

# **VIRUSES**

**BC530**

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# VIRUSES

- Are nucleo-protein or lipid-nucleo-protein complexes which use living cells for their proliferation.
- Affect bacterial, plant and animal cells. Those attacking bacteria are called “bacteriophages” or “phages”.
- Are very diverse in content: they contain either ds or ss DNA or RNA.
- Have EITHER a protein outer shell and contain NO lipids, OR contain lipids which surround as a membrane a core of nucleic acids and proteins. These membranes contain specialized surface proteins.
- Have very different sizes and shapes. Some form long rod-like structures, others are spherical with high symmetry and again others can assume a number of different shapes.

# VIRUSES

## HAVE VERY DIFFERENT SIZE GENOMES

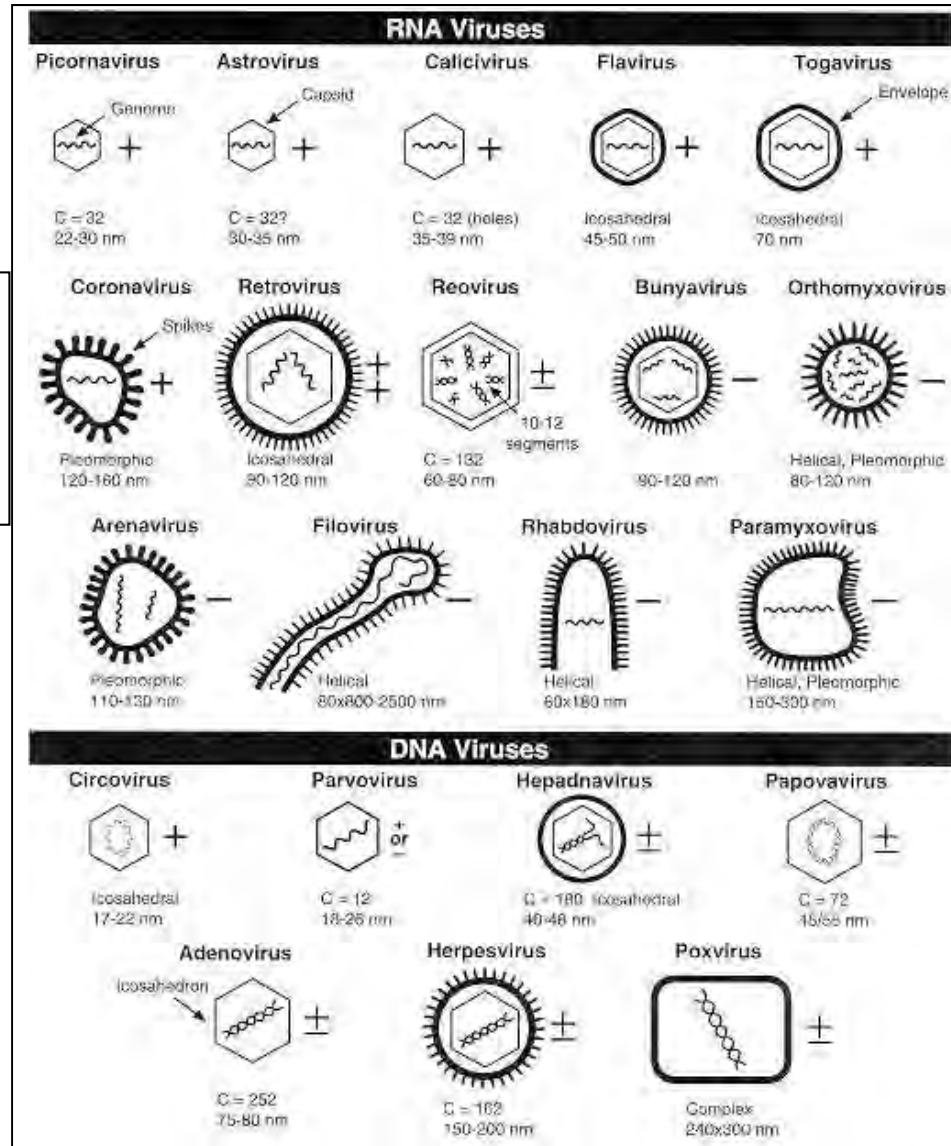
- ✓ Tobacco Mosaic Virus (TMV) has about **4** genes
- ✓ Ebola virus codes for ~ **8** proteins
- ✓ Hepatitis C Virus (HCV) has about **10** protein-encoding genes
- ✓ Rotavirus codes for **11** proteins
- ✓ Influenza virus has ~ 8 genes encoding ~**10** proteins
- ✓ Picornaviruses (like TBSV, SBMV, Poliovirus, Foot and Mouth Disease Virus (FMDV), Rhino virus) have about **12** protein products
- ✓ Bacteriophage T4 has ~ **300** protein encoding genes
- ✓ Some large icosahedral algae-infecting viruses, like the *Emiliana huxleyi* Virus-86 from the English channel, has just over 400 Kbases and is predicted to encode **472** proteins (Wilson, Science 309,1090-1092 (2005))
- ✓ Mimivirus is even larger: 1.2 Mb with **1262** open reading frames. It infects amoebae (Raoult, Science 308, 1114- (2004))
- ✓ Pandoraviruses are as large as small bacteria....

A general website with info about viruses:  
<http://www.microbiology.wustl.edu/training/med/micpath99/04huang/>

# The Diversity of Viruses is Enormous

<http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=mmed.figgrp.2270>

Book Edited by Samuel Baron.  
Title: Medical Microbiology  
Medical Microbiology Section 2.  
Virology  
41. Structure and Classification of Viruses



Schemes of 21 virus families infecting humans showing a number of distinctive criteria: presence of an envelope or (double-) capsid and internal nucleic acid genome.

+: Sense strand;  
- : antisense strand;  
± : dsRNA or DNA;  
0 : circular DNA;  
C : number of capsomeres or holes, where known;  
nm: dimensions of capsid, or envelope when present;

The hexagon designates the presence of an isometric or icosahedral outline.

