Structure of viruses

Lecture 4
Biology 3310/4310
Virology
Spring 2017

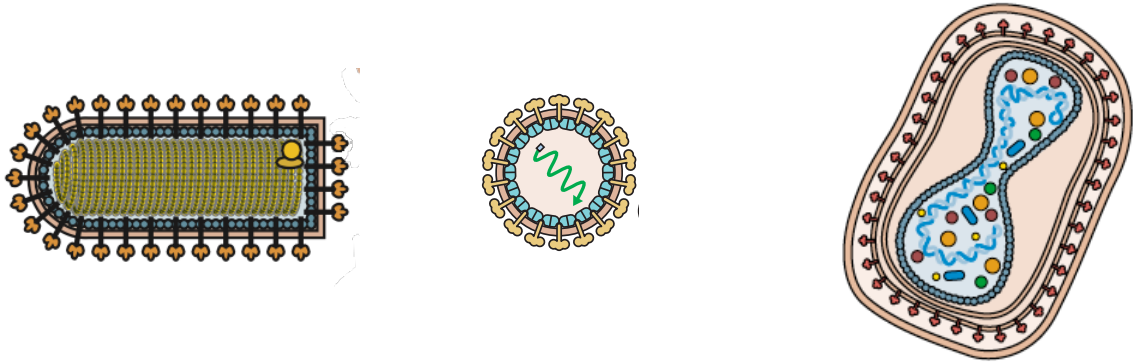
In order to create something that functions properly - a container, a chair, a house - its essence has to be explored, for it should serve its purpose to perfection, i.e., it should be durable, inexpensive, and beautiful.
- WALTER GROPIUS

Functions of structural proteins

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

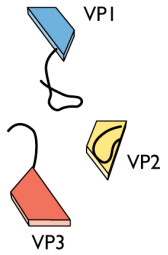
Functions of structural proteins

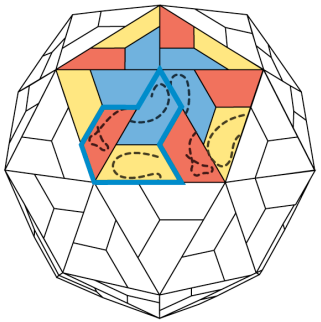


Delivery of the genome

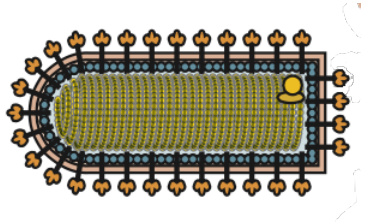
- Bind host cell receptors
- Uncoating of the genome
- Fusion with cell membranes
- Transport of genome to the appropriate site

Definitions

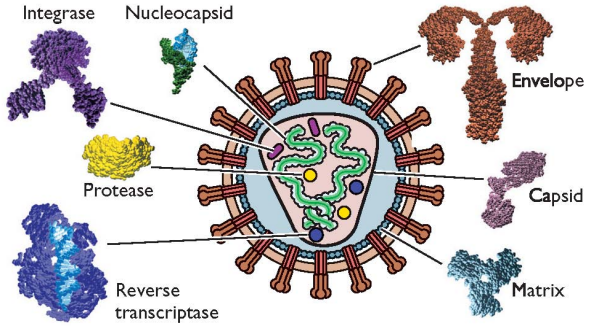




- *Subunit*
  - Single folded polypeptide chain
- *Structural unit* (protomer, asymmetric unit)
  - Unit from which capsids or nucleocapsids are built; one or more subunits
- *Capsid* (*capsa* = Latin, box)
  - Protein shell surrounding genome

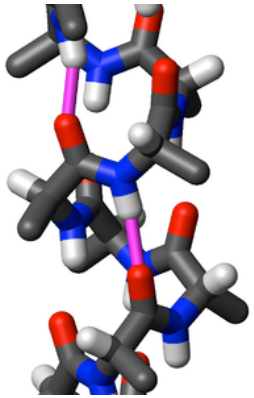


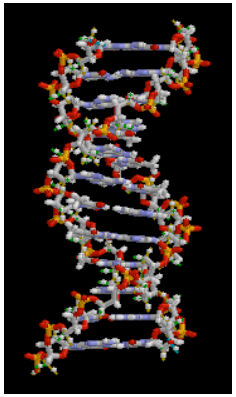
- *Nucleocapsid* (core)
  - Nucleic acid - protein assembly within particle; used when is a discrete substructure
- *Envelope* (viral membrane)
  - Host cell-derived lipid bilayer
- *Virion*
  - Infectious virus particle

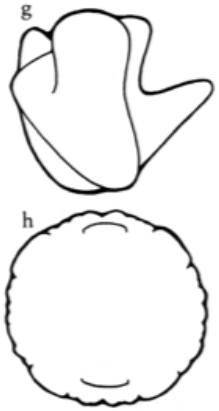


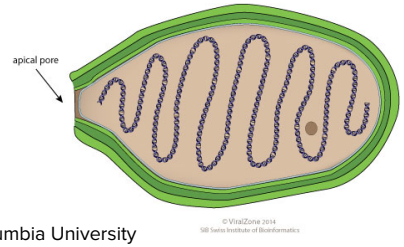
Putting virus particles into perspective

- 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



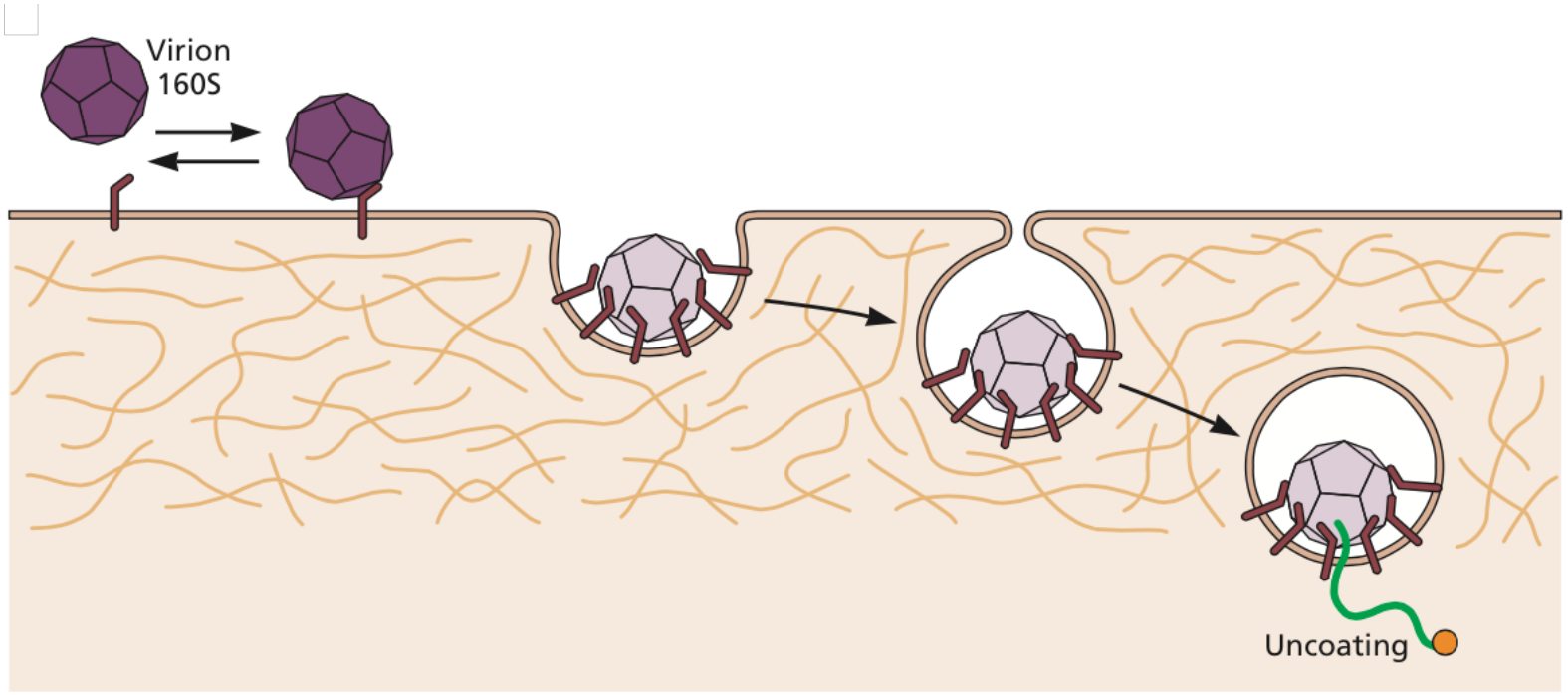






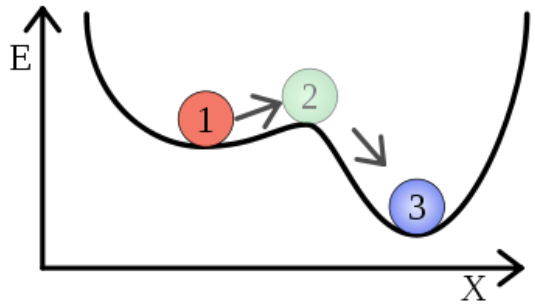
Virus particles are metastable

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



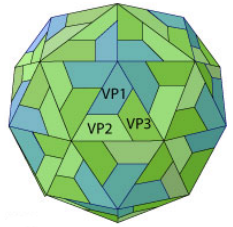
Virions are metastable

- 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

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room number: virus

Viral capsids are metastable because:

A. They must protect the viral genome 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

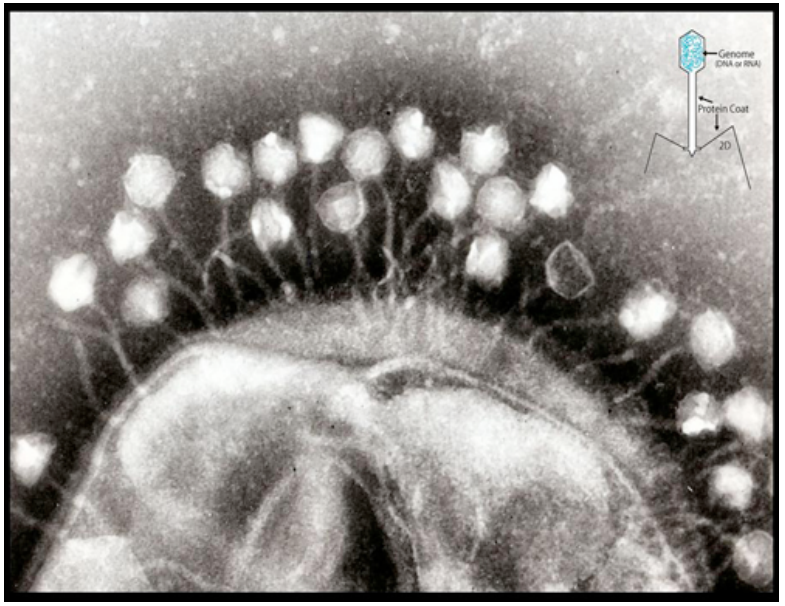
- Electron microscopy
- X-ray crystallography
- Cryo-electron microscopy (cryoEM) & cryo-electron tomography
- Nuclear magnetic resonance spectroscopy (NMR)

Flint volume I, chapter 4

Beginning of the era of modern structural virology

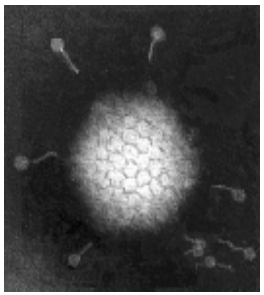
1940: Helmuth Ruska used an electron microscope to take the first pictures of virus particles

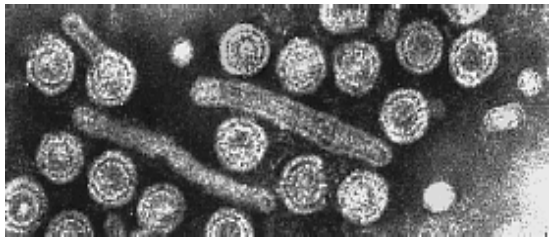
Ruska, H. 1940. Die Sichtbarmachung der BakteriophagenLyse im Ubermikroskop. Naturwissenschaaften. 28:45-46).

