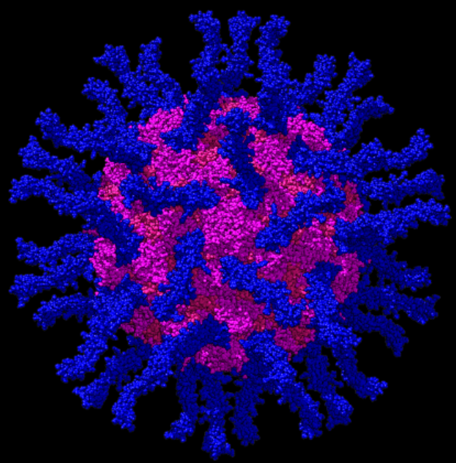


VIROLOGY LIVE

WITH VINCENT RACANIELLO

Structure of viruses

Session 4

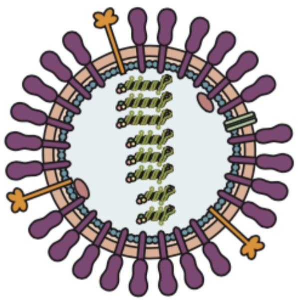
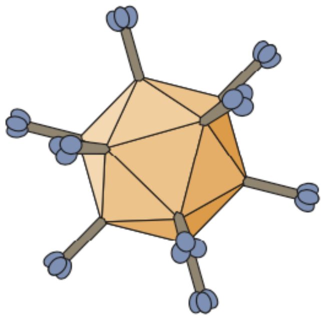
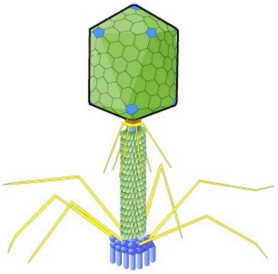
Virology Live

Fall 2021

In order to create something that functions properly - a container, a chair, a house - its essence has to be explored, for it should serve its purpose to perfection, i.e., it should be durable, inexpensive, and beautiful.

- Walter Gropius

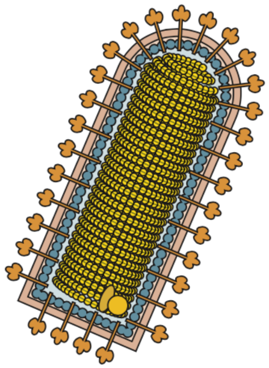
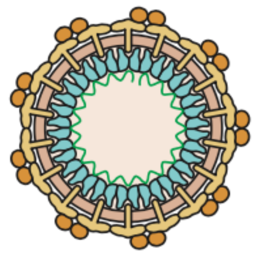
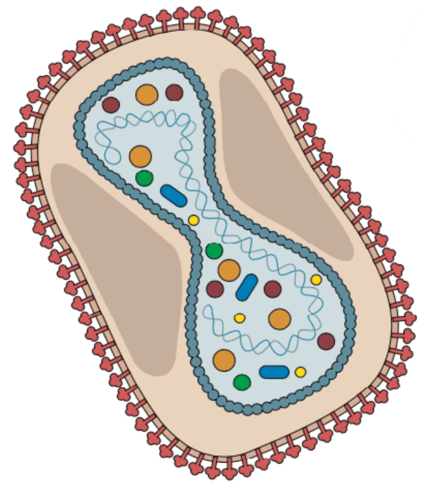
Functions of structural proteins of virus particles

Protection of the genome

- Assembly of a stable protective protein shell
- Specific recognition and packaging of the nucleic acid genome
- Interaction with host cell membranes to form the envelope

Functions of structural proteins of virus particle

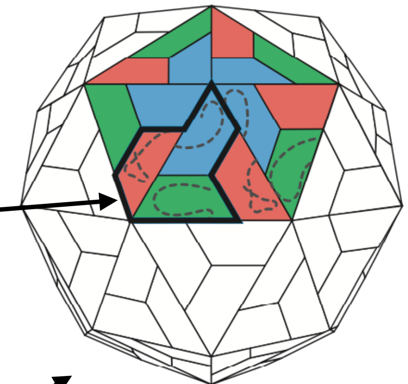




Delivery of the genome

- Bind host cell receptors
- Uncoating of the genome
- Fusion with cell membranes
- Transport of genome to the appropriate site

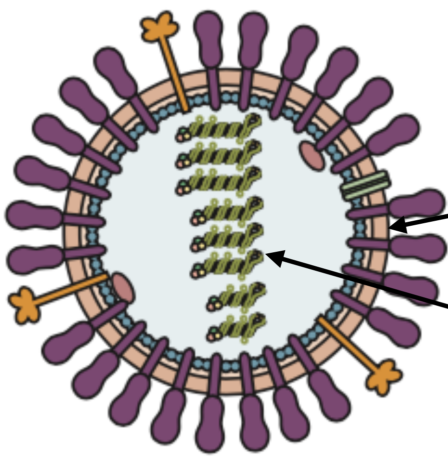
Definitions

Subunit - *Single folded polypeptide chain*



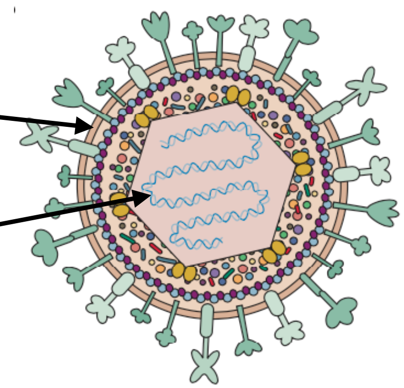
Structural unit (protomer, asymmetric unit) - Unit from which capsids or nucleocapsids are built; one or more subunits

Capsid (*capsa* = Latin, box) - Protein shell surrounding genome



Envelope (viral membrane) - Host cell-derived lipid bilayer

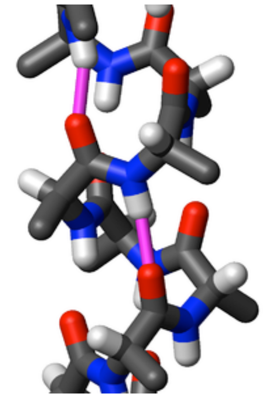
Nucleocapsid (core) - Nucleic acid - protein assembly within particle; used when is a discrete **sub**structure

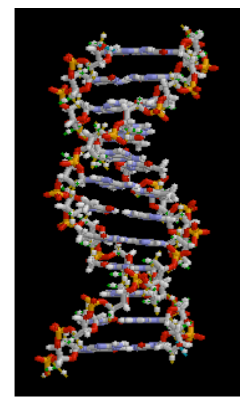


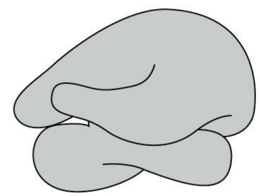
Virion - Infectious virus particle

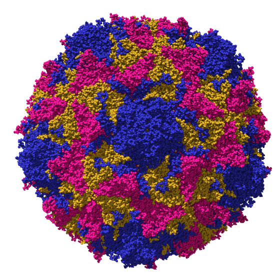
Putting virus particles into perspective

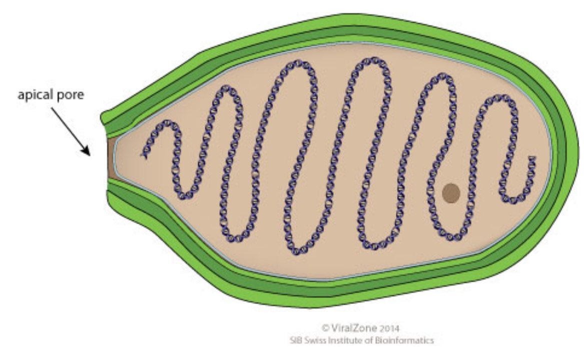
- Nanometer: 10^{-9} meters = 10 Å = 0.001 microns
- Alpha helix in protein: 1 nm diameter
- DNA: 2 nm diameter
- Ribosome: 20 nm diameter
- Poliovirus: 30 nm
- Pandoravirus: 1000 nm





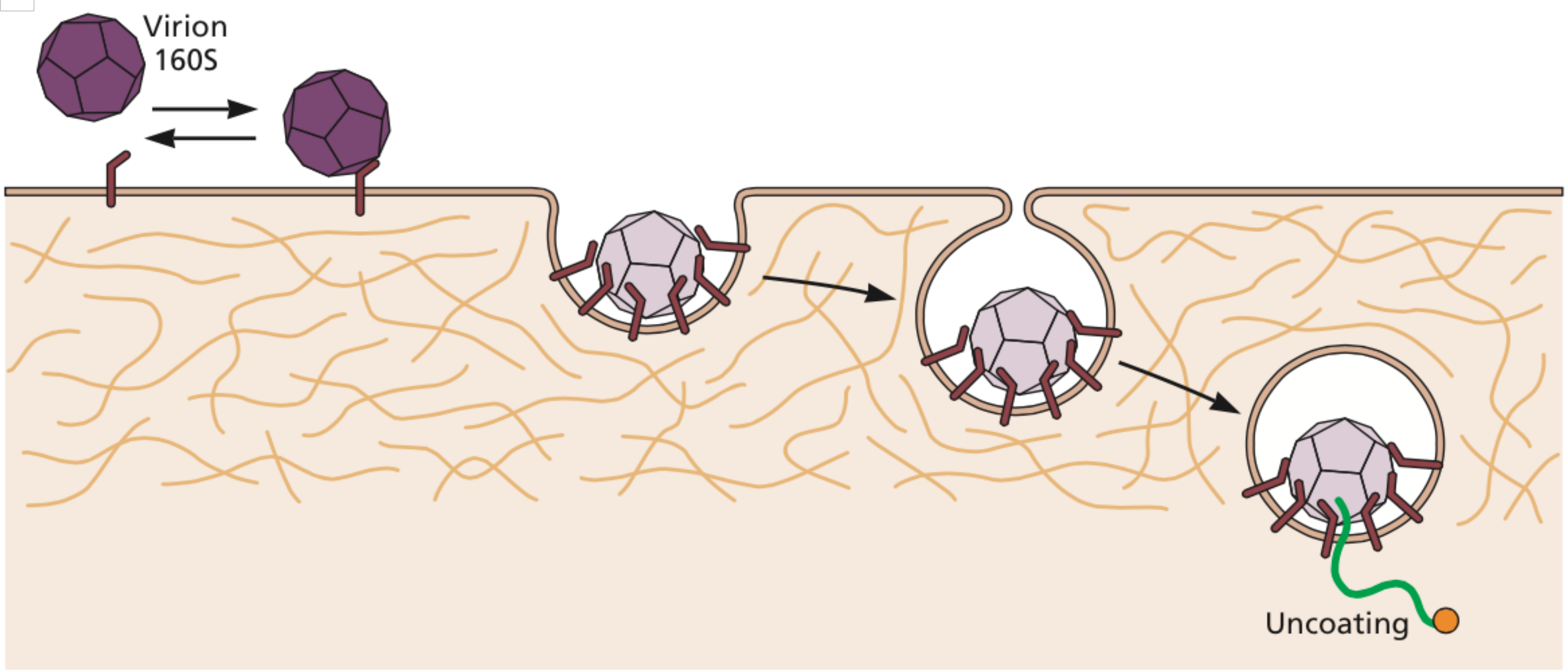






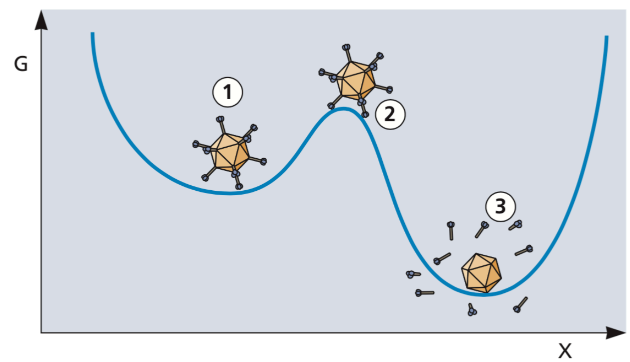
Virus particles are metastable

- Must protect the genome (stable)
- Must come apart on infection (unstable)



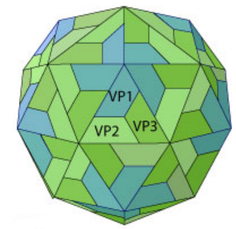
Virus particles are metastable

- Virus particles have not attained minimum free energy conformation
- Unfavorable energy barrier must be surmounted



- Energy put into virus particle during assembly (*spring loaded*)
- Potential energy used for disassembly if cell provides proper signal

How is metastability achieved?



- *Stable structure*
  - Created by symmetrical arrangement of many identical proteins to provide maximal contact
- *Unstable structure*
  - Structure is not usually permanently bonded together
  - Can be taken apart or loosened on infection to release or expose genome

Go to:

b.socrative.com/login/student

room number: virus

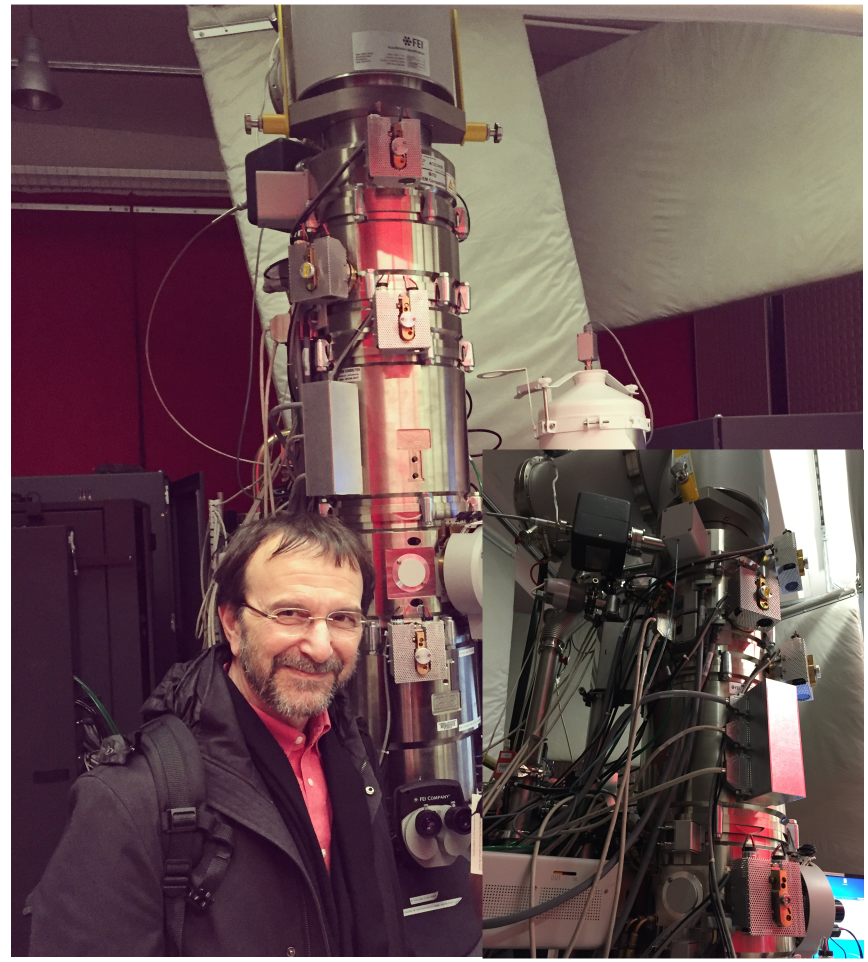
Viral capsids are metastable because:

A. They must protect the viral genome when the particle is outside of the cell
B. They must come apart and release the genome into a cell
C. They have not obtained a minimum free energy conformation
D. They are spring-loaded
E. All of the above

The tools of viral structural biology

- Electron microscopy
- X-ray crystallography
- Cryo-electron microscopy (cryoEM) & cryo-electron tomography
- Nuclear magnetic resonance spectroscopy (NMR)

Flint volume I, chapter 4



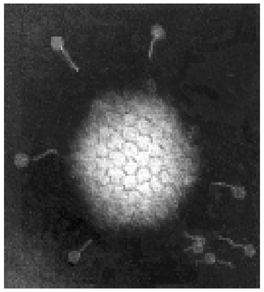
Beginning of the era of modern structural virology

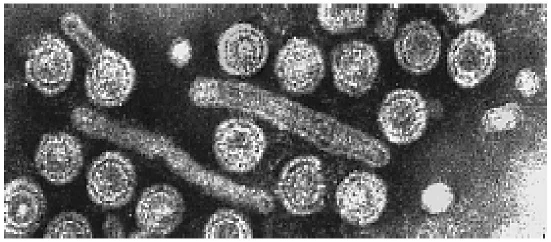
1940: Helmuth Ruska used an electron microscope to take the first pictures of virus particles

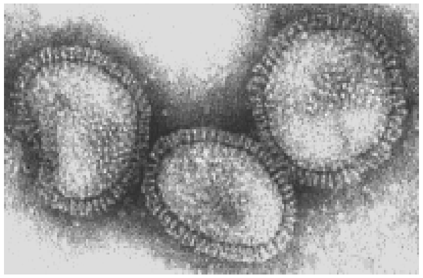
Ruska, H. 1940. Die Sichtbarmachung der BakteriophagenLyse im Ubermikroskop. Naturwissenschaaften. 28:45-46).