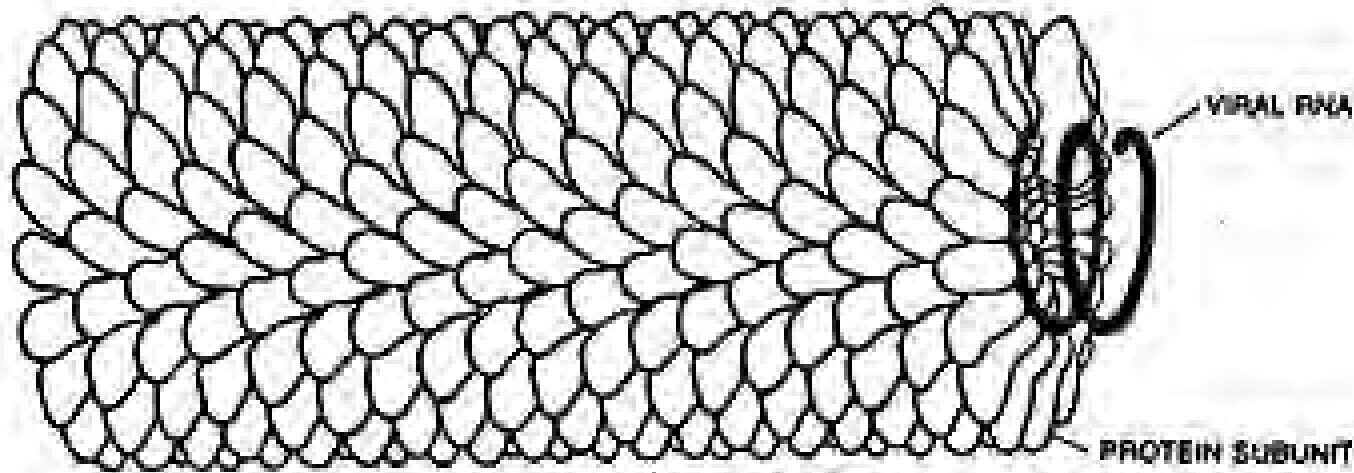
**A selection of viruses affecting humans.**

For formal classification see:

The International Committee on Taxonomy of Viruses (<http://www.ncbi.nlm.nih.gov/ICTVdb/Images/>)  
(And: <http://www.mcb.uct.ac.za/ictv/ICTV.html>)

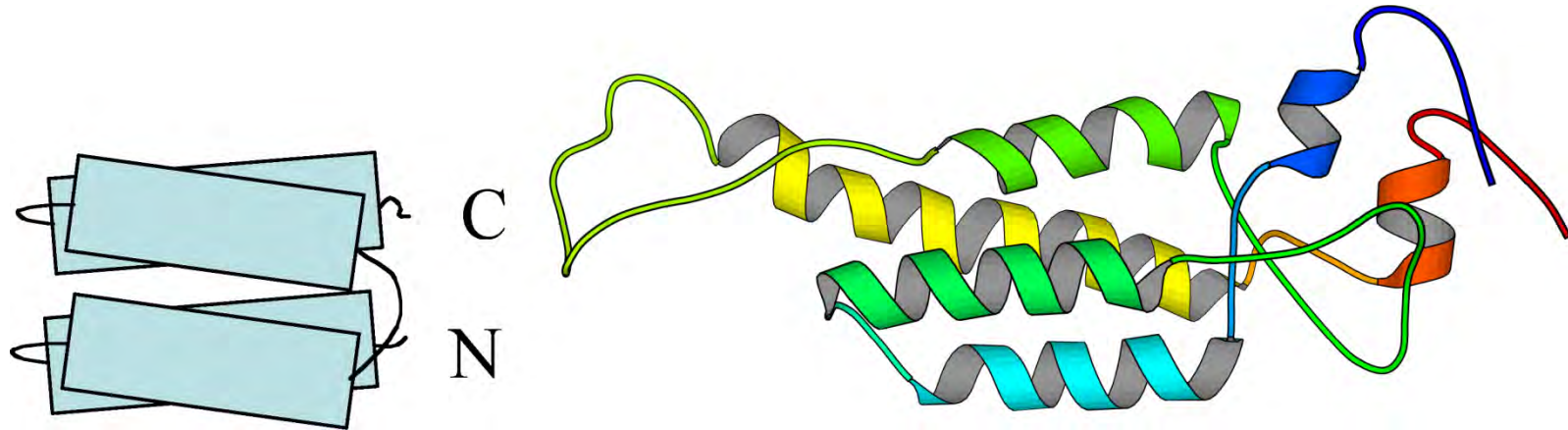
# Tobacco Mosaic Virus (TMV)

A Rod-shaped virus



- **HELICAL STRUCTURE** of the tobacco-mosaic virus is apparent in this drawing, which shows about a sixth of the entire length of the rod-shaped virus particle.
- The virus consists of a single long strand of RNA (black), representing perhaps four genes, packed between the turns of a helical protein coat made up of **2,130 identical protein subunits**.
- The rod is about 3000 Å long and 90 Å in radius, with a central hole of 20 Å diameter.
- The final length of the rod is determined by the length of the RNA. The viral RNA is 6,400 nucleotides long.
- Until the virus infects its host cell the protein helix protects the RNA from damage; after infection the RNA is released from the protein and the viral genes are expressed by the host's enzymes.
- The central hole in the rod of the virus particle, once thought to be a trivial consequence of protein packing, plays an essential role in assembly of the virus.