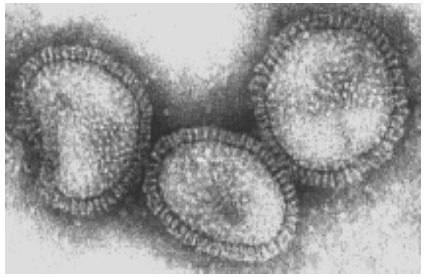


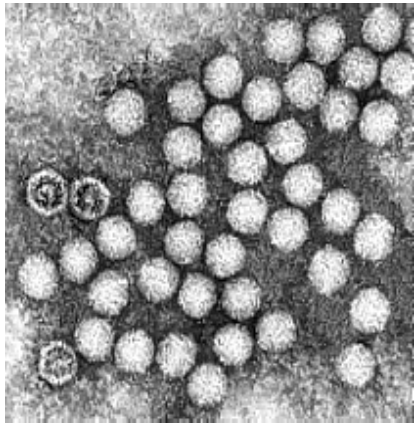
Electron microscopy

- 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



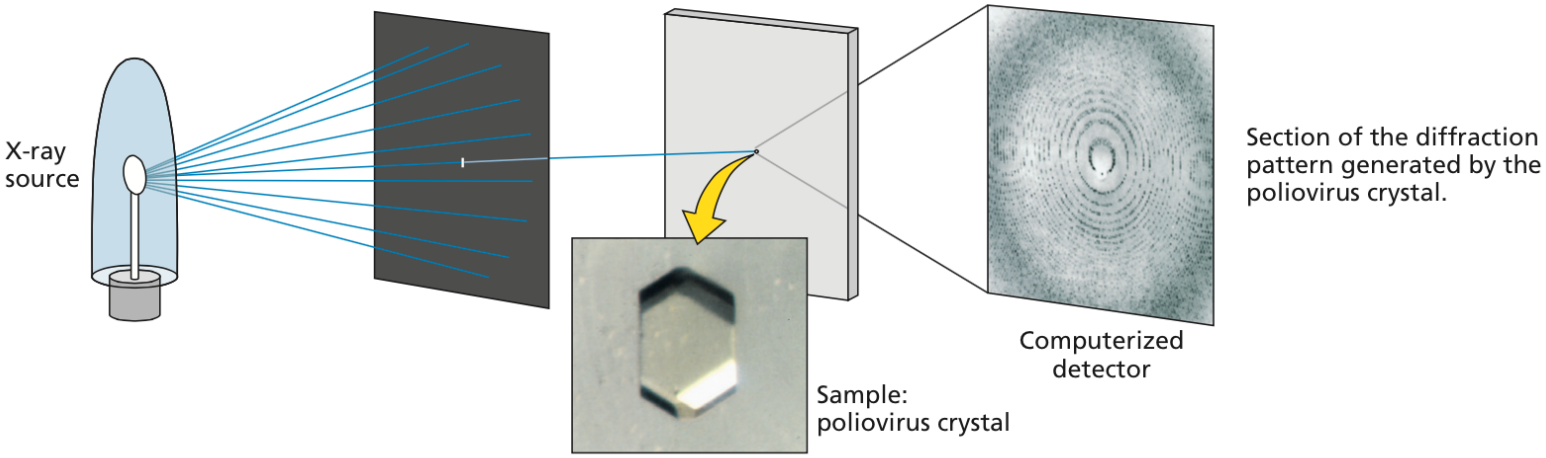




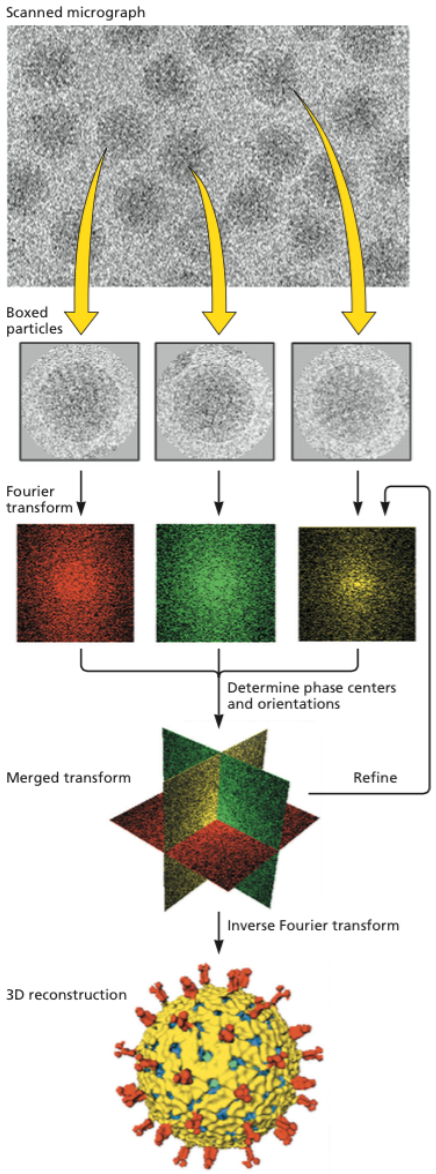


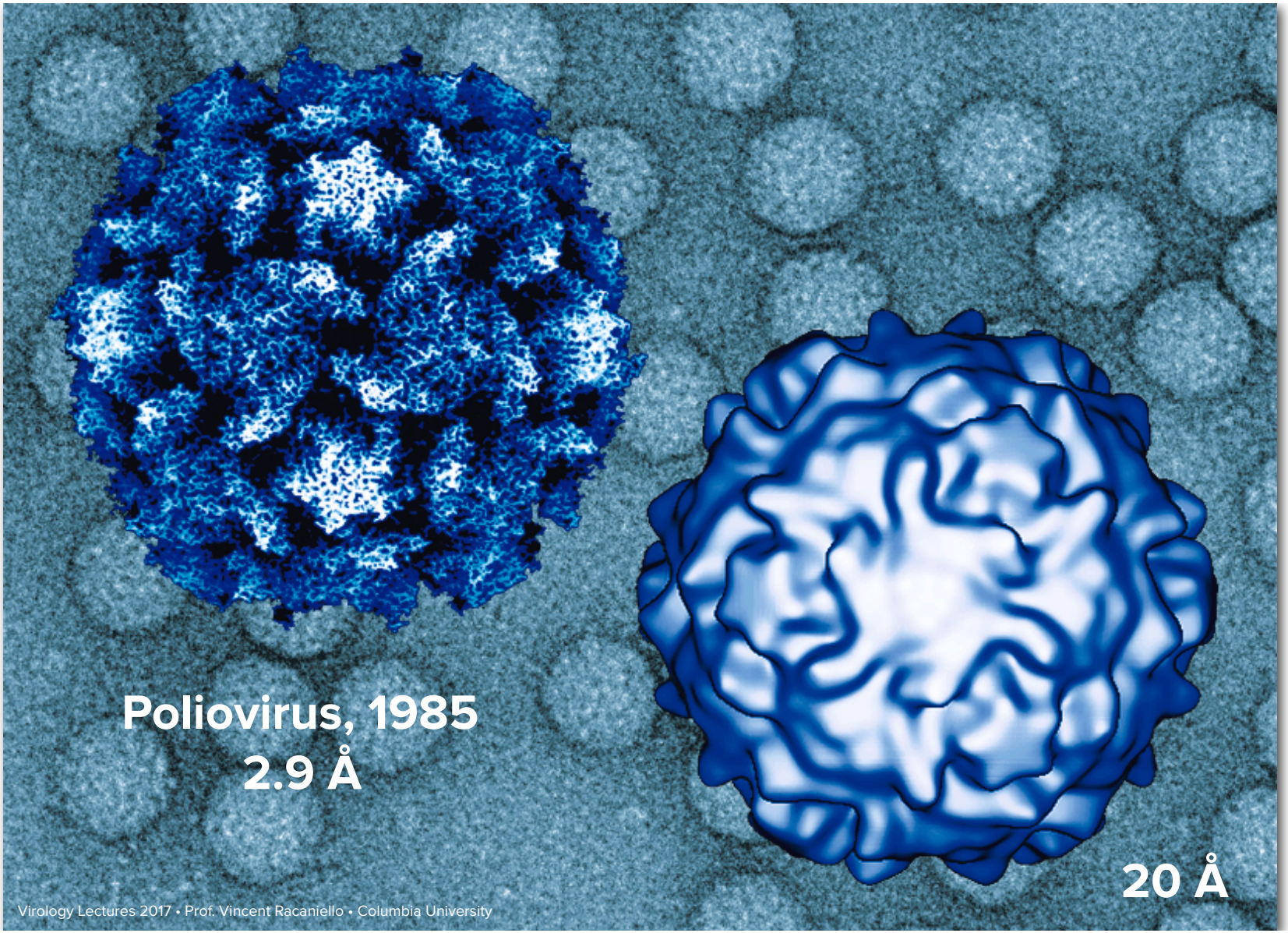
Linda Stannard, University of Cape Town
http://web.uct.ac.za/depts/mmi/stannard/linda.html