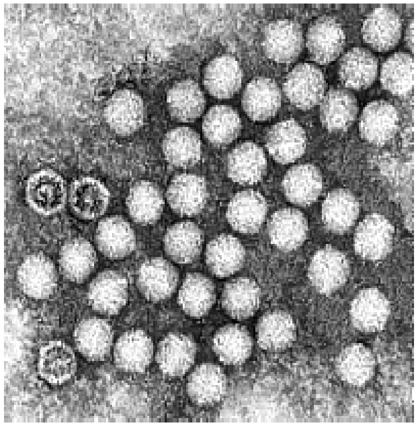
Electron microscopy

- Biological materials have little inherent contrast: need to be stained
- Negative staining with electron-dense material (uranyl acetate, phosphotungstate), scatter electrons (1959)
- Resolution 50-75 Å (alpha helix 10 Å dia; 1 Å = 0.1 nm)
- Detailed structural interpretation impossible



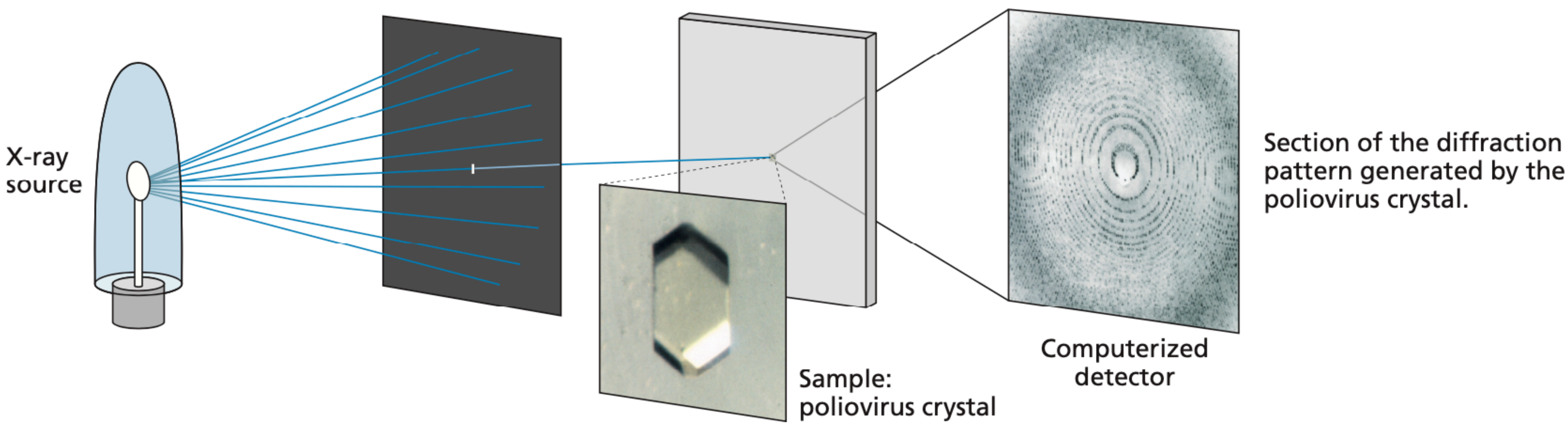




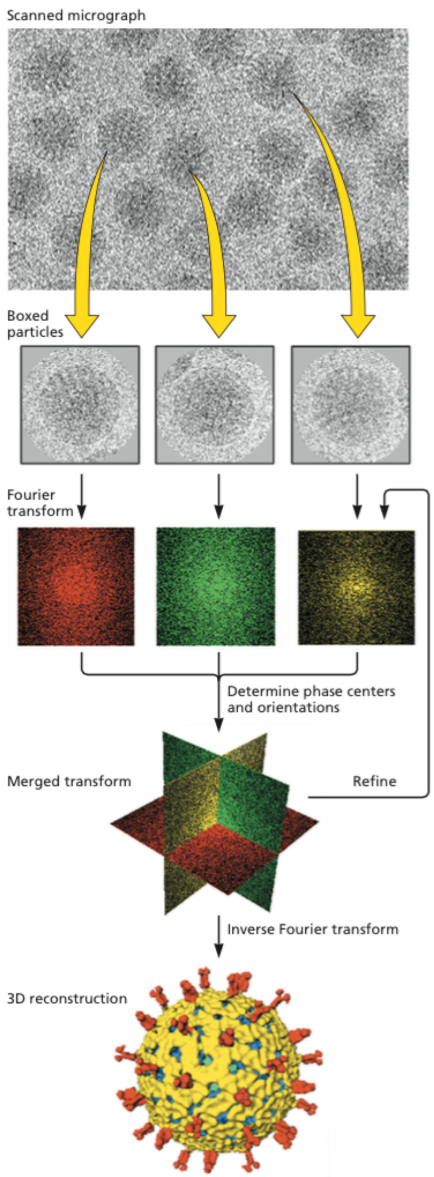


Linda Stannard, University of Cape Town

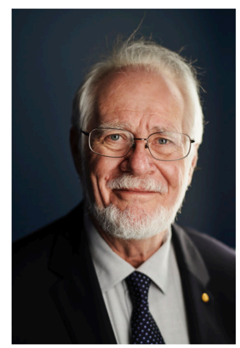
X-ray crystallography
(2-3 Å for viruses)



Cryo-electron microscopy (cryoEM)

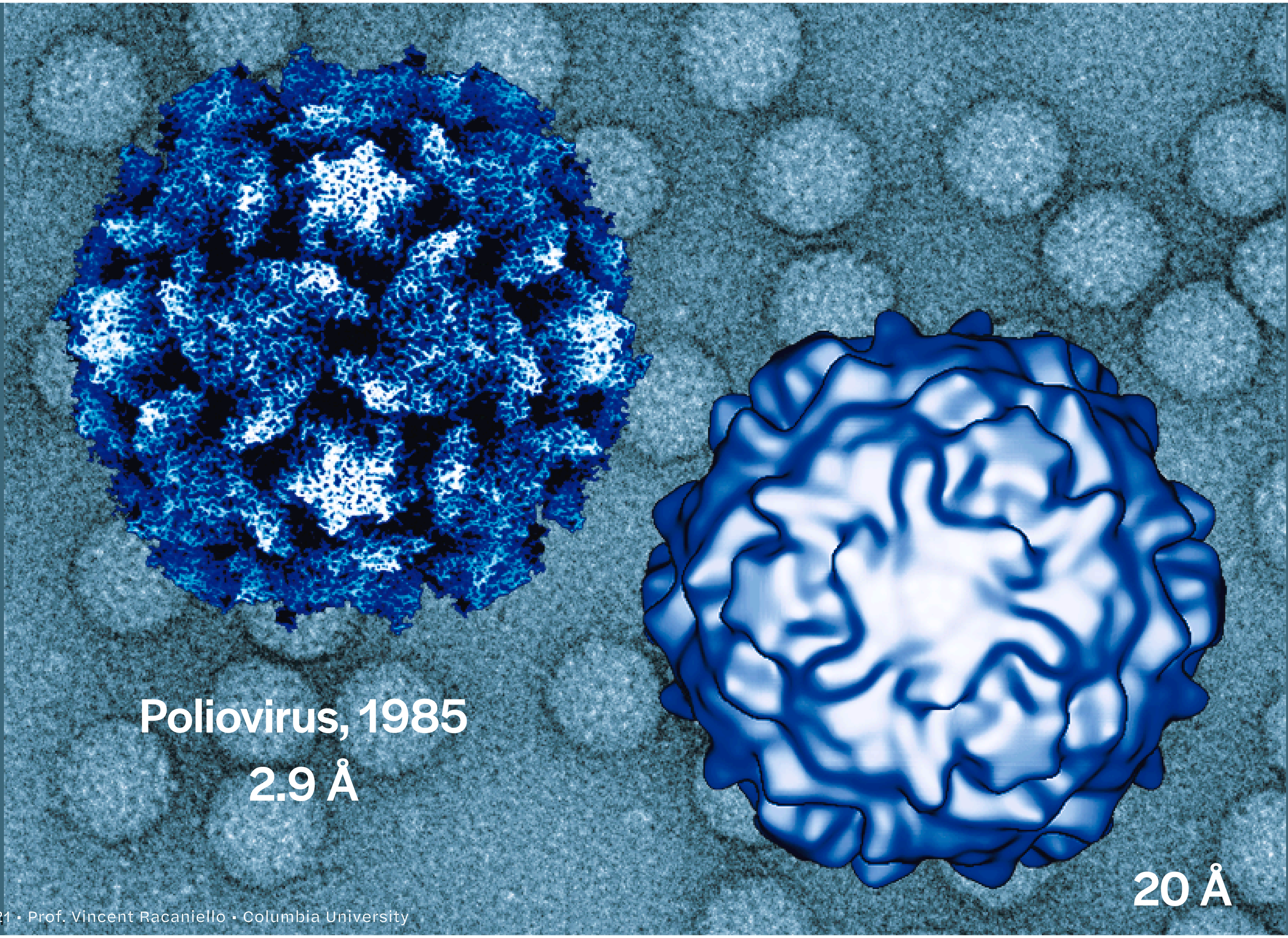


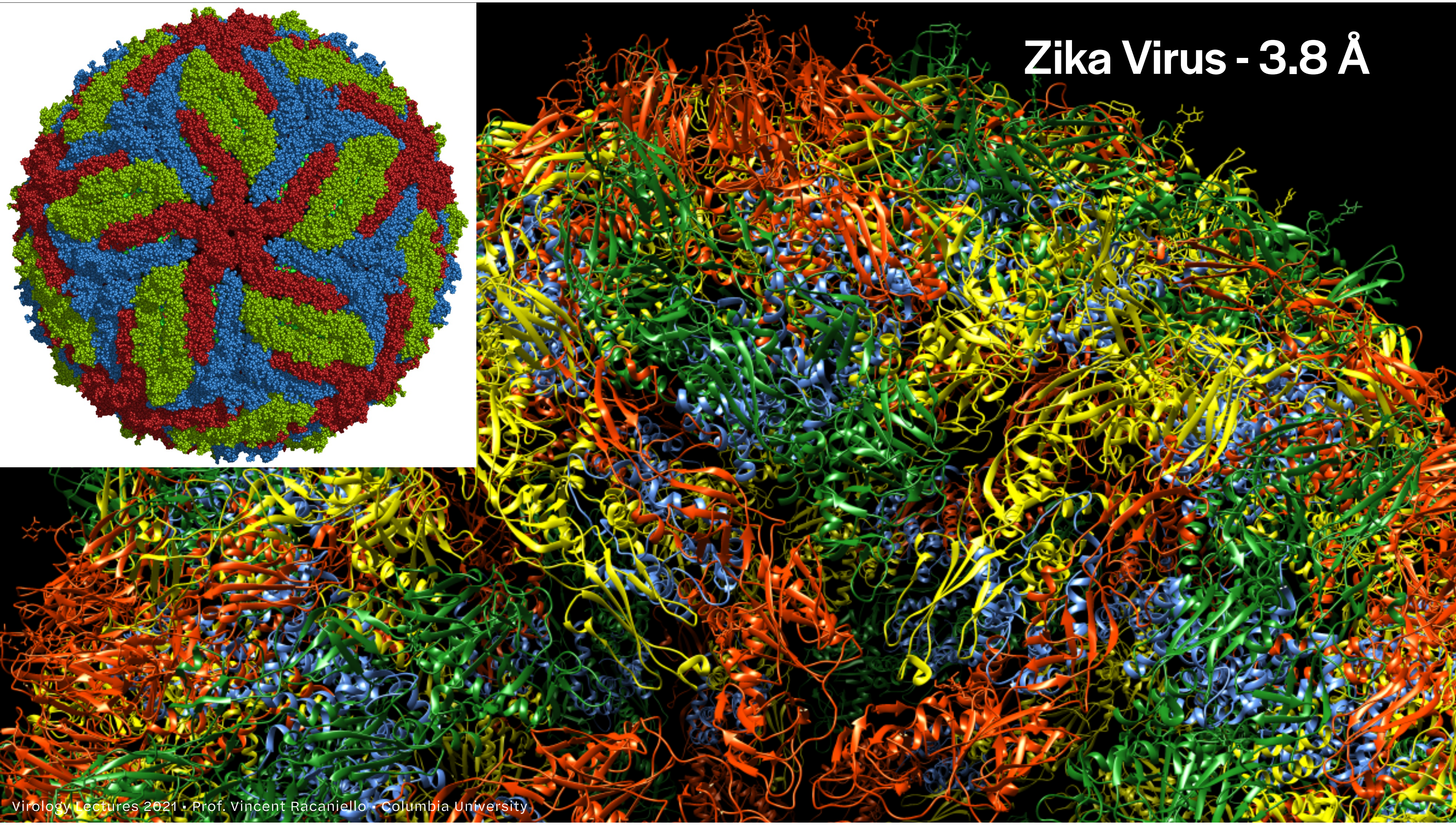
The Nobel Prize in Chemistry 2017 was awarded jointly to Jacques Dubochet, Joachim Frank and Richard Henderson "for developing cryo-electron microscopy for the high-resolution structure determination of biomolecules in solution."