## TMV: The coat protein



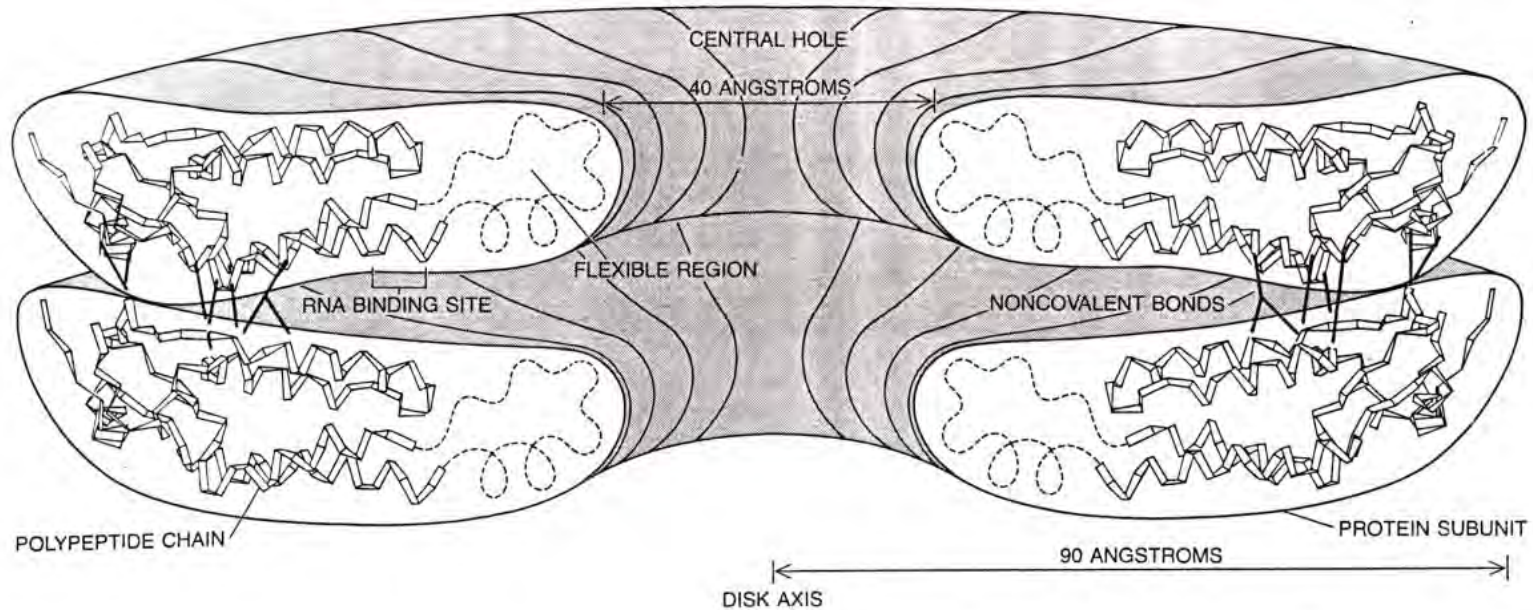
**Left: Schematic drawing of an up-and-down four-helix bundle.**

**Right: The coat protein of tobacco mosaic virus (PDB: 2TMV).**

**Each subunit consists of 158 residues (17,500 daltons)**



## TMV : DOUBLE DISK without RNA



**CROSS SECTION THROUGH A DISK** was reconstructed from the results of an X-ray diffraction analysis to a resolution of 2.8 Å.

**Each ring of a disk contains 17 subunits.**

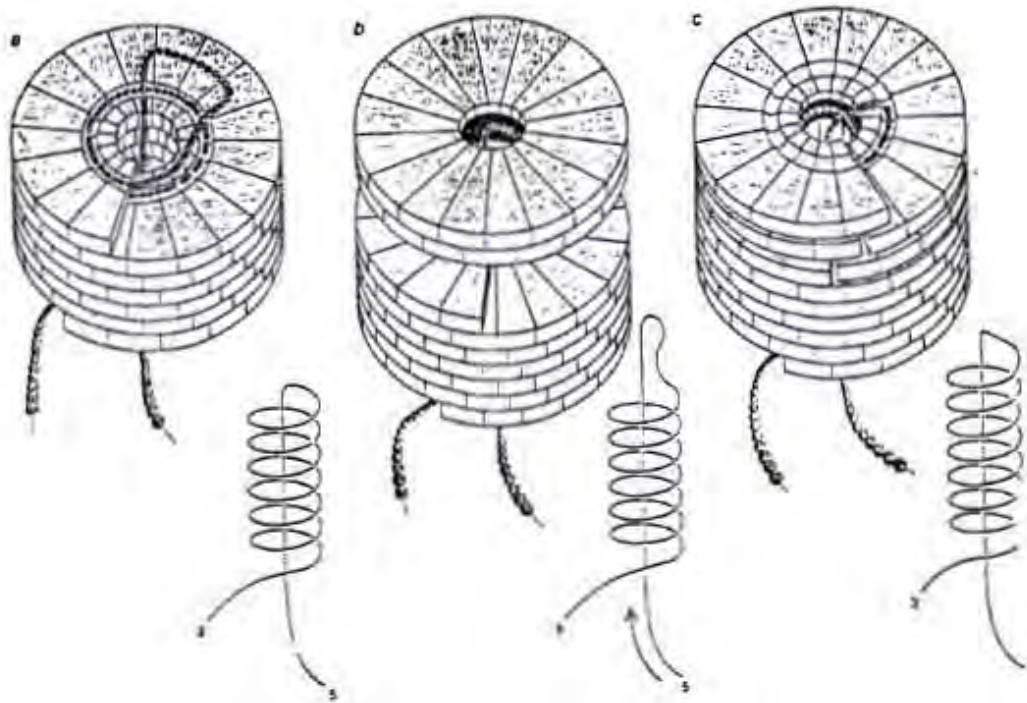
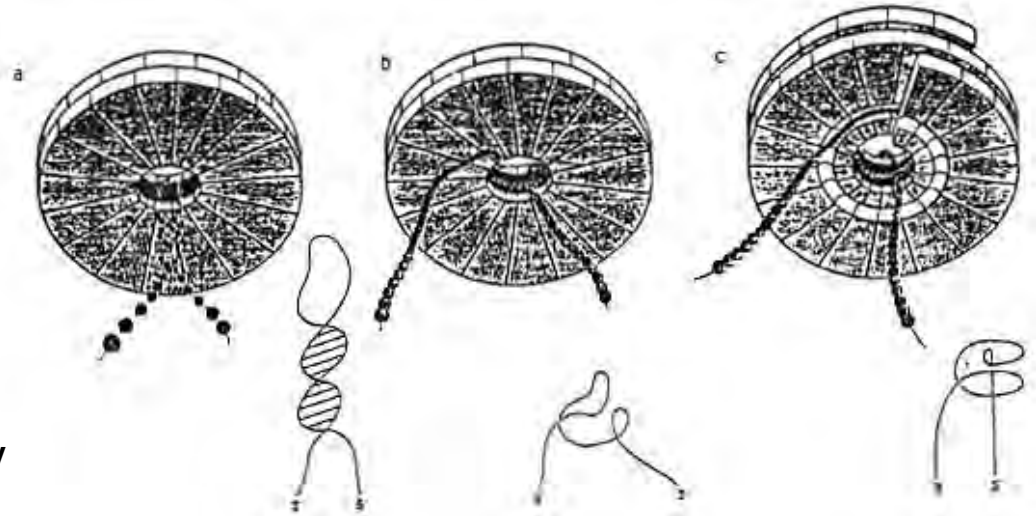
Bent ribbons indicate the polypeptide chains that make up the protein subunits. The two-layered structure of the disk is evident. The subunits of the two stacked rings touch over a small area near the outer rim of the disk but open up toward the center like a pair of jaws. During the assembly of the virus the viral RNA binds within the jaws.