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

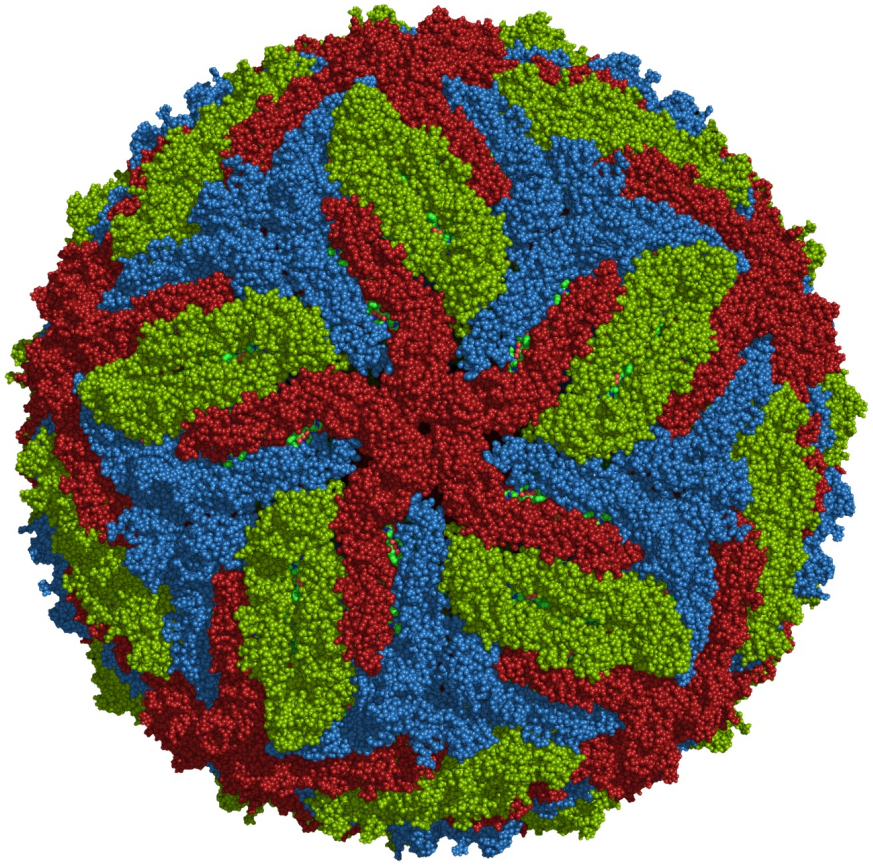


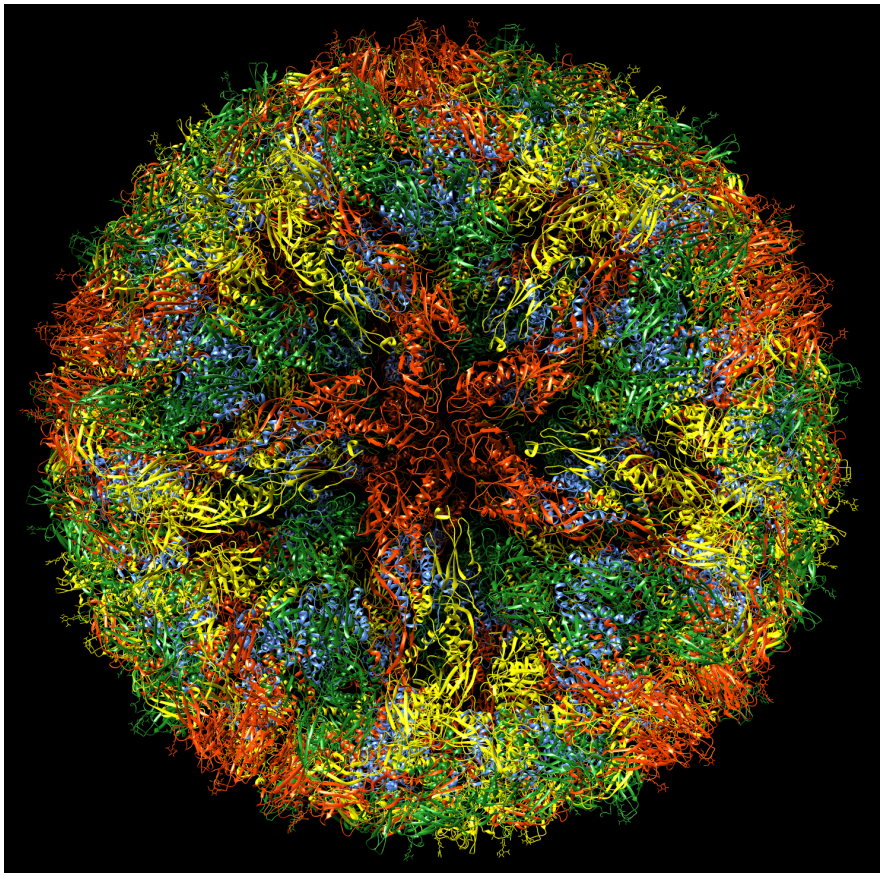
Cryo-electron microscopy (cryoEM)



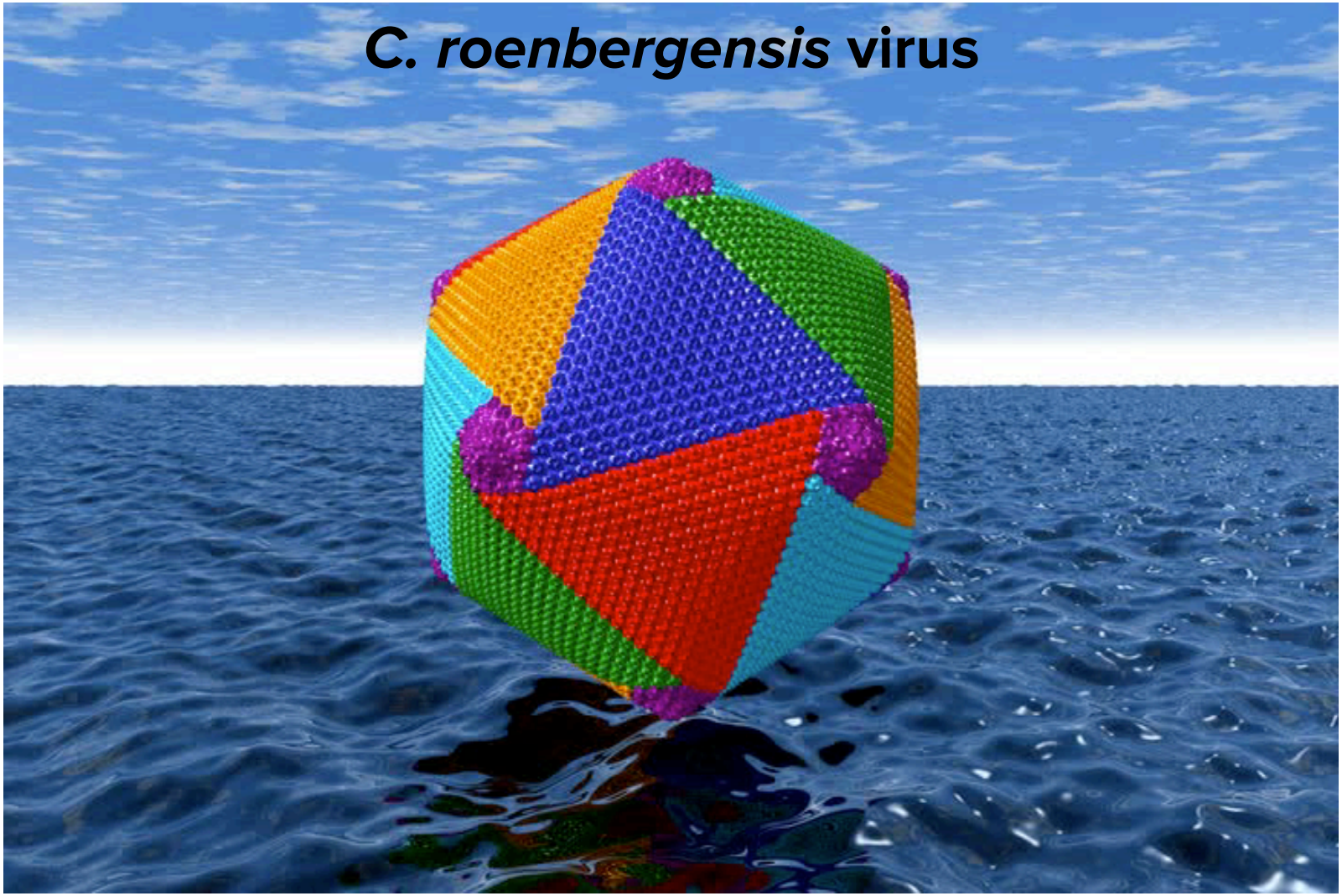

Poliovirus, 1985
2.9 Å
20 Å
Virology Lectures 2017 • Prof. Vincent Racaniello • Columbia University

Zika Virus - 3.8 Å





Science March 2016 doi: 10.1126/science.aaf5316

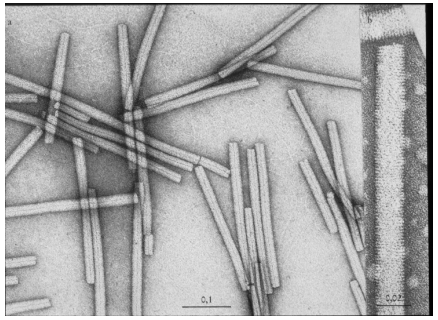


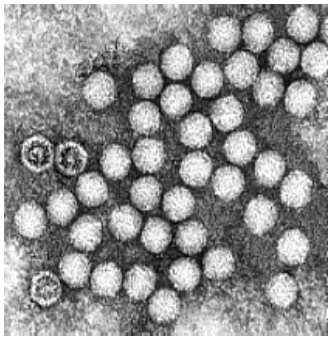
300 nm, >15,000 capsid proteins

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

Building virus particles: Symmetry is key

- Watson and Crick did more than discover DNA structure





- Their seminal contribution to virology:
  - Identical protein subunits are distributed with *helical symmetry* for rod-shaped viruses
  - *Platonic polyhedra symmetry* for round viruses

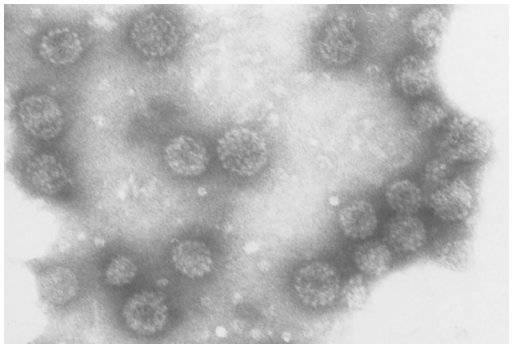
The symmetry rules are elegant in their simplicity

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

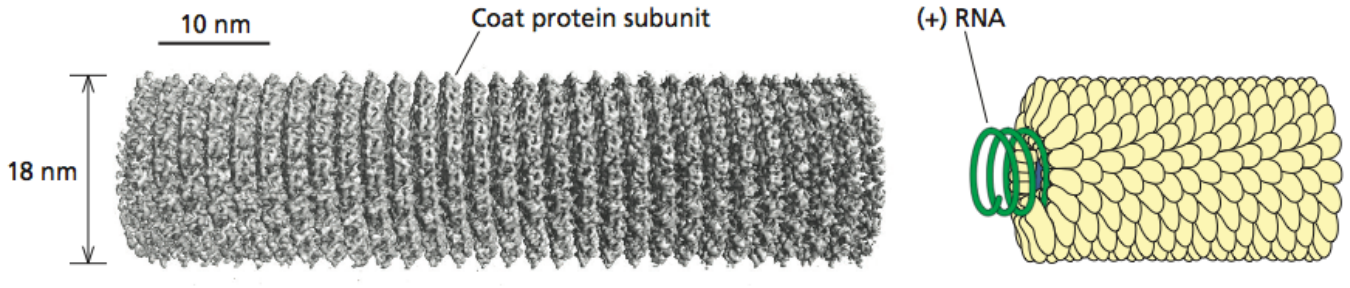
Symmetry and self-assembly

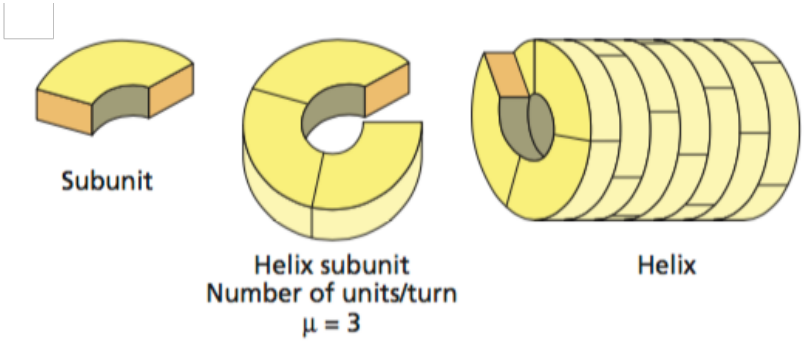
- Many capsid proteins can self assemble into virus-like particles (VLPs)
- The HBV and HPV vaccines are VLPs made in yeast