© Nobel Media AB. Photo: A. Mahmoud
Jacques Dubochet

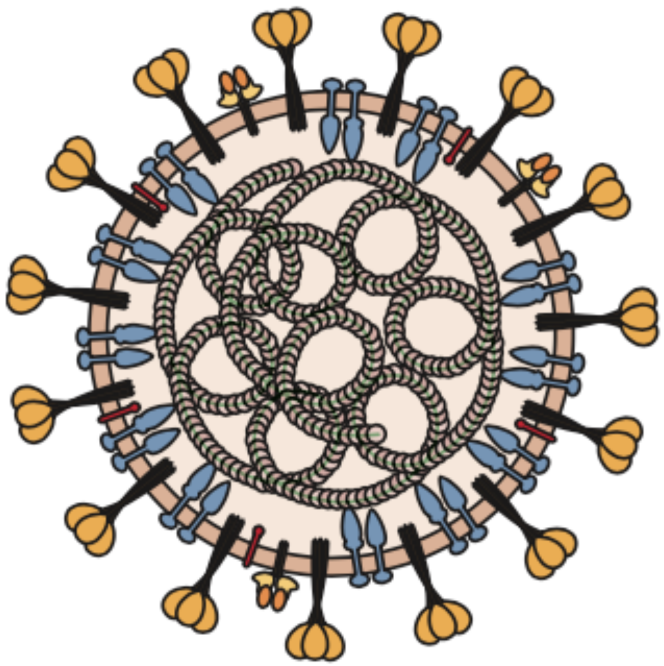

© Nobel Media AB. Photo: A. Mahmoud
Joachim Frank

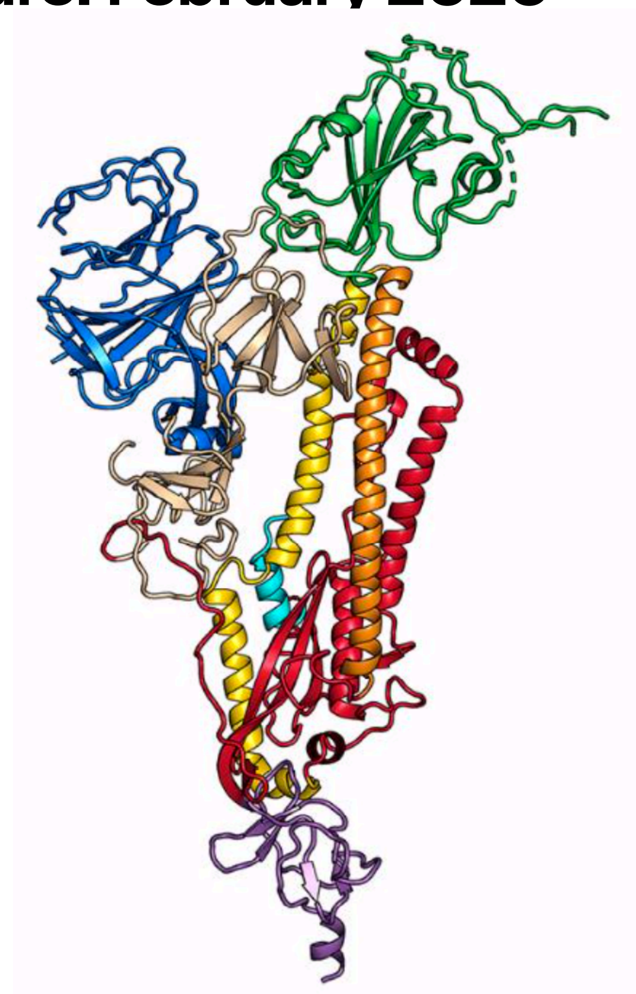

© Nobel Media AB. Photo: A. Mahmoud
Richard Henderson


Poliovirus, 1985
2.9 Å
20 Å

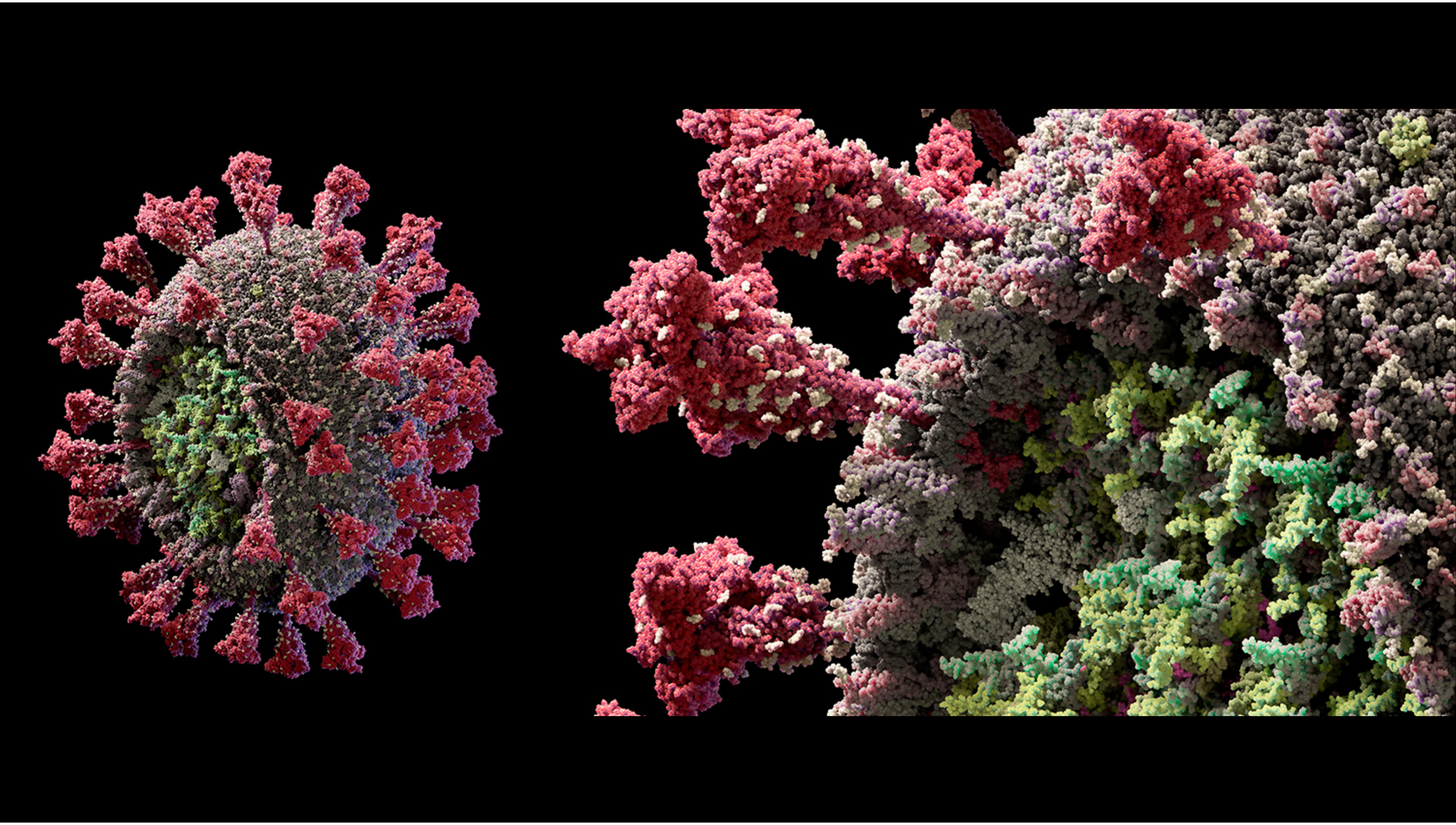

Zika Virus - 3.8 Å

SARS-CoV-2 spike structure: February 2020

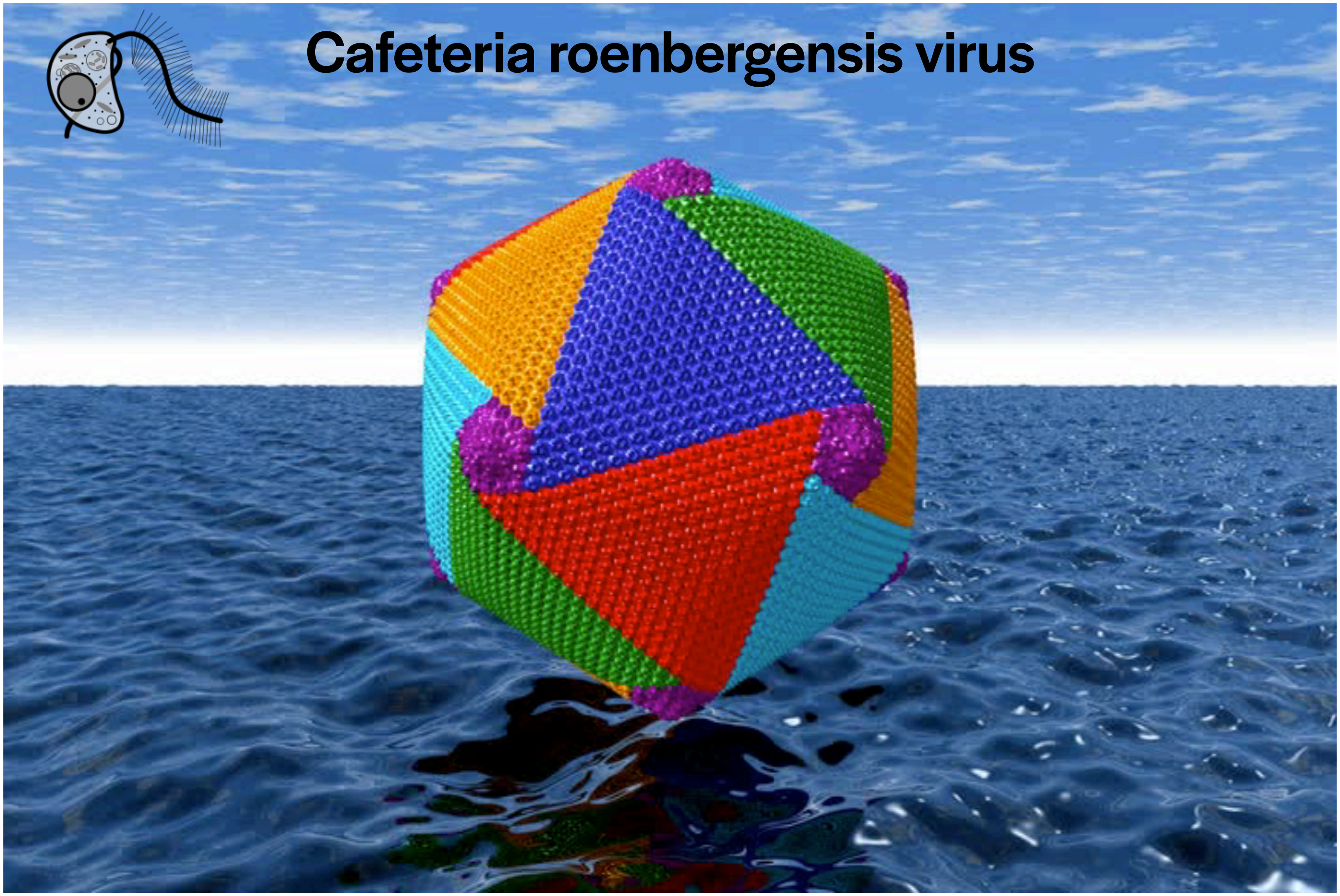




10.1126/science.abb2507 (2020)



Cafeteria roenbergensis virus



300 nm, >15,000 capsid proteins, 3 million CPU hours

Chuan Xiao http://utminers.utep.edu/cxiao/#4

Genomes

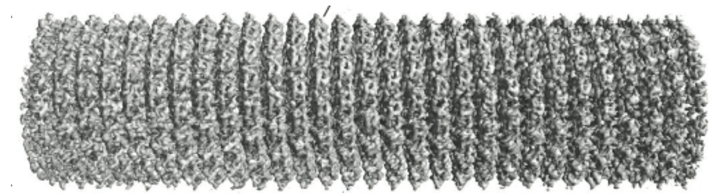
7

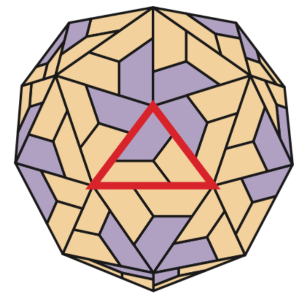
Virus particles

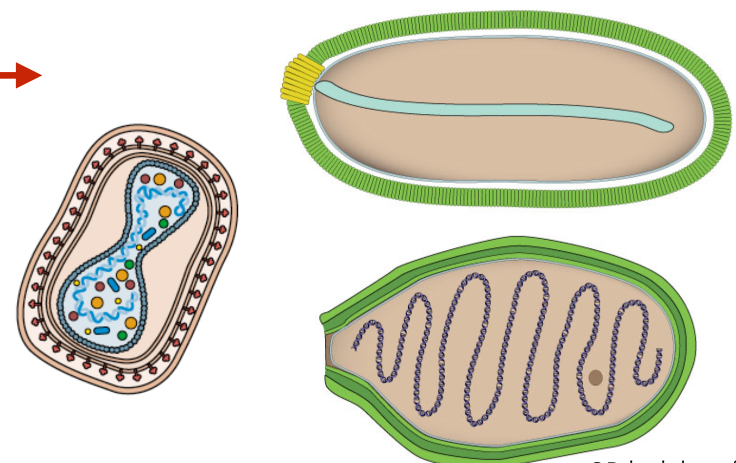
3

3

- Helical
- Icosahedral
- Complex

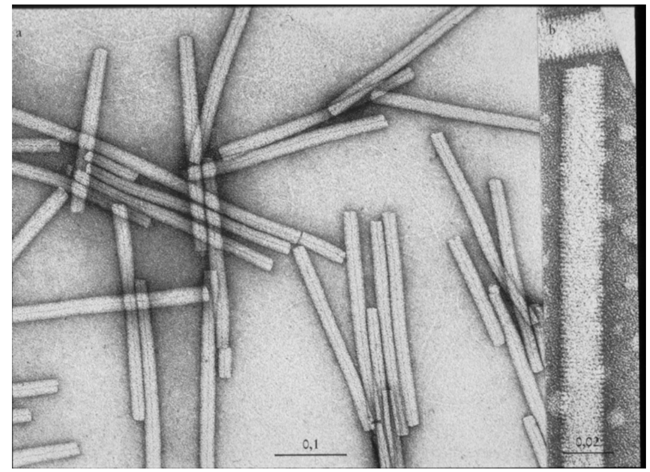


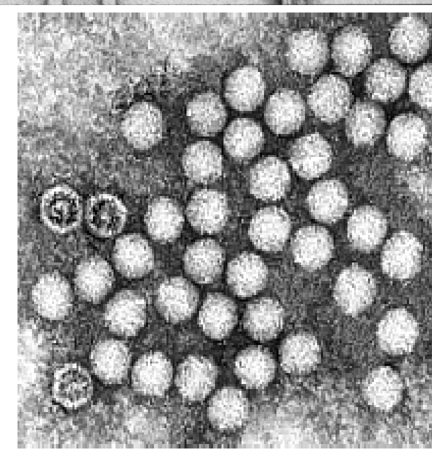




Building virus particles: Symmetry is key

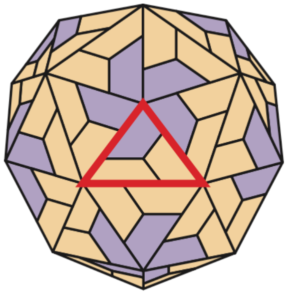
- Watson and Crick did more than discover DNA structure
- Their seminal contribution to virology:
  - Noted that most virus particles were spherical or rod-shaped
  - Idea: as virus genomes are small (!) particles would be built with many copies of a few proteins (genetic economy)
  - Identical protein subunits are distributed with *helical symmetry* for rod-shaped viruses
  - *Icosahedral symmetry* for round viruses





The symmetry rules are elegant in their simplicity

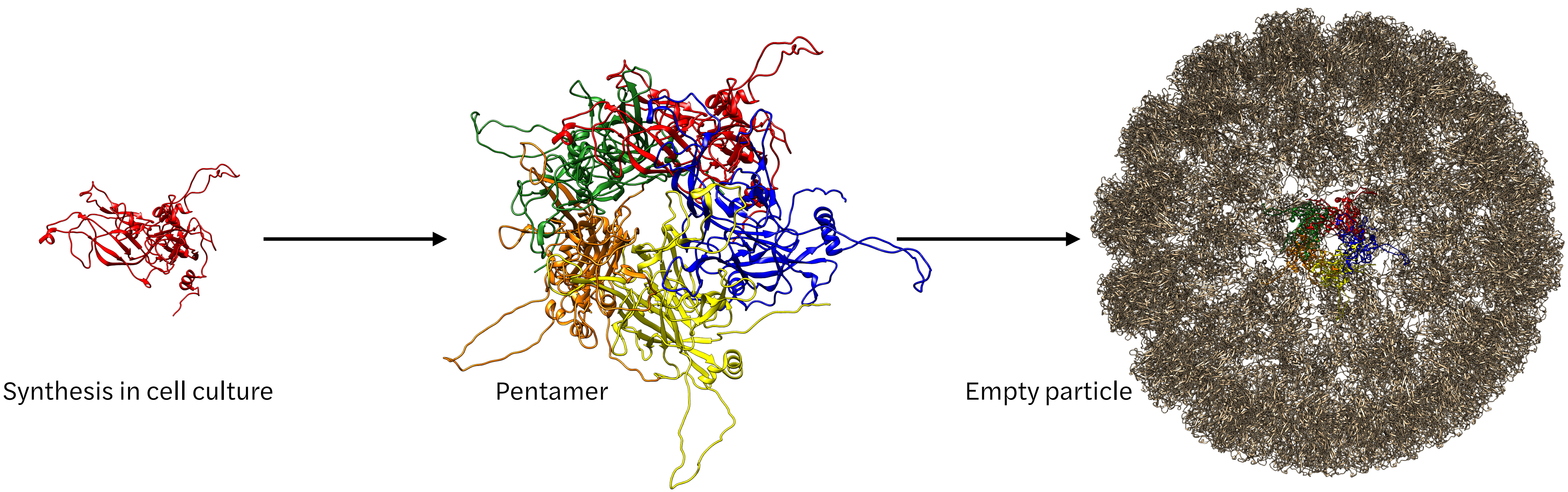
They provide rules for "self-assembly"



- **Rule 1:** Each subunit has 'identical' bonding contacts with its neighbors
  - Repeated interaction of chemically complementary surfaces at the subunit interfaces naturally leads to a symmetric arrangement
- **Rule 2:** These bonding contacts are usually non-covalent
  - Reversible; error-free assembly

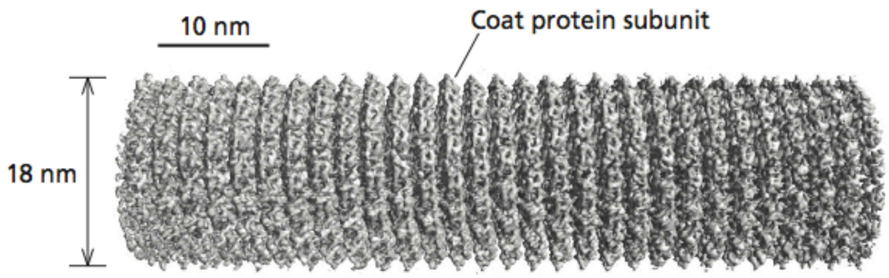
Symmetry and self-assembly

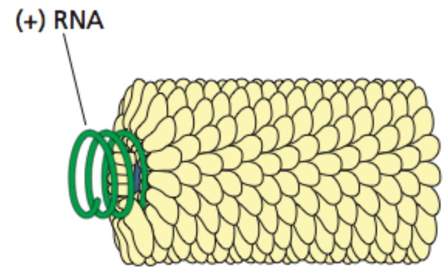
- Many capsid proteins self assemble into virus-like particles (VLPs)
- The HBV and HPV vaccines are VLPs made in yeast