The broken lines indicate the flexible portion of the protein chains extending up from the RNA binding site. Because this chain segment is in constant motion its structure cannot be resolved.

# TMV-assembly

## NUCLEATION of Tobacco Mosaic Virus

- RIGHT:** It begins with the insertion of the hairpin loop formed by the initiation region of the viral RNA into the central hole of the between the two layers of subunits and binds to the protein disk
- The loop intercalates around the first turn of the disk, opening up the base-paired stem as it does so
- some feature of the interaction causes the disk to dislocate into the helical lock-washer form. This structural transformation closes the jaws made by the rings of subunits, trapping the viral RNA inside.

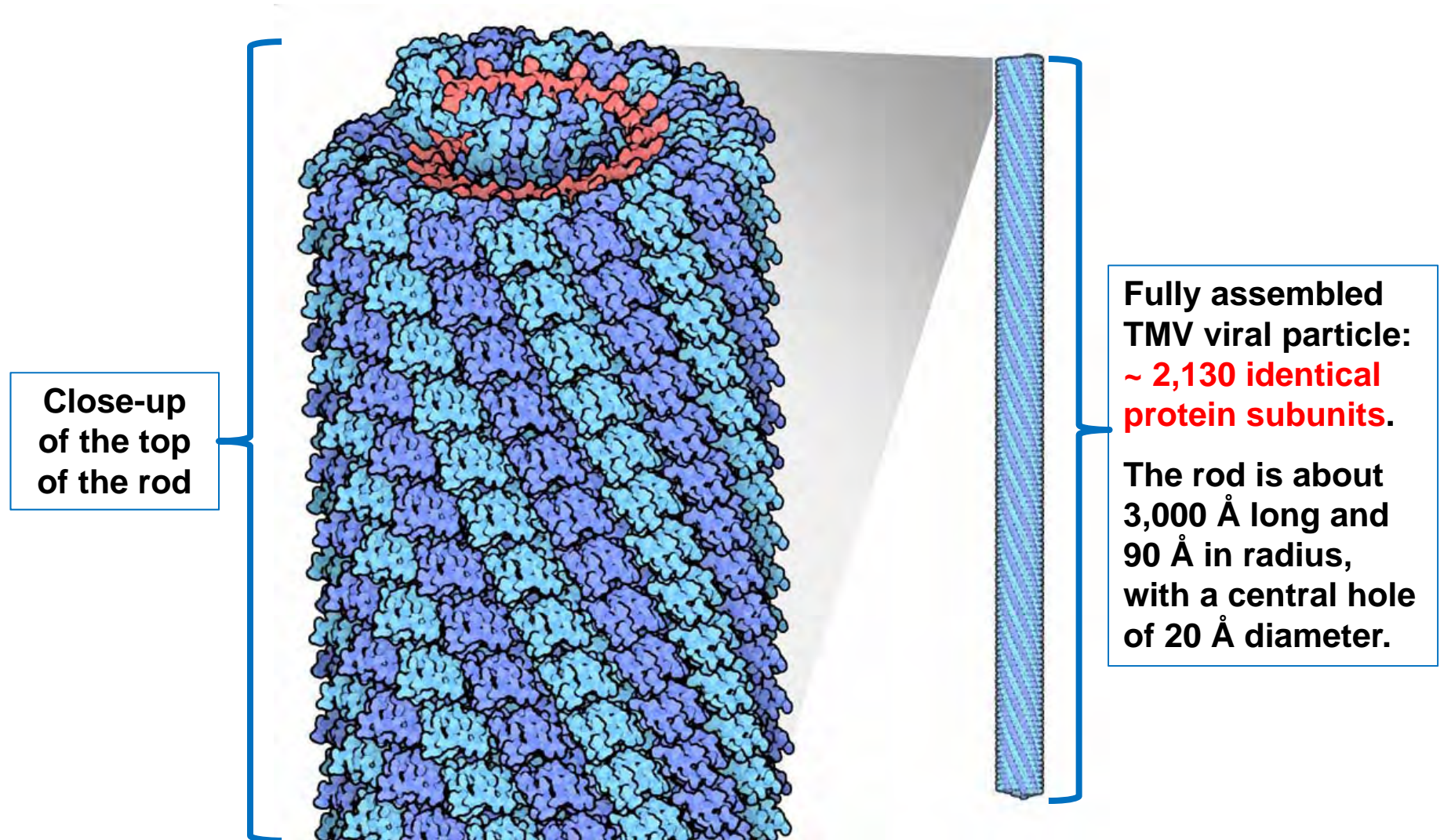


**LEFT: ELONGATION** of the virus proceeds by the addition of protein disks.

- As a result of the mode of initiation the longer RNA tail is doubled back through the central hole of the growing rod, forming a traveling loop at the growing end of the particle
- The loop inserts itself into the center of an incoming disk and binds within open jaws of the rings
- This interaction converts the new disk into a helical lock washer.
- Once all RNA is inserted into disks the assembly is complete.



# TMV



## Tobacco Mosaic Virus in the PDB

The helix contains 49 subunits in three turns, or  $16 \frac{1}{3}$  subunit per turn. Red is viral RNA.

# TMV

## References Tobacco Mosaic Virus (TMV):

Structural Biology of Viruses (Eds. W. Chiu, R.M. Burnett & R. L. Garcia), Oxford University Press (1997).

P.J.G. Butler and A. Klug, *Scientific American* 239, 62-69 (1978). The assembly of a virus.

A.C. Bloomer, J.N. Champness, G. Bricogne, R. Staden and A. Klug, *Nature* 276, 362-373 (1978). Protein disk of tobacco mosaic virus at 2.8 Å resolution showing the interactions within and between subunits.

