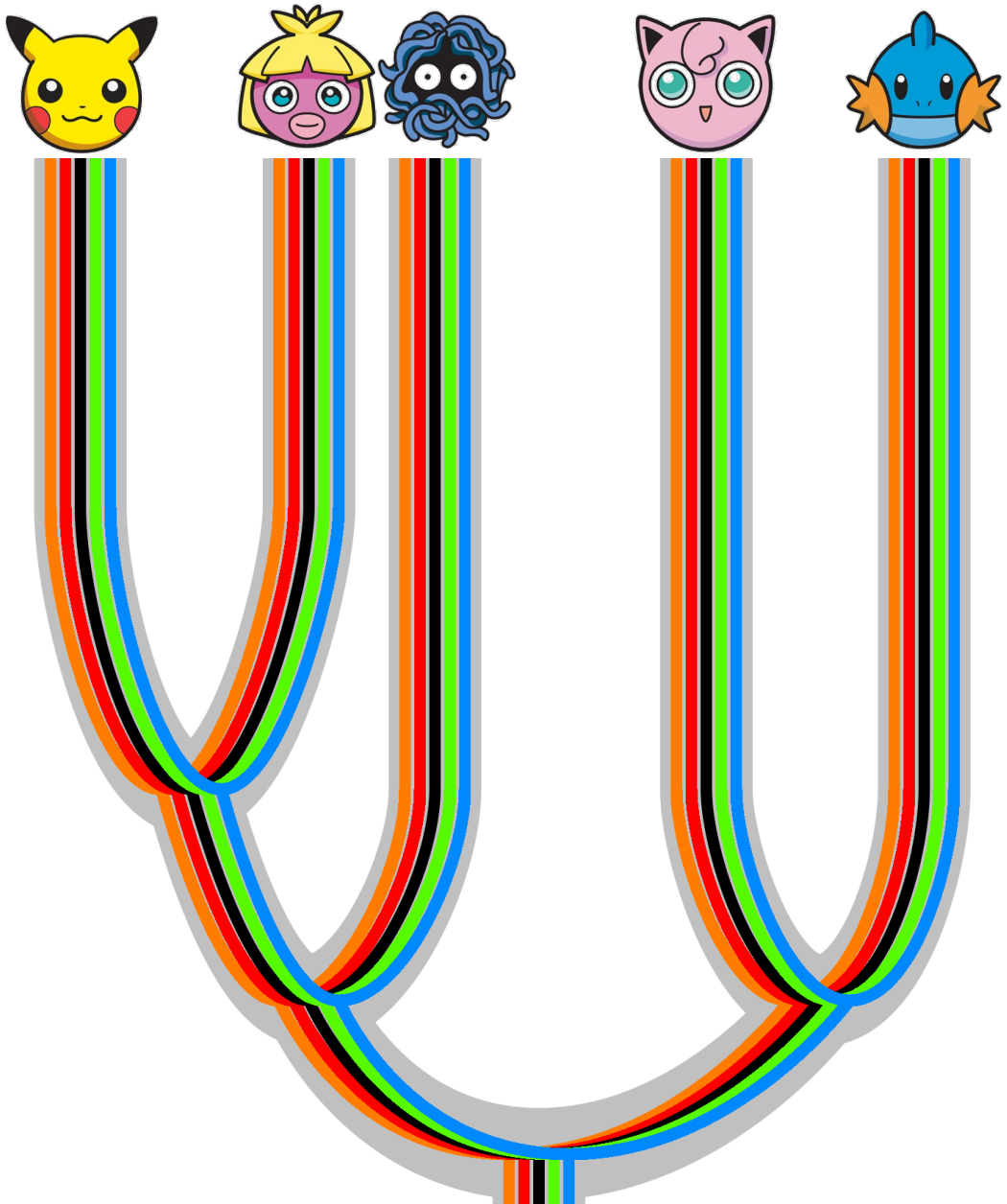
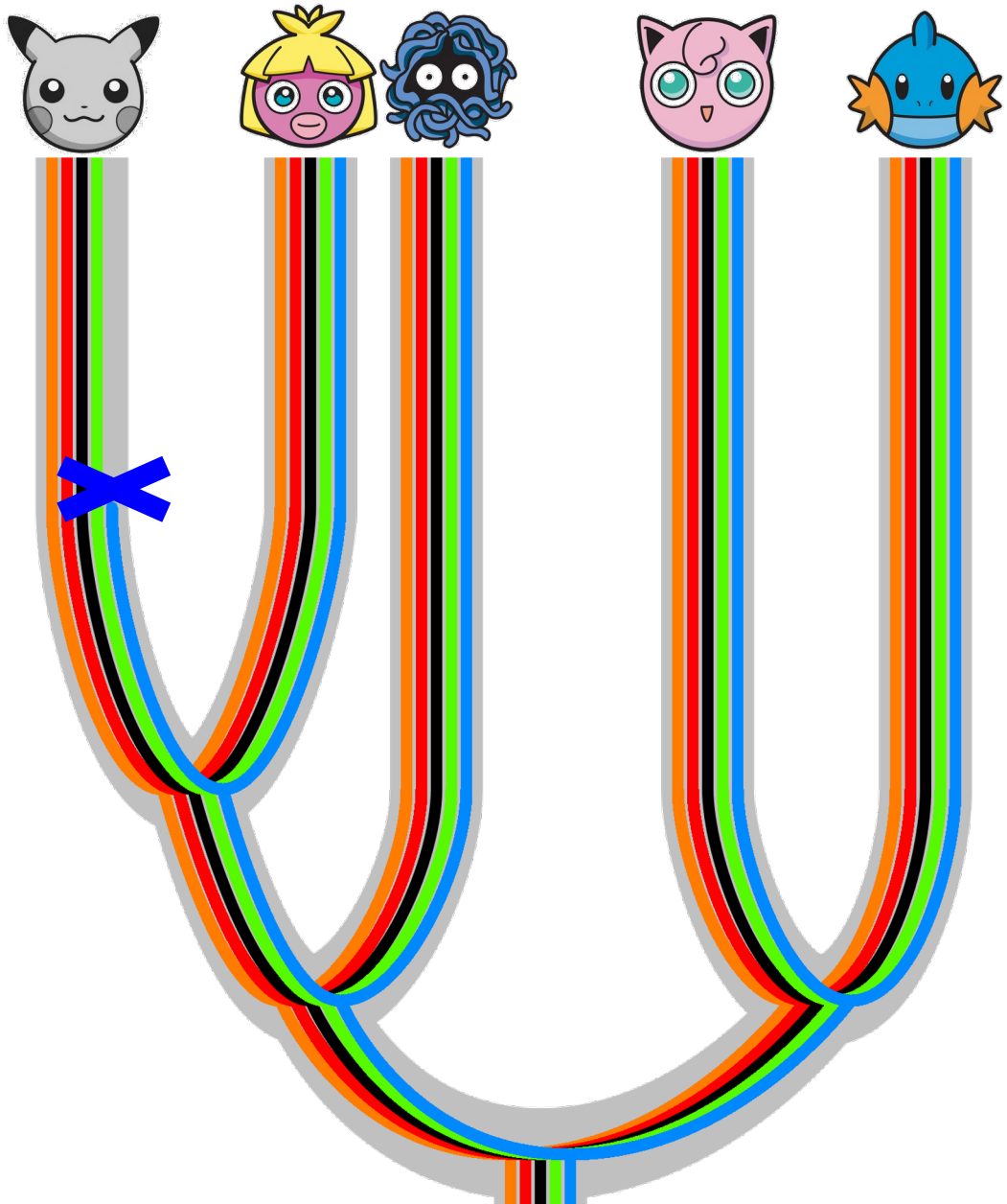
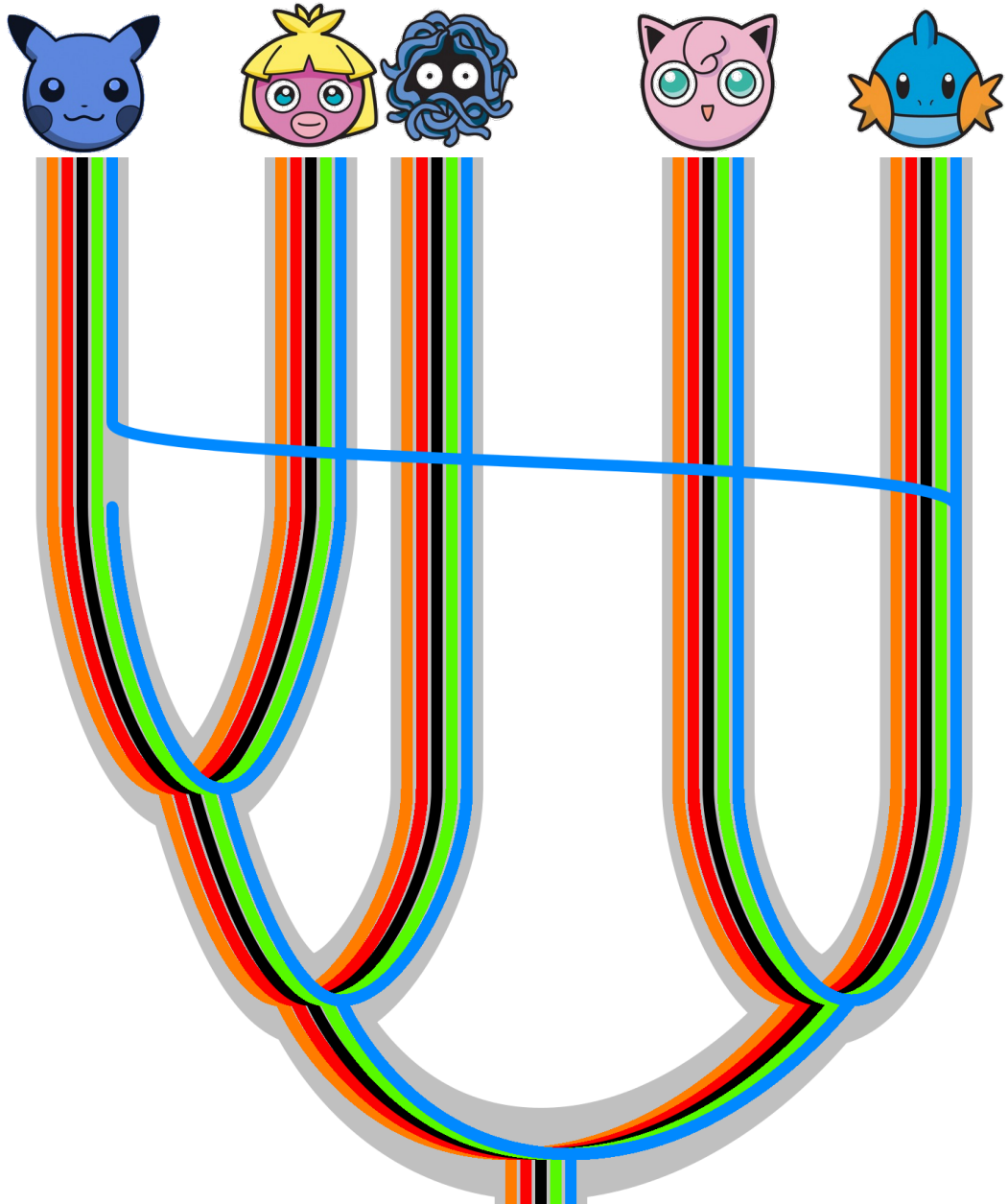
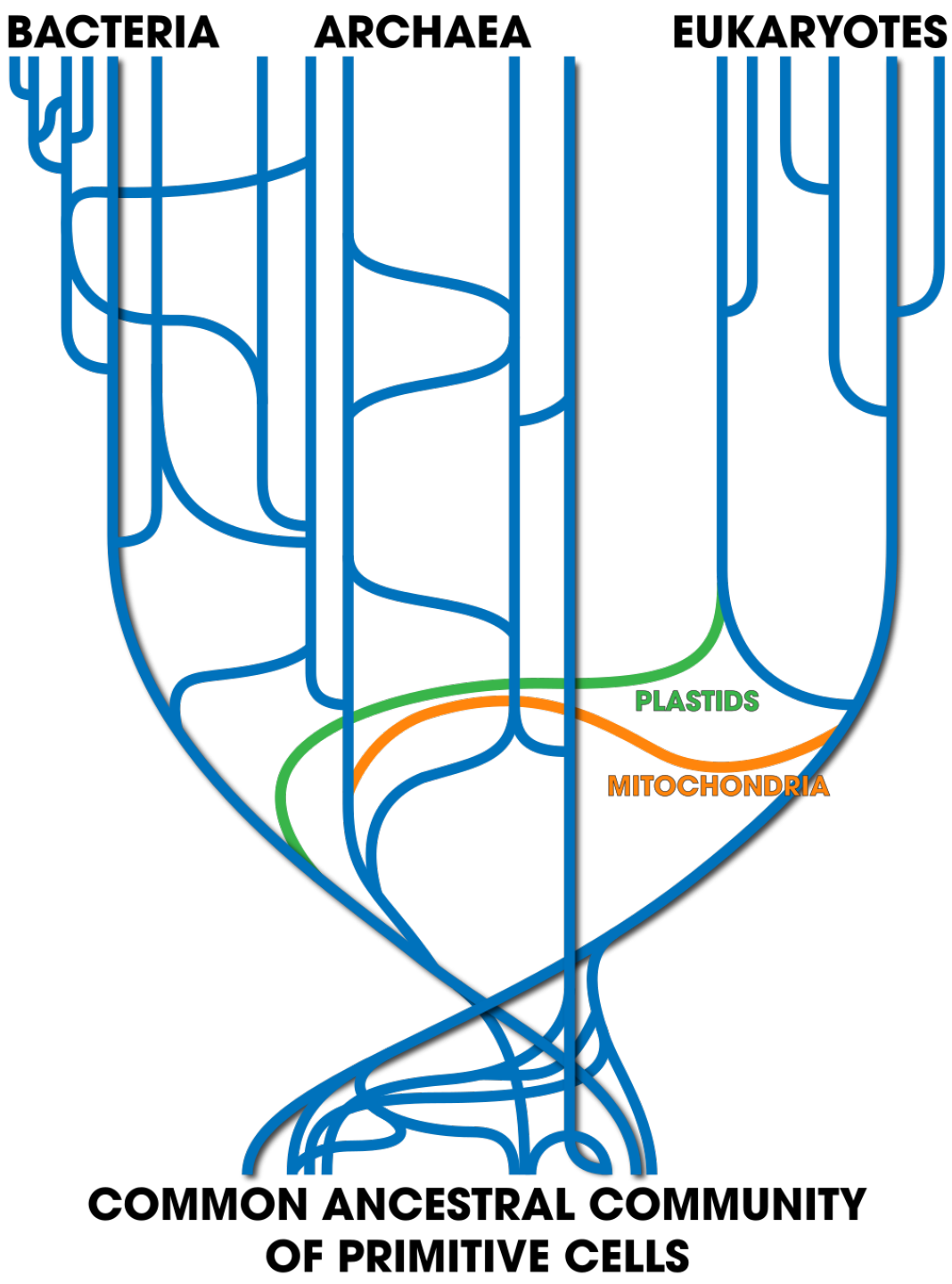
Tail fibers
Baseplate
Extracellular space
Bacterial cell wall
Cytoplasm