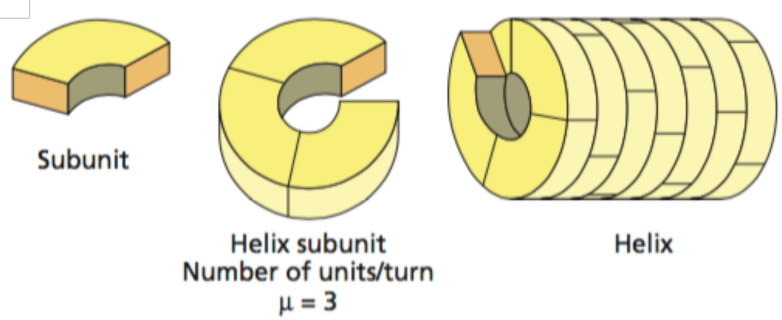


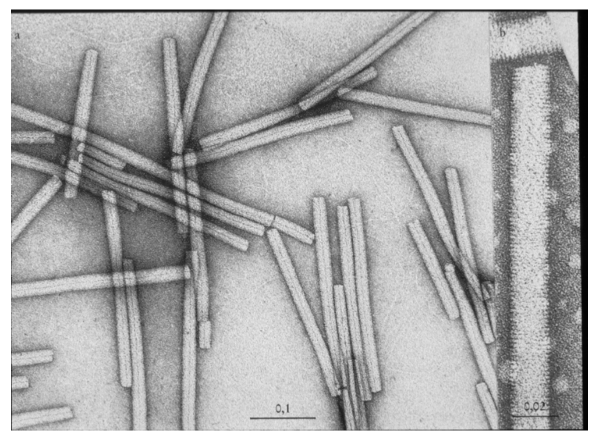
Helical symmetry

Coat protein molecules engage in identical, equivalent interactions with one another and with the viral genome to allow construction of a large, stable structure from a single protein subunit



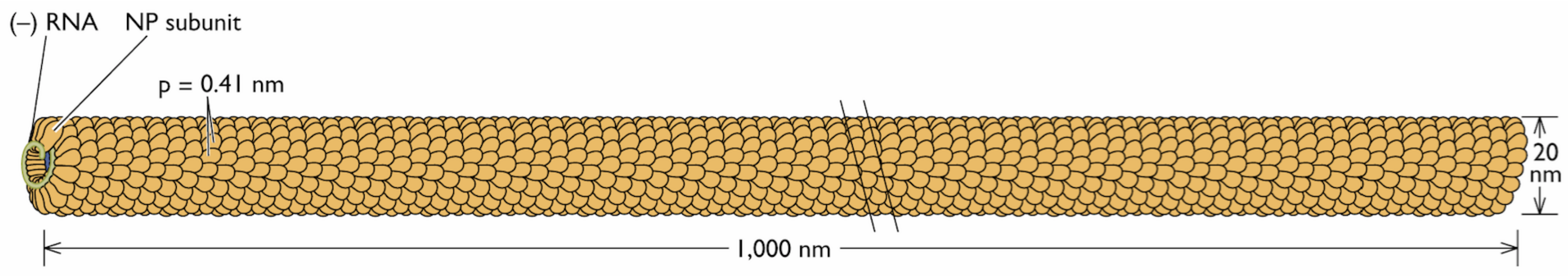


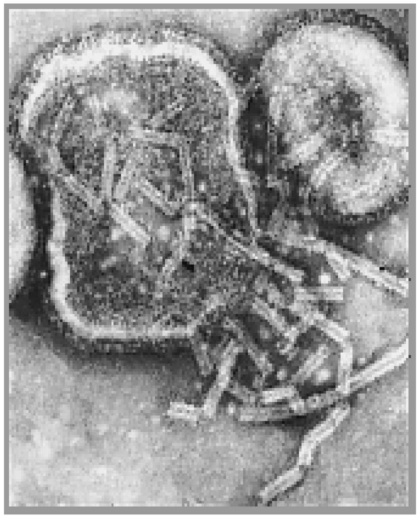




Helical symmetry

Sendai virus (paramyxovirus) nucleocapsid

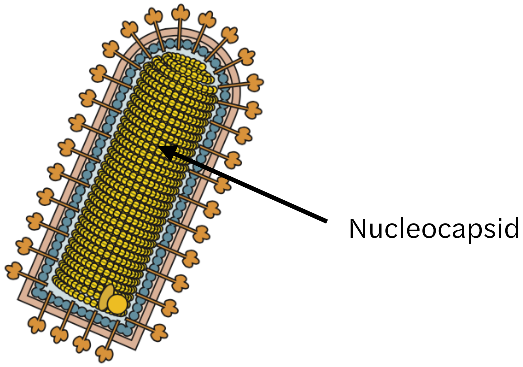


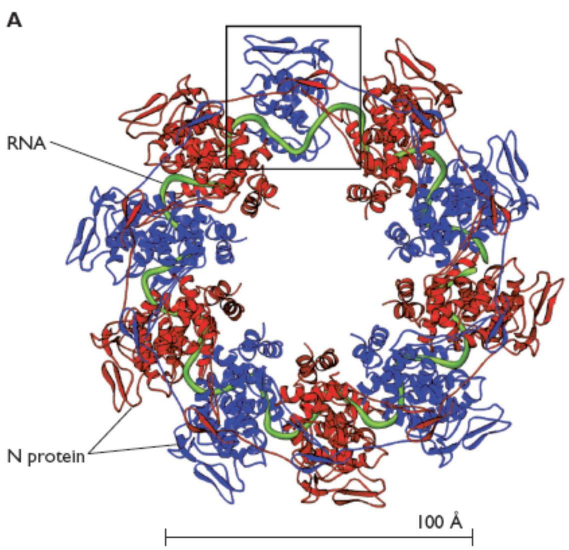


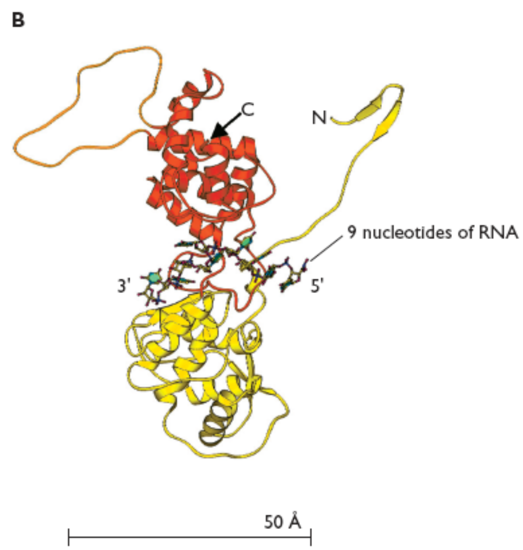
Animal viruses with helical symmetry are always enveloped!

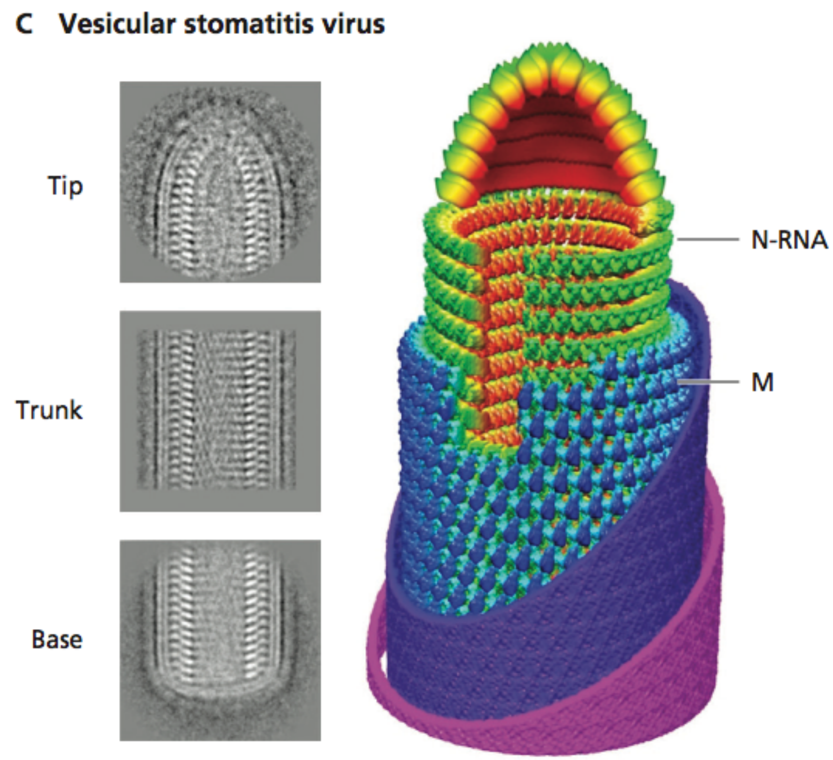
Helical symmetry

Vesicular stomatitis virus



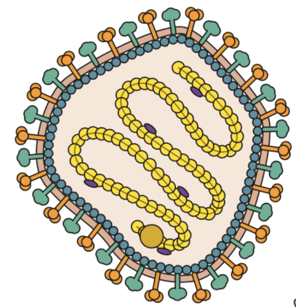


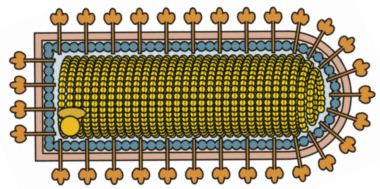


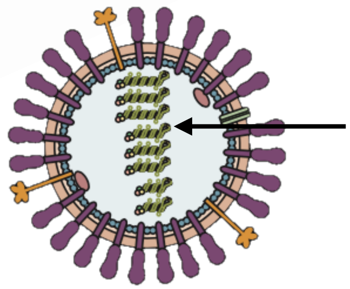


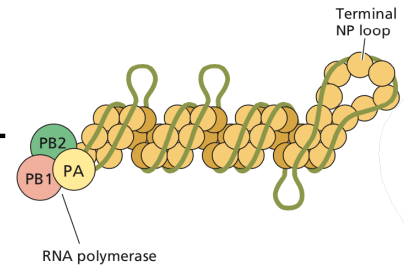
Enveloped RNA viruses with (-) ssRNA and helical capsids

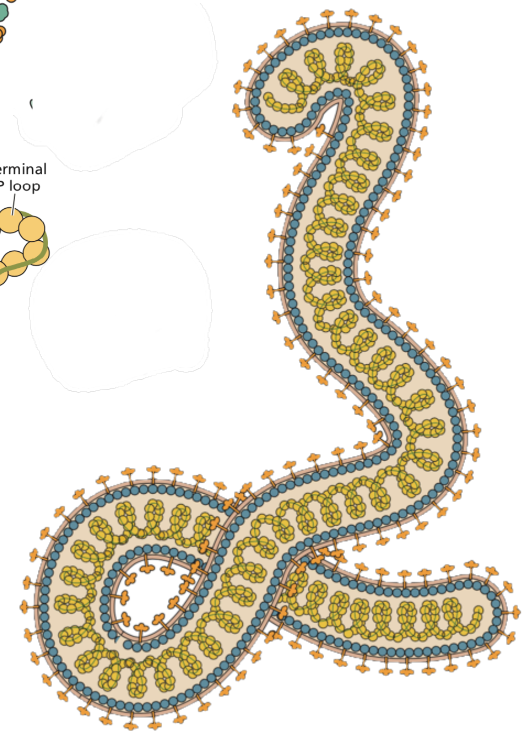
- *Paramyxoviridae* (measles virus, mumps virus)
- *Rhabdoviridae* (rabies virus)
- *Orthomyxoviridae* (influenza virus)
- *Filoviridae* (ebolaviruses)
- The *nucleocapsid* is the nucleic acid-protein assembly that is packaged within the virus particle





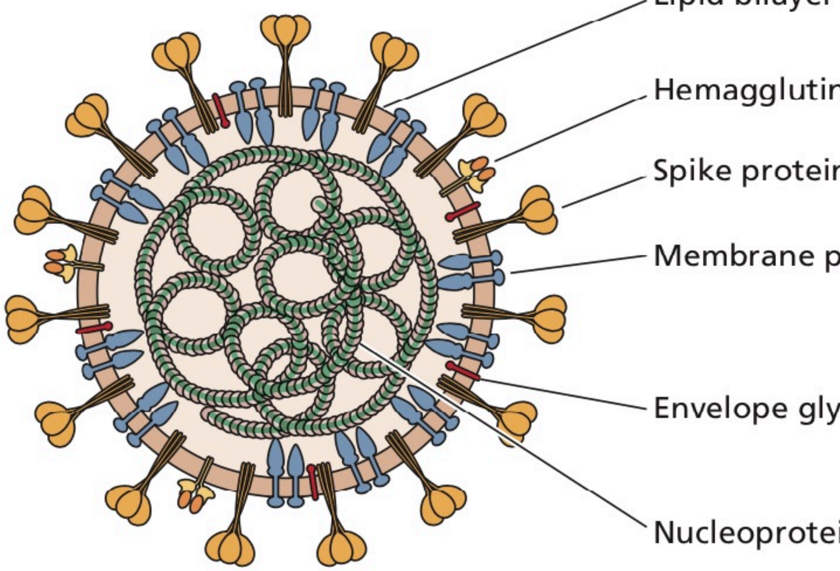




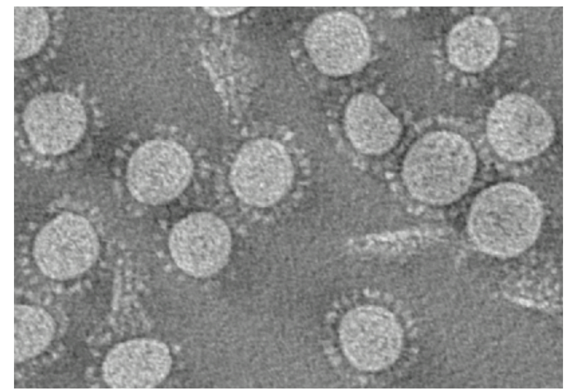


Enveloped RNA viruses with (+) ssRNA and helical capsids

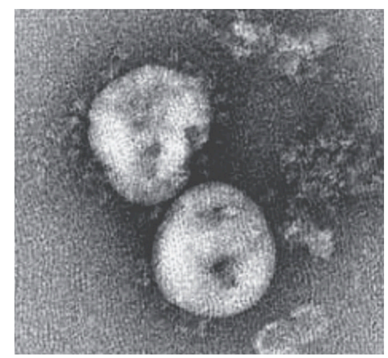
Coronaviridae (SARS-CoV, MERS-CoV, SARS-CoV-2)



= nucleocapsid



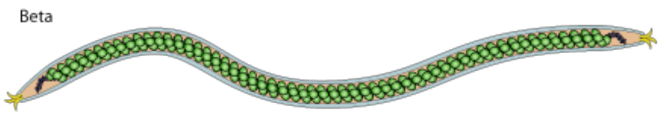
Halo of spikes = solar corona
~100 nm diameter



DOI: 10.1056/NEJMoa2001017

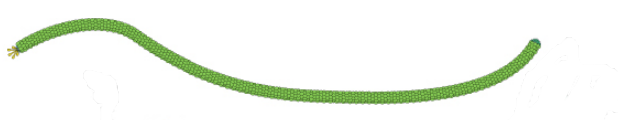
DNA and RNA viruses with helical symmetry

dsDNA viruses of Archaea



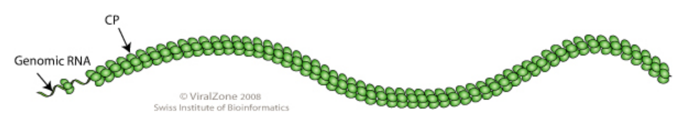
(enveloped)

ssDNA viruses of Bacteria



Rod-like + and - RNA viruses of plants

Flexuous +RNA viruses of plants

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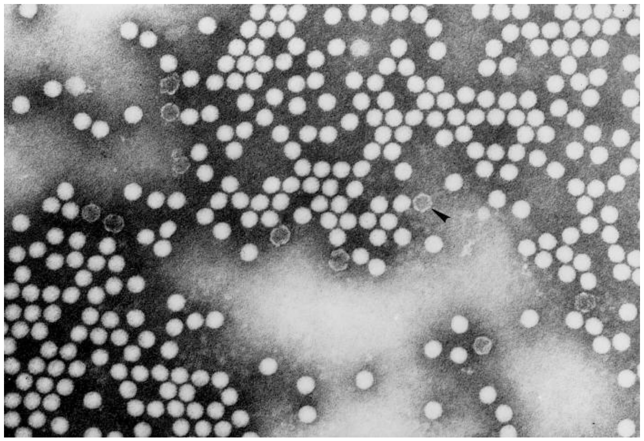
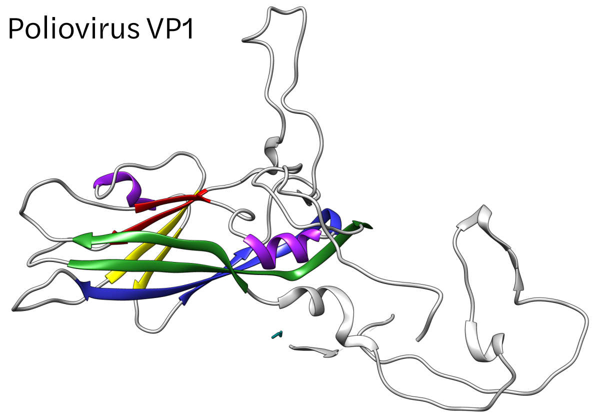
room number: virus

Which of the following describe virus symmetry and self assembly?

A. The bonding contacts of subunits are usually covalent
B. The bonding contacts of subunits are usually non-covalent
C. Each subunit has different bonding contacts with its neighbors
D. Self-assembly of virus particles does not occur
E. None of the above

How can you make a round capsid from proteins with irregular shapes?