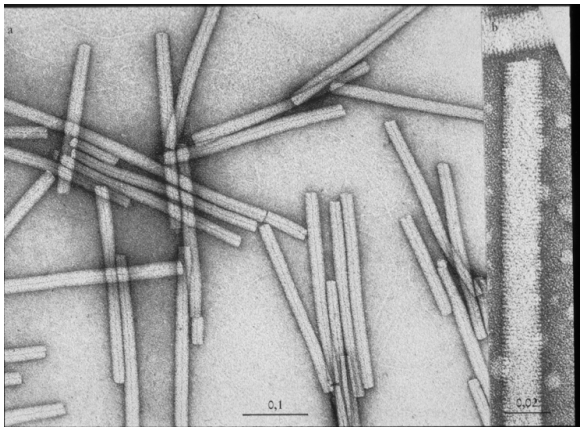


Helical symmetry

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



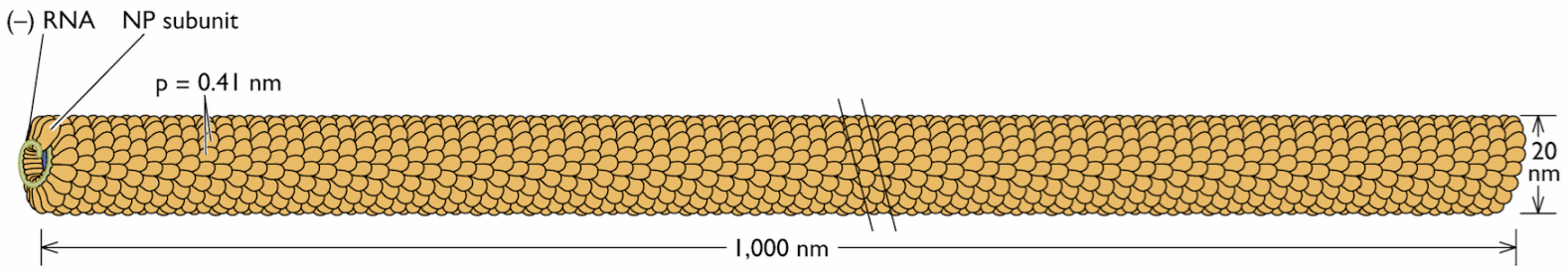


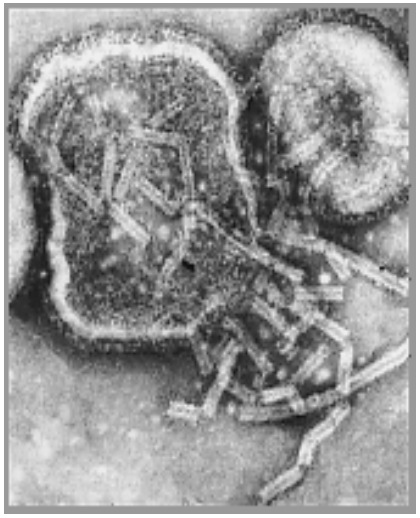


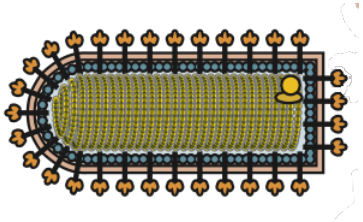

The Amazing
Magnetic Toy
You Can't Put Down!
Buckyballs

Helical symmetry

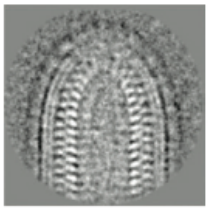
Sendai virus nucleocapsid

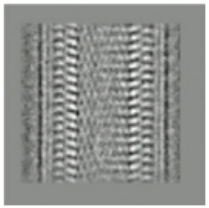


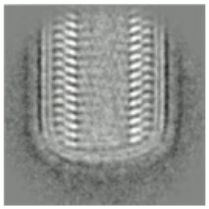


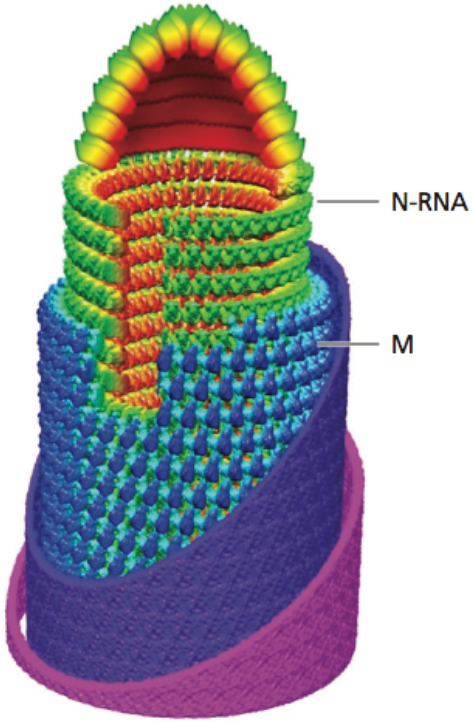


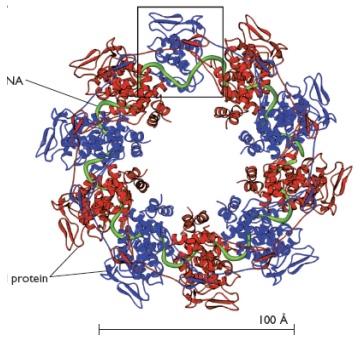
C Vesicular stomatitis virus

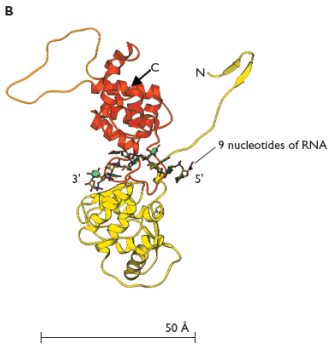




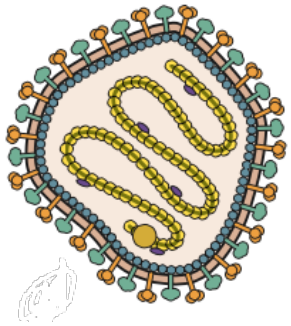


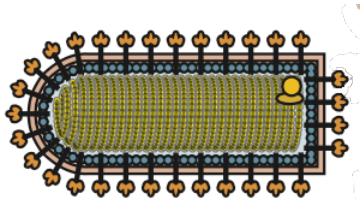


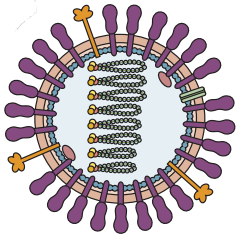


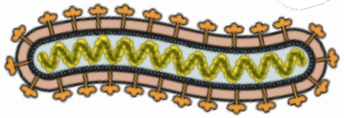


Enveloped RNA viruses with (-) ssRNA and helical capsids









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

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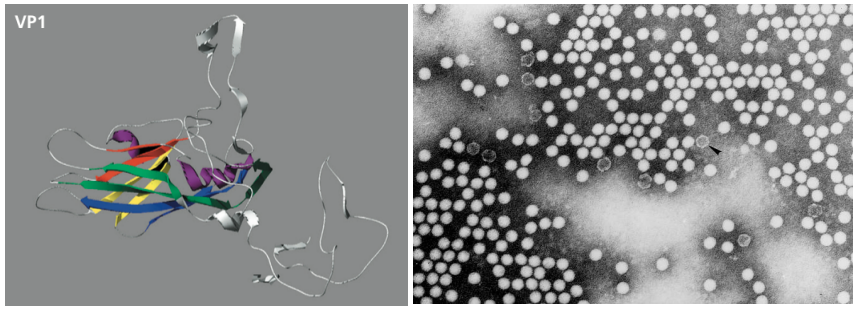
room number: virus

Which of the following describe virus symmetry and self assembly?

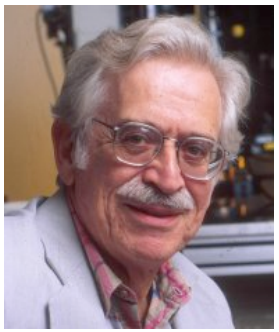
A. The bonding contacts of subunits are usually covalent
B. The bonding contacts of subunits are usually non-covalent
C. Each subunit has different bonding contacts with its neighbors
D. Self-assembly of virus particles does not occur
E. None of the above

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

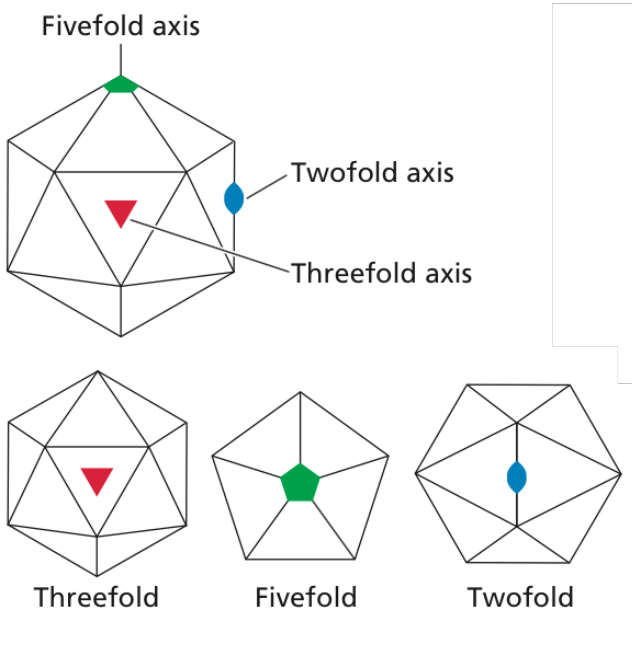
Caspar & Klug's 1962 solution



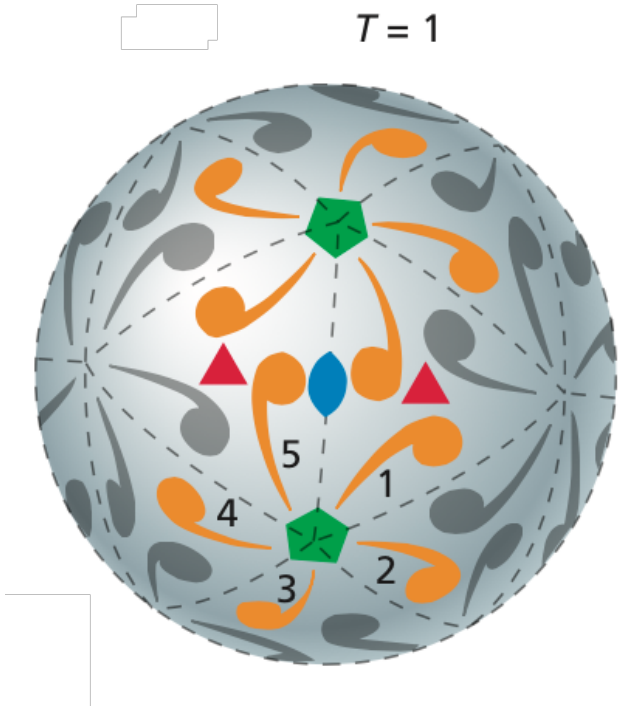
- They knew from Watson & Crick's work that round capsids are icosahedrons - no other Platonic solids were used
- Capsid subunits tended to be arranged as hexamers and pentamers