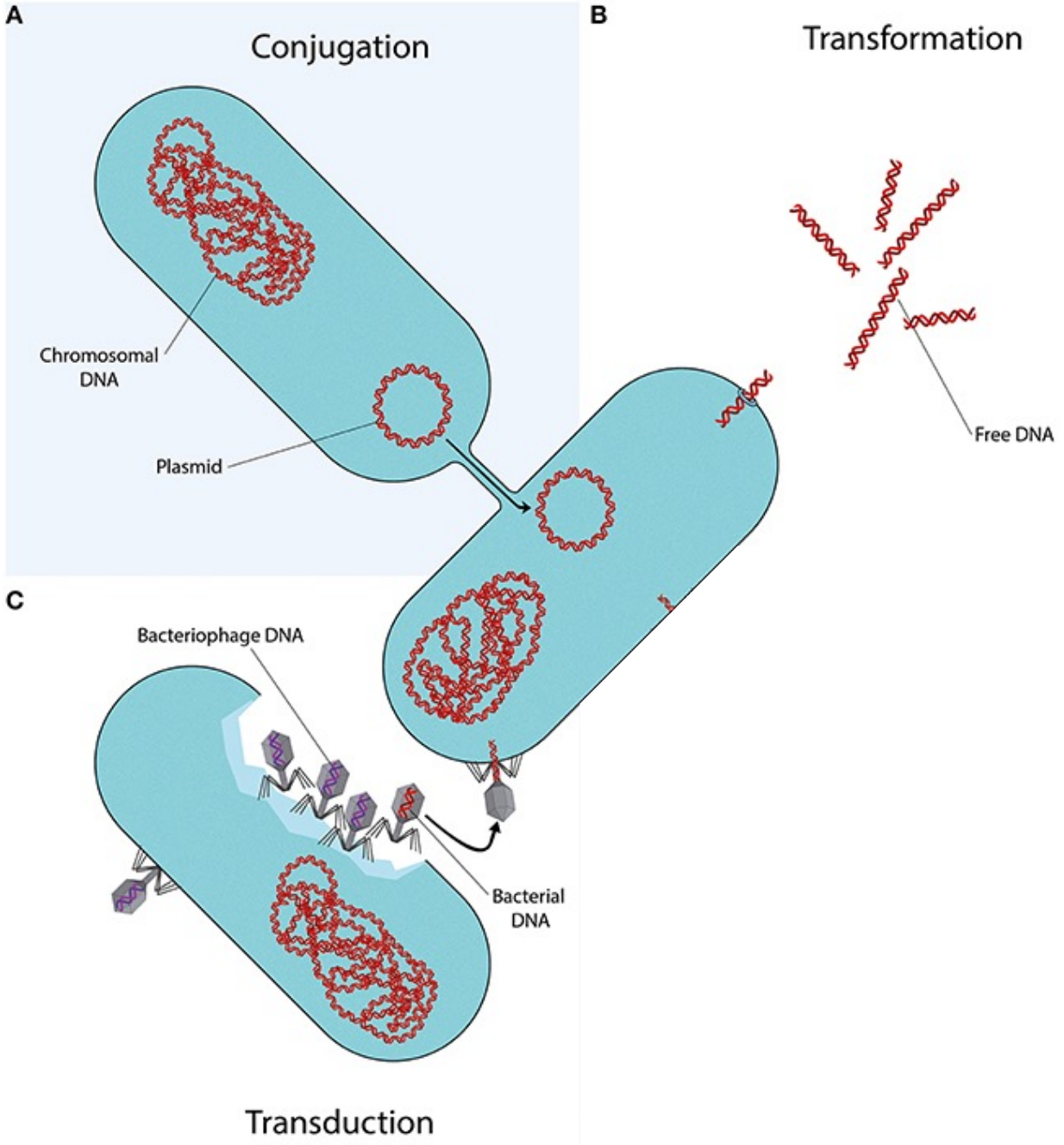






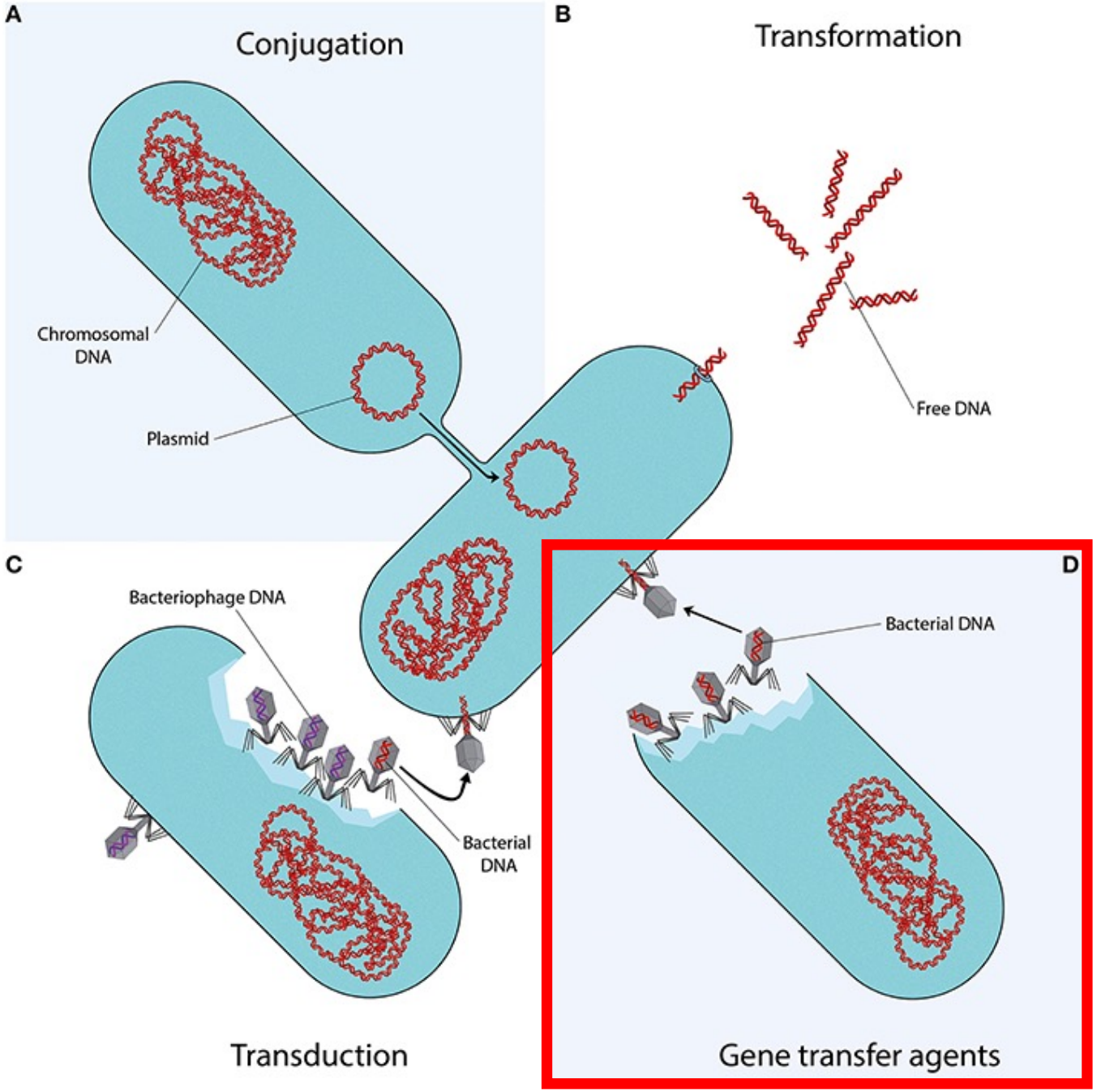

BACTERIA
ARCHAEA
EUKARYOTES
PLASTIDS
MITOCHONDRIA
COMMON ANCESTRAL COMMUNITY
OF PRIMITIVE CELLS

Mechanisms of horizontal gene transfer



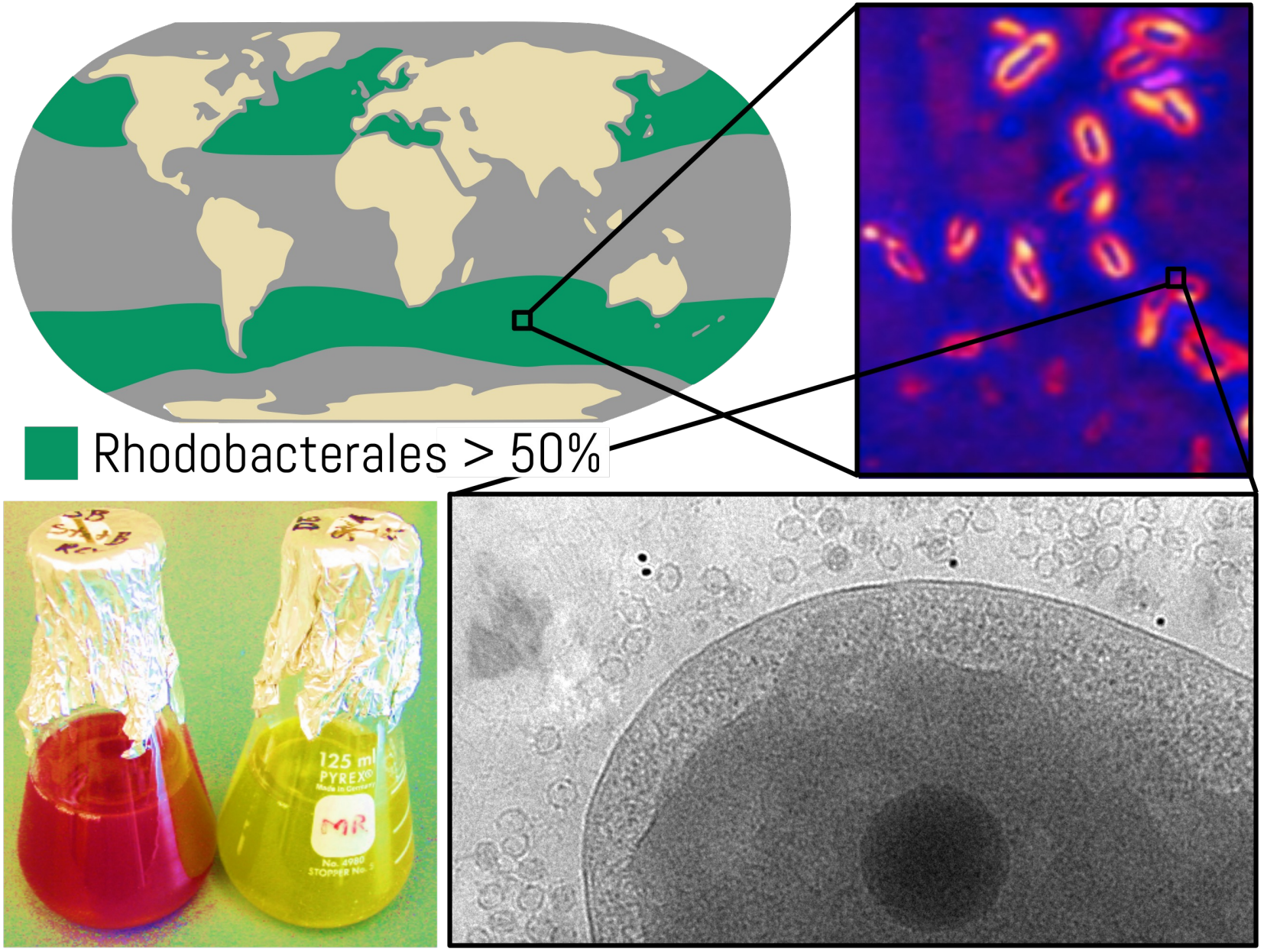
Wintersforf et al. Front. Microbiol. 2016

Mechanisms of horizontal gene transfer



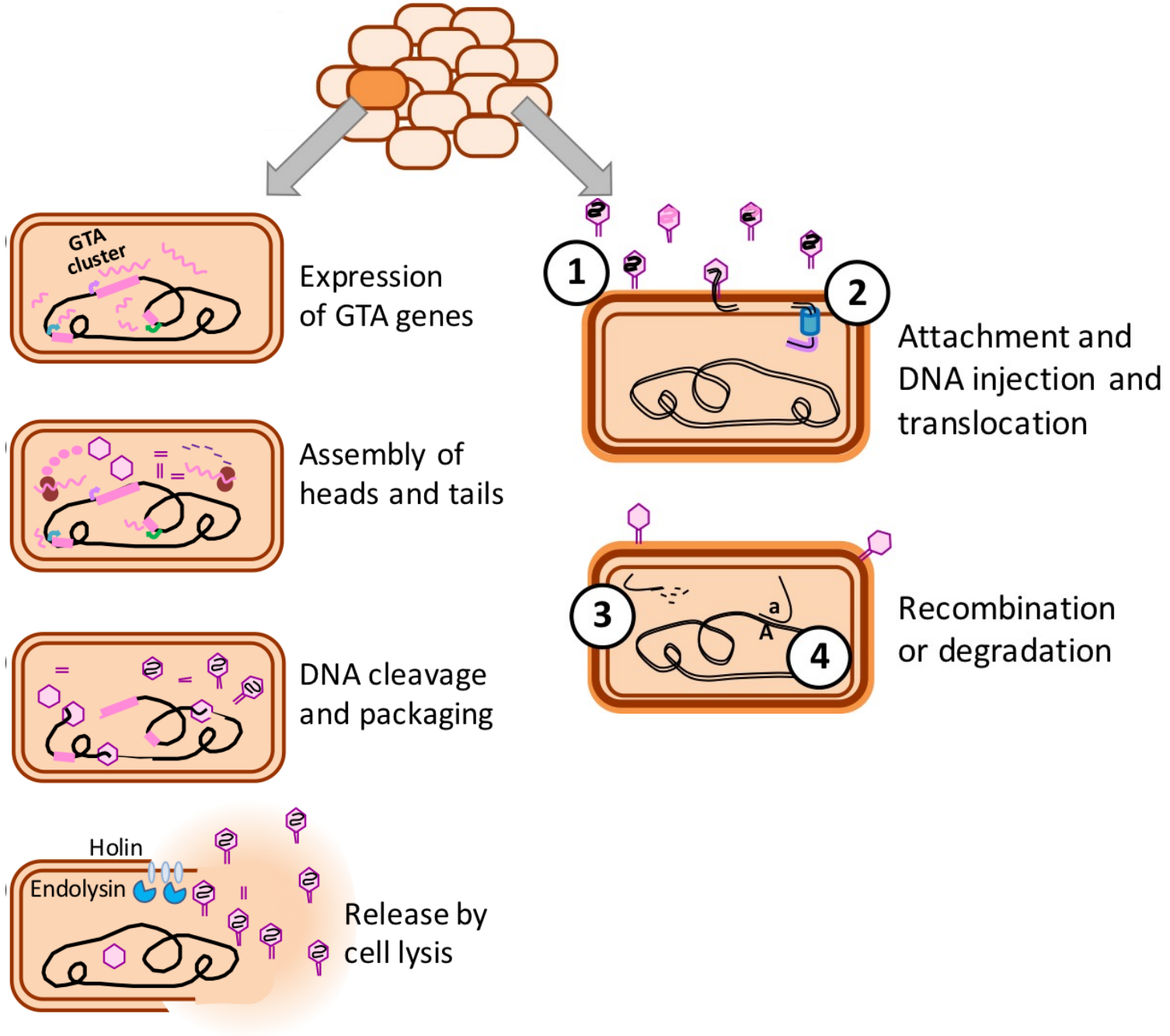
Wintersforf et al. Front. Microbiol. 2016

Rhodobacter capsulatus gene transfer agent

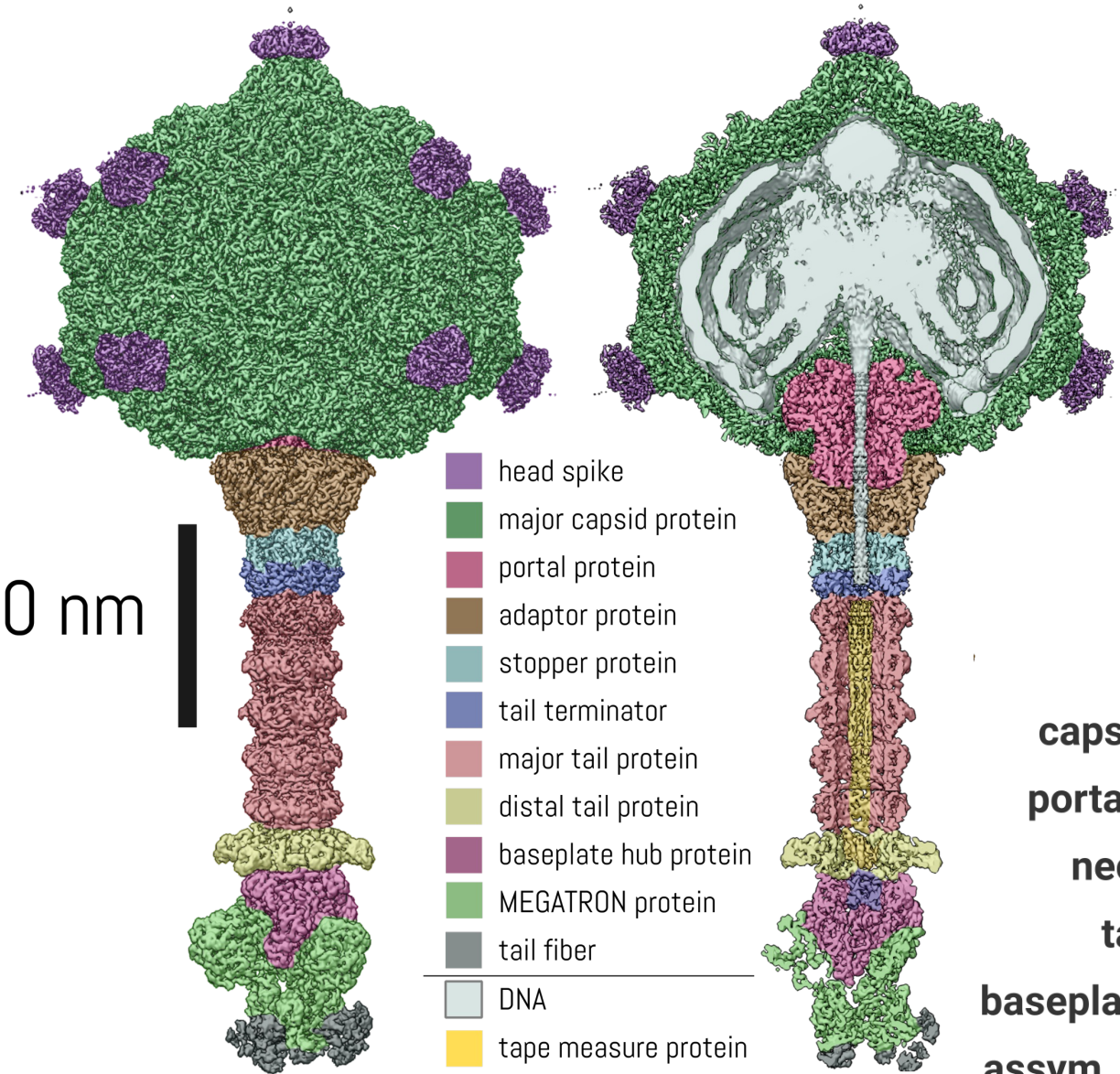


Dang et al., App Env Microbiol 2008, Fogg et al., Nat Comm 2019, Brimacombe et al., Mol Microbiol 2013

Function of gene transfer agents



Virion of the GTA particle

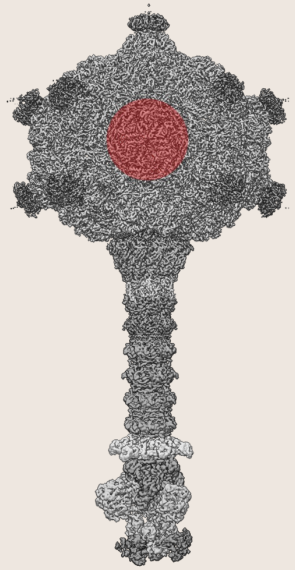


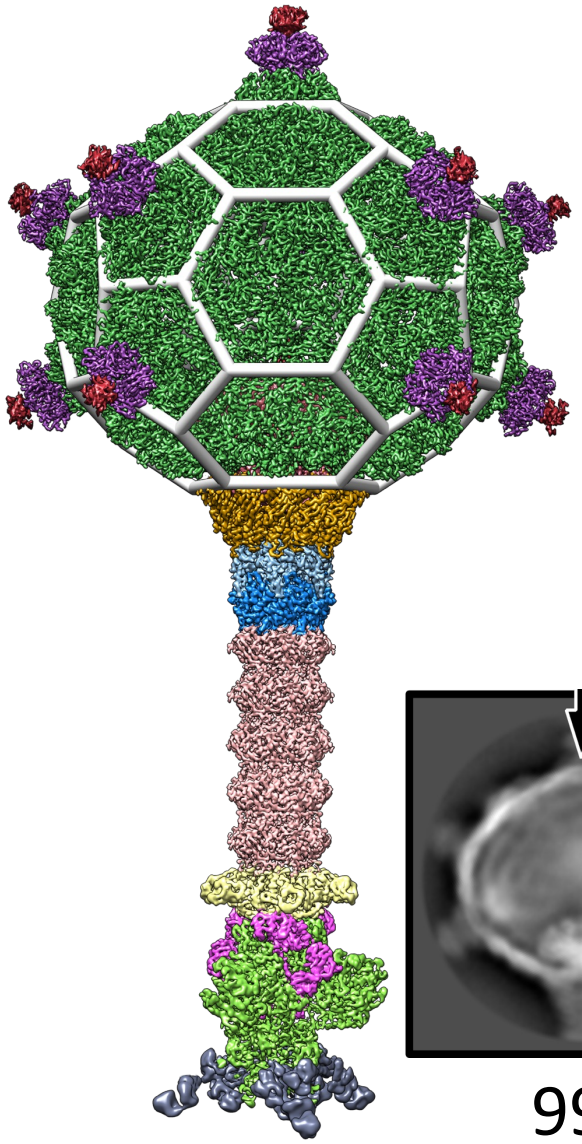
20 nm

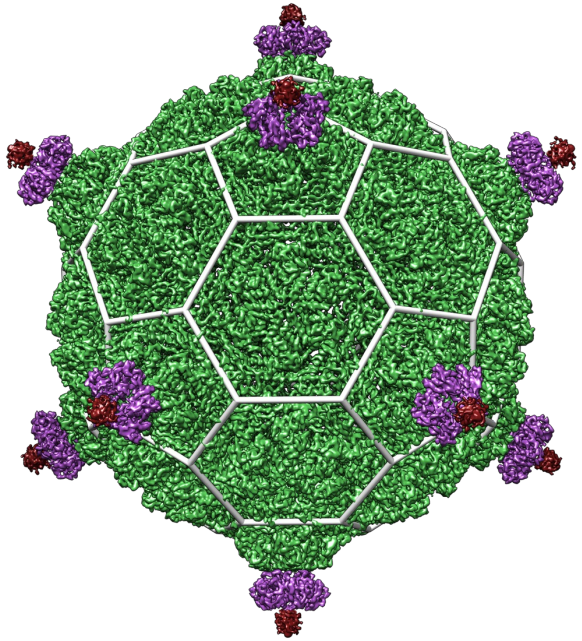
- head spike
- major capsid protein
- portal protein
- adaptor protein
- stopper protein
- tail terminator
- major tail protein
- distal tail protein
- baseplate hub protein
- MEGATRON protein
- tail fiber
- DNA
- tape measure protein
- cell wall peptidase