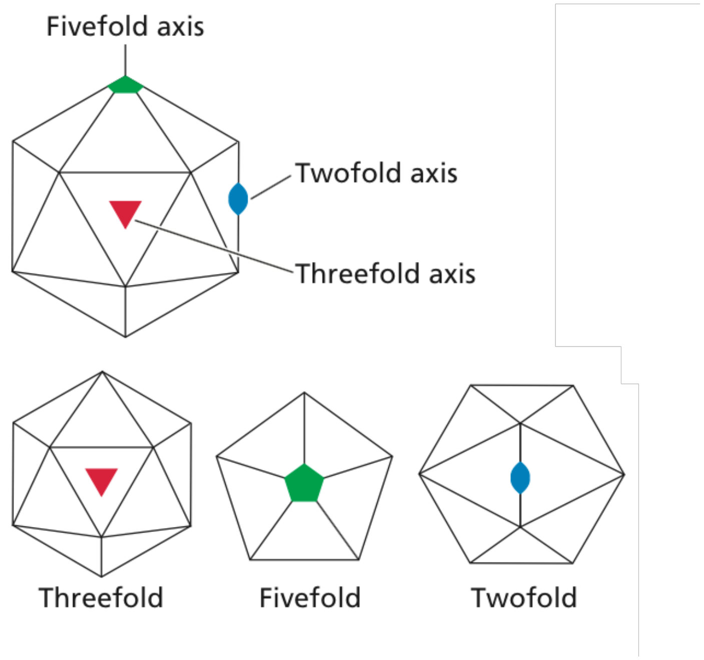




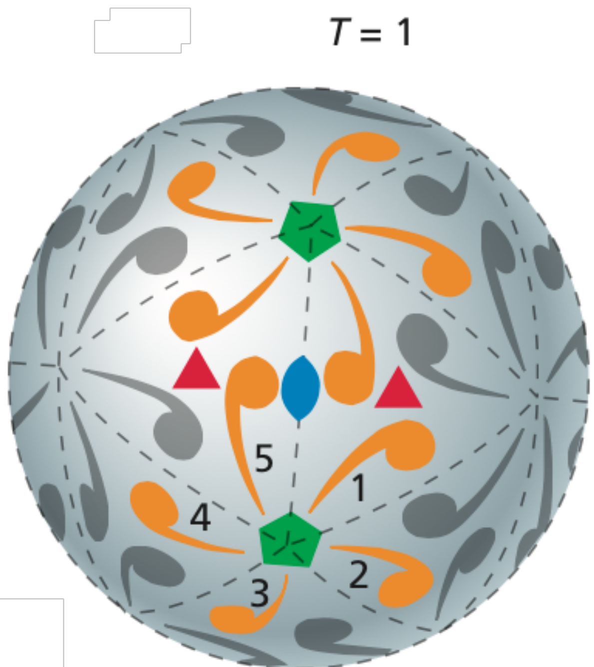
- **Clue 1:** All round capsids have precise numbers of proteins; multiples of 60 are common (60, 180, 240, 960)
- **Clue 2:** Spherical viruses come in many sizes, but capsid proteins are 20-60 kDa average
- Watson & Crick concluded that these are built with *icosahedral symmetry*

Icosahedral symmetry

- Icosahedron: solid with 20 faces, each an equilateral triangle
- 5x, 3x, 2x axes of symmetry (12 each)
- Allows formation of a closed shell with smallest number (60) of identical subunits



Simple icosahedral capsids

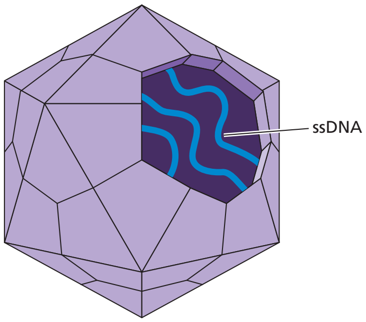


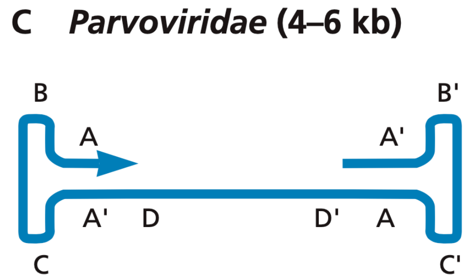
- Made of 60 identical protein subunits
- The protein subunit is the structural unit
- Interactions of all molecules with their neighbors are identical (head-to-head, tail-to-tail)
- The particles are spherical, not icosahedra!

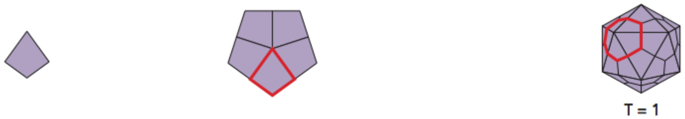
Adeno-associated virus 2 (parvovirus)

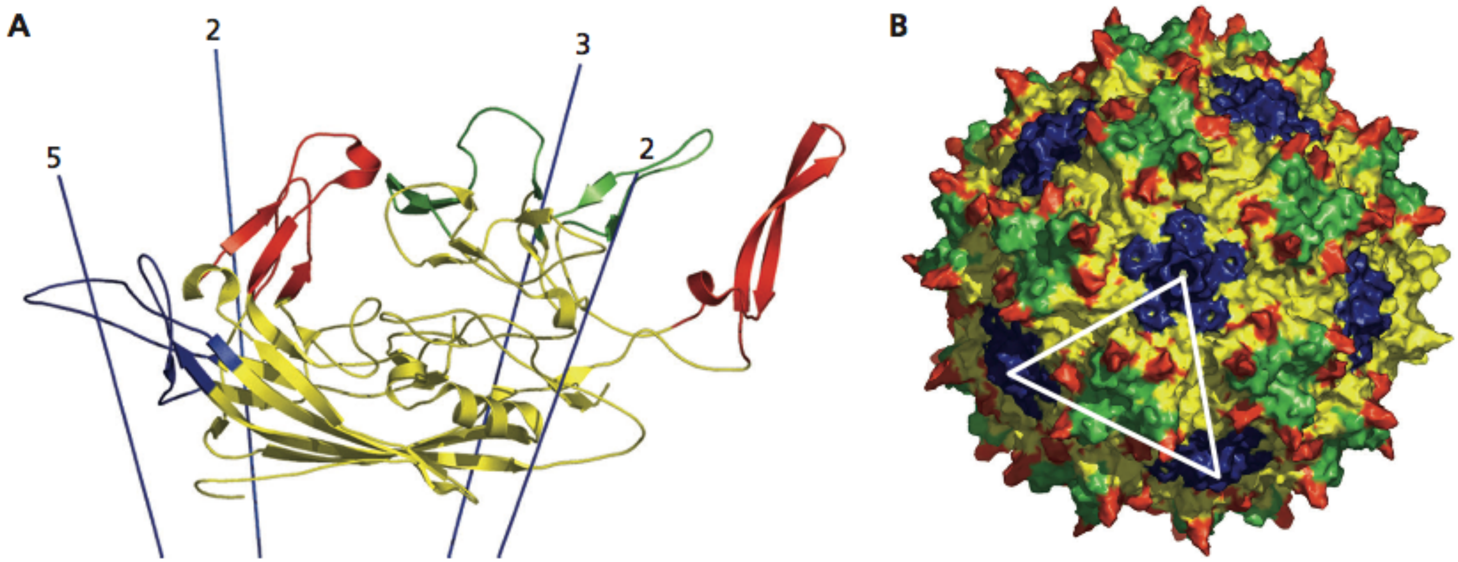
25 nm

60 copies of a single capsid protein



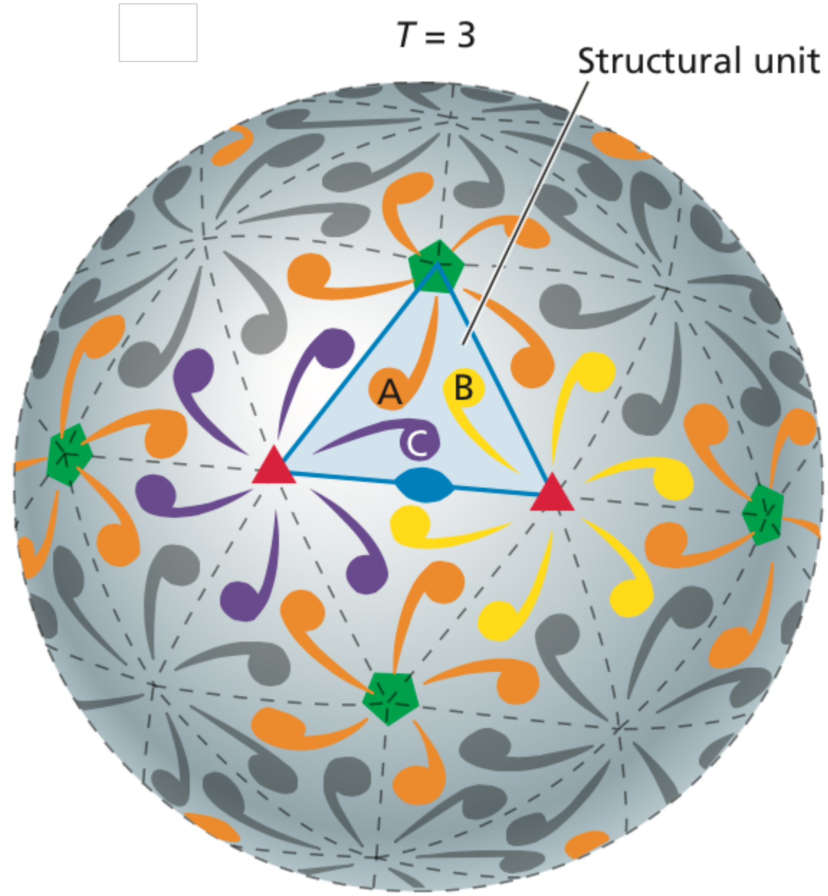






How are larger virus particles built? By adding more subunits

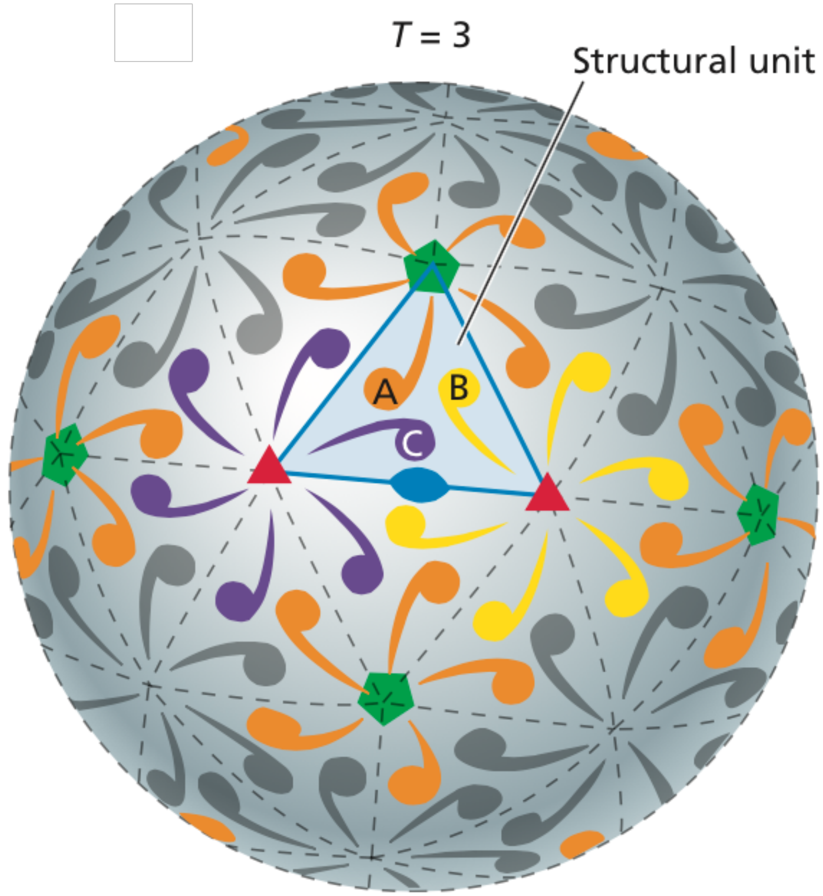
- Pentamers & hexamers
- Three modes of subunit packing (orange, yellow, purple)
- Bonding interactions are *quasiequivalent*: all engage tail-to-tail and head-to-head



180 identical protein subunits

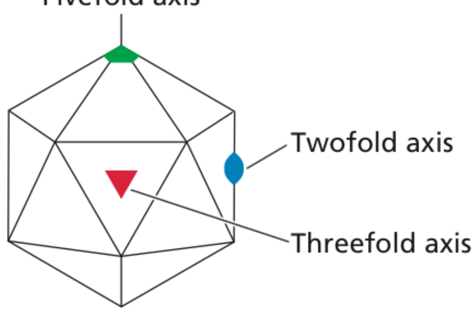
Quasiequivalence

- When a capsid contains more than 60 subunits, each occupies a *quasiequivalent* position
- The noncovalent binding properties of subunits in different structural environments are similar, but not identical

