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

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

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

**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
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

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
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.”
Zika Virus - 3.8 Å
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

RNA
N protein

Scale: 100 Å

Scale: 50 Å

Tip
Trunk
Base

N-RNA
M

Diagram A: Nucleocapsid

Diagram B: N protein and RNA interactions

Diagram C: Vesicular stomatitis virus structure
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, 2019-nCoV)

- Lipid bilayer
- Hemagglutinin (HE)
- Spike protein (S)
- Membrane protein (M)
- Envelope glycoprotein (E)
- Nucleoprotein (N) + RNA = nucleocapsid
DNA and RNA viruses with helical symmetry

- dsDNA viruses of Archaea
  - (enveloped)

- ssDNA viruses of Bacteria

- Rod-like -RNA viruses of plants

- Flexuous +RNA viruses of plants
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
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

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

<table>
<thead>
<tr>
<th>Structural unit</th>
<th>Organization at 5-fold axes</th>
<th>Capsid</th>
<th>Total number of subunits (60T)</th>
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</tr>
</tbody>
</table>

Virology Lectures 2020 • Prof. Vincent Racaniello • Columbia University

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Buckyball Viruses
https://youtu.be/qLAEUvlVmqY
Poliovirus (*Picornaviridae*)

30 nm

60 promoters 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
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)
Complex capsids with two icosahedral protein layers

Reoviruses
• $T=13$
• 70 - 90 nm
• two concentric shells

VP7 trimers, $T=13$
VP3 monomers, $T=2$
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
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

A  Influenza virus HA trimer

Globular head

Hinge

Stem

B  Flavivirus E dimer

Lipid membrane

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Helical vs icosahedral nucleocapsids

- Influenza virus
- Ebolavirus
- Herpesvirus
- Togavirus (rubella virus)
Complex virus particles

Poxvirus

Pithovirus

Pandoravirus

apical pore
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
Next time: Attachment and Entry