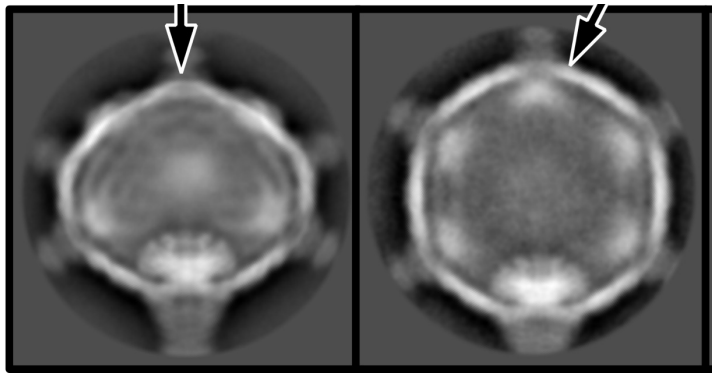
	full	empty
capsid C5	3.6 Å	3.4 Å
portal C12	3.3 Å	3.4 Å
neck C6	3.6 Å	3.4 Å
tail C6	3.9 Å	3.8 Å
baseplate C3	4.0 Å	4.5 Å
assym. p. C1	4.2 Å	4.4 Å

Capsid of the GTA





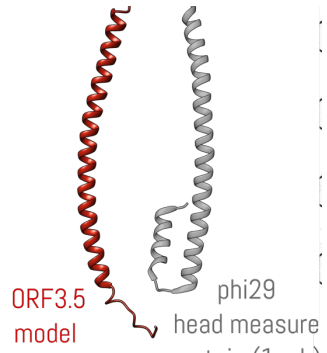




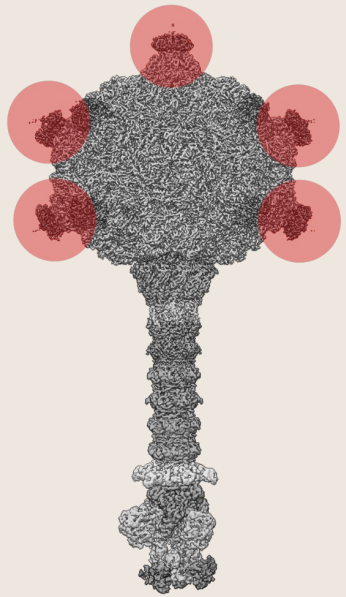
99%

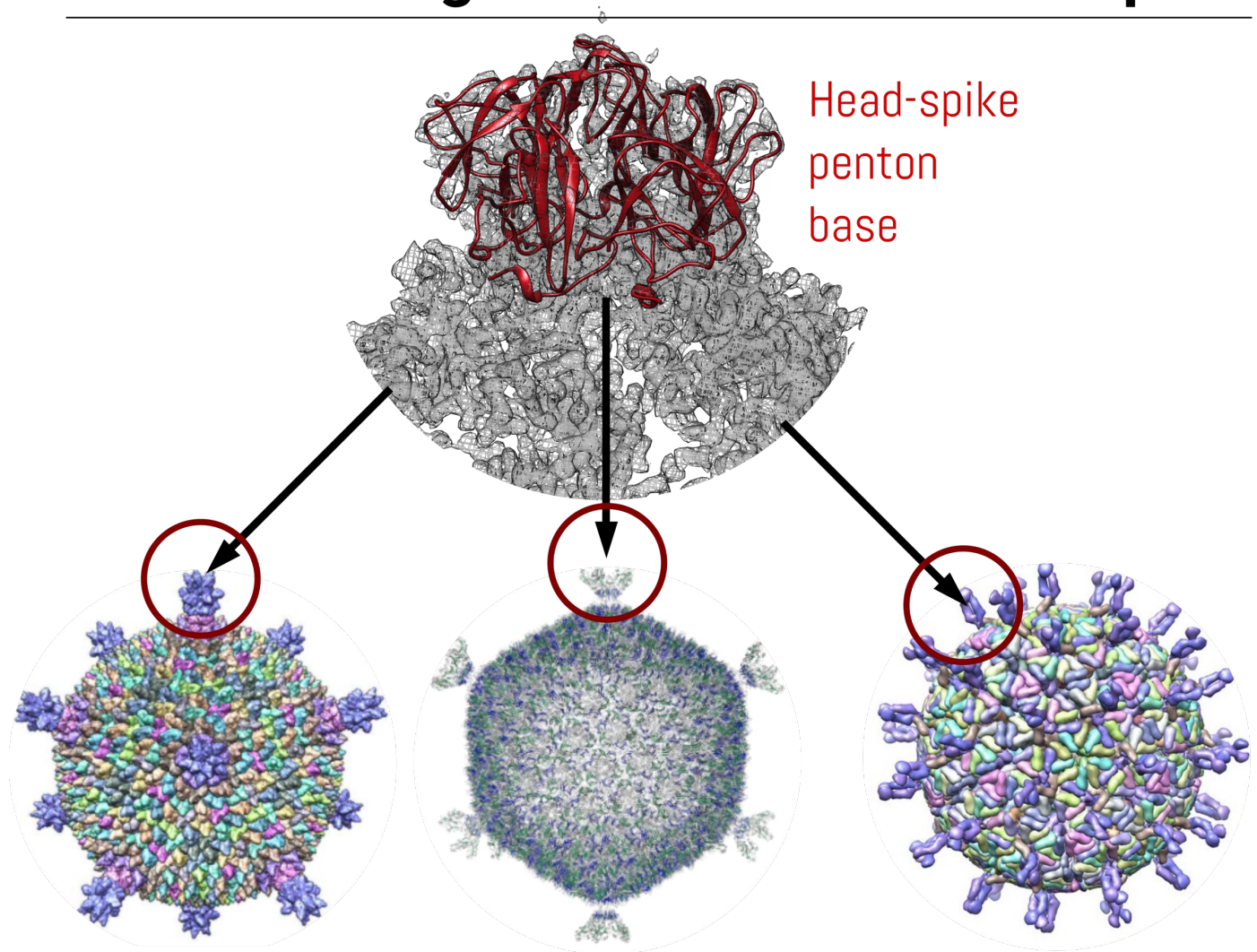
1%

putative head-measure protein ORF3.5



Structural alignement of GTA head-spike



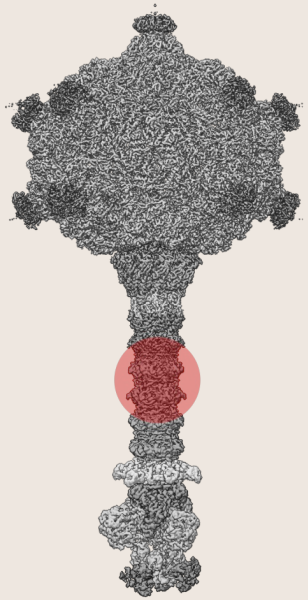


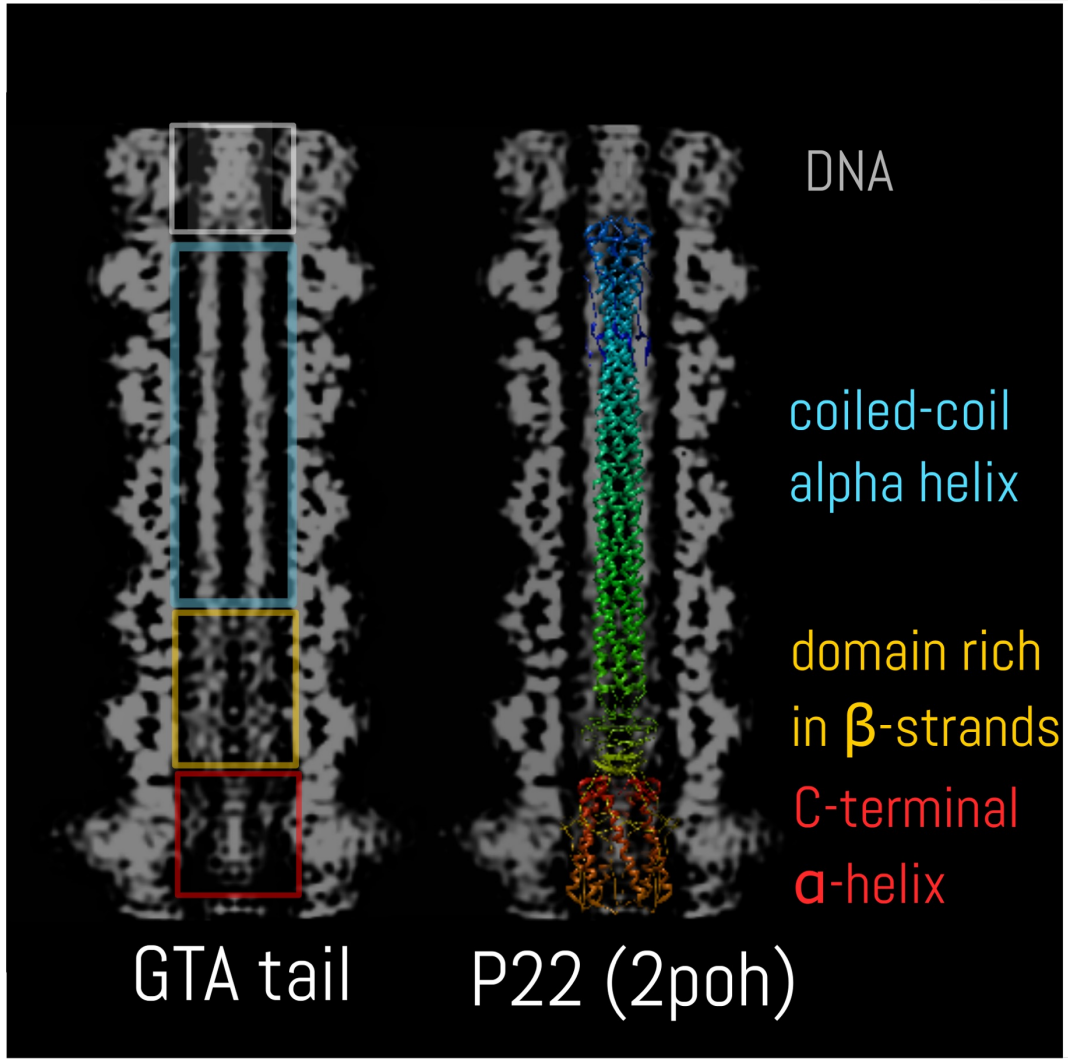
Archeal virus
Sulfolobus turreted IV1
(3j31)

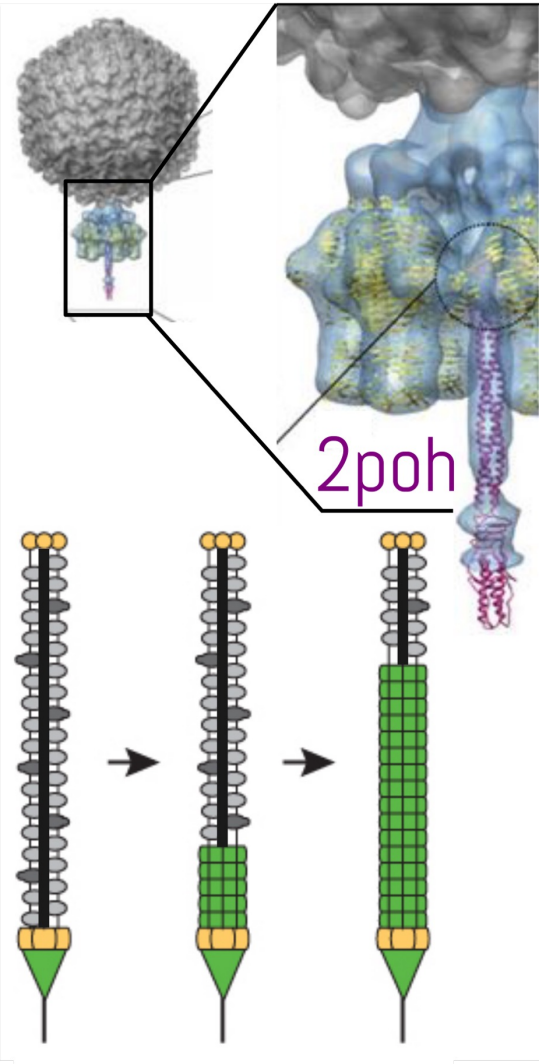
Lipid phage PM2
(2woc)

eukaryotic
dsRNA Simian rotavirus SA11
(4v7q)

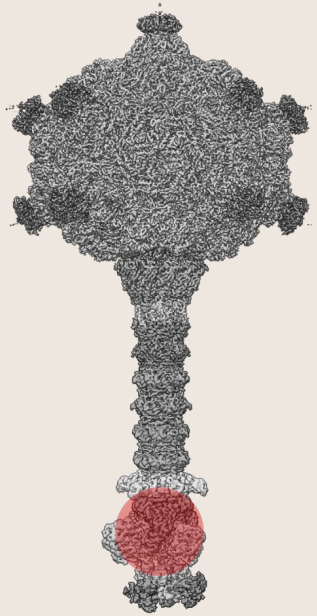
Tape-measure protein of the GTA





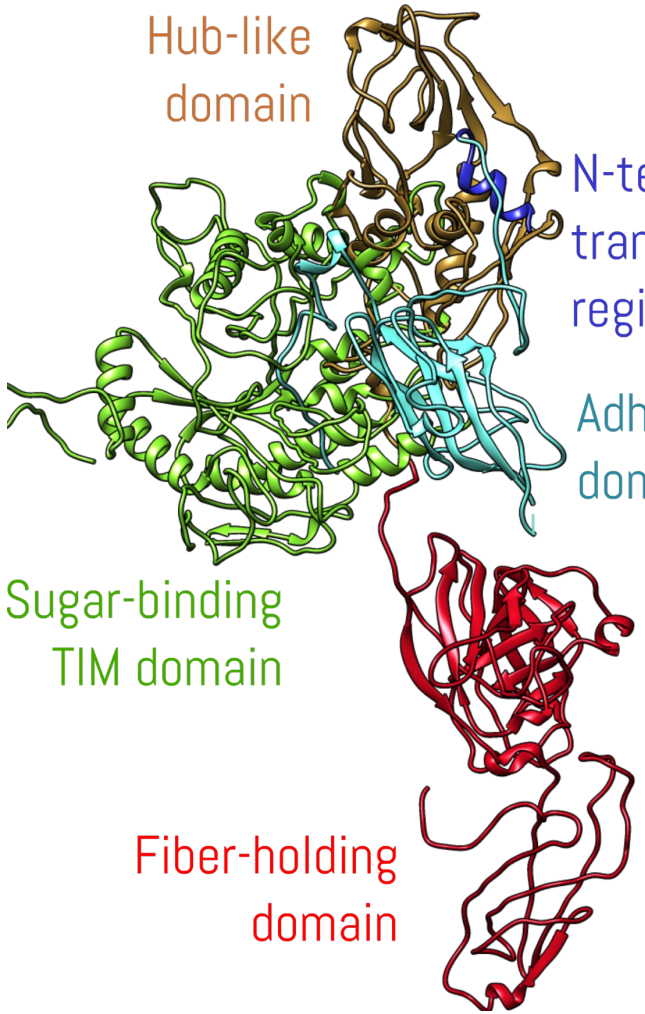


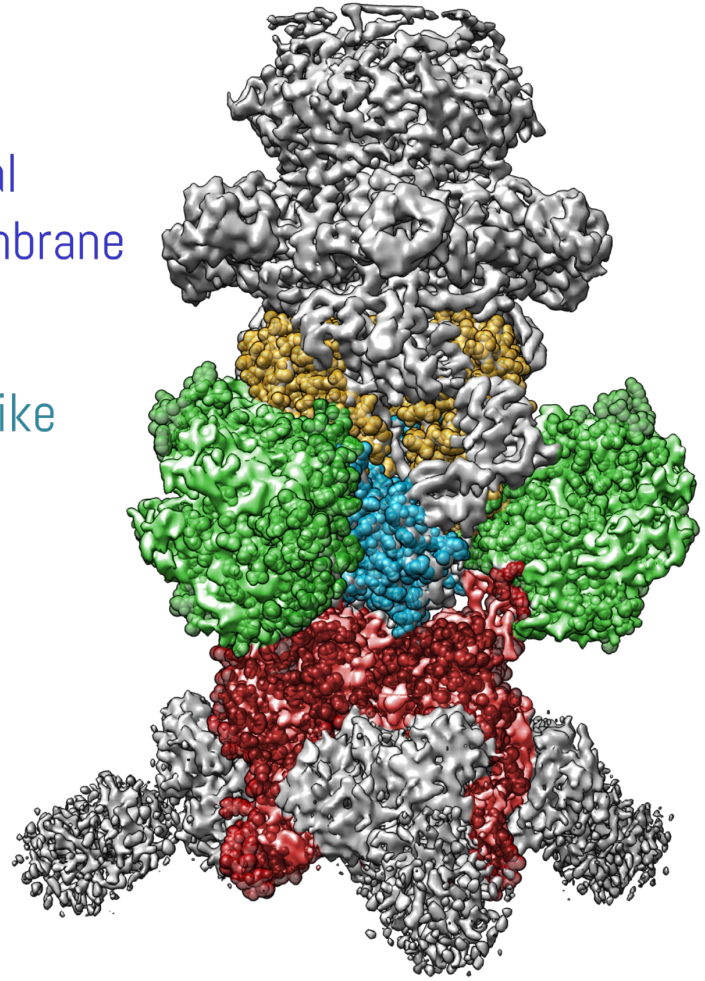
Olia et al., Nat Struct Mol Biol 2007; www.viralexpasy.org