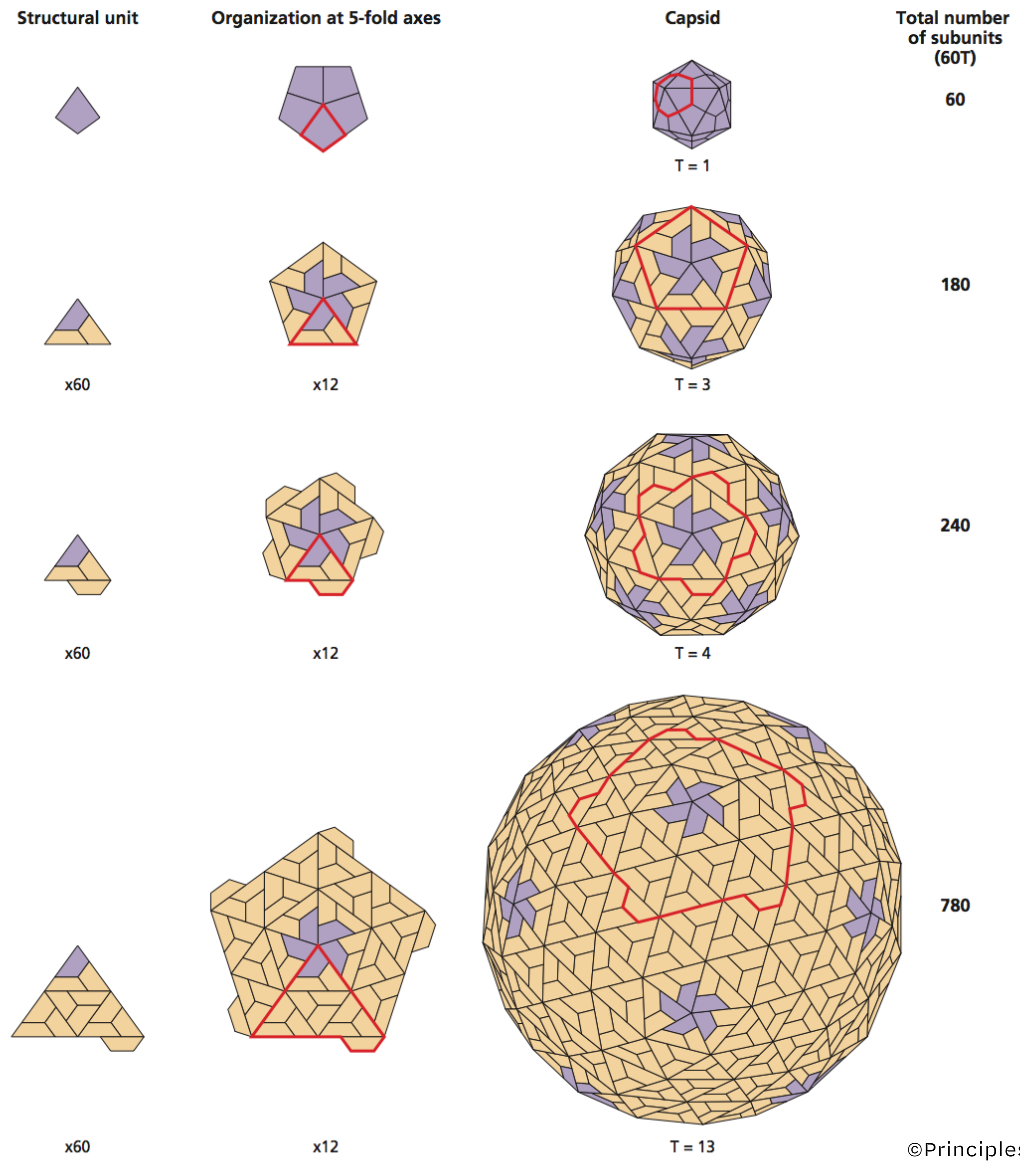


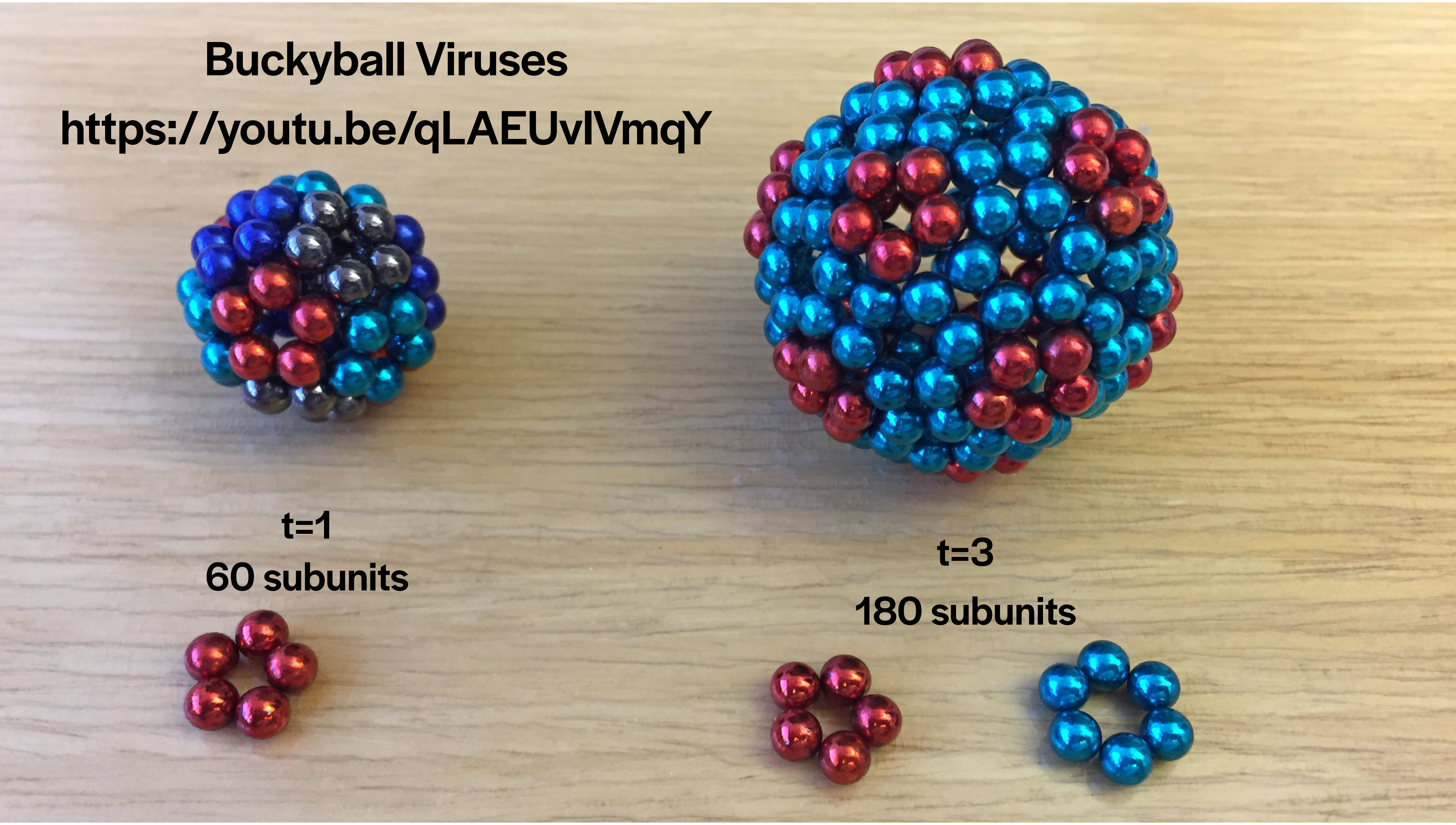
T, triangulation number

The number of smaller triangles (facets) of the triangular face (equilateral triangle)



Capsids with T>1 have a 6-fold axis of symmetry

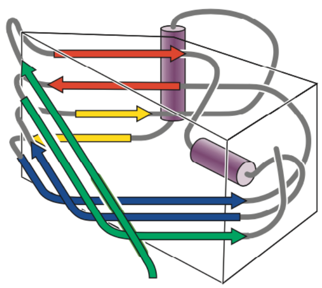


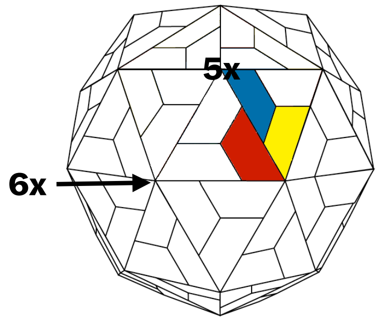

Buckyball Viruses
https://youtu.be/qLAEUvlVmqY
t=1
60 subunits
t=3
180 subunits

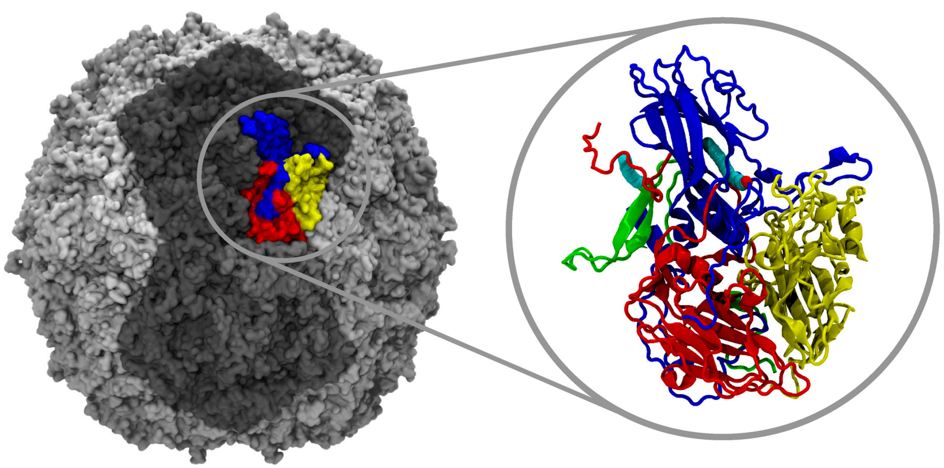
Poliovirus (Picornaviridae)

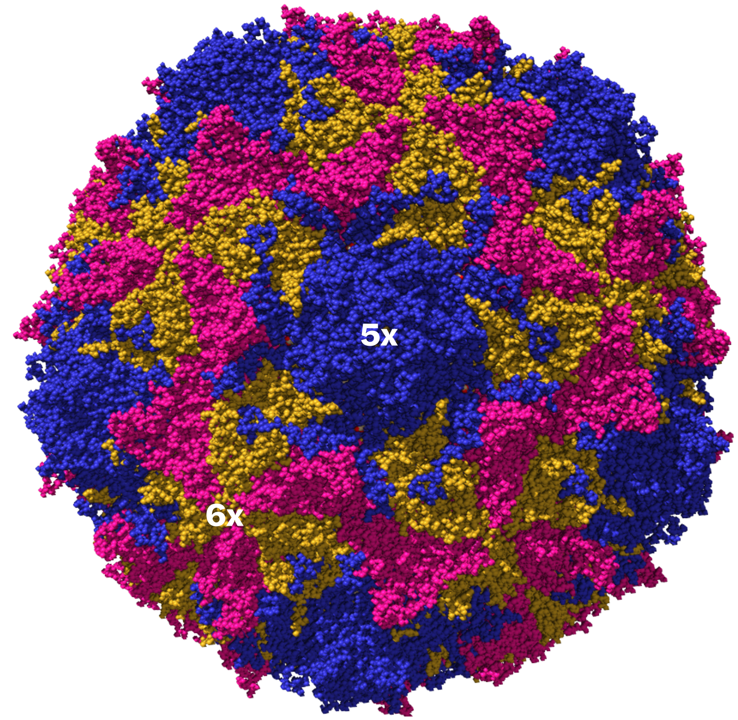
30 nm

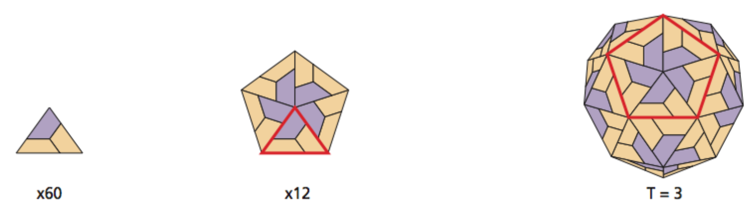
60 protomers of VP1, VP2, VP3 = 180 subunits







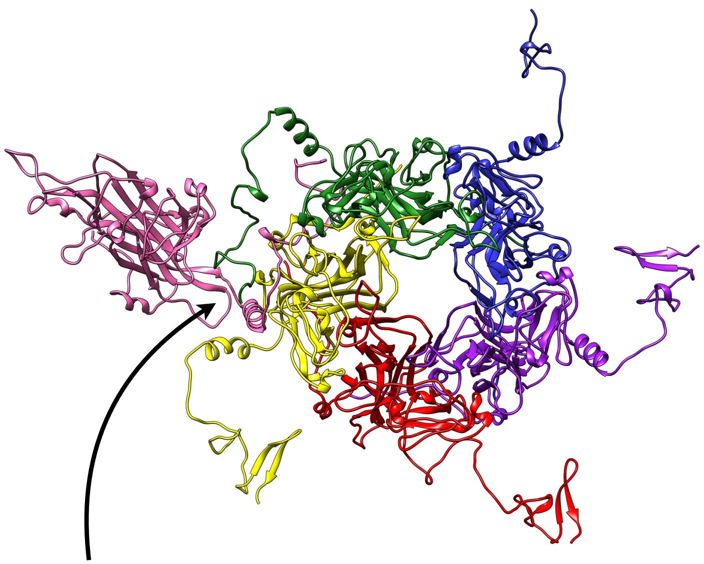




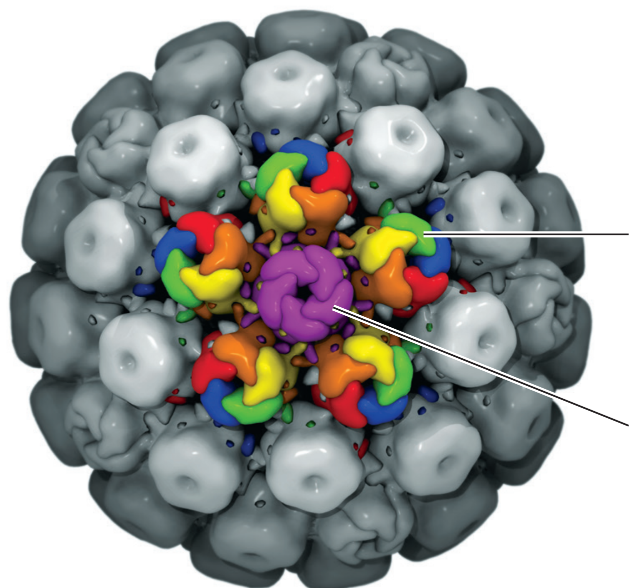
SV40 (polyomavirus)

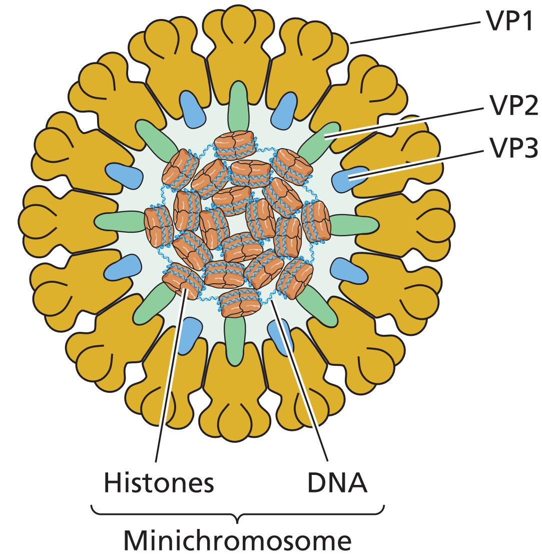
50 nm

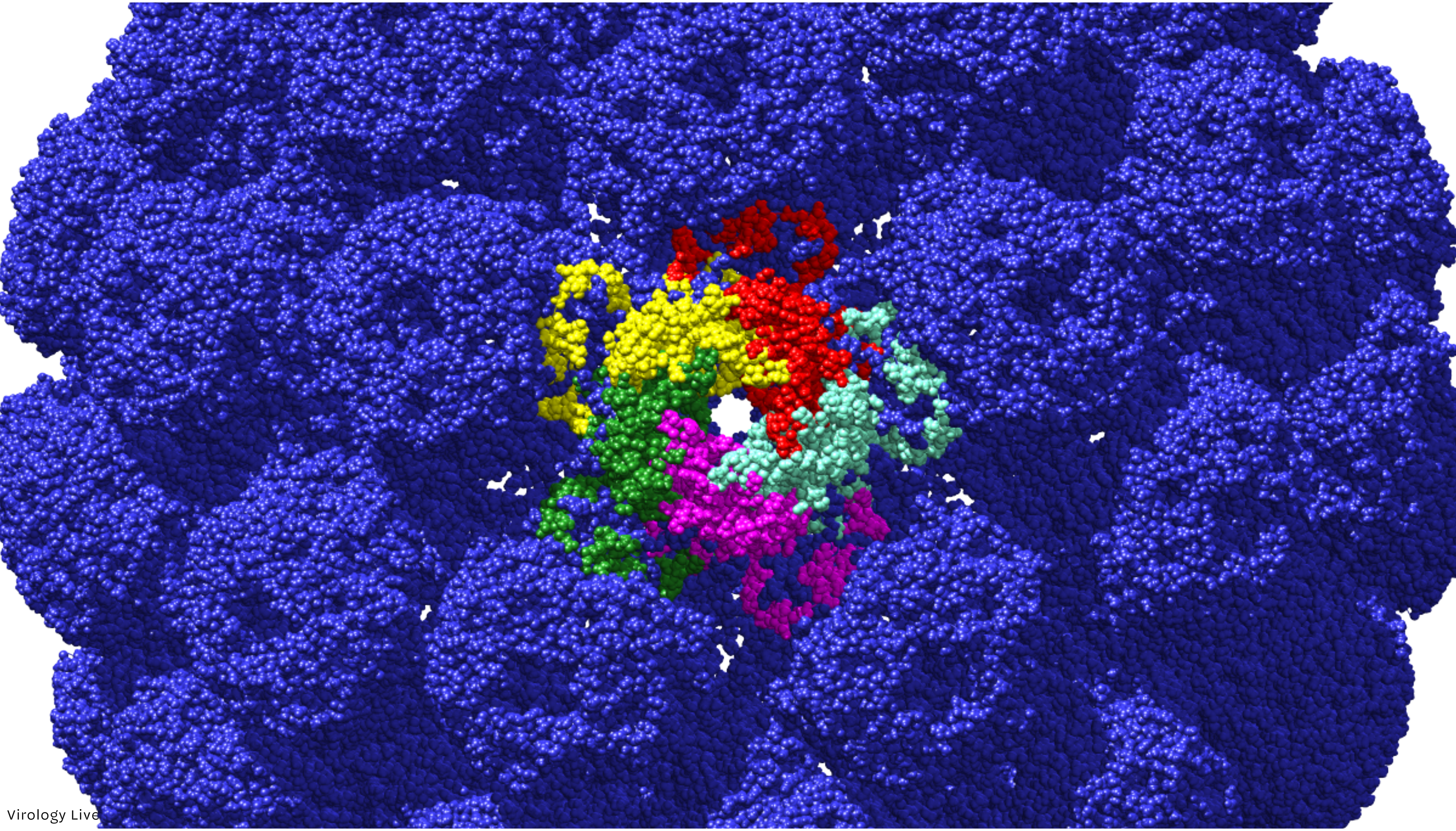
72 pentamers of VP1 = 360 subunits



N-terminal extensions of VP1 engage VP1 of neighboring pentamer







Virology Live

Go to:

b.socrative.com/login/student

room number: virus

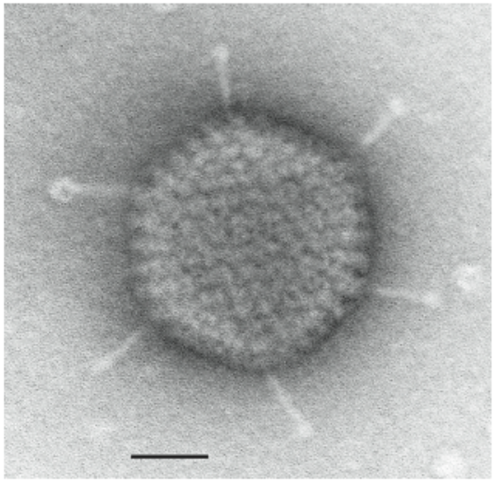
Which of the following are characteristics of icosahedral symmetry in viral capsids?