G. Stubbs, S. Warren and K. Holmes, *Nature* 267, 216-221 (1977). Structure of RNA and RNA binding site in tobacco mosaic virus from 4 Å map calculated from X-ray fibre diagrams.

A nice impression of the TMV disk and the TMV particle can be found on a Website from the Protein Data Bank:

<http://www.rcsb.org/pdb/101/motm.do?momID=109>

# Icosahedral Viruses

## Icosahedral symmetry and “quasi-equivalence”

Small spherical virus particles appear to contain numerous subunits.

The number of subunits of the icosahedral capsid can be 60 but is usually a multiple of 60.

An icosahedron has “point group symmetry” **532**.

- That means that it contains five-fold, three-fold, and two-fold symmetry axes which all intersect in one point.
- That point is the center of the icosahedron.

The symmetry operations are such that 60 equivalent “icosahedral asymmetric units” are generated. This means that icosahedral assemblies have 60 repeats of whatever is the icosahedral asymmetric unit .

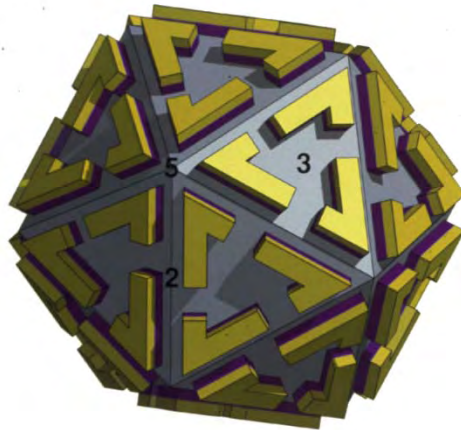
An intriguing question is: how are spherical viruses with multiples of 60 subunits organized? Well, before any information at the atomic level was obtained, Caspar and Klug, in 1962, came up with a possible explanation based on the principle of “quasi-equivalence.”

### Reference:

D.L.D Caspar and A. Klug, Cold Spring Symposium on Quantitative Biology 27, 1-24 (1962). Physical principles in the construction of regular viruses.

# ICOSAHERDRAL SYMMETRY

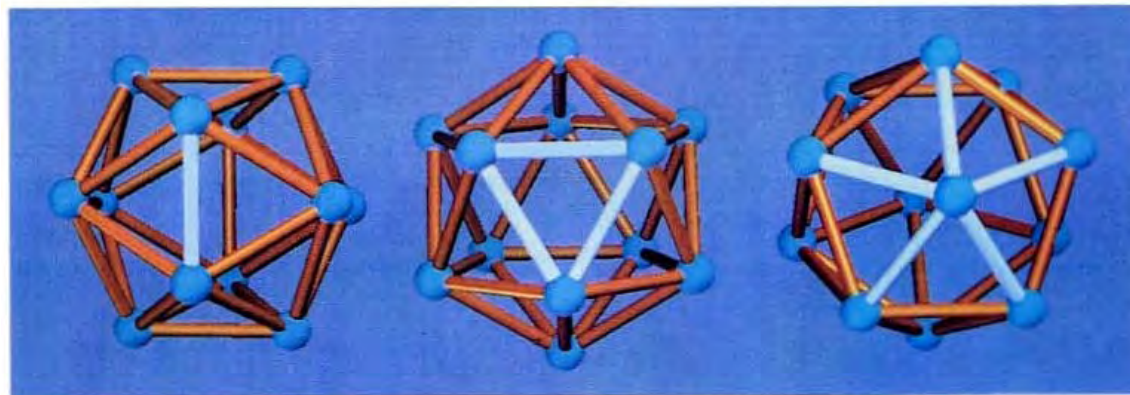
Underlies the architecture of several important viruses



## ICOSAHERDRON

- 20 equilateral triangles as its faces
- 12 five-fold axes – at the corner of each triangle
- 20 three-fold axes i.e. there are 20 “triangles”
- 30 two-fold axes

These axes generate an object with 60 “units” (e.g. gold L’s). More precise: 60 icosahedral asymmetric units.