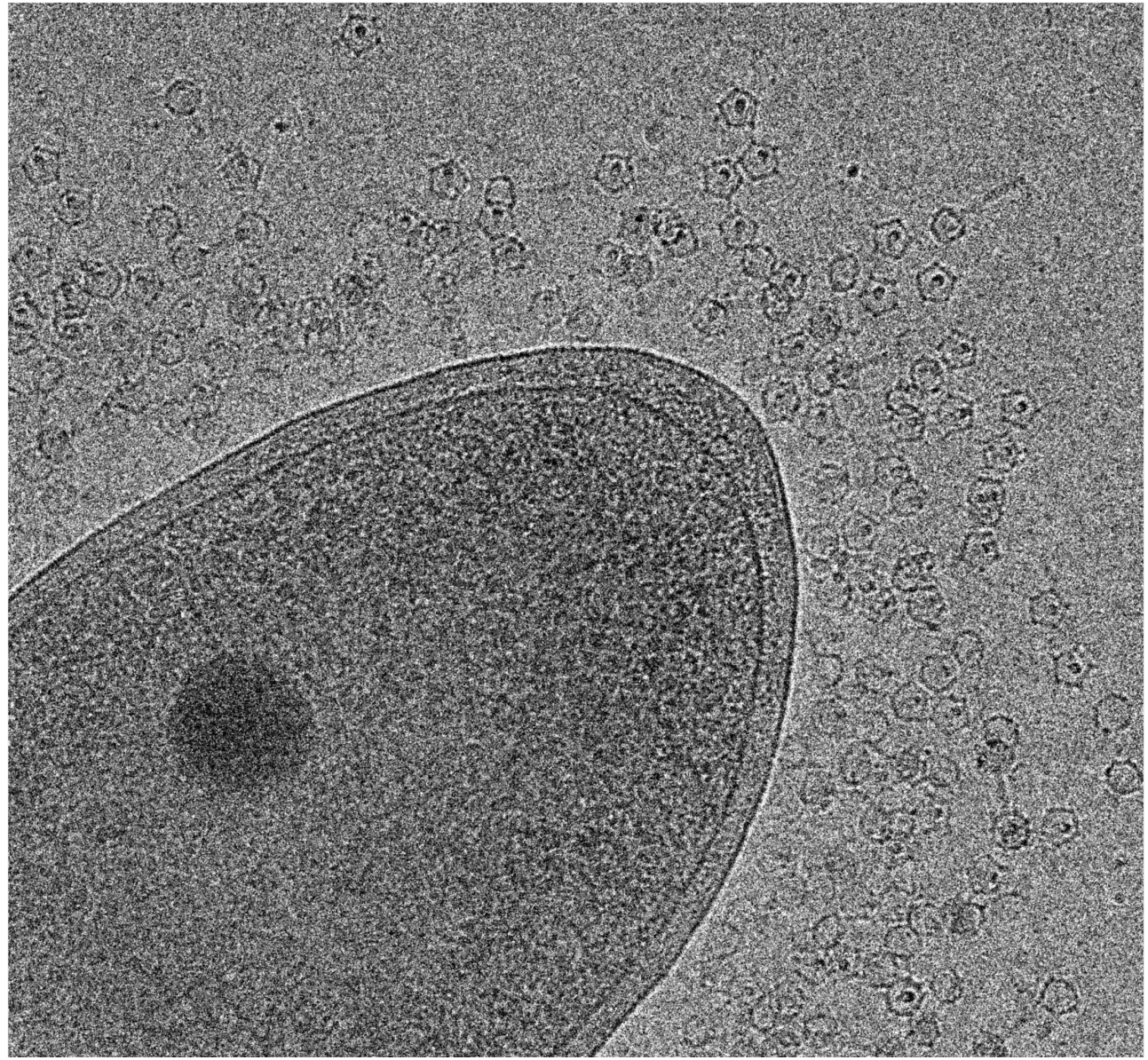
RESULTS

MEGATRON protein

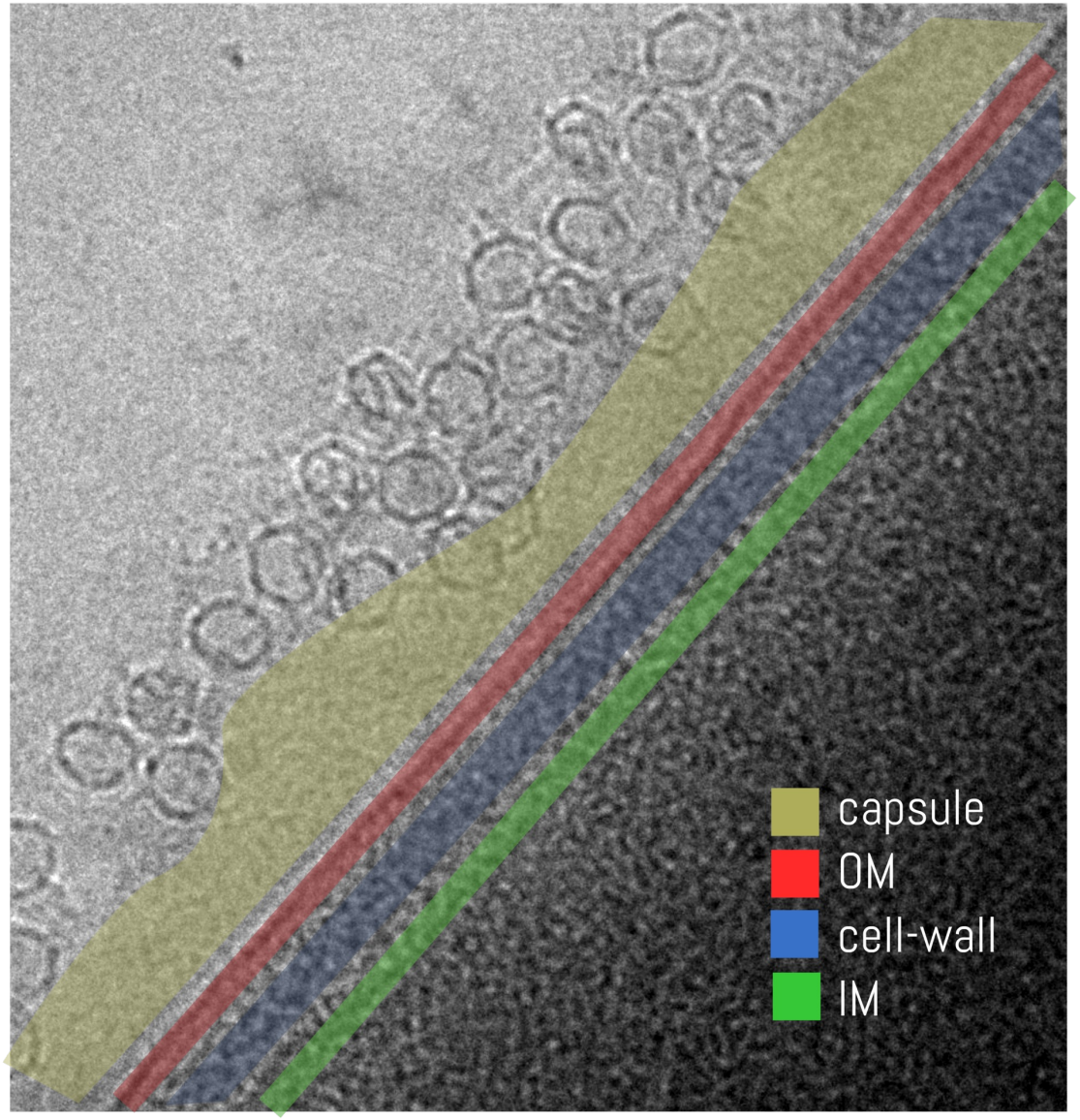




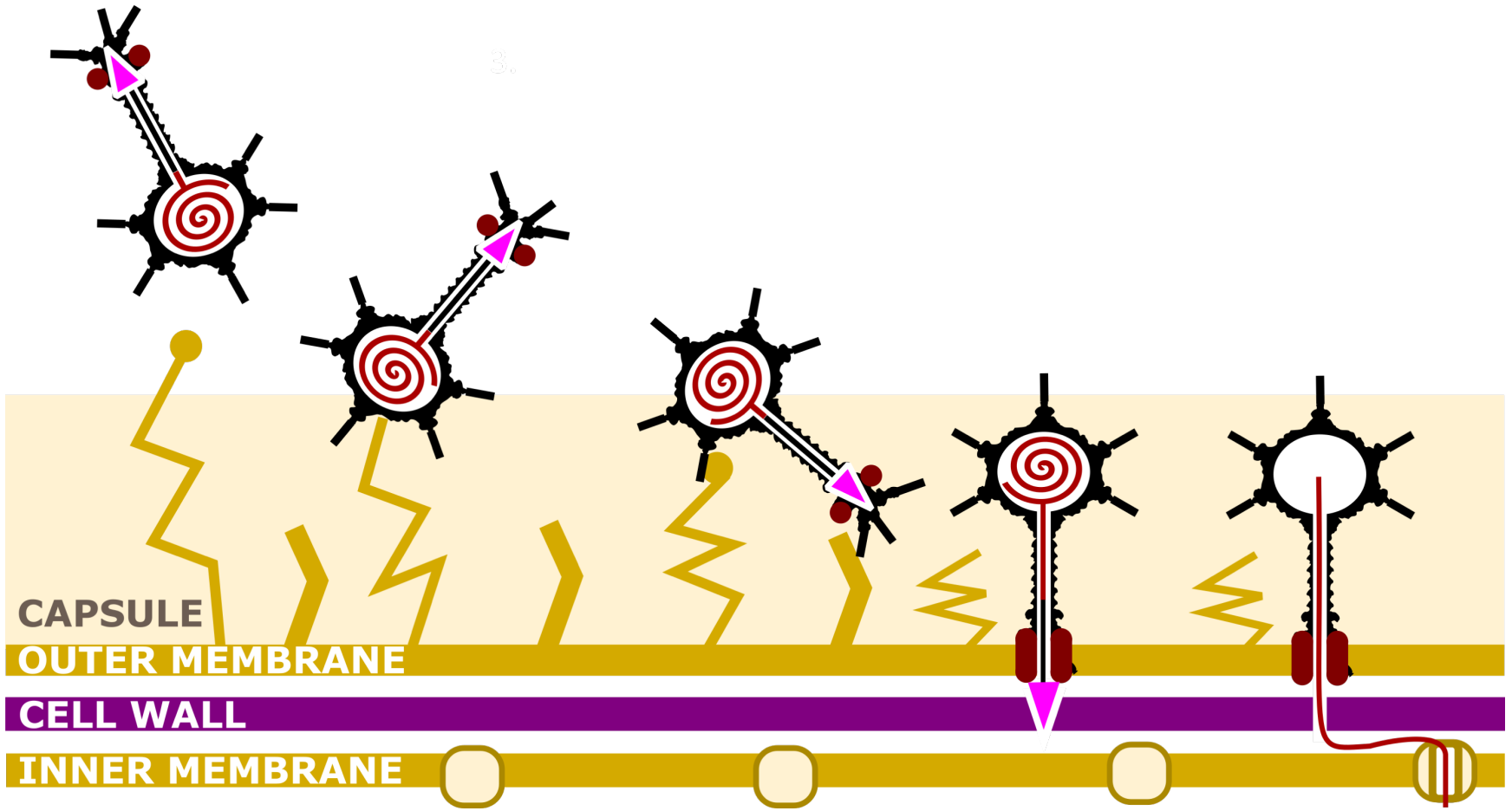
DNA-delivery mechanism of the GTA

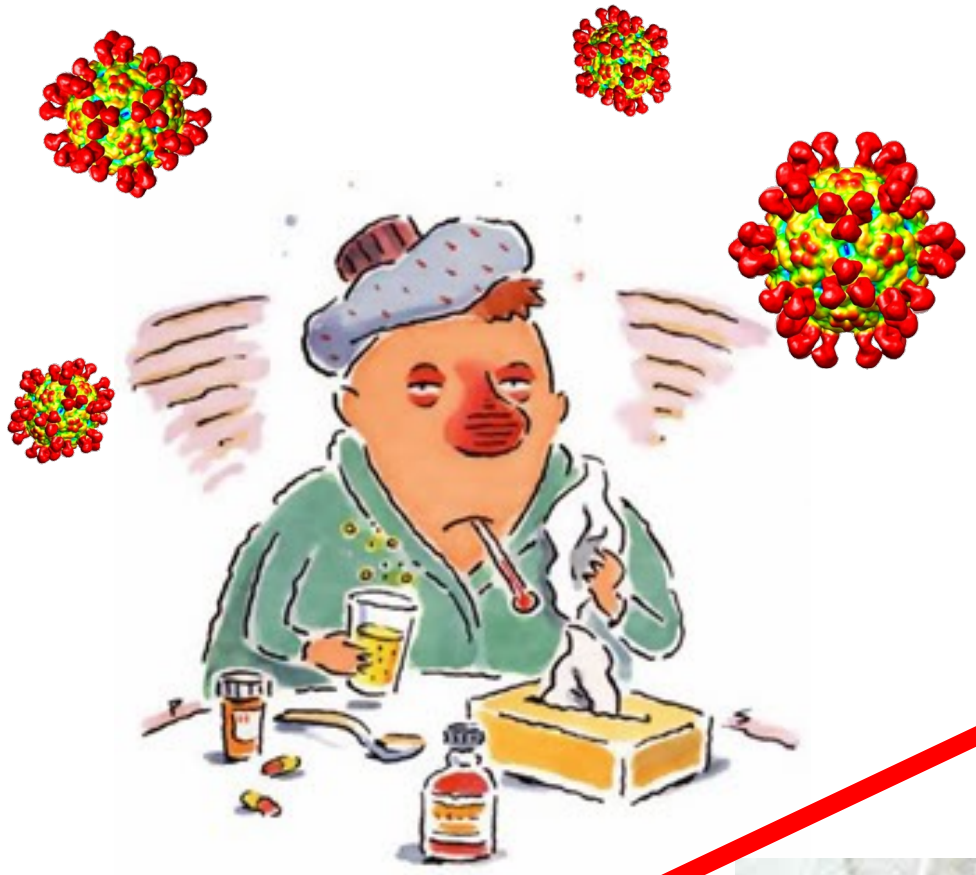


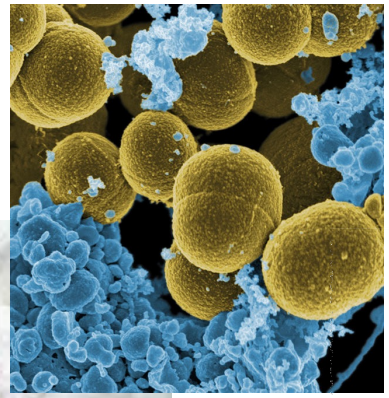
DNA-delivery mechanism of the GTA

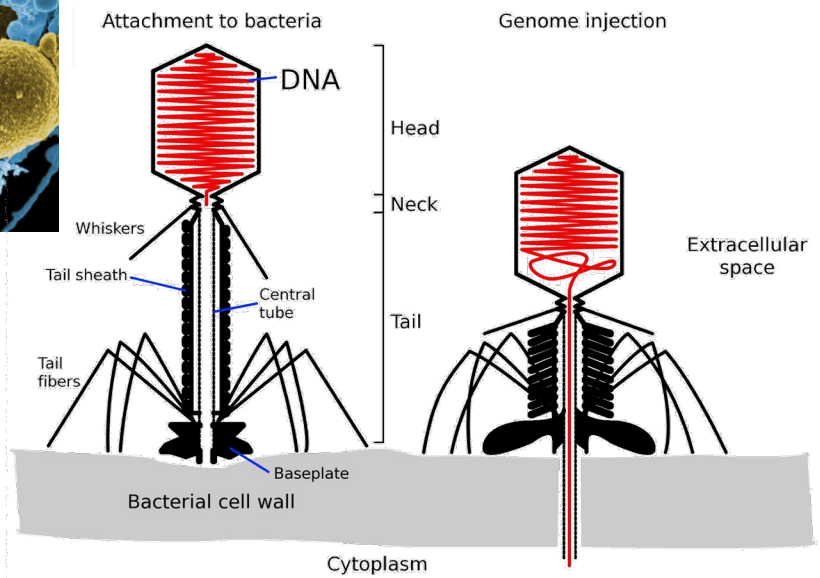


DNA-delivery mechanism of the GTA

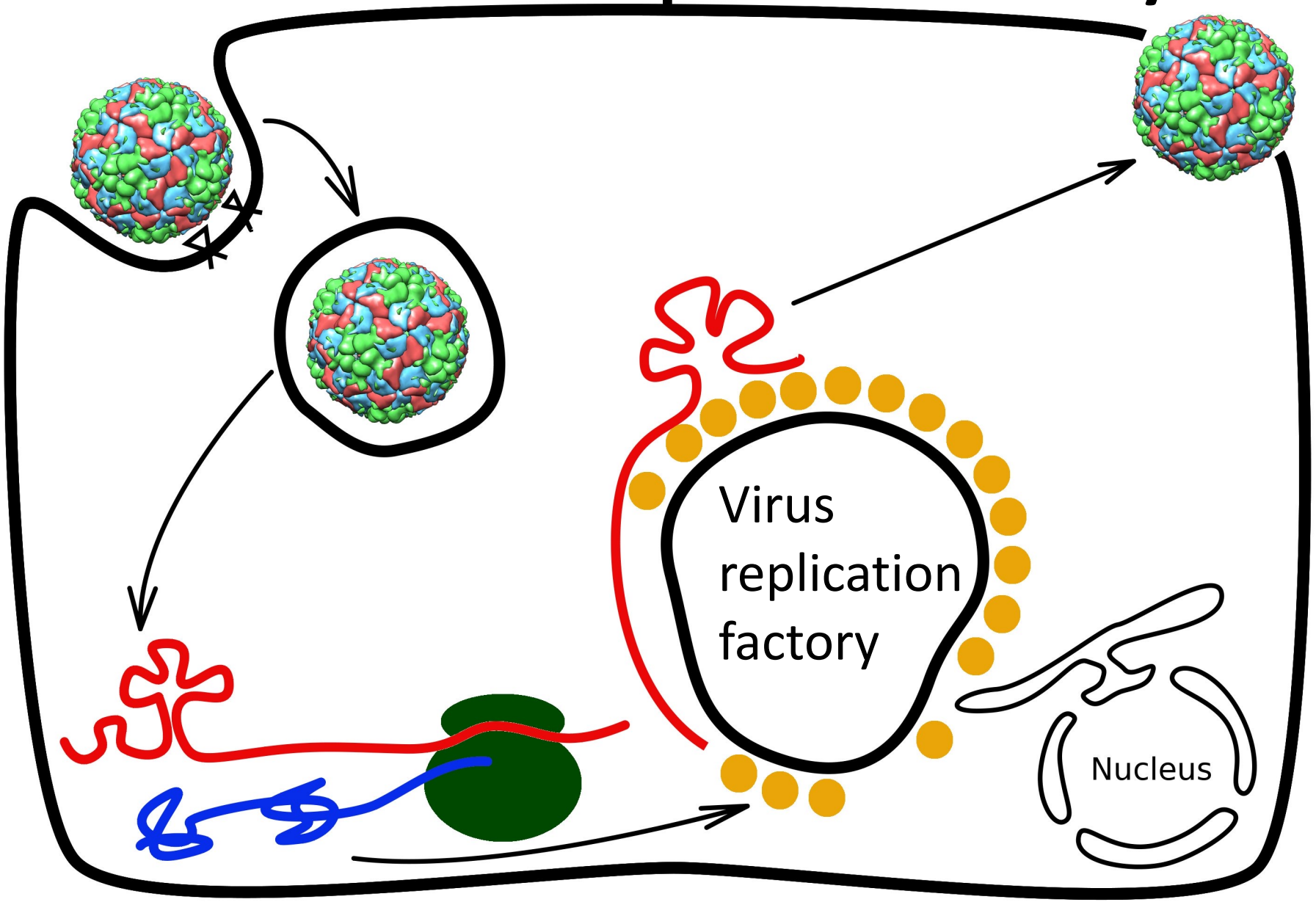




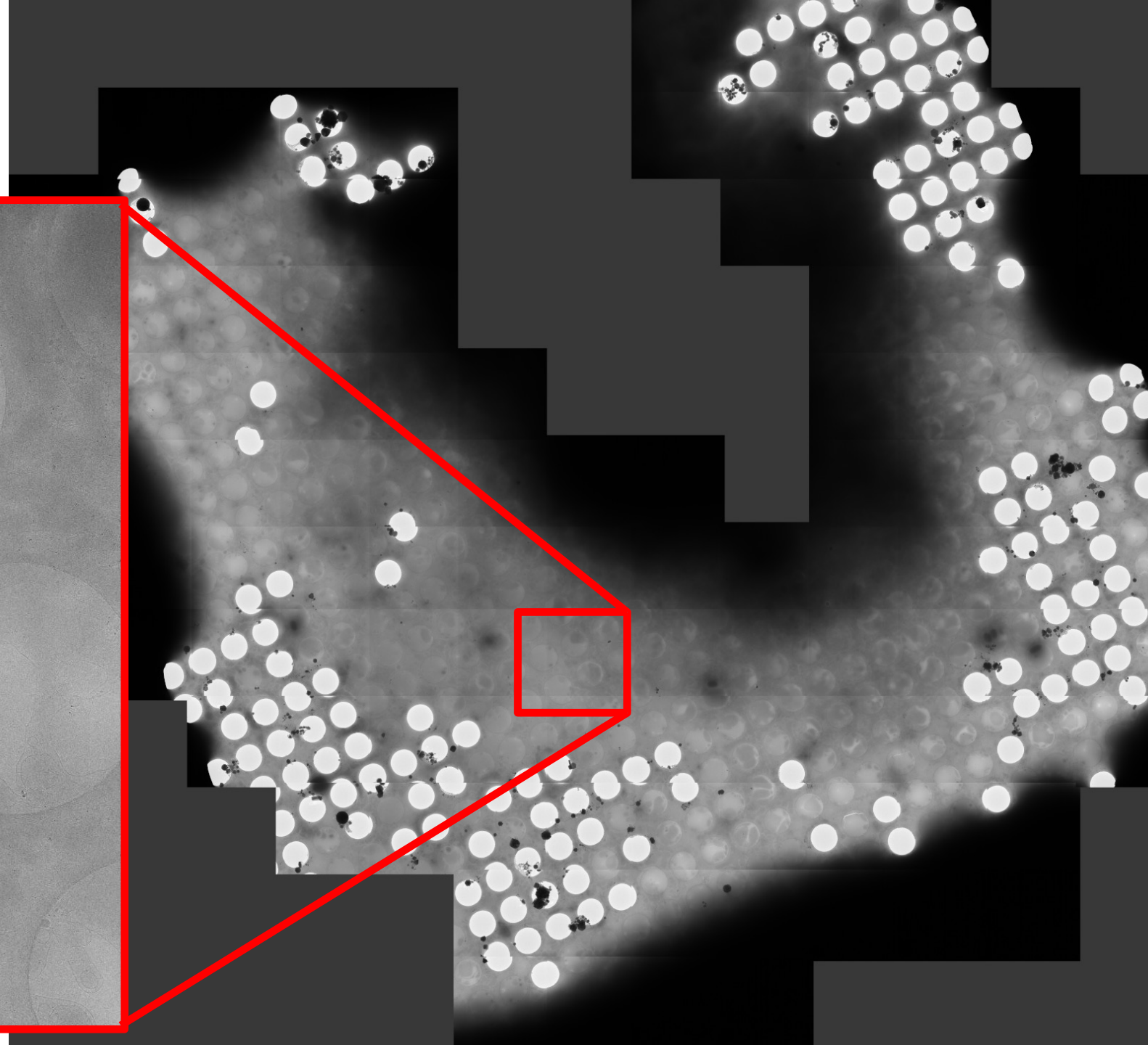



Attachment to bacteria
Genome injection
DNA
Head
Neck
Whiskers
Tail sheath
Central tube
Tail
Tail fibers
Baseplate
Extracellular space
Bacterial cell wall
Cytoplasm