Icosahedral symmetry

- Icosahedron: solid with 20 faces, each an equilateral triangle
- Allows formation of a closed shell with smallest number (60) of identical subunits



Simple icosahedral capsids



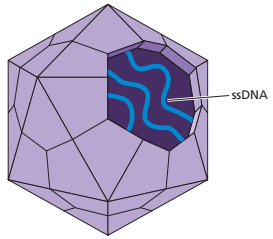
- Made of 60 identical protein subunits
- The protein subunit is the structural unit
- Interactions of all molecules with their neighbors are identical (head-to-head, tail-to-tail)

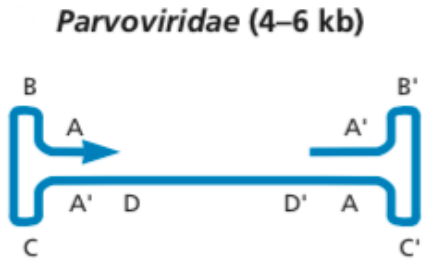
Adeno-associated virus 2 (parvovirus)

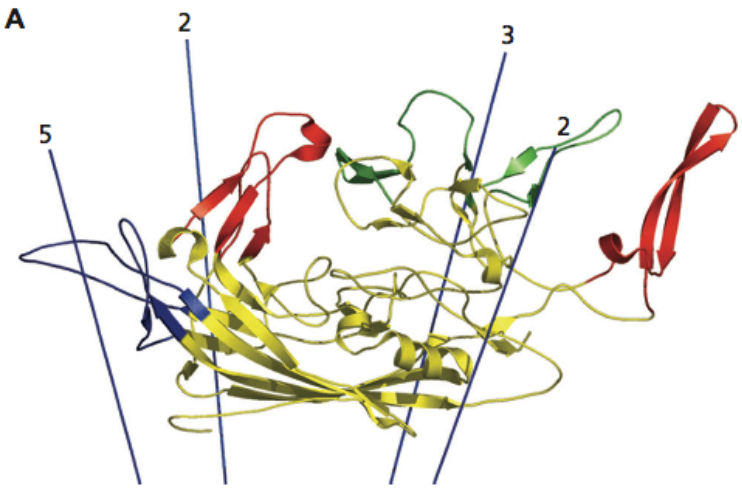
25 nm

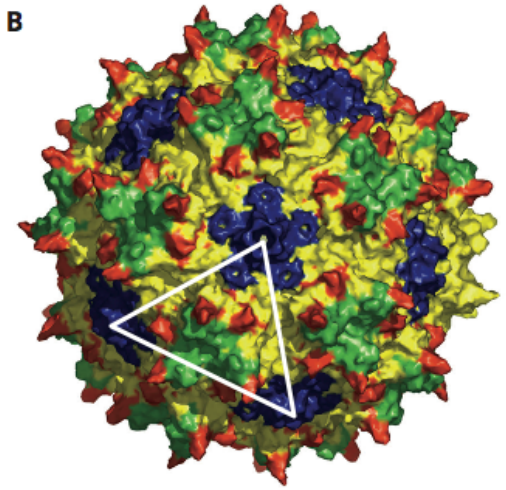
T=1

60 copies of a single capsid protein



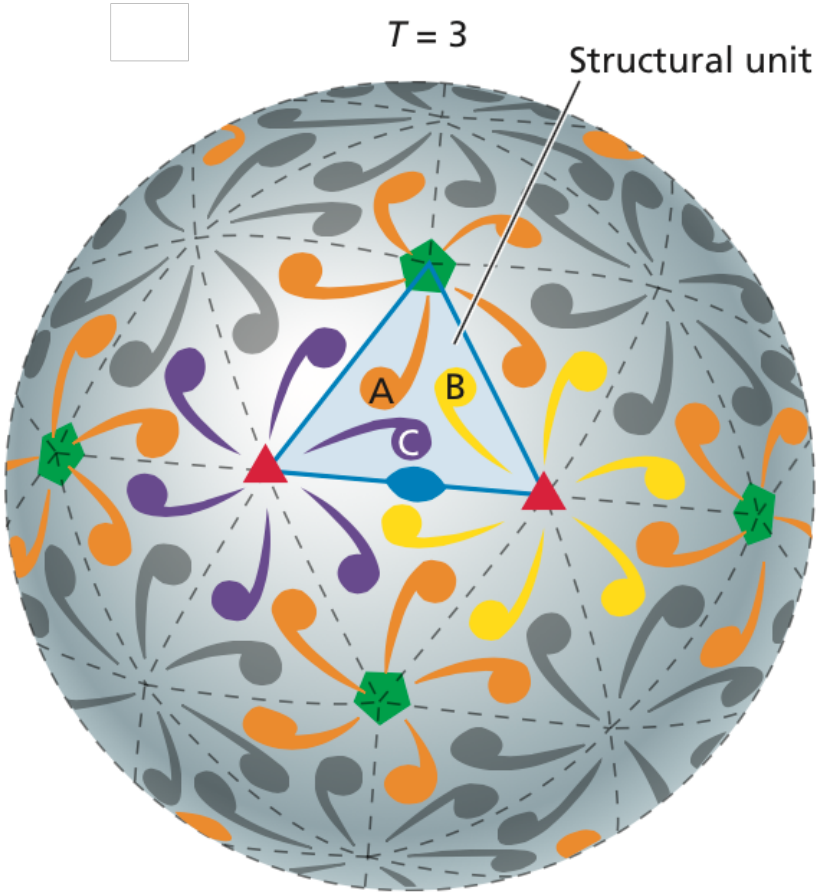






How are larger virus particles built? By adding more subunits

- 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

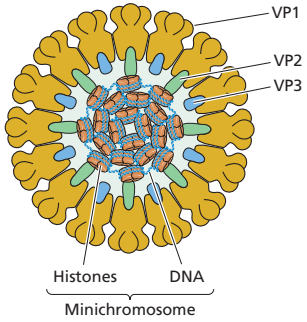
- When a capsid contains more than 60 subunits, each occupies a *quasiequivalent* position
- The noncovalent binding properties of subunits in different structural environments are similar, but not identical

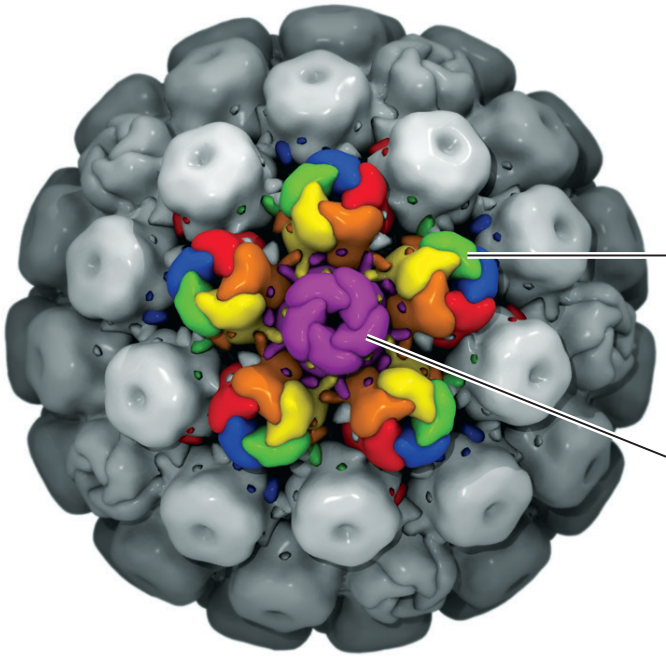
SV40 (polyomavirus)