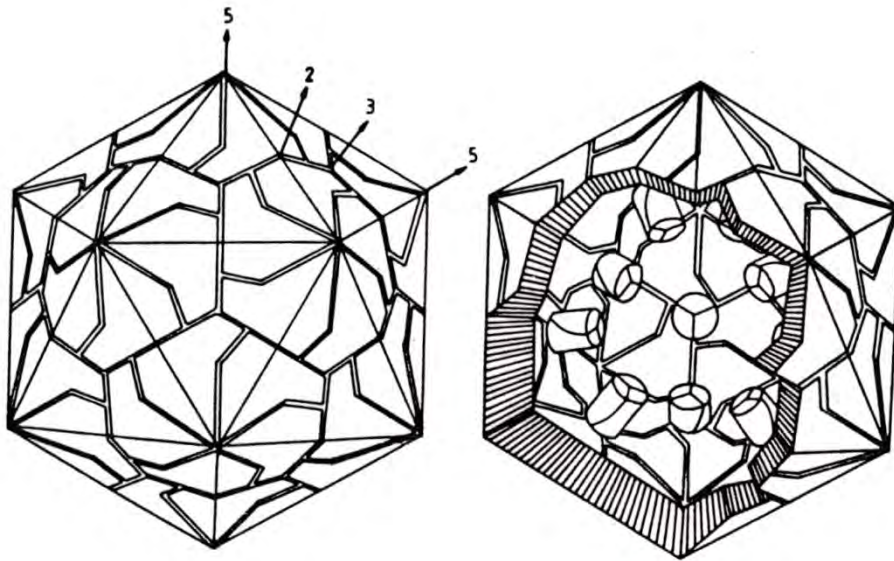
View (almost)  
along a 2-fold

View  
along a 3-fold

View (almost)  
along a 5-fold



# Satellite Tobacco Necrosis Virus (STNV)



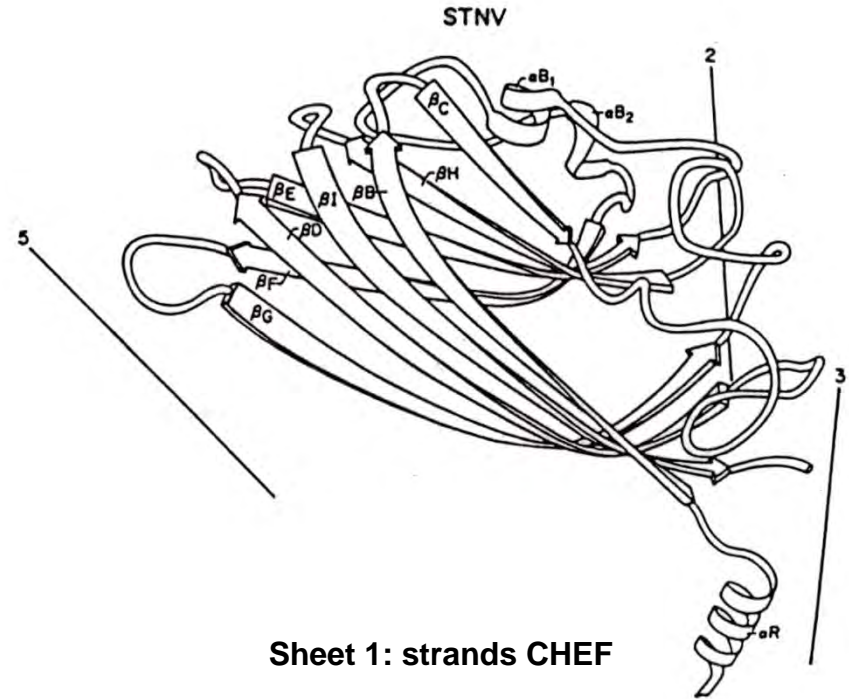
Drawing of the subunit packing in STNV.

**There are 60 subunits per STNV capsid.**

This is a  $T=1$  virus capsid.  
With 60 subunits per capsid.

T is the "triangulation number"

The geometric meaning of "T" will be explained in the next slides.



Sheet 1: strands CHEF

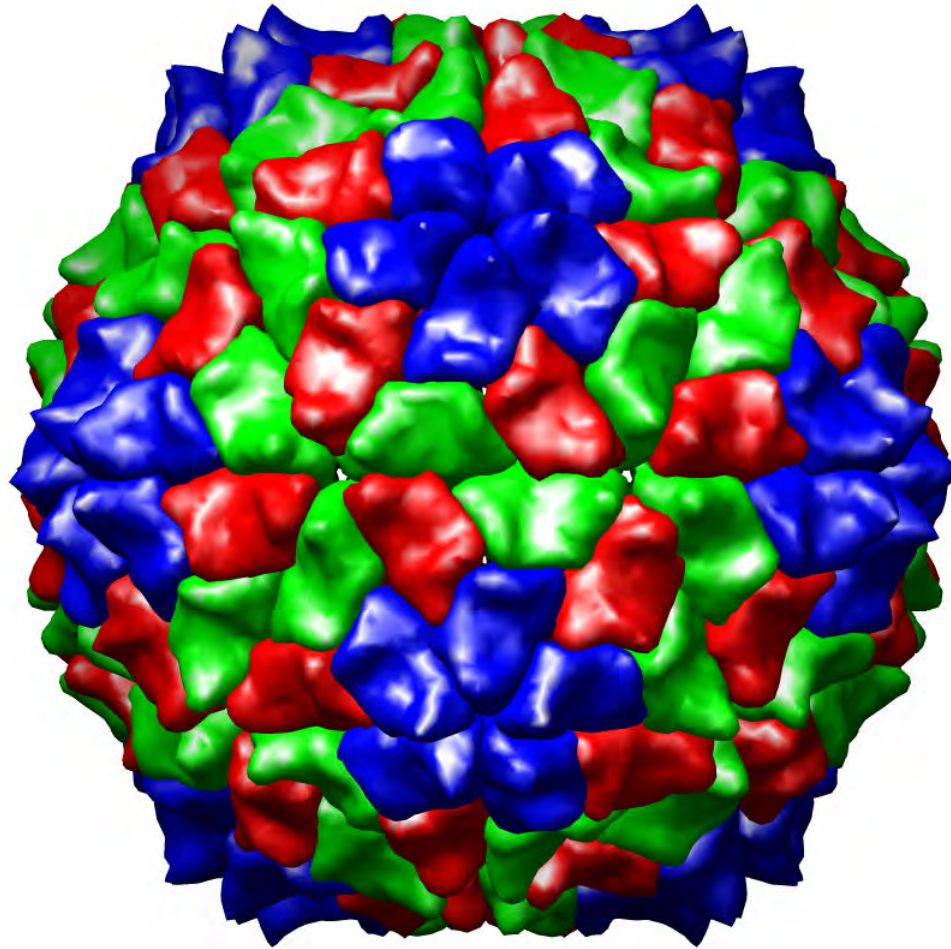
Sheet 2: strands BIDG

Schematic drawing of the STNV protein subunit.

The fold is similar to the capsid proteins of plant viruses like TBSV and SBMV.



# Southern Bean Mosaic Virus (SBMV) Capsid



SBMV, a simple plant icosahedral

There are 180 copies the same capsid protein.

But there are three different conformations of the capsid protein – shown in green, red and blue.