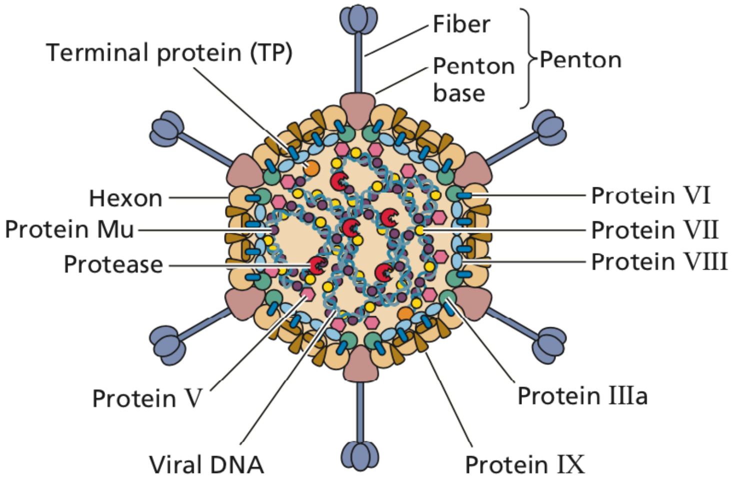
A. Produces a solid with 20 faces, each an equilateral triangle
B. Allows formation of a closed shell with 60 identical subunits
C. Fivefold, threefold, and twofold axes of symmetry
D. The T number describes the number of facets per icosahedral face
E. All of the above

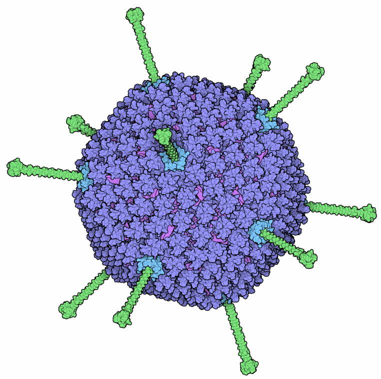
Large complex capsids

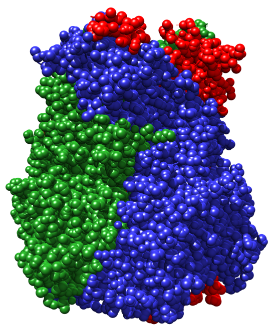
Adenovirus

- 150 nm
- T=25 capsid, 720 copies viral protein II
- Fibers at 12 vertices
- Proteins with specialized roles
- Protein IX = cement (penton-hexon mismatches are weak)

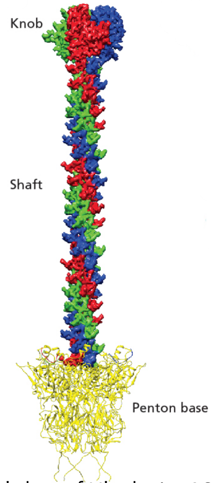




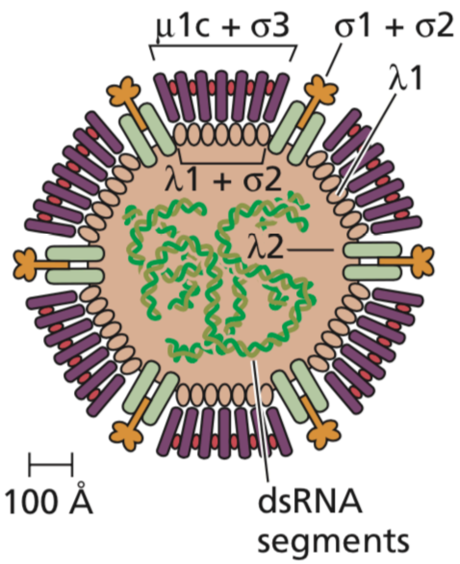




Hexon trimer

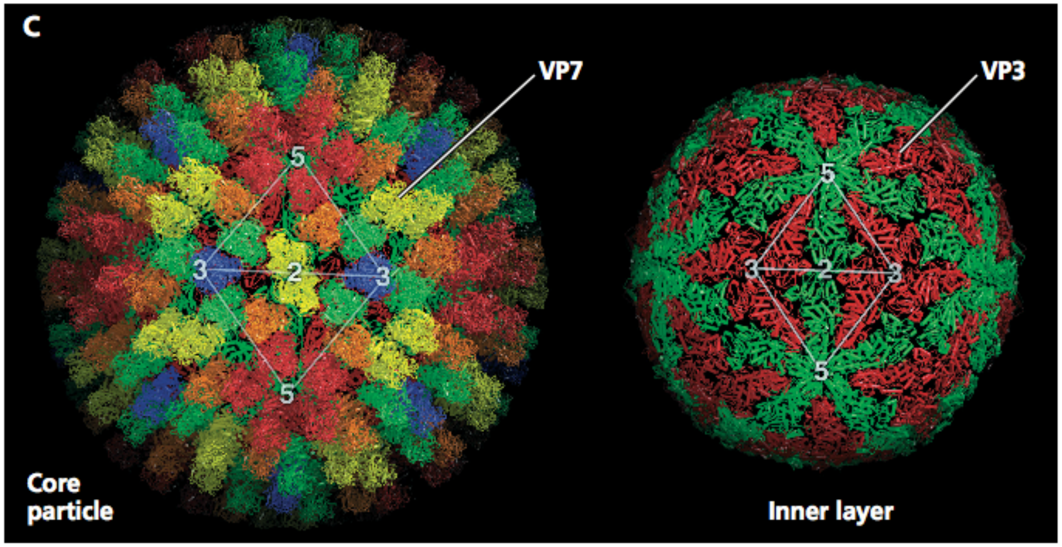


Complex capsids with two icosahedral protein layers



Reoviruses

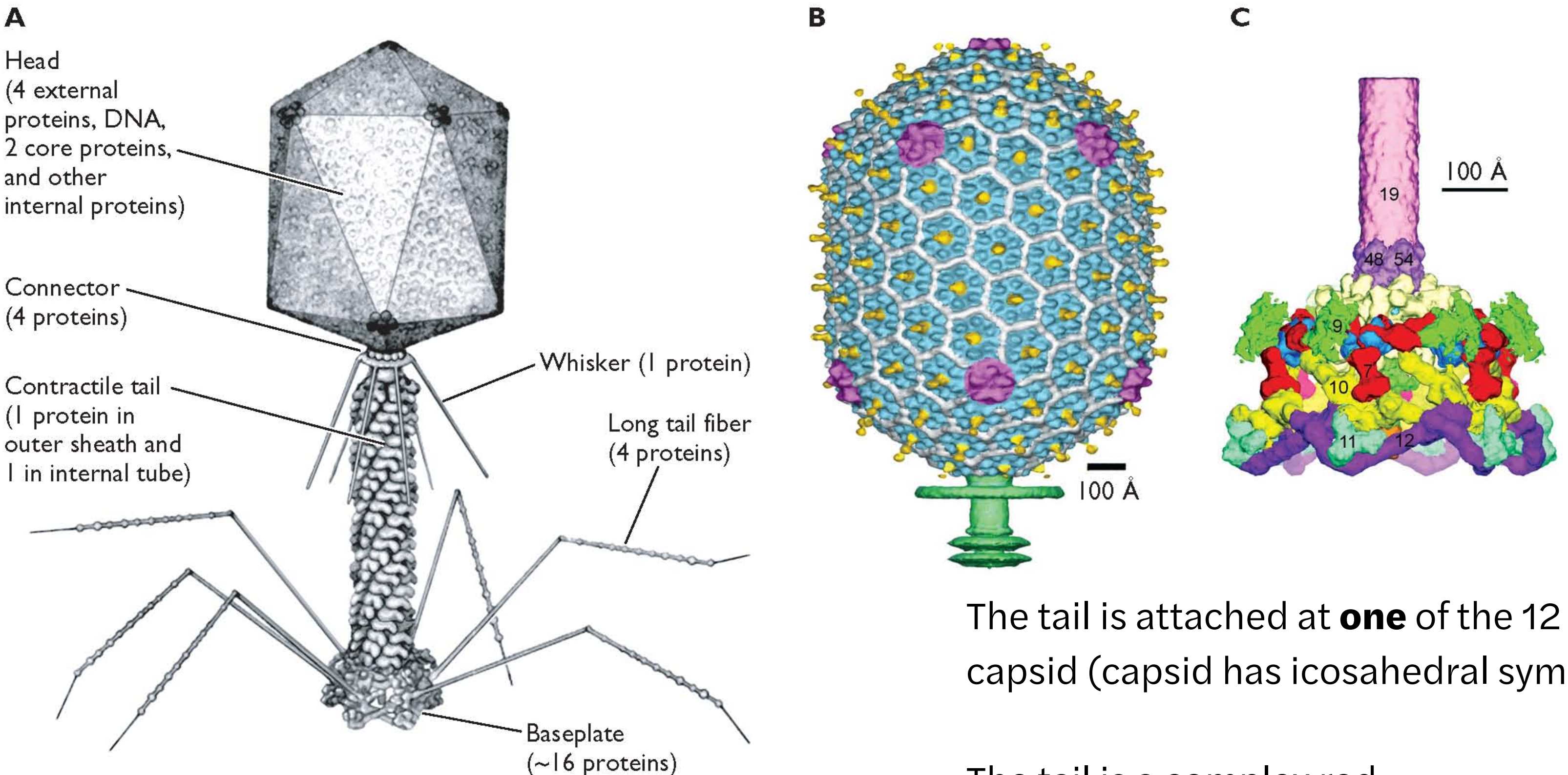
- T=13
- 70 - 90 nm
- two concentric shells



VP7 trimers, T=13

VP3 monomers, T=2

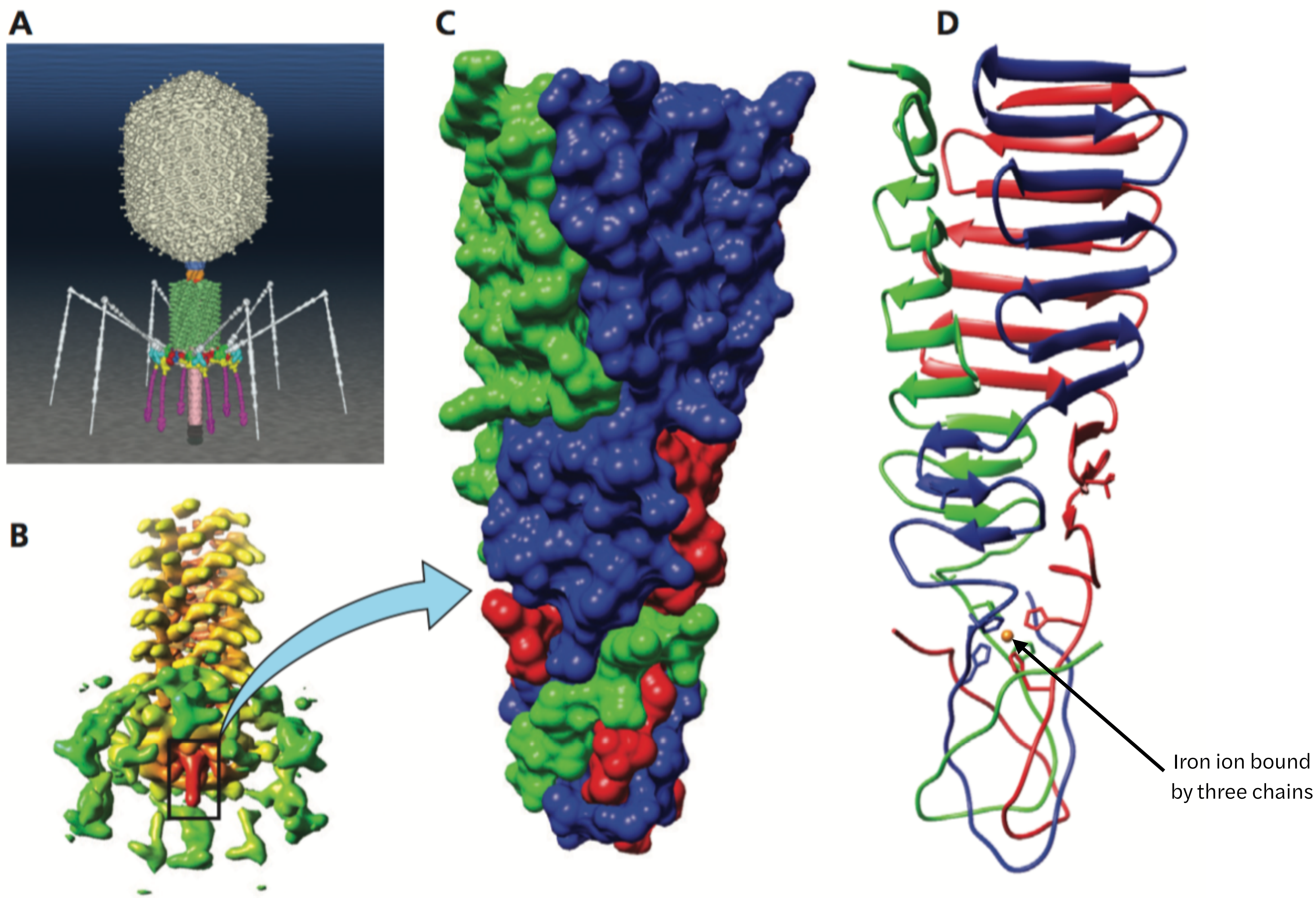
Tailed bacteriophages



The tail is attached at **one** of the 12 vertices of the capsid (capsid has icosahedral symmetry).

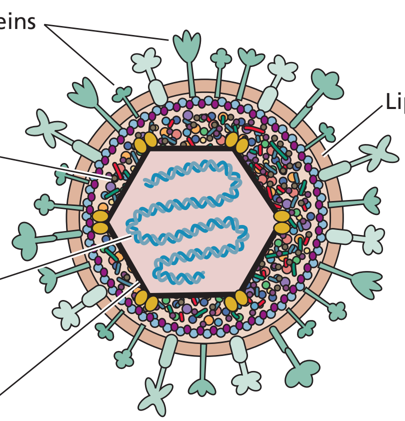
The tail is a complex rod

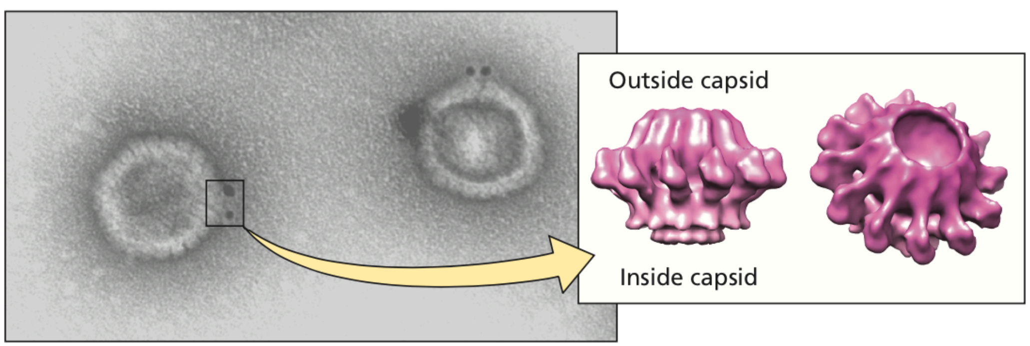
- *uses helical symmetry in many places*
- *some tails are contractile*

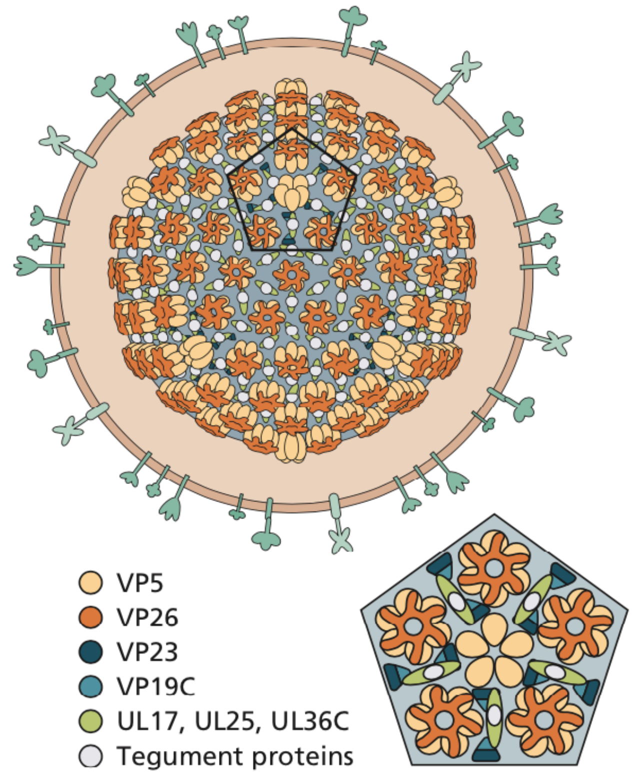

A
B
C
D
Iron ion bound
by three chains

Herpes simplex virus capsid

Holes for entry and exit of DNA



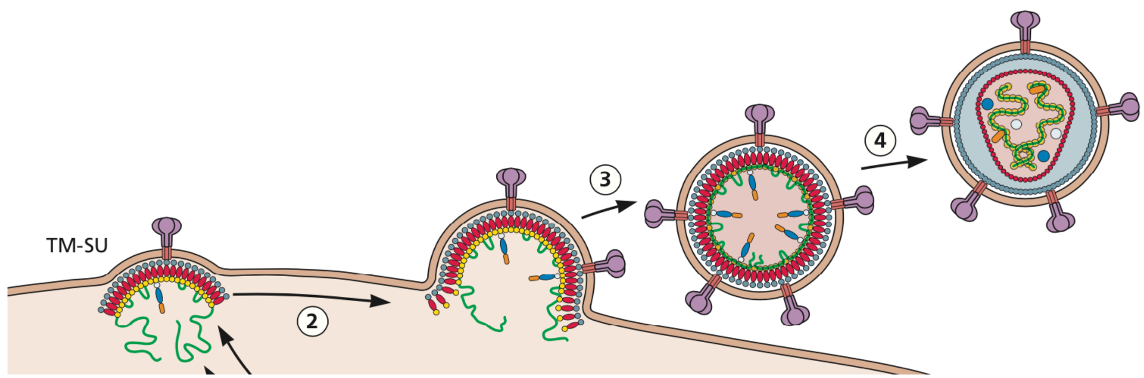




The portal or opening for viral DNA is built at ONE of the 12, 5-fold vertices of the T=16 200 nm herpesvirus capsid