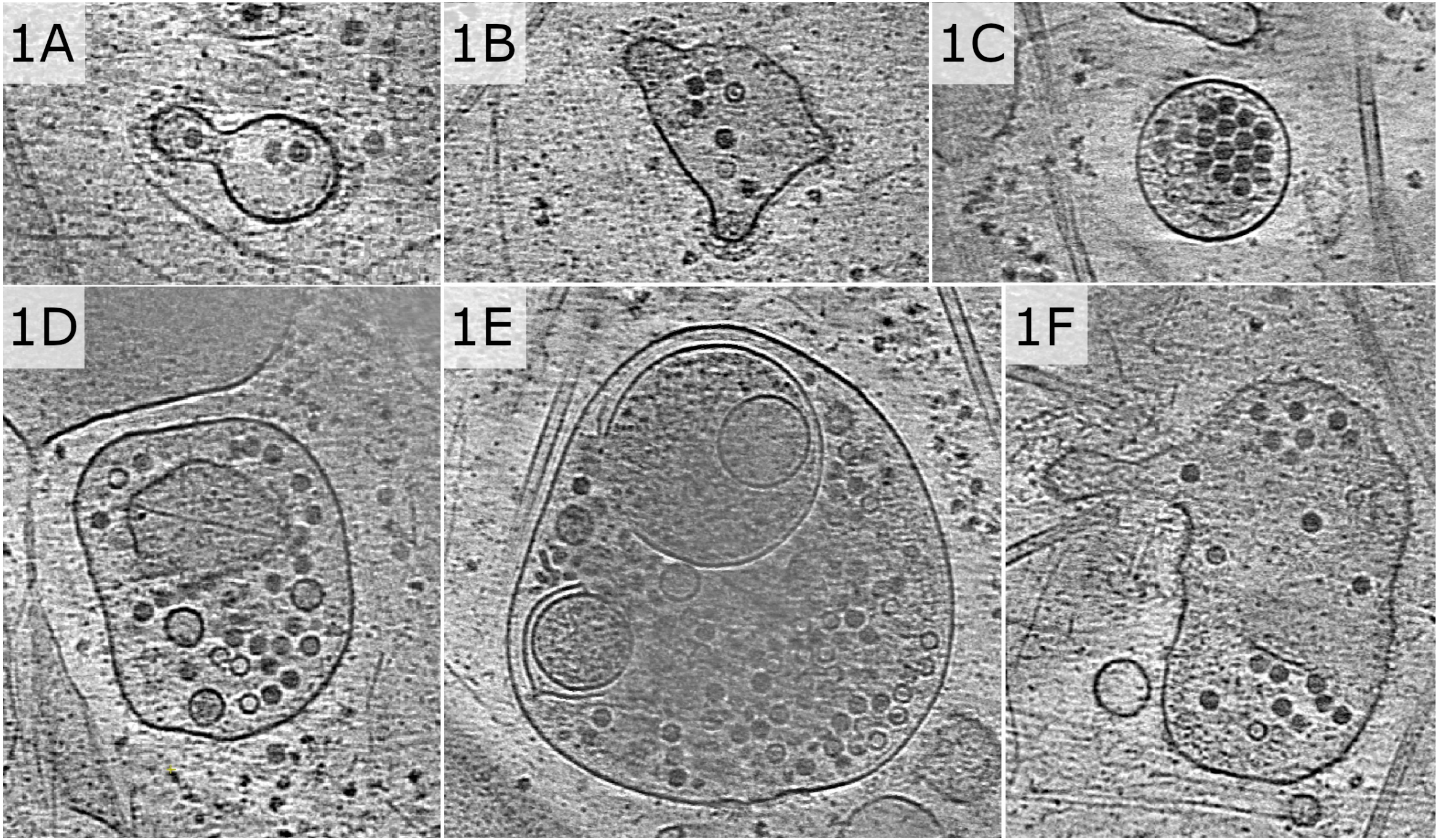
Enterovirus replication cycle



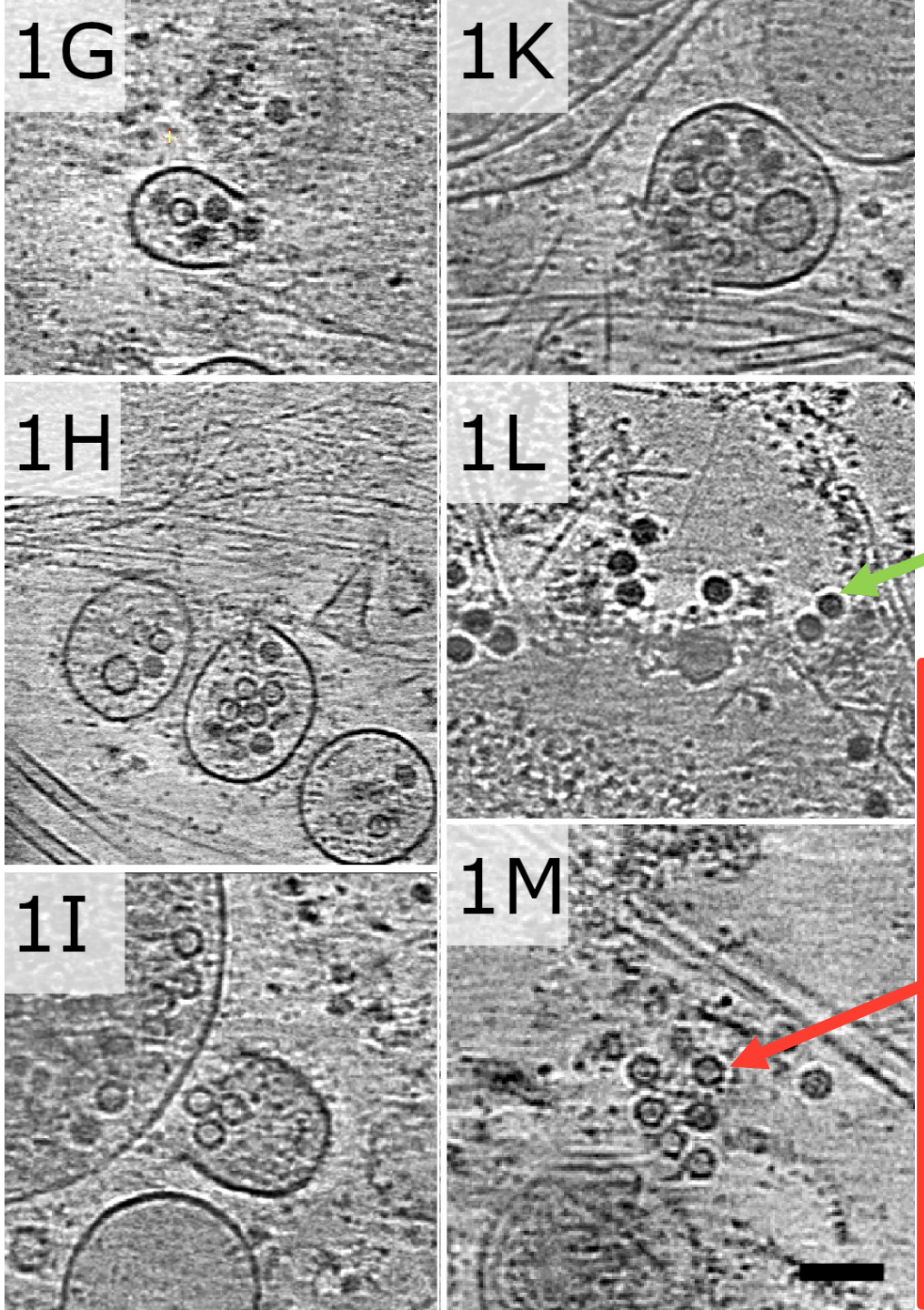
in situ cryo-electron tomography

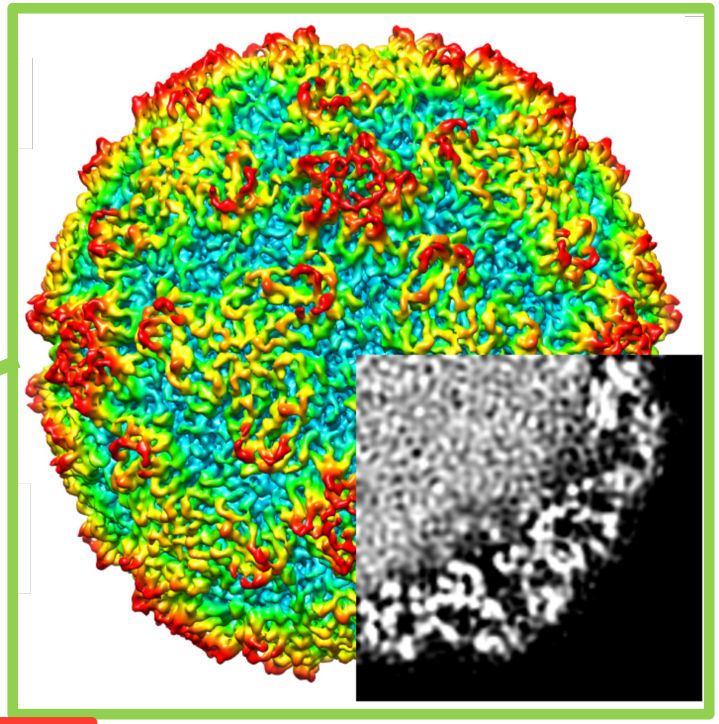


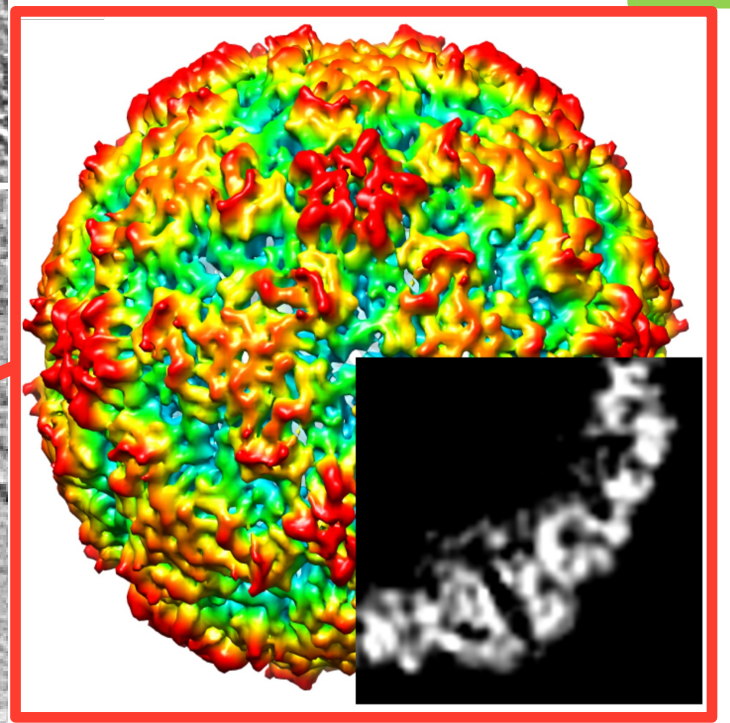
Cell entry of rhinovirus 2



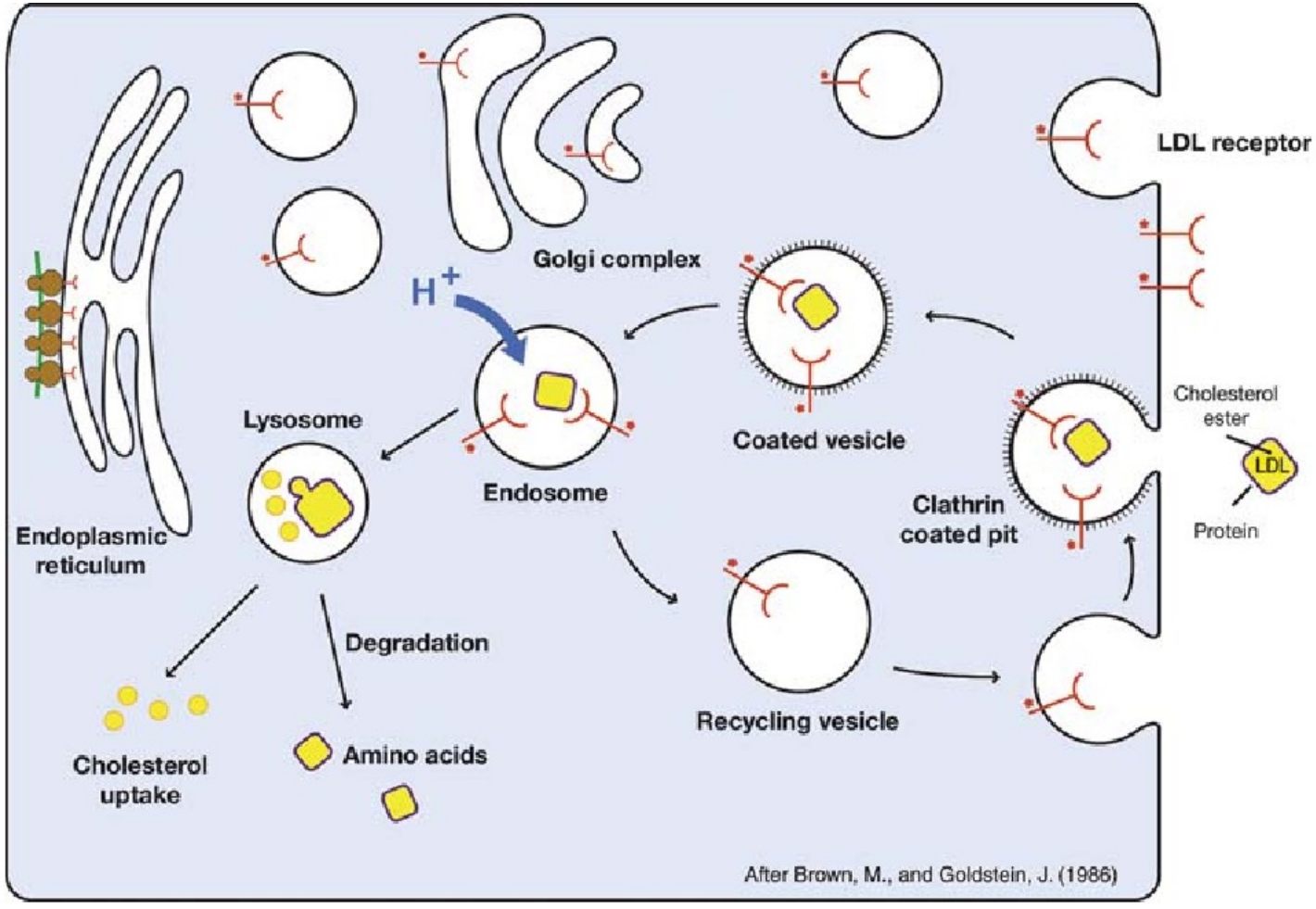