50 nm

T=6

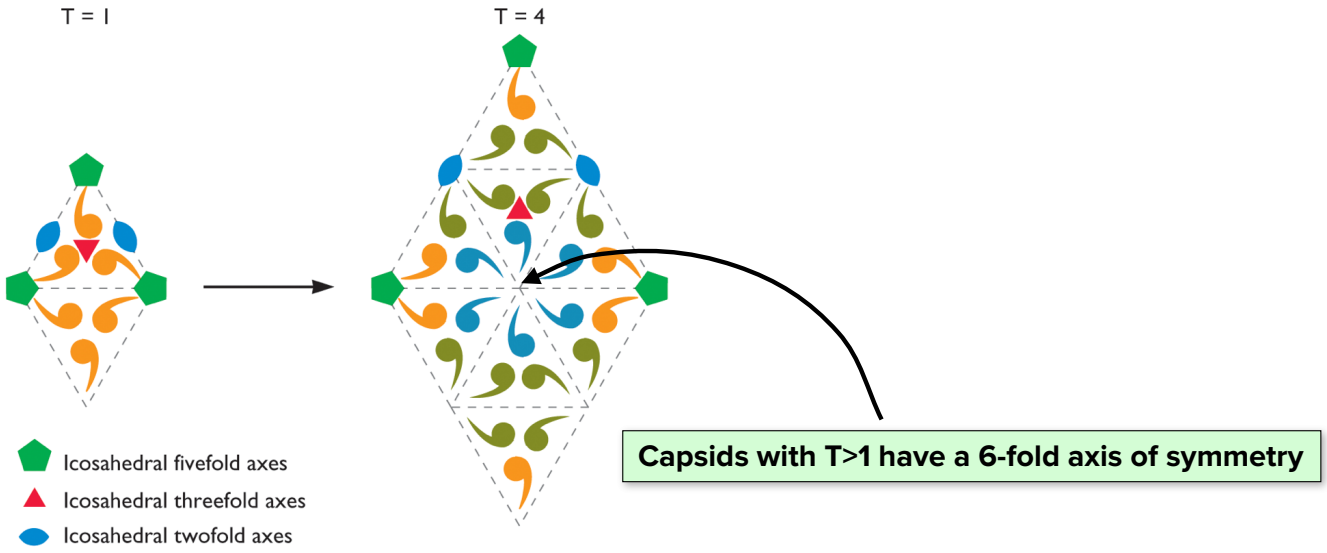
72 pentamers of VP1 = 360 subunits

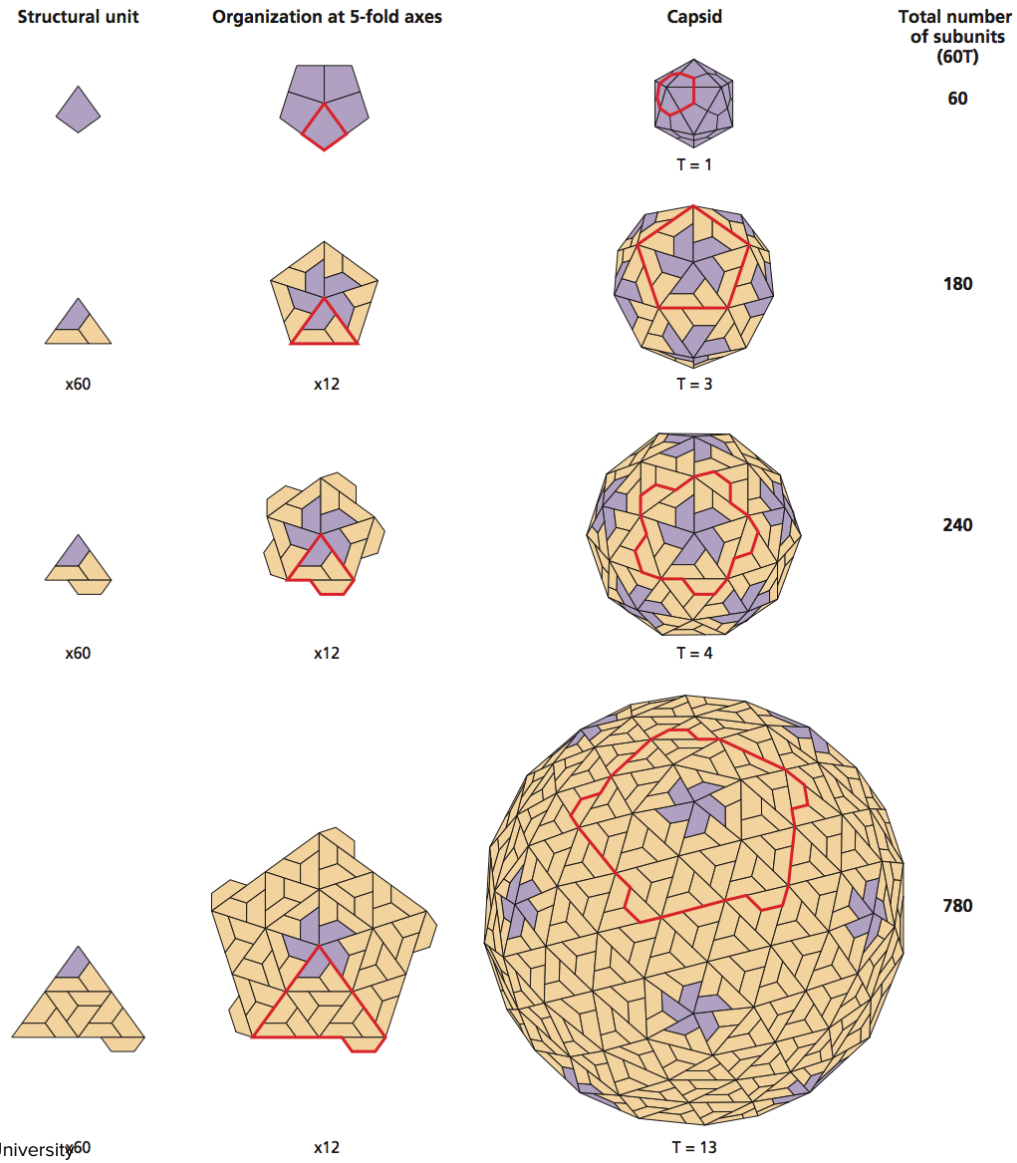




Triangulation number, T

- The number of facets per triangular face of an icosahedron
- Combining several triangular facets allows assembly of larger face from same structural unit





Go to:

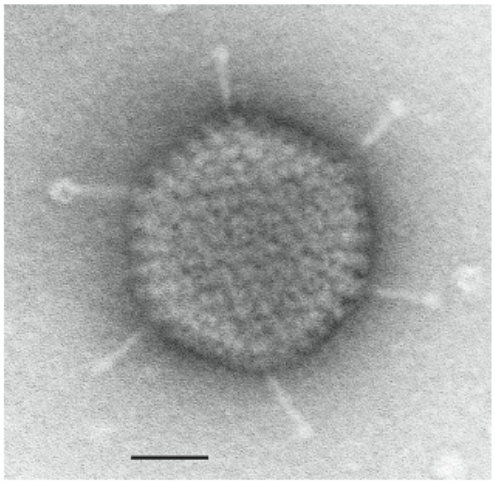
b.socrative.com/login/student
room number: virus

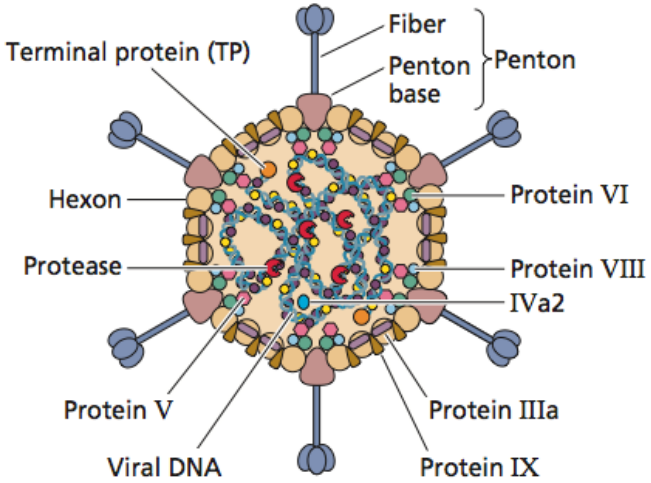
Which of the following are characteristics of icosahedral symmetry in viral capsids?

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

- Distinct components with different symmetries
- Presence of proteins devoted to specialized roles

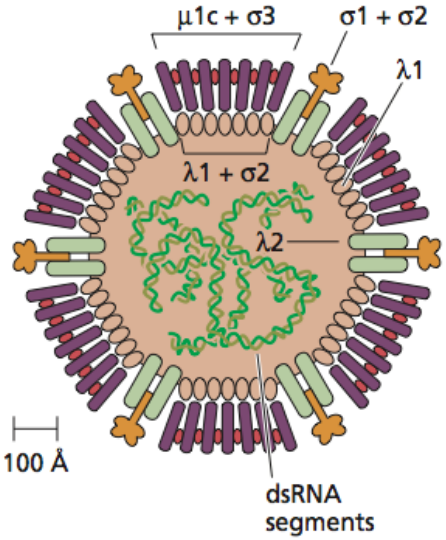


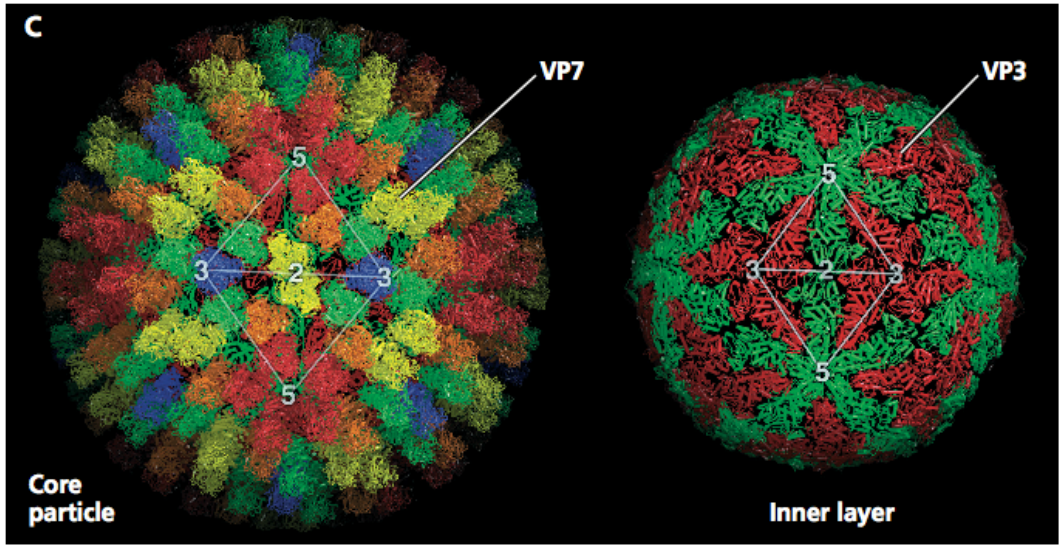


Adenovirus

- 150 nm
- T=25 capsid, 720 copies viral protein II + 60 copies of protein III
- Fibers at 12 vertices