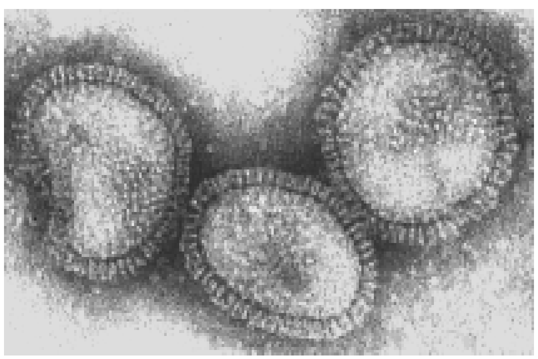
Capsids can be covered by host membranes: enveloped virions

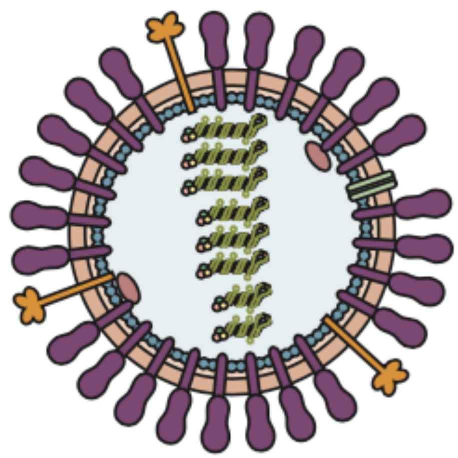
- Envelope is a lipid bilayer derived from host cell
  - Viral genome does not encode lipid synthetic machinery
- Envelope acquired by budding of nucleocapsid through a cellular membrane
  - Can be any cell membrane, but is virus-specific
- Nucleocapsids inside the envelope may have helical or icosahedral symmetry

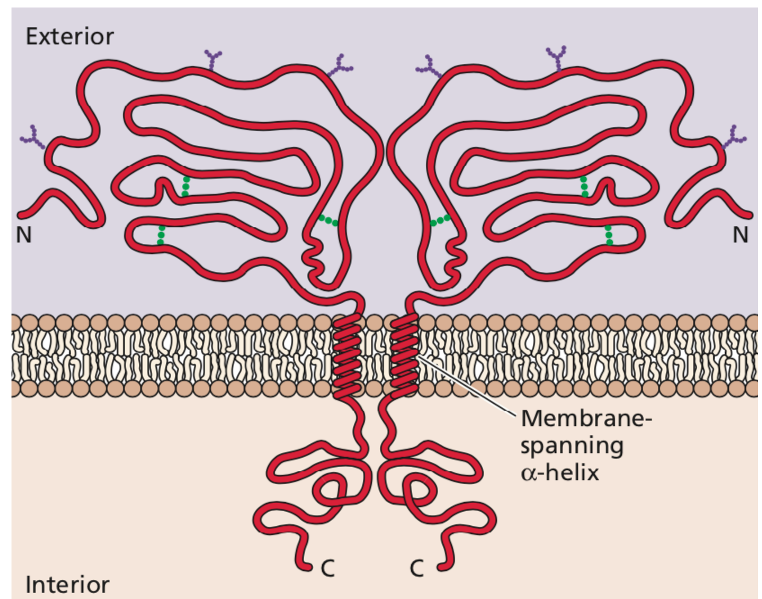


Viral envelope glycoproteins

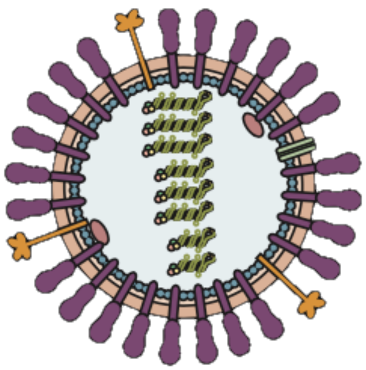
- Integral membrane glycoproteins
- Ectodomain: attachment, antigenic sites, fusion
- Internal domain: assembly
- Oligomeric: spikes



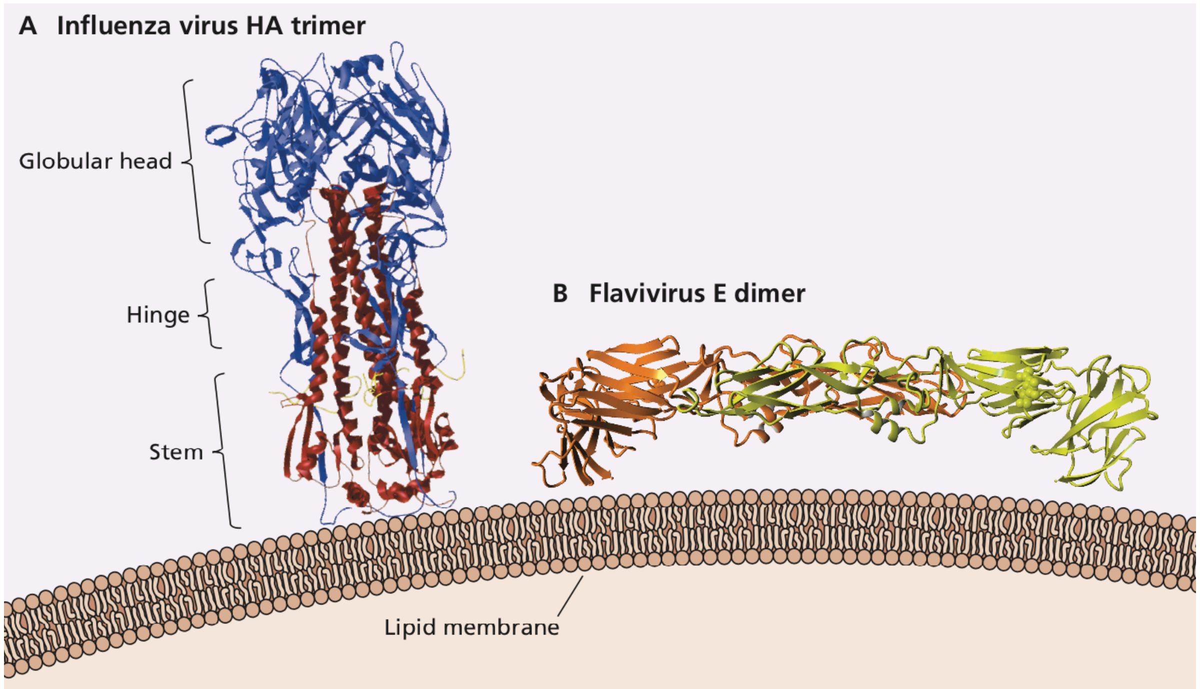


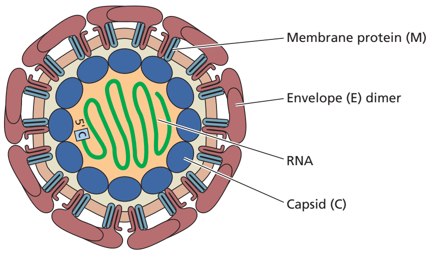


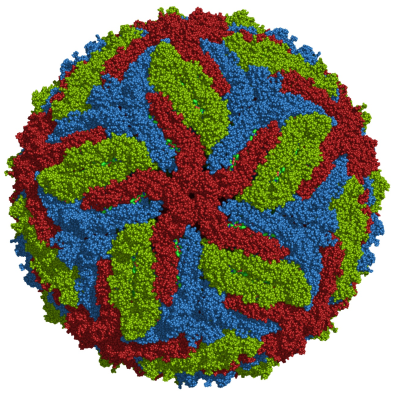
Viral envelope glycoproteins



Unstructured envelope

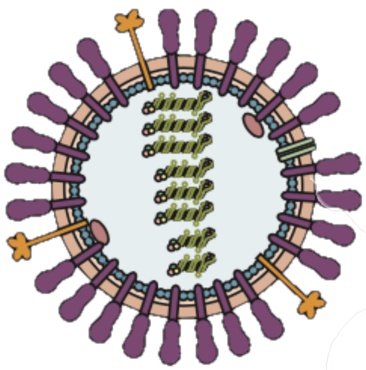




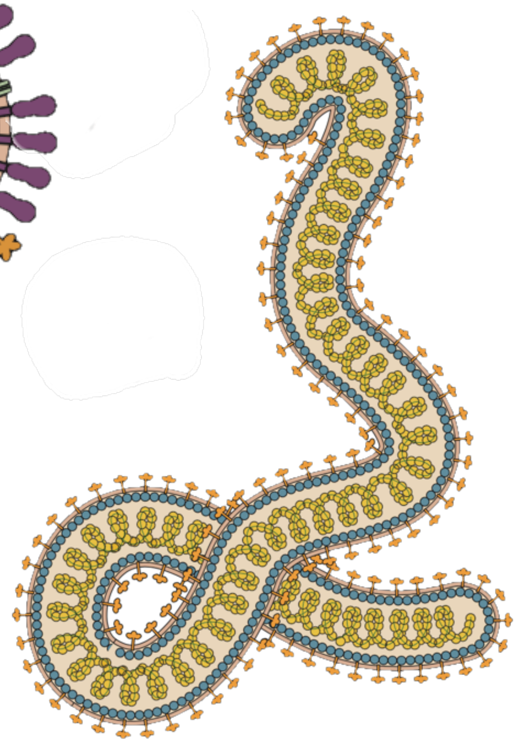


Structured envelope - proteins are icosahedral ordered

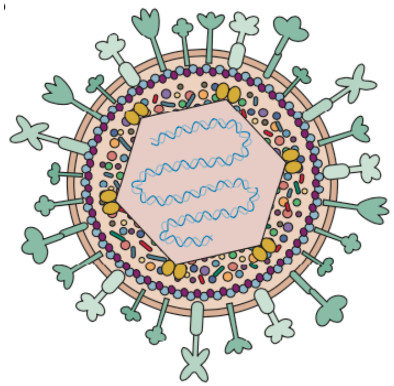
Helical vs icosahedral nucleocapsids



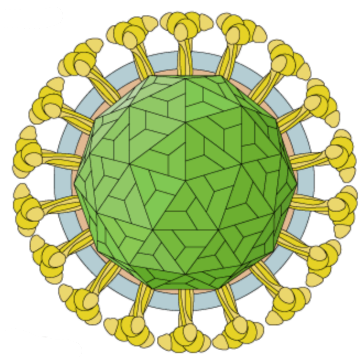
Influenza virus



Ebolavirus



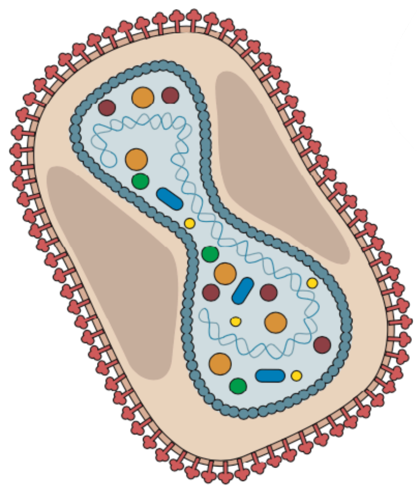
Herpesvirus

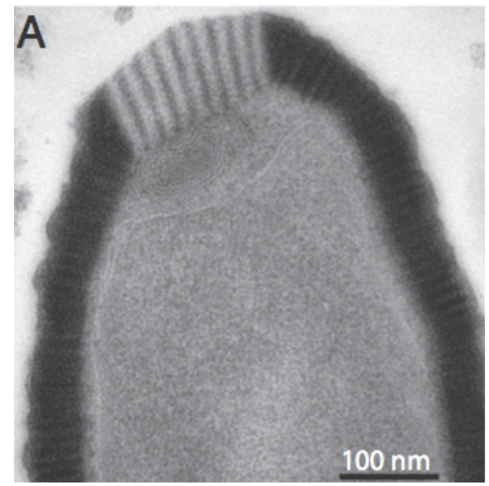


Togavirus (rubella virus)

Complex virus particles

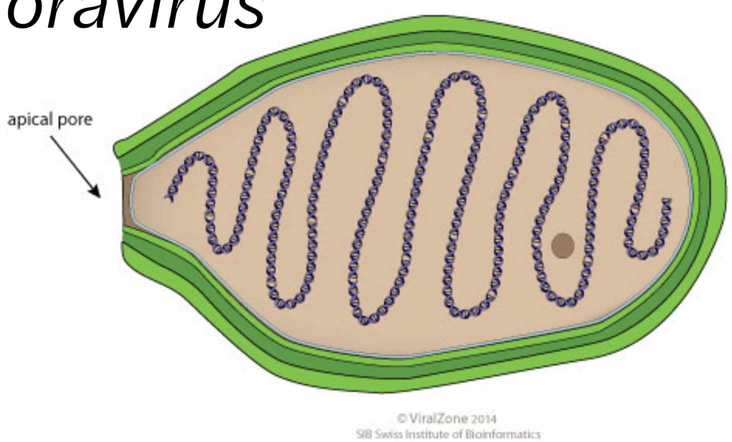
Poxvirus

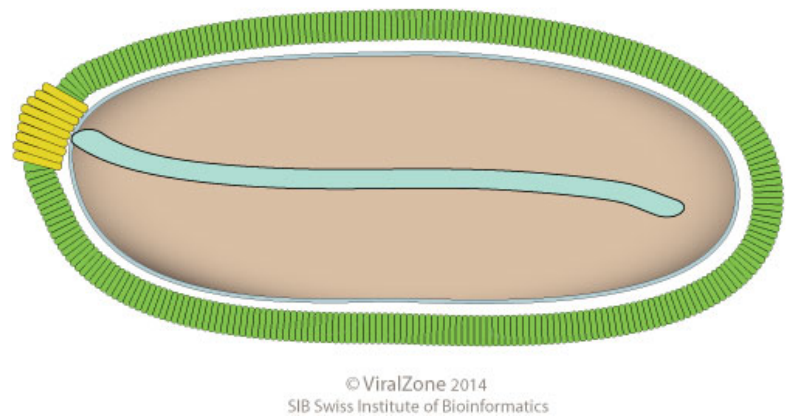




Pithovirus

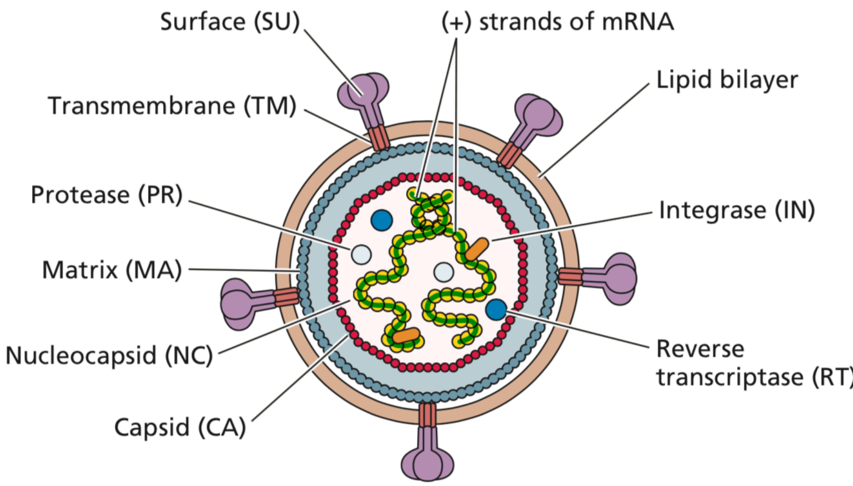
Pandoravirus





Other components of the virus particle

- Enzymes
  - polymerases, integrases, associated proteins
  - proteases
  - poly(A) polymerase
  - capping enzymes
  - topoisomerase



- Activators, mRNA degradation, required for efficient infection, mRNAs
- Cellular components - histones, tRNAs, myristate, lipid, cyclophilin A, and many more

VIROLOGY LIVE

WITH VINCENT RACANIELLO

Next time: Attachment and Entry