Cell entry of rhinovirus 2

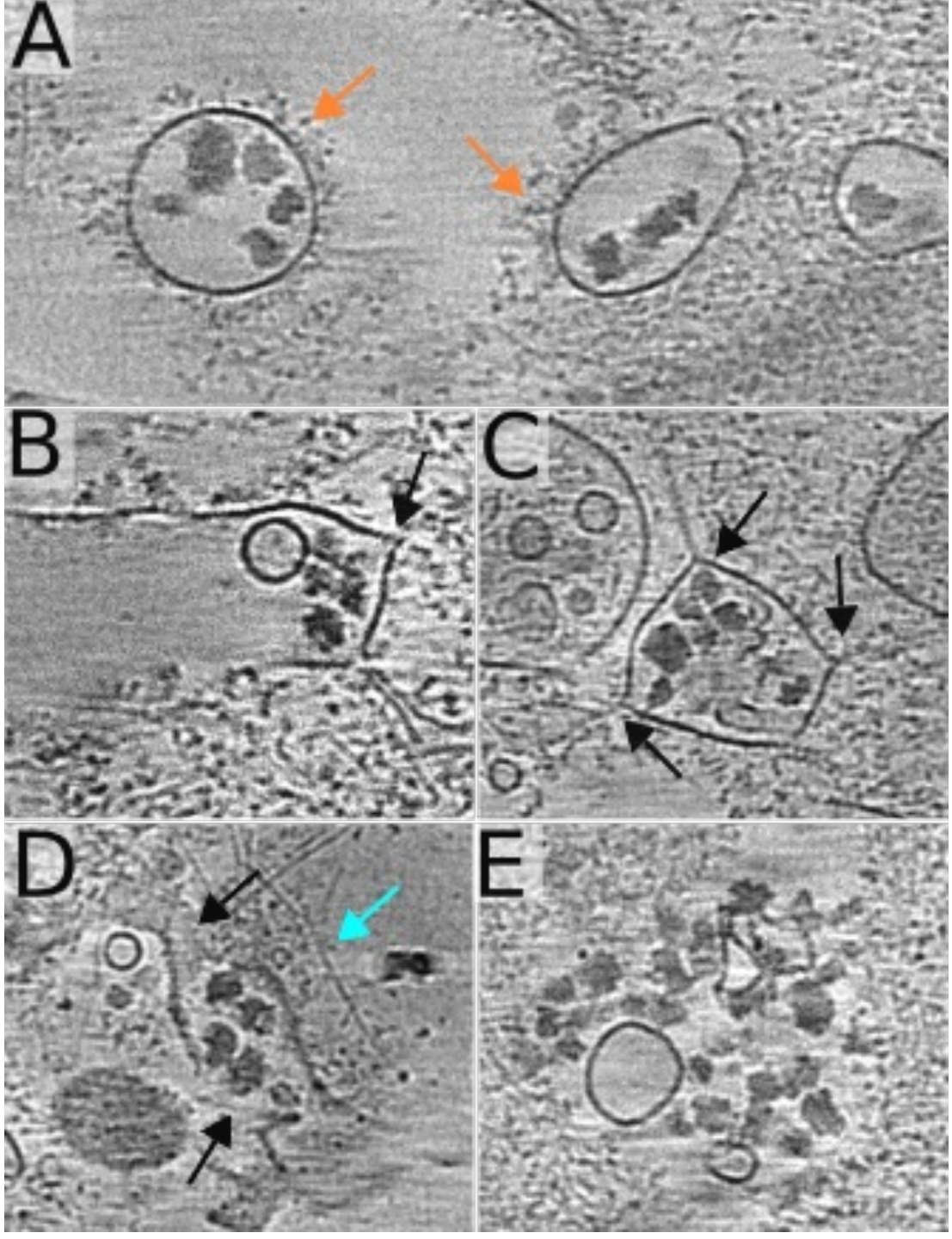






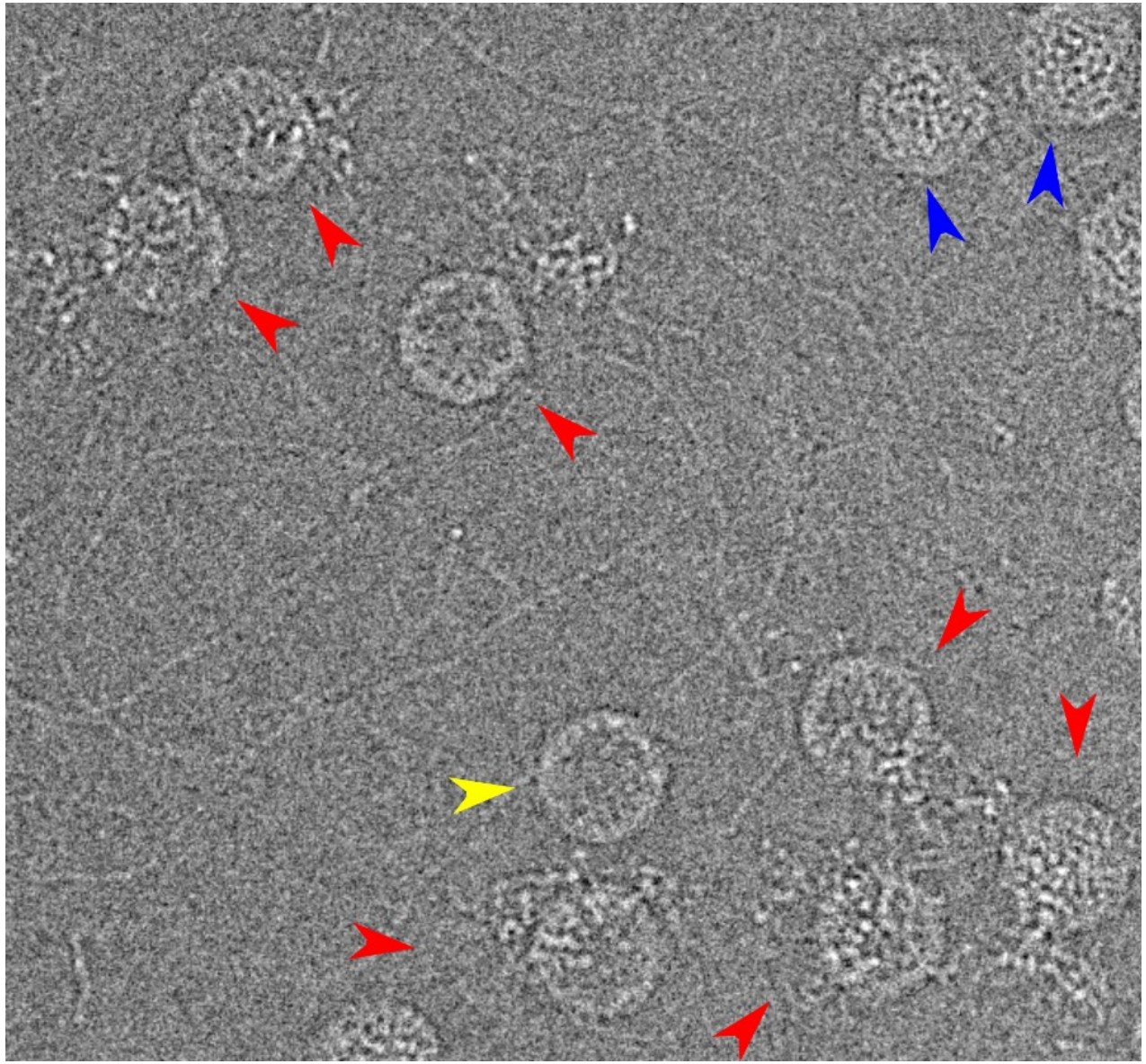
Endocytosis of VLDL - native cargo of VLDLR

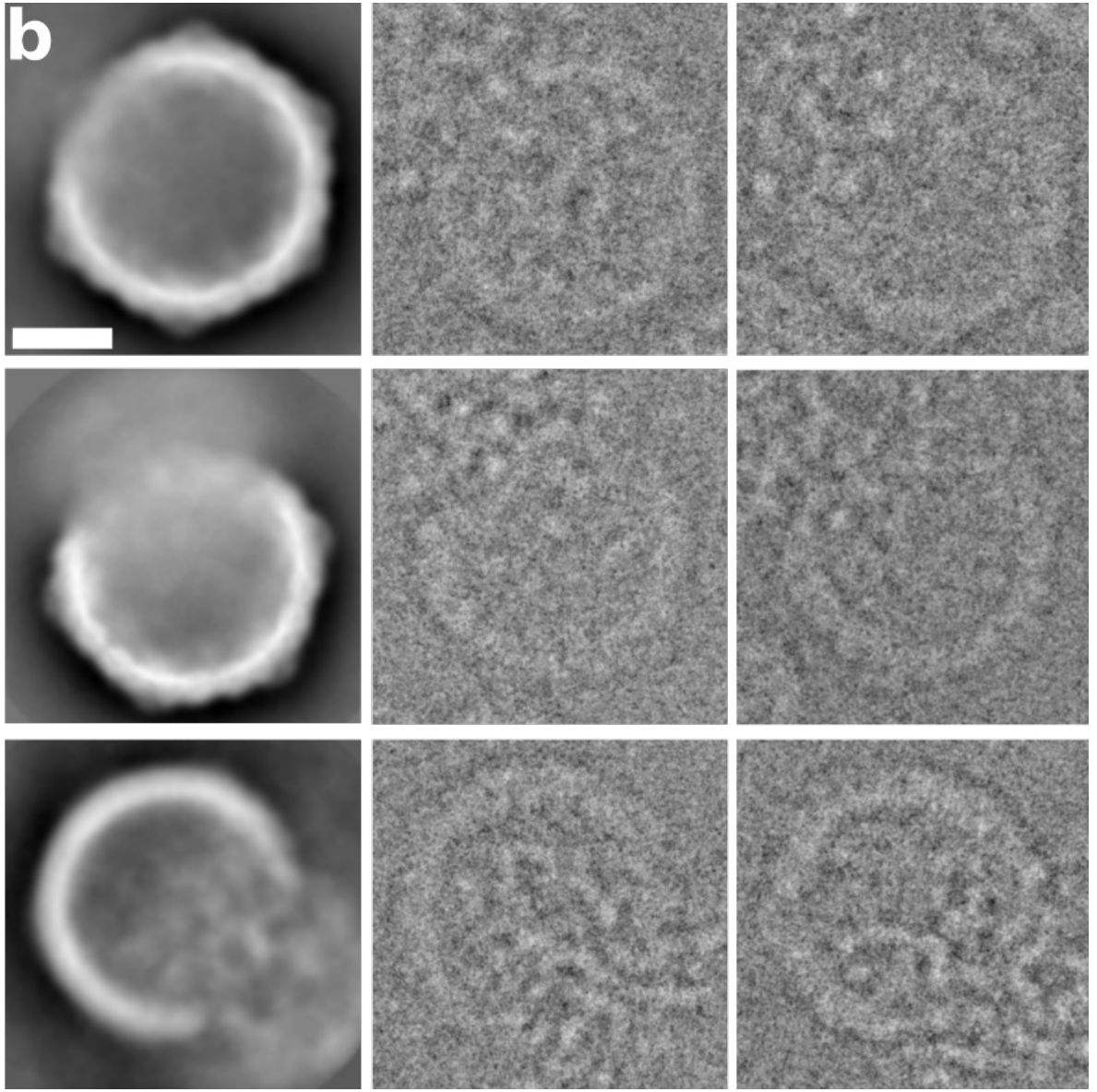




Jeov et al. 2005

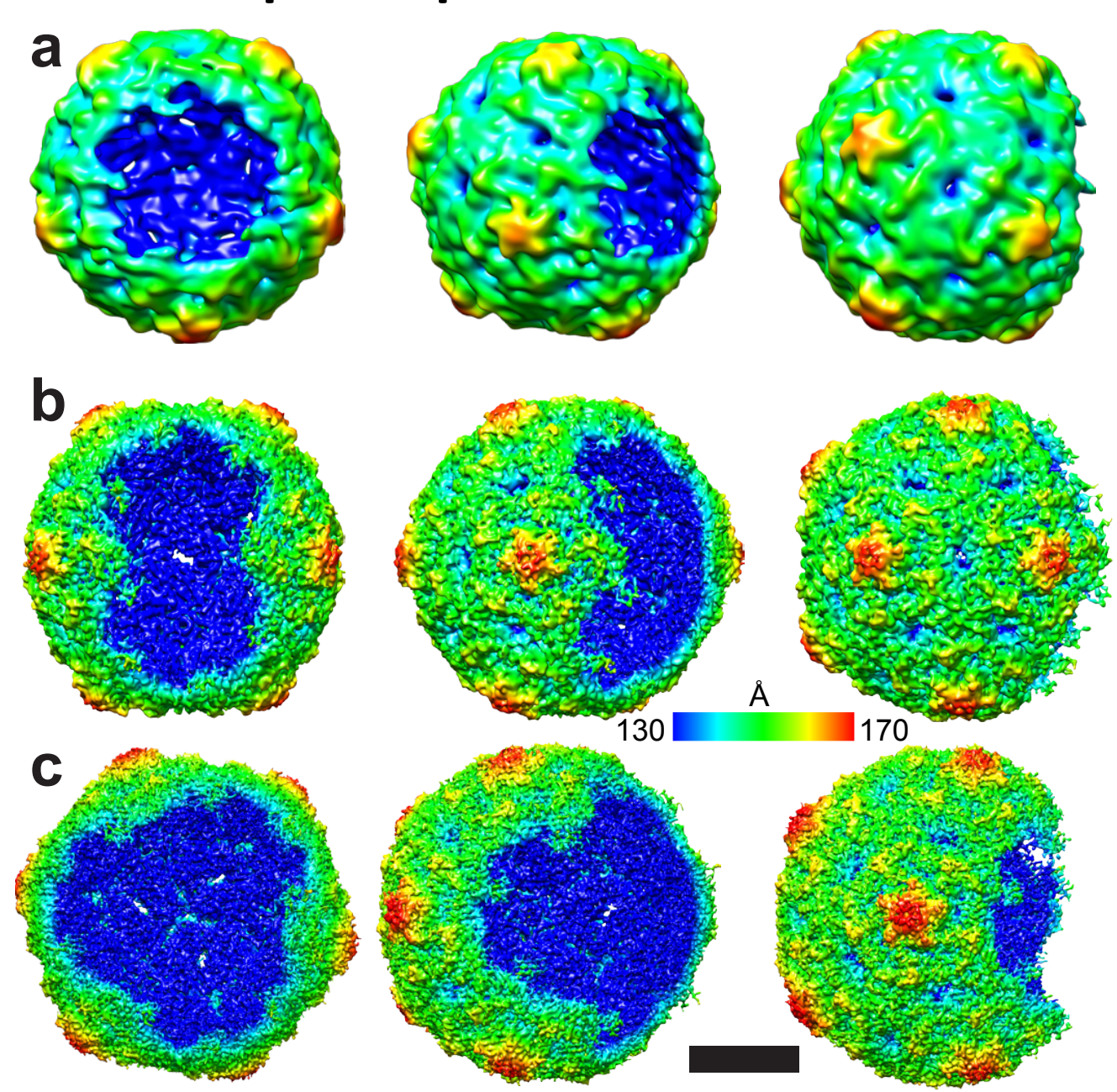
Genome release intermediates of echovirus 18