Complex capsids with two icosahedral protein layers





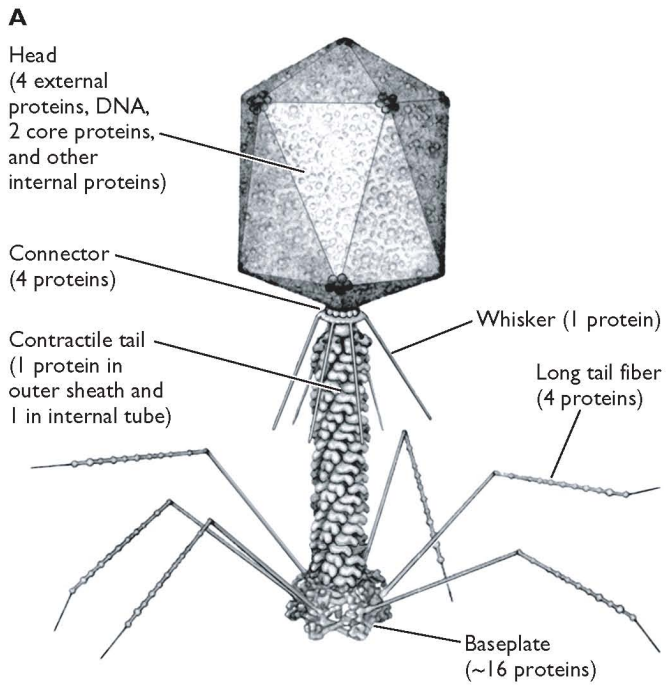
VP7 trimers, T=13

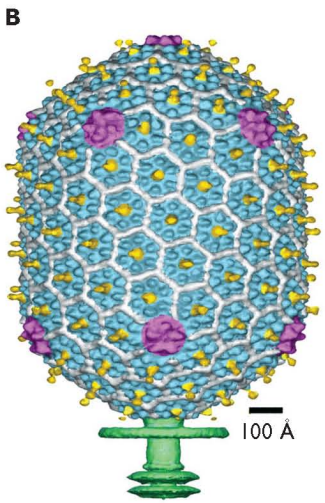
VP3 monomers, T=2

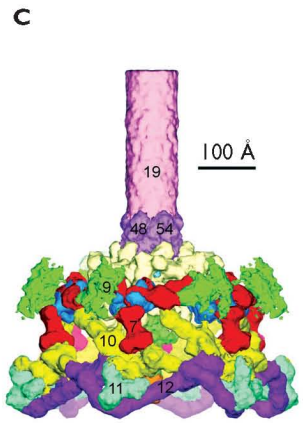
Reoviruses

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

Tailed bacteriophages



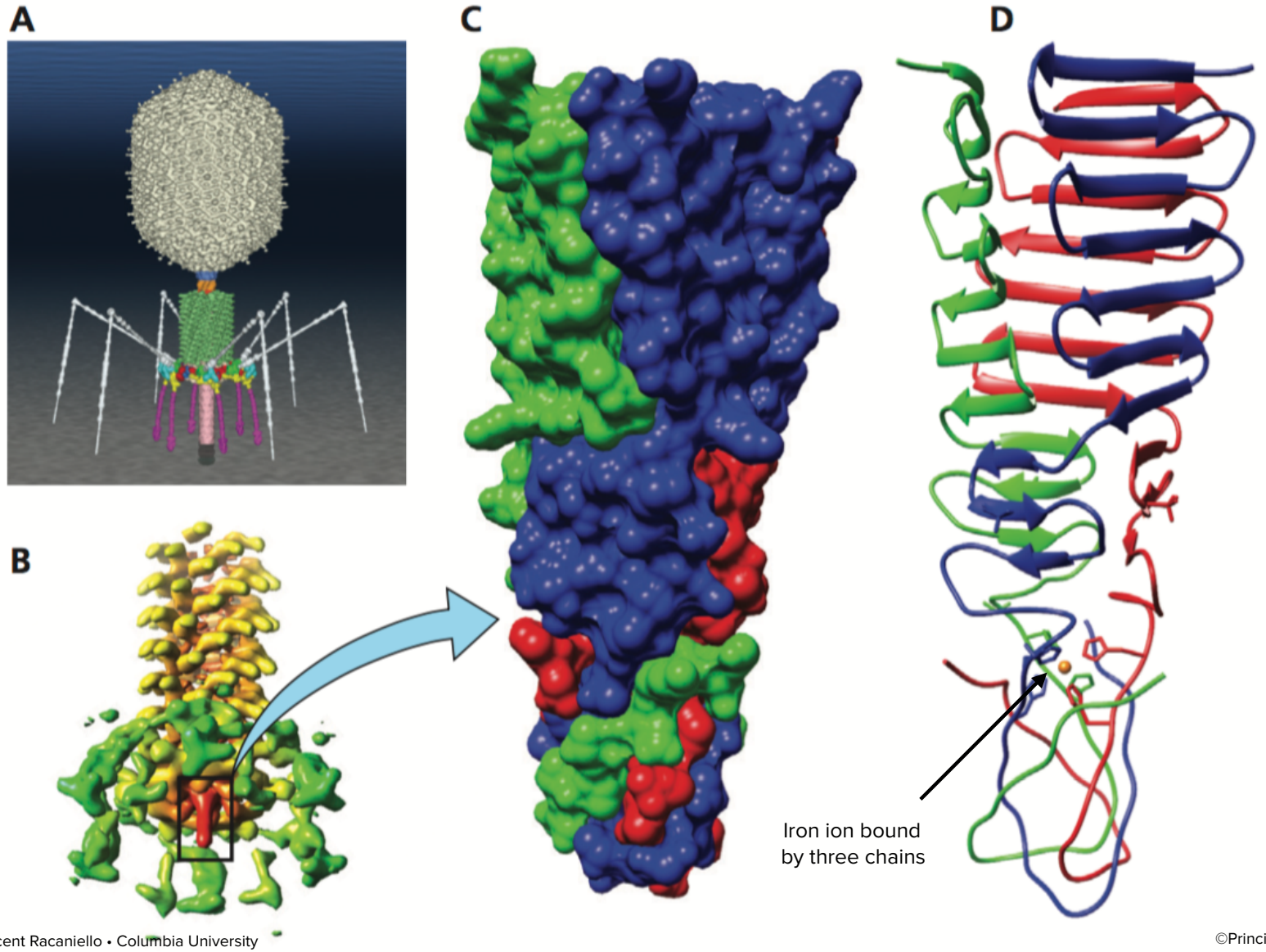




The tail is attached at **one** of the 12 vertices of the capsid (capsid has icosahedral symmetry).

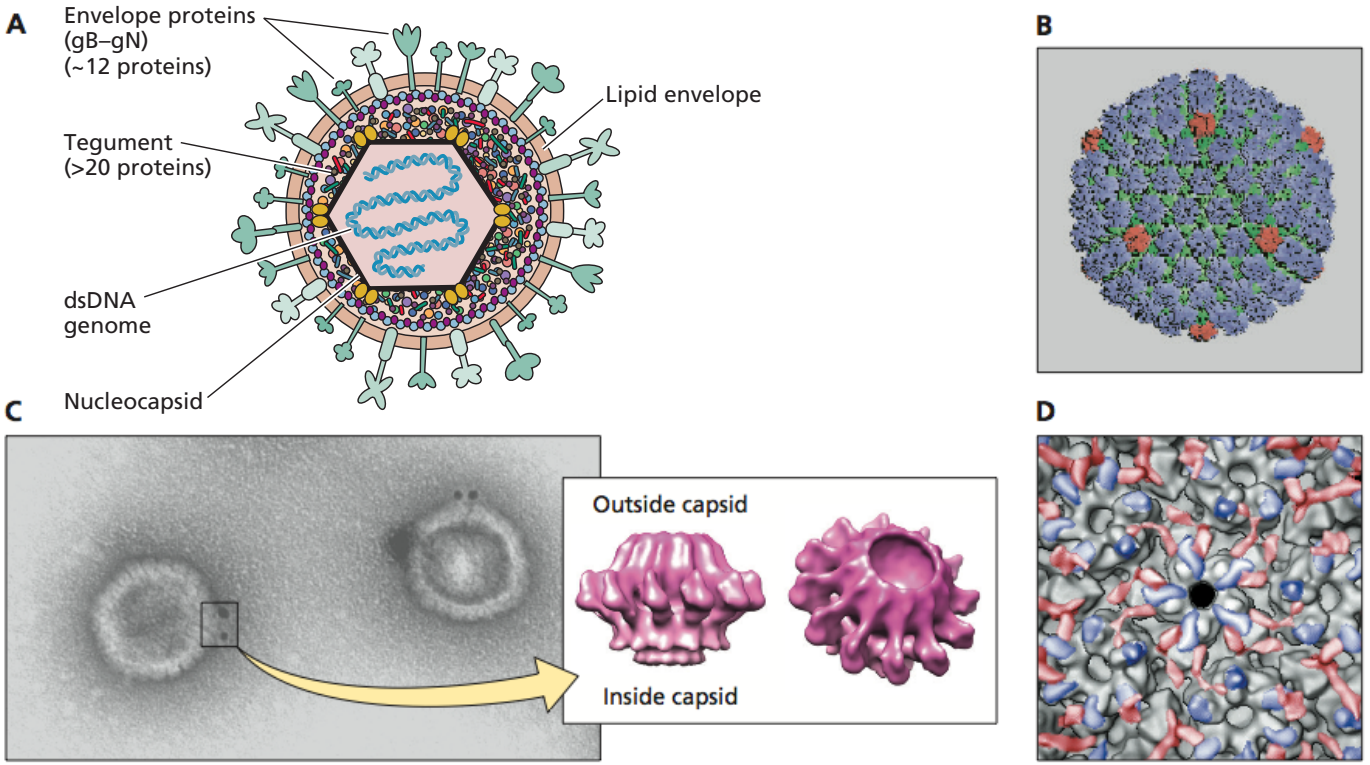
The tail is a complex rod

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



Herpes simplex virus capsid

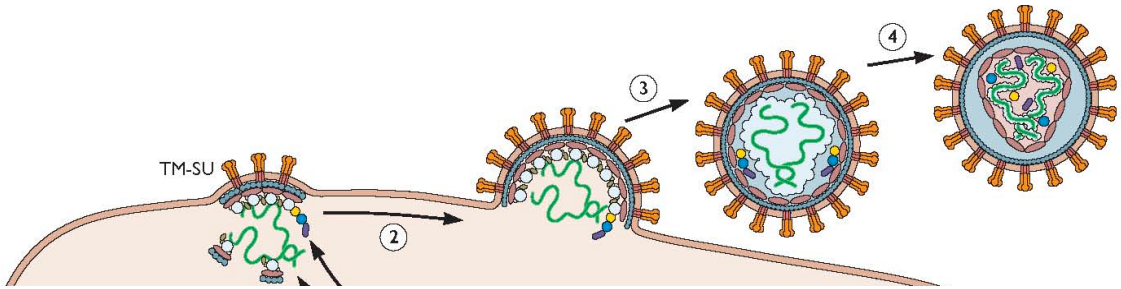
Holes for entry and exit of DNA



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

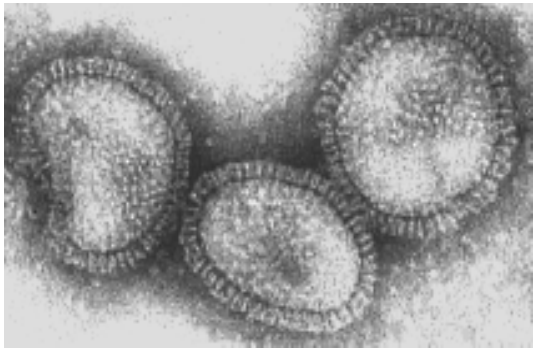
Capsids can be covered by host membranes: enveloped virions

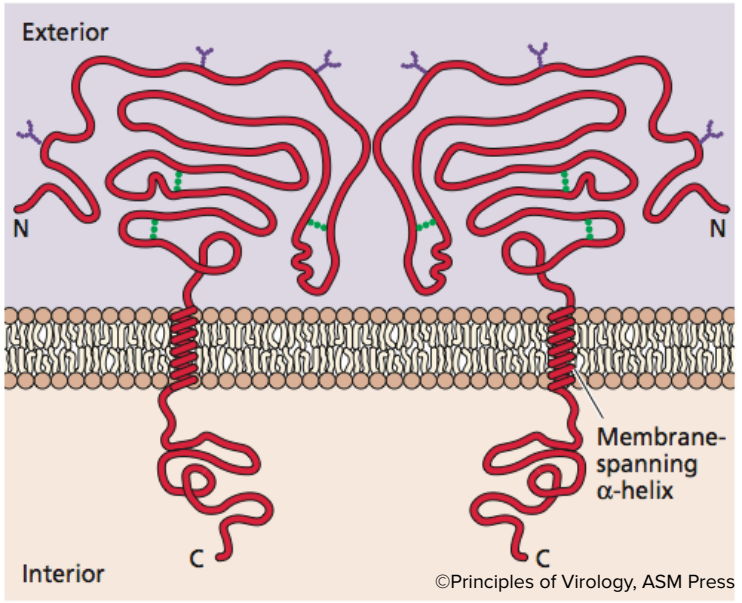
- Envelope is a lipid bilayer derived from host cell
  - Viral genome does not encode lipid synthetic machinery
- Envelope acquired by budding of nucleocapsid through a cellular membrane
  - Can be any cell membrane, but is virus-specific
- Nucleocapsids inside the envelope may have helical or icosahedral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