The blue subunits form a pentamer. The red and green subunits form a hexamer

# Quasi Equivalence

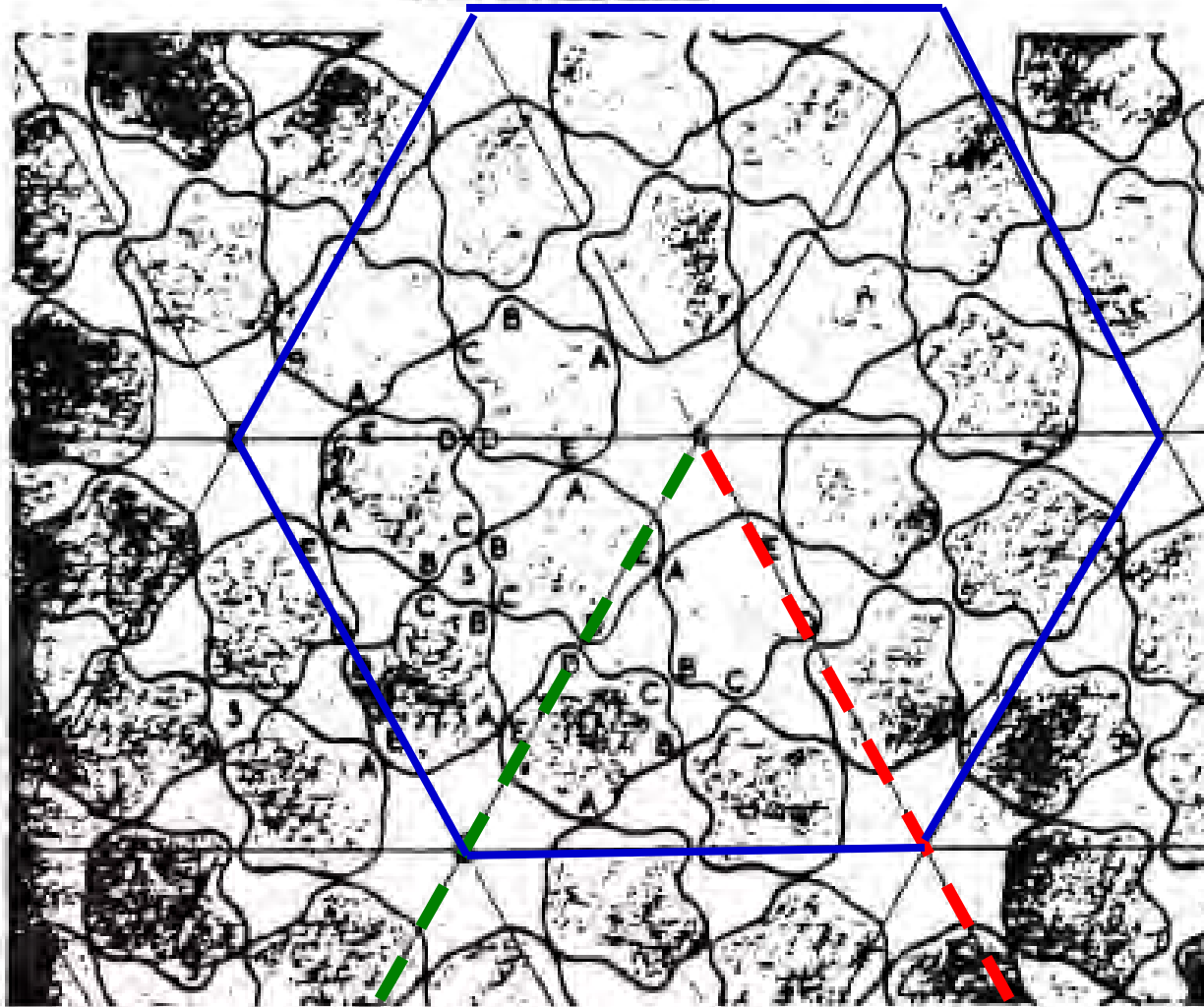
Quasi-equivalence is based on the assumption that the protein shell of an icosahedral virus is held together by the same type of subunit-subunit interactions throughout, but that these interactions may differ slightly in different, non-symmetry related, environments.

Caspar and Klug (1962) proposed that hexamers deviate sufficiently little from pentamers, so that by a judicious mixture of hexamers and pentamers at the surface of the icosahedron each subunit could be given an environment which was almost the same everywhere.

The Caspar and Klug theory implies that only spherical icosahedral viruses with 1 x 60, 3 x 60, 4 x 60, 7 x 60, 9 x 60, etc. identical subunits should occur, but that e.g. 2 x 60 and 5 x 60 would *not* occur.

In their terminology, this means that viruses with so-called “triangulation numbers”  $T = 1, 3, 4, 7$  and 9 do exist, but not with  $T=2$  or 5.

## Triangulation



Asymmetric units arrayed in an equilateral-triangular plane net. Besides having translations, a along the hexagonal axes, the lattice has 6-fold rotational axes of symmetry. Each unit here is equipped with five “bond” sites, A, B, C, D and E, forming three different “bonds”, namely a hexamer bond AE, a trimer bond BC, and a dimer bond DD.

Cut the paper along the red line, then bend so that red touches green=> a pointed five-fold arrangement is obtained

The AE, EA and DD contacts in the original hexamer and in the new pentamer are “quasi-equivalent”.

The sixfold axis at the center of the current blue hexamer shown becomes a five fold axis.



**Where H and K are steps along the hexagonal axes of the grid.**



**Triangular nets where points of six-fold symmetry have been selected in a regular manner to be replaced by five-folds.**

Twenty triangles (in green; tough to find except for  $T=1$ !) correspond to the surfaces of the icosahedron, where each corner of the triangles corresponds to a five-fold axis.

The icosahedral asymmetric unit is shown as the blue-ish triangle.