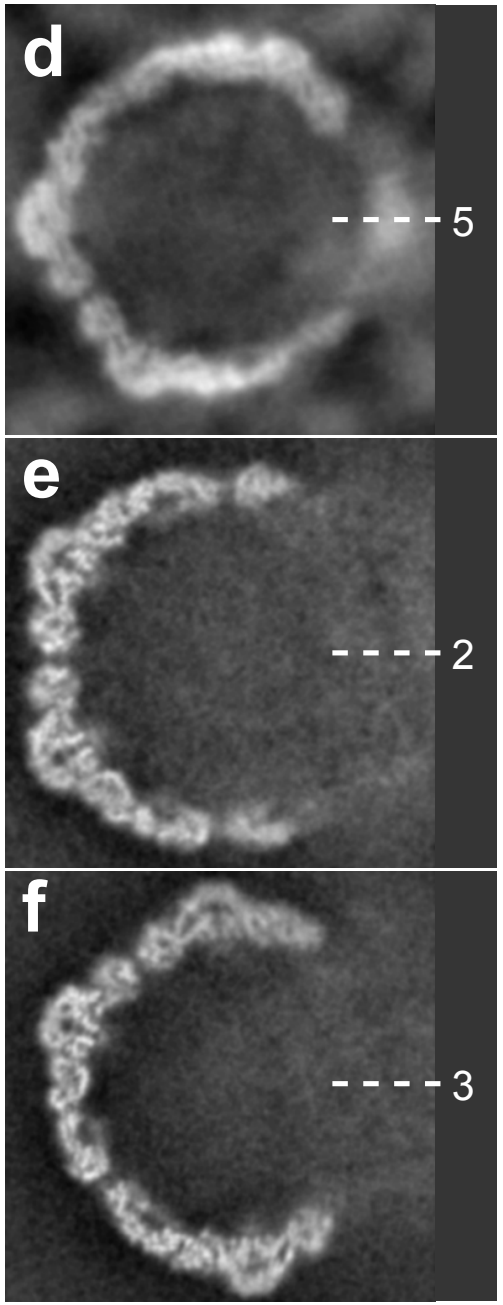


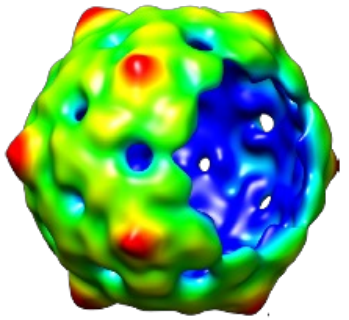


Buchta et al. 2019

Open particles of echovirus 18





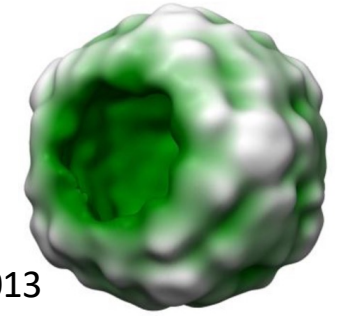


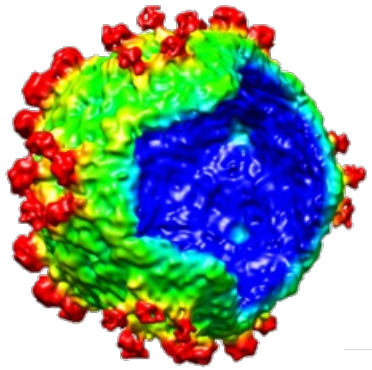
Echovirus 30
Picornaviridae

Buchta et al. 2019

Human rhinovirus 2
Picornaviridae

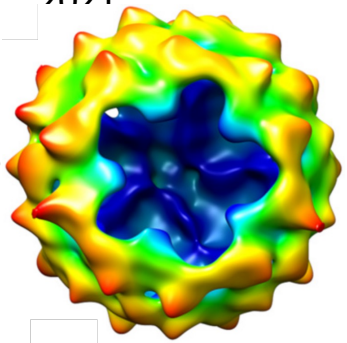
Harutyunyan et al. 2013





Slow bee paralysis virus
Iflaviridae

Škubník et al. 2021



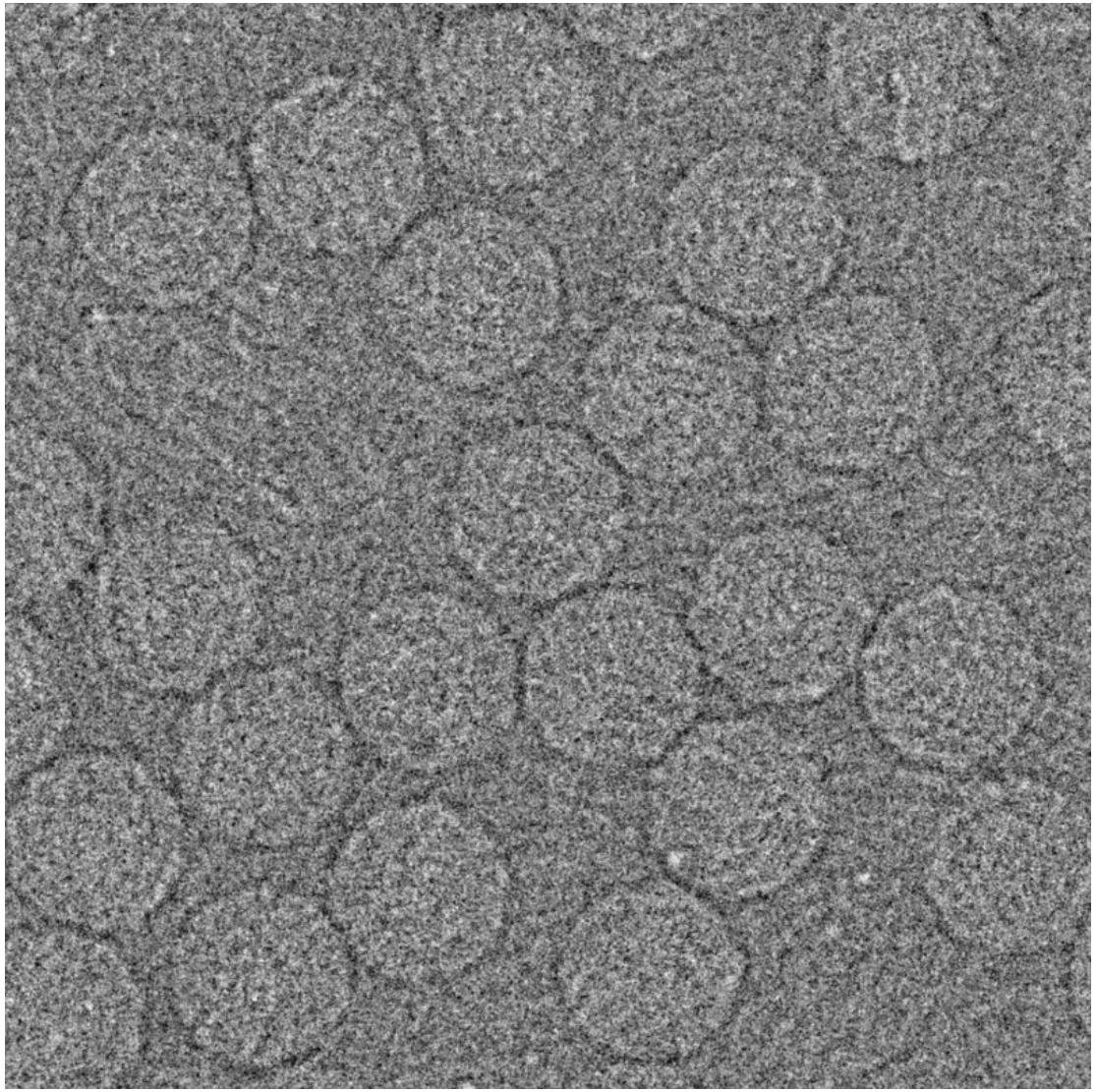
Kashmir bee virus
Dicistroviridae

Mukhamedova et al. 2021

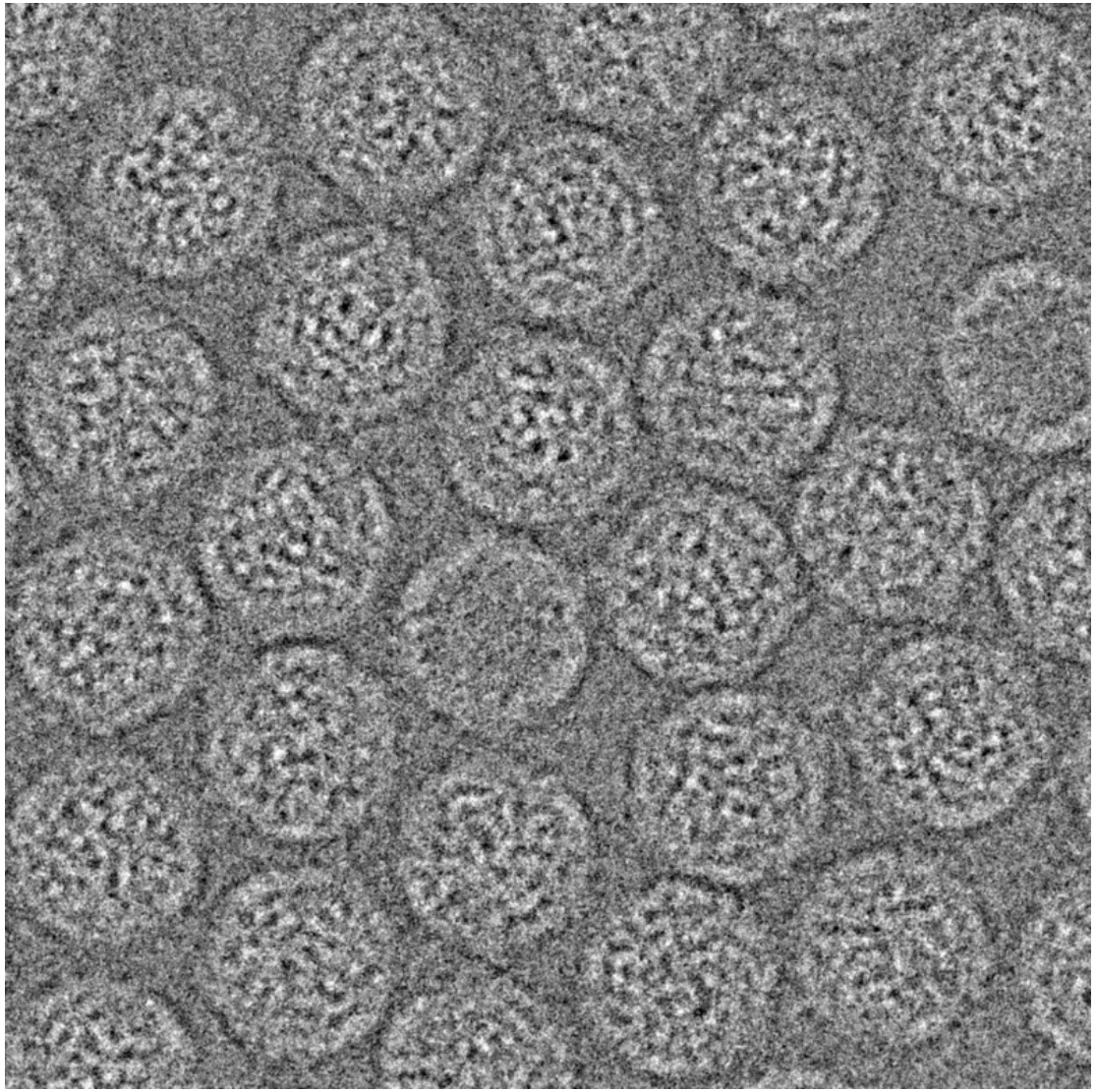
Buchta et al. 2019

Acidic pH induces genome reorganization

Virions at neutral pH



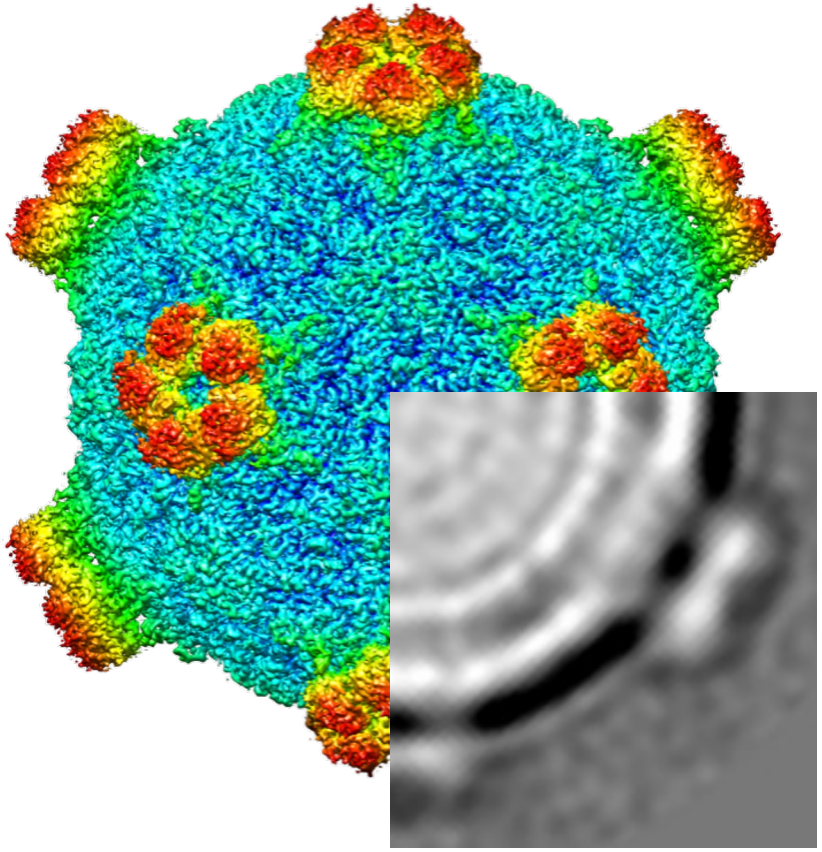
Activated particles at acidic pH

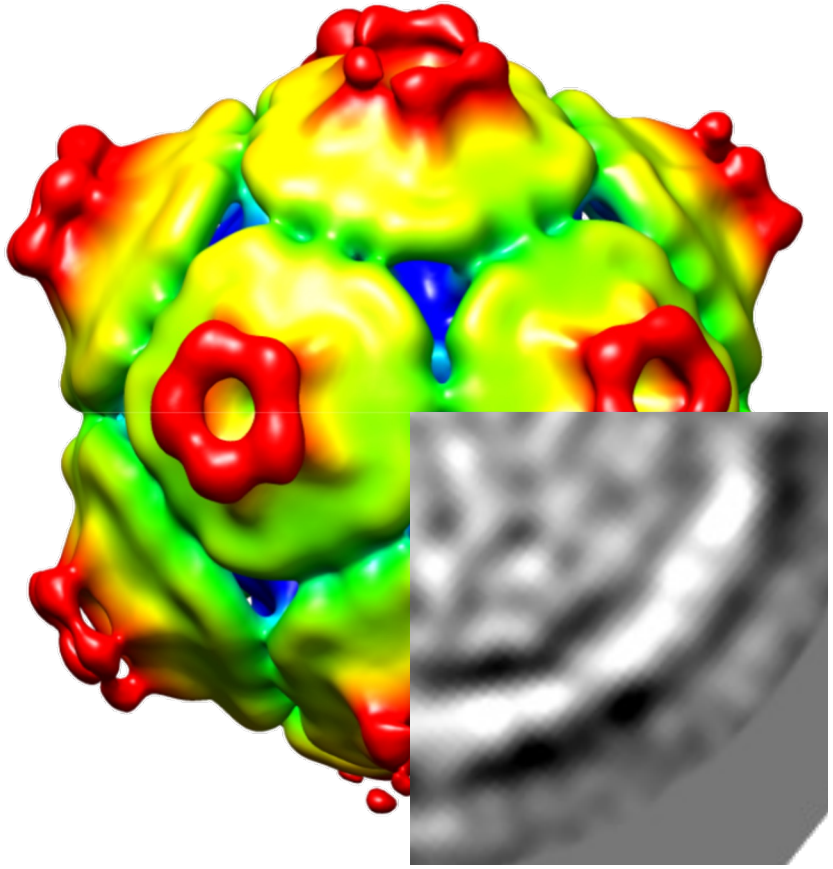


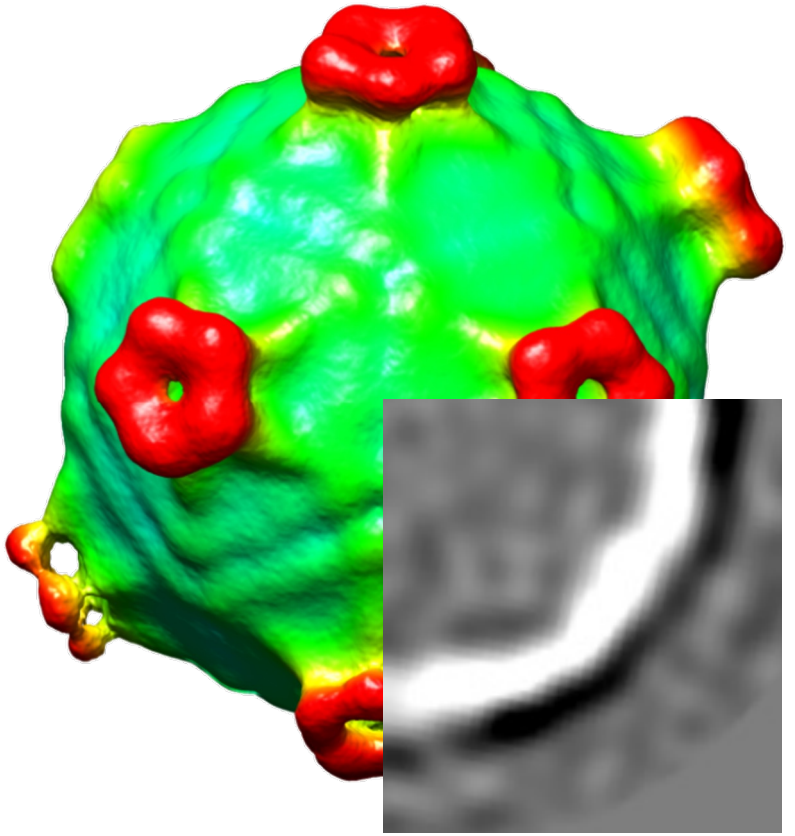
Deformed wing virus (*Iflaviridae*)