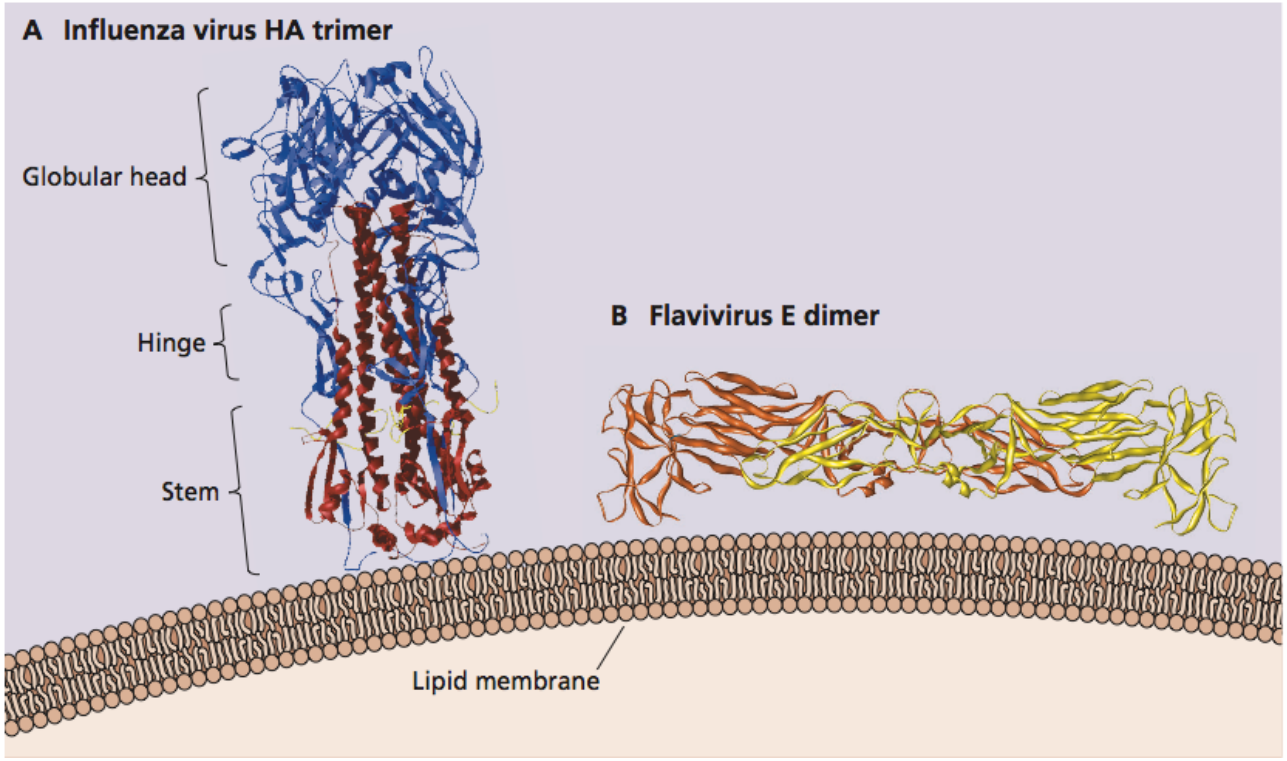


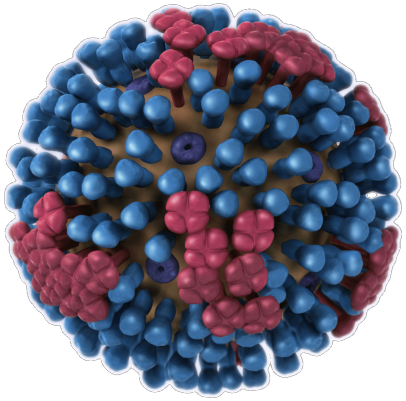
Viral envelope glycoproteins

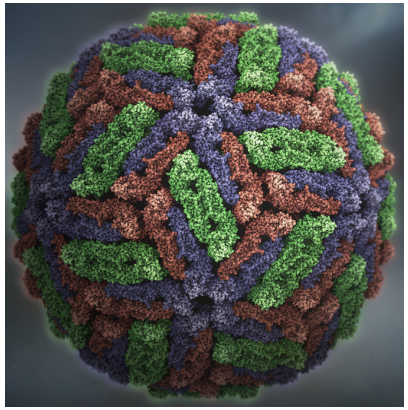
- Integral membrane glycoproteins
- Ectodomain: attachment, antigenic sites, fusion
- Internal domain: assembly
- Oligomeric: spikes



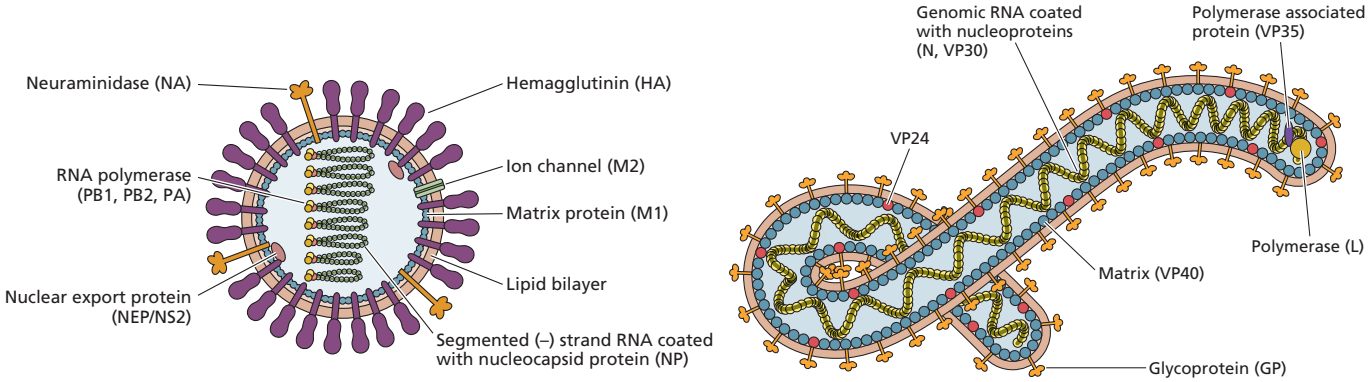




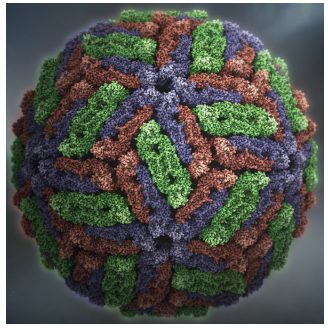


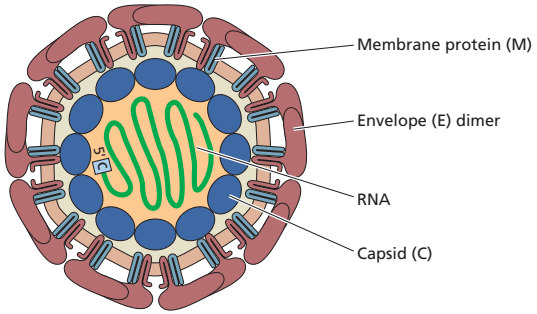


Helical nucleocapsids - unstructured envelopes

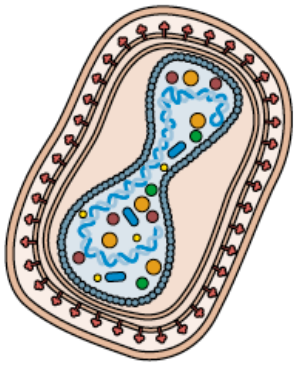


Icosahedral nucleocapsids - structured envelopes

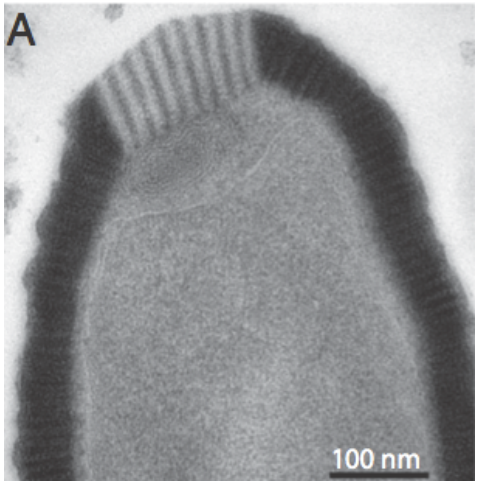




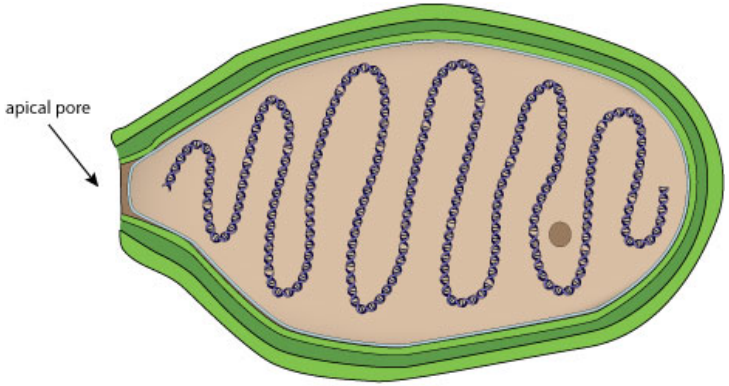
Poxvirus

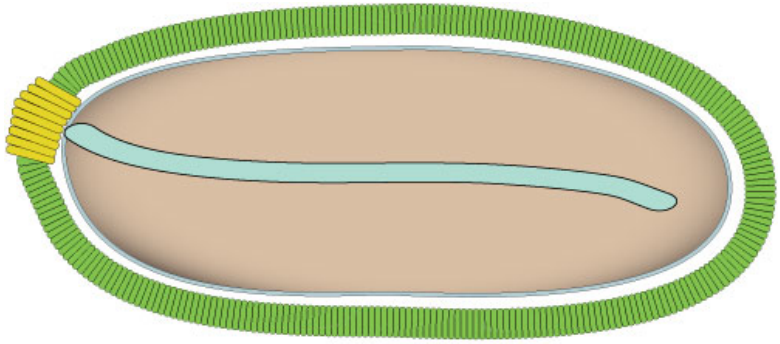


Pithovirus



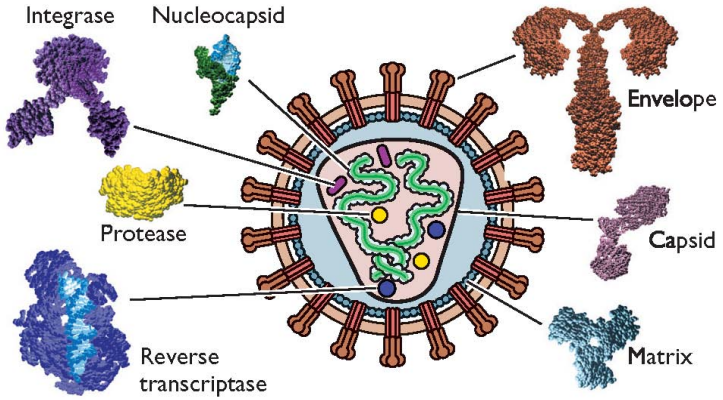
Pandoravirus





Other virion components

- 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