Neutral pH

Acidic pH





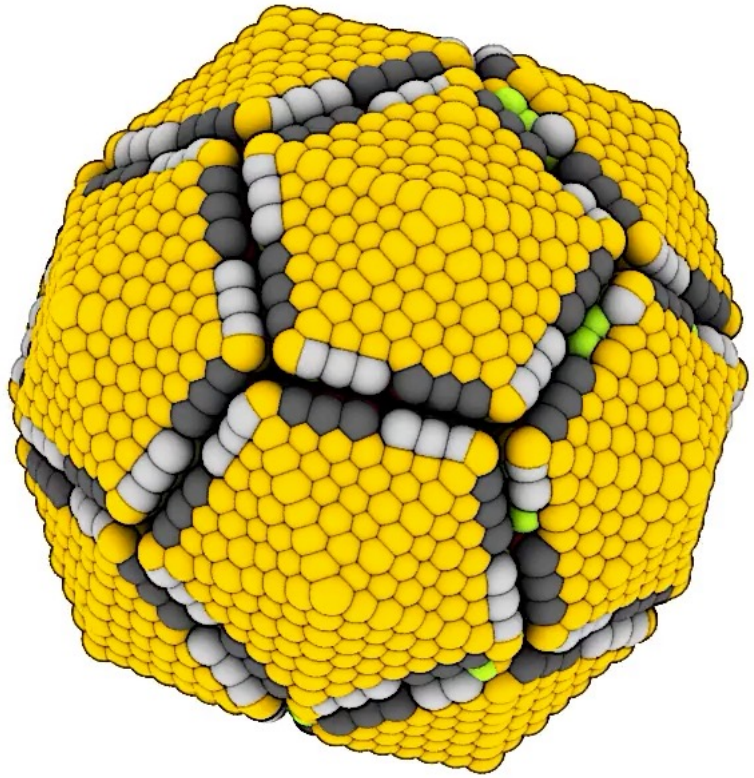


Virion

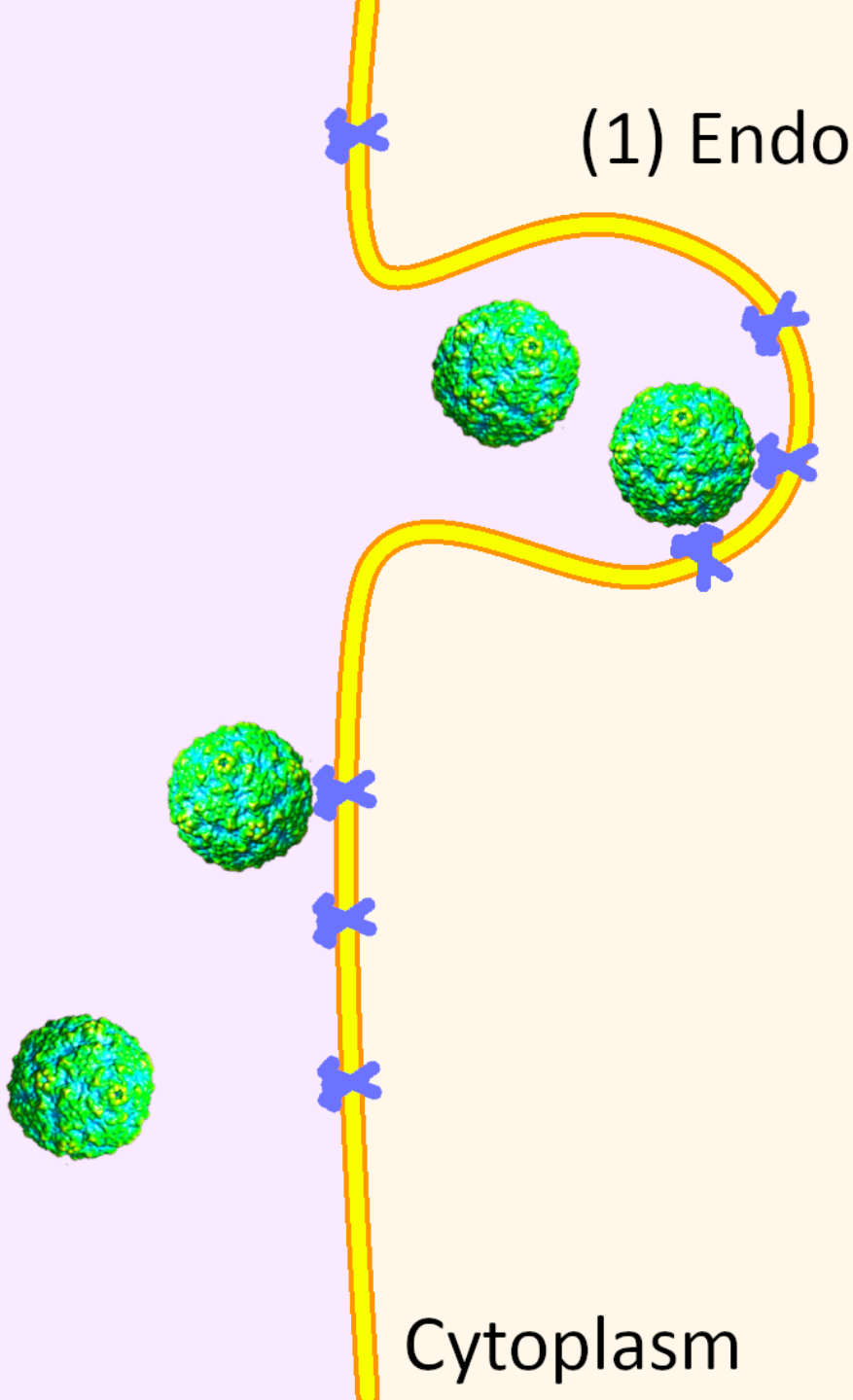
Full particle

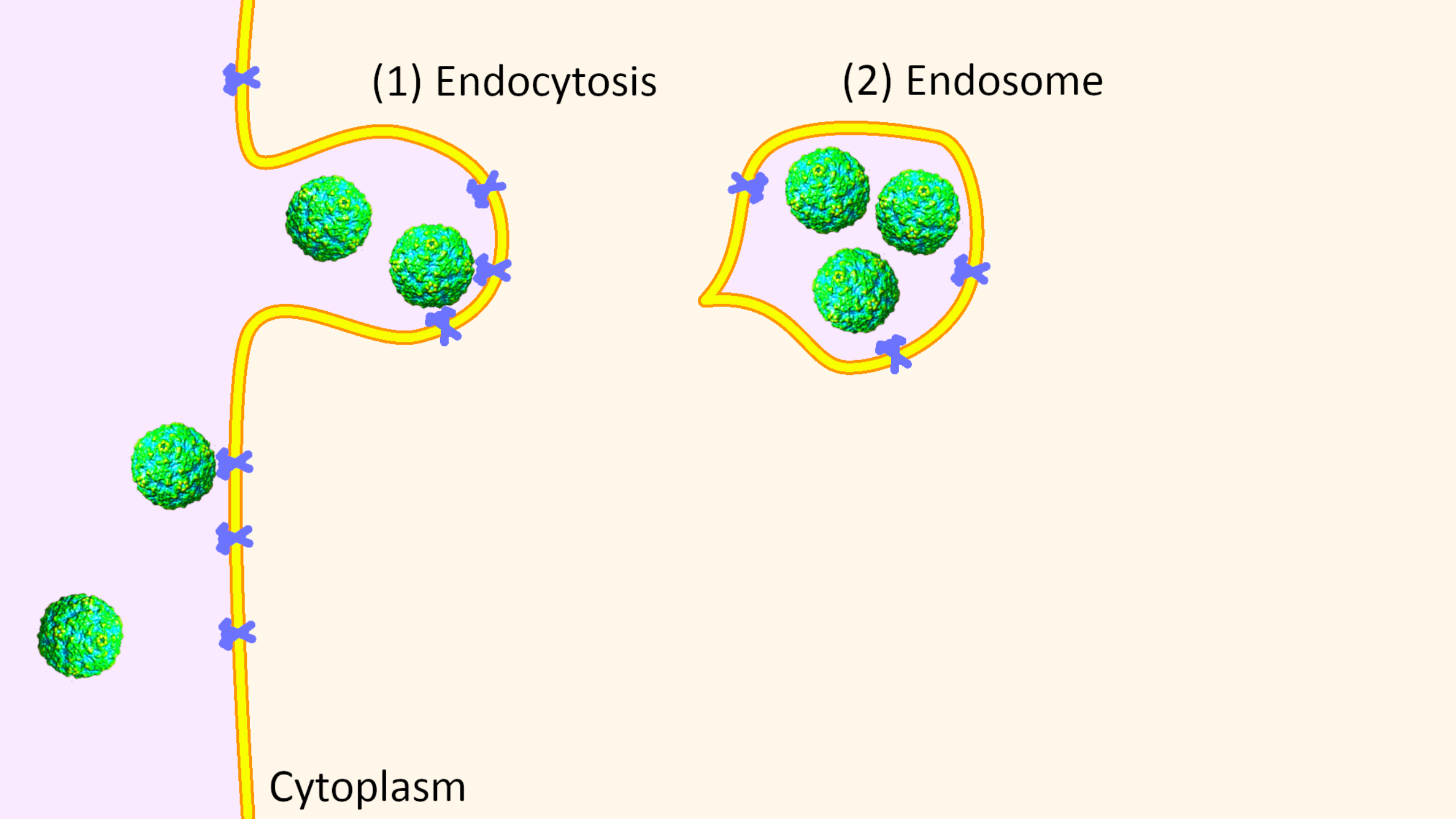
Empty particle

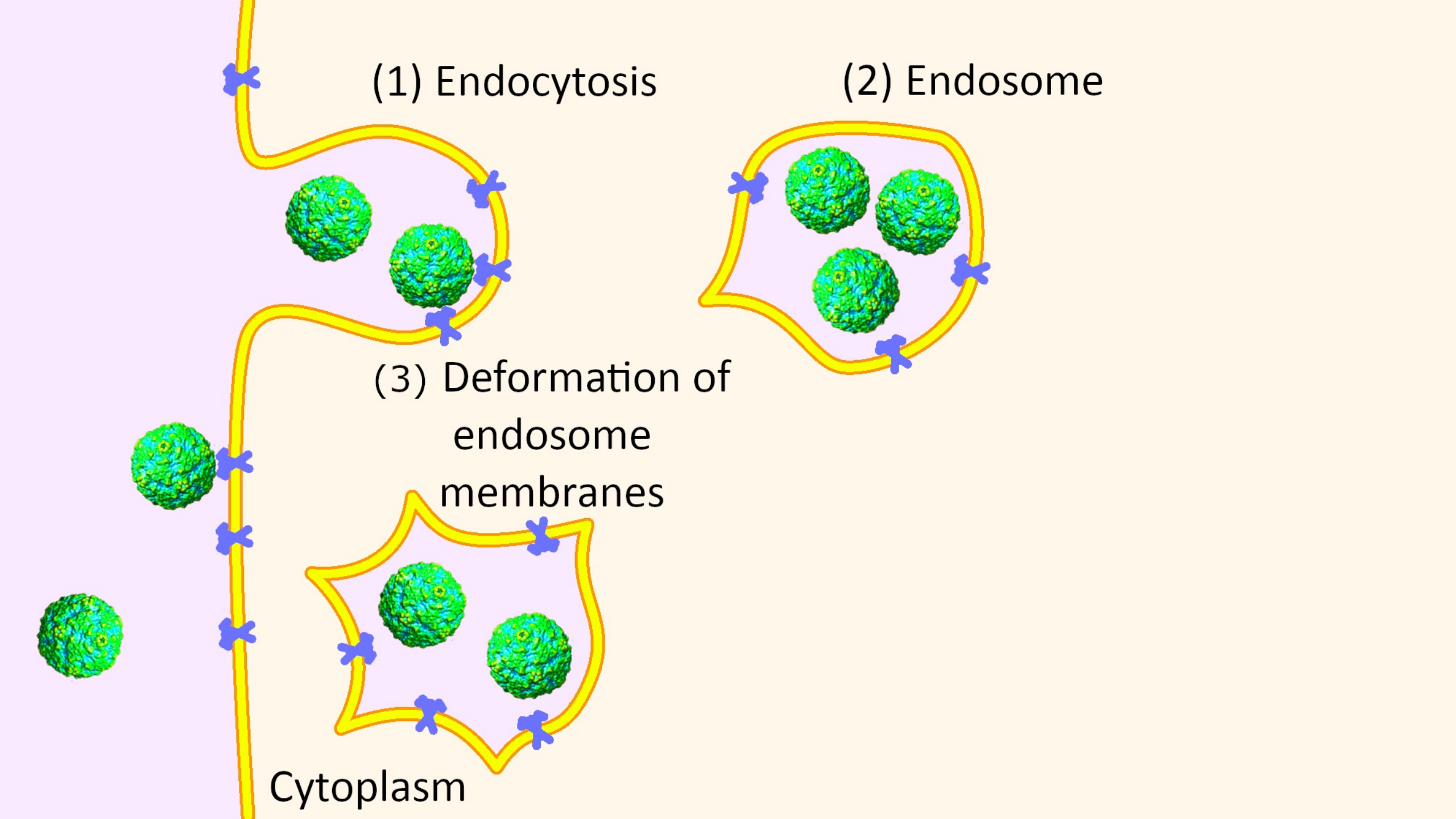
Škubník et al. 2021

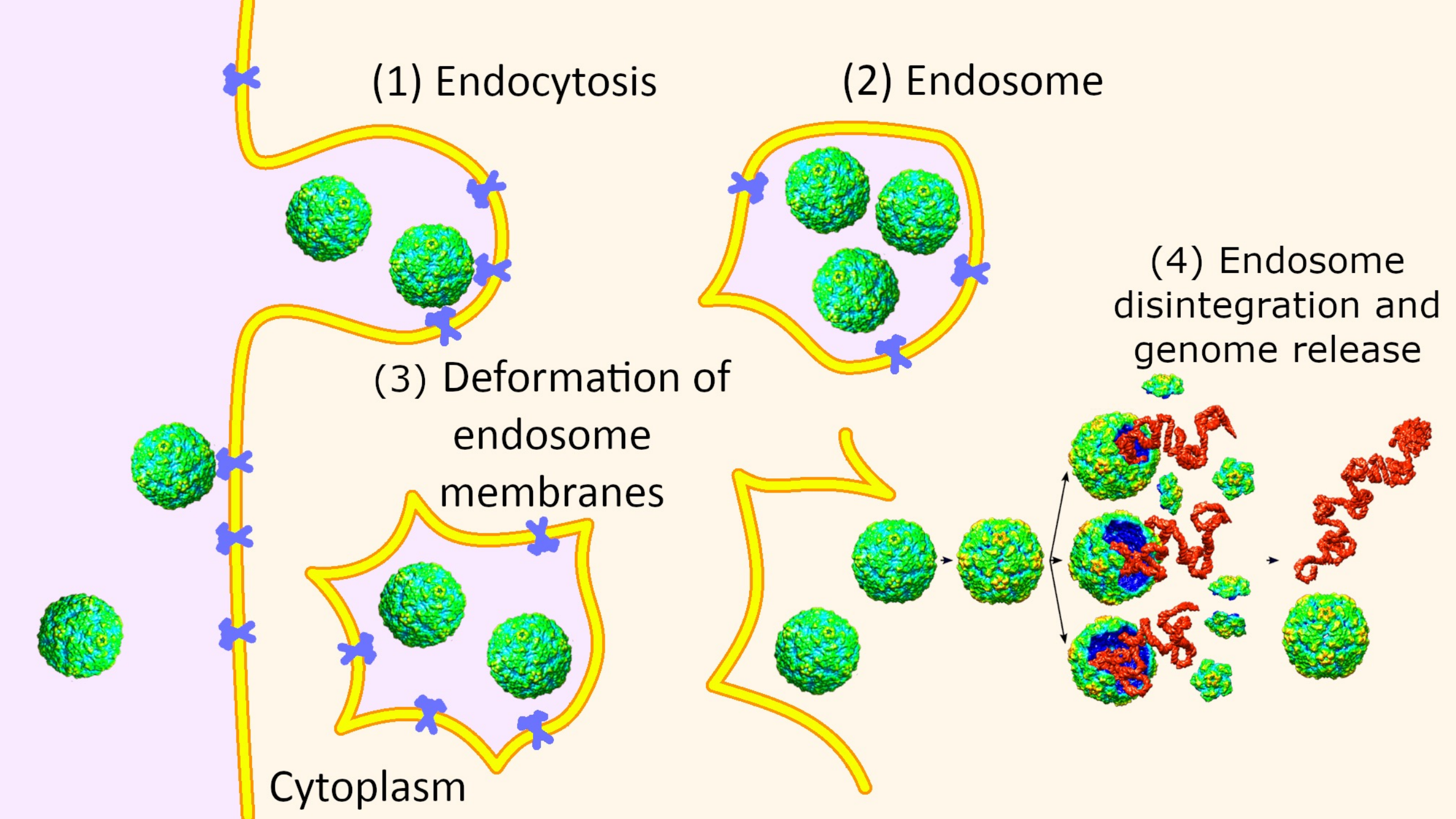


Buchta et al. 2019, Sukeník et al. 2021


(1) Endocytosis
Cytoplasm


(1) Endocytosis
(2) Endosome
Cytoplasm


(1) Endocytosis
(2) Endosome
(3) Deformation of endosome membranes
Cytoplasm


(1) Endocytosis
(2) Endosome
(3) Deformation of endosome membranes
(4) Endosome disintegration and genome release
Cytoplasm

Acknowledgments:

Robert Vácha, Lukáš Sukeník, CEITEC Masaryk University, Czech Republic
Antonín Přidal, Mendel University, Czech Republic
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Roman Pantůček, Martin Benešík, Masaryk University, Czech Republic

What is a virus?

Origins of viruses

Virus structures and infection processes

Extra

I'm in college!

What is important?

Lectures × Bachelor's and Master's theses

- Lectures are the theoretical basis, but you will gain practical skills by solving real problems.
- In a bachelor's/master's thesis, you will encounter questions without a known solution for the first time.

A good supervisor is the basis for your career

The thesis topic and the supervisor are an indivisible whole.

The topic of your bachelor's thesis will define your study specialisation and thus determine your employment.

How to choose a supervisor and topic for your bachelor's and master's thesis

- Think and invest time in the choice.
- Internet and MU website.
- Ask **sensible** colleagues and teachers.

Choosing the right supervisor requires information

Starting to work on BC work from the first semester means less stress, better results, and more experience.

What NOT to choose by

- academic degrees
- I like her/him (how she/he lectures)
- I attended his/her lecture
- I have a friend working for him

Desirable traits of a supervisor

- receives individual research funding from GAČR, TAČR, and EU
  - Read CV
- publishes as corresponding author in international journals
  - Read CV
- students and post-docs are at the first place of their supervisor's publications
- studied or worked abroad

Structure of deformed wing virus, a major honey bee pathogen

Karel Škubník[a], Jiří Nováček[a], Tibor Füzik[a], Antonín Přidal[b], Robert J. Paxton[c], and Pavel Plevka[a,1]

[a]Structural Virology, Central European Institute of Technology, Masaryk University, 62500 Brno, Czech Republic; [b]Department of Zoology, Fishery, Hydrobiology, and Apidology, Faculty of Agronomy, Mendel University in Brno, 613 00 Brno, Czech Republic; and [c]Institute of Biology/Zoology, Martin Luther University Halle-Wittenberg, 06120 Halle, Germany

Edited by Wolfgang Baumeister, Max Planck Institute of Chemistry, Martinsried, Germany, and approved February 10, 2017 (received for review September 20, 2016)

The worldwide population of western honey bees (*Apis mellifera*) is under pressure from habitat loss, environmental stress, and pathogens, particularly viruses that cause lethal epidemics. Deformed wing virus (DWV) from the family *Iflaviridae*, together with its vector, the mite *Varroa destructor*, is likely the major threat to the world's honey bees. However, lack of knowledge of the atomic structures of iflaviruses has hindered the development of effective treatments against them. Here, we present the virion structures of DWV determined to a resolution of 3.1 Å using cryo-electron microscopy and 3.8 Å by X-ray crystallography. The C-terminal extension of capsid protein VP3 folds into a globular protruding (P) domain, exposed on the virion surface. The P domain contains an Asp-His-Ser catalytic triad that is, together with five residues that are spatially close, conserved among iflaviruses. These residues may participate in receptor binding or provide the protease, lipase, or esterase activity required for entry of the virus into a host cell. Furthermore, nucleotides of the DWV RNA genome interact with VP3 subunits. The capsid protein residues involved in the RNA binding are conserved among honey bee iflaviruses, suggesting a putative role of the genome in stabilizing the virion or facilitating capsid assembly. Identifying the RNA-binding and putative catalytic sites within the DWV virion structure enables future analyses of how DWV and other iflaviruses infect insect cells and also opens up possibilities for the development of antiviral treatments.

colony collapse disorder | virus | structure | *Apis mellifera* | honey bee

The western honey bee (*Apis mellifera*) plays a vital role in world agriculture by providing pollination services to diverse commercial crops, a service valued at US$ 215 billion annually (1). In addition, honey bees pollinate numerous wild flowering plants, thereby supporting biodiversity (2, 3). However, over the past two decades, honey bees have suffered from elevated mortality in North America and Europe (4, 5). Colony losses have been associated with the exotic ectoparasitic mite *Varroa destructor*, which feeds on honey bee hemolymph, thereby vectoring numerous honey bee viral pathogens, in particular the iflavirus deformed wing virus (DWV). In the absence of varroa, DWV levels are low, and the virus causes asymptomatic infections. Varroa-infested colonies show elevated levels of DWV (6, 7). Symptoms associated with acute DWV infections include the death of pupae, as well as deformed wings, shortened abdomen, and cuticle discoloration of adult bees that die soon after pupation, causing colony collapse (6, 8). Indeed, winter colony mortality is strongly correlated with the presence of DWV, irrespective of the levels of varroa infestation (8, 9). DWV-induced loss of honey bees, coupled with a long-term decline in beekeeping, has become a serious threat to adequate provision of pollination services, threatening food security and ecosystem stability (1).

Viruses from the order *Picornavirales*, including the family *Iflaviridae*, have nonenveloped icosahedral virions that are about 30 nm in diameter (10). Iflavirus capsids protect 10,000-nt-long ssRNA genomes, which are translated into polyproteins that are cotranslationally and posttranslationally cleaved by viral proteases to produce structural (capsid-forming) and nonstructural proteins (11). The major capsid proteins VP1, VP2, and VP3 originating from a single polyprotein form a protomer, the basic building block of the pseudo-T3 icosahedral capsid. The entire capsid consists of 60 such protomers, arranged in 12 pentamer units of 5 protomers each.

Previously, the structure of the iflavirus Chinese sacbrood virus was characterized to a resolution of 25 Å by cryo-electron microscopy. The structure confirmed the pseudo-T3 icosahedral symmetry of its capsid and a smooth outer surface of the virion (12). Recently, we determined the structure of the iflavirus slow bee paralysis virus (SBPV) to a resolution of 2.6 Å by X-ray crystallography (13). Despite its efficient transmission by *V. destructor*, SBPV infection is a rare disease of honey bees (14). The structure revealed that the C-terminal extension of capsid protein VP3 of SBPV forms a globular protruding (P) domain positioned at the virion surface. The P domain is anchored to the core of the VP3 subunit by a 23-residue-long flexible linker that allows the P domain to attach to different areas of the capsid (13). In addition, the P domain contains the putative active site Asp-His-Ser, which is conserved among several iflaviruses (13). Iflaviruses were also proposed to harbor short VP4 subunits consisting of only about 20 residues (11, 14); however, electron density

Significance

Honey bee populations in Europe and North America have been decreasing since the 1950s. Deformed wing virus (DWV), which is undergoing a worldwide epidemic, causes the deaths of individual honey bees and collapse of whole colonies. We determined three-dimensional structures of DWV at different conditions and show that the virus surface is decorated with protruding globular extensions of capsid proteins. The protruding domains contain a putative catalytic site that is probably required for the entry of the virus into the host cell. In addition, parts of the DWV RNA genome interact with the inside of the virus capsid. Identifying the RNA binding and catalytic sites within the DWV virion offers prospects for the development of antiviral treatments.

Author contributions: P.P. designed research; K.Š. and A.P. performed research; R.J.P. contributed new reagents/analytic tools; K.Š., J.N., T.F., and P.P. analyzed data; and K.Š., R.J.P., and P.P. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

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Data deposition: Cryo-EM maps of the DWV virions from different conditions have been deposited in the Electron Microscopy Data Bank (EMDB) (accession nos. EMD-4014, EMD-3574, EMD-3570, and EMD-3575); the corresponding coordinates and structure factors have been deposited in the Protein Data Bank (PDB), www.pdb.org (PDB ID codes 5LBQ, 5MV5, 5MUP, and 5MV6). The crystal structures of the DWV virion and P domain have been deposited under PDB ID codes 5GS2 and 5GS1.

[1]To whom correspondence should be addressed. Email: pavel.plevka@ceitec.muni.cz.

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MICROBIOLOGY

Structure of deformed wing virus, a major honey bee pathogen

Karel Škubník[a], Jiří Nováček[a], Tibor Füzik[a], Antonín Přidal[b], Robert J. Paxton[c], and Pavel Plevka[a,1]

[a]Structural Virology, Central European Institute of Technology, Masaryk University, 62500 Brno, Czech Republic; [b]Department of Zoology, Fishery, Hydrobiology, and Apidology, Faculty of Agronomy, Mendel University in Brno, 613 00 Brno, Czech Republic; and [c]Institute of Biology/Zoology, Martin Luther University Halle-Wittenberg, 06120 Halle, Germany

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colony collapse disorder | virus | structure | *Apis mellifera* | honey bee

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Significance

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truding domains
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Author contributions: P.
contributed new reage
K.Š., R.J.P., and P.P. wrot
The authors declare no c
This article is a PNAS Dire
Freely available online th
Data deposition: Cryo-EM
deposited in the Electron
3574, EMD-3570, and EM
have been deposited in
5L8Q, 5MV5, 5MUP, and
have been deposited und
[1]To whom correspondenc
This article contains suppo
1073/pnas.1615695114/-/D

Author contributions: P.P. designed research; K.Š. and A.P. performed research; R.J.P. contributed new reagents/analytic tools; K.Š., J.N., T.F., and P.P. analyzed data; and K.Š., R.J.P., and P.P. wrote the paper.

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Freely available online through the PNAS open access option.

Data deposition: Cryo-EM maps of the DWV virions from different conditions have been deposited in the Electron Microscopy Data Bank (EMDB) (accession nos. EMD-4014, EMD-3574, EMD-3570, and EMD-3575); the corresponding coordinates and structure factors have been deposited in the Protein Data Bank (PDB), www.pdb.org (PDB ID codes 5L8Q, 5MV5, 5MUP, and 5MV6). The crystal structures of the DWV virion and P domain have been deposited under PDB ID codes 5G52 and 5G51.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1615695114/-/DCSupplemental.

Plan

- at least 3 personal interviews with potential supervisors
- the supervisors will be happy if you come to talk to them casually about job opportunities
- get to know the members of the lab (students, technicians, post-docs)
- internship or short project (2 months)

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