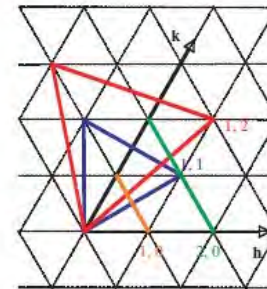
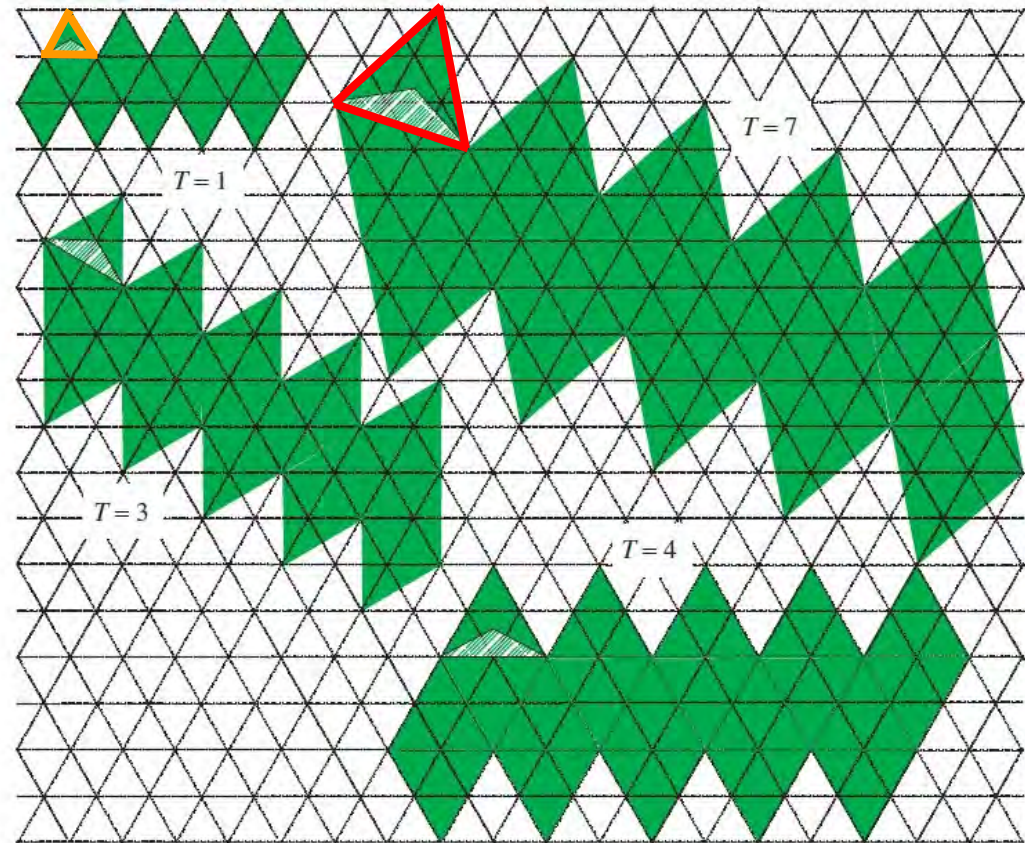
The arrangements for triangulation numbers 1, 3, 4 and 7 are shown.

The six-fold symmetry becomes a quasi-six-fold that coincides with the icosahedral three-fold ( $T=3$ ) or two-fold ( $T=4$ ).

In  $T=1$ , only five-fold symmetry is found and in  $T=7$  the quasi-six-fold does not coincide with any of the icosahedral symmetry axes.

*Bottom:* The  $h, k$  coordinate system in the net, showing one triangle for each of these triangulation numbers (orange:  $T=1$ , blue:  $T=3$ , green:  $T=4$ , red:  $T=7$ ).

## Triangulation

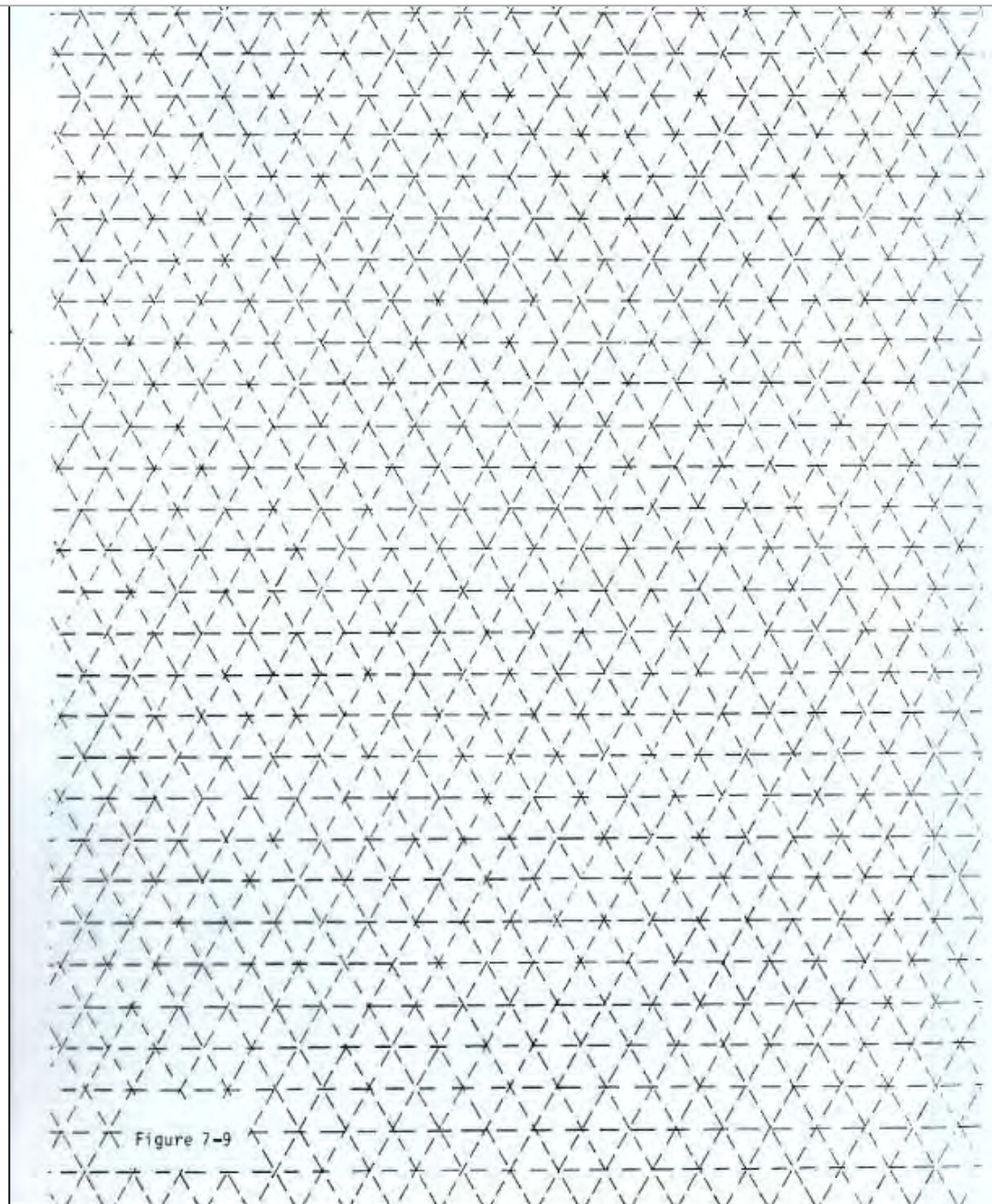


**Nice origami exercise to make these icosahedrons yourself.**  
Best to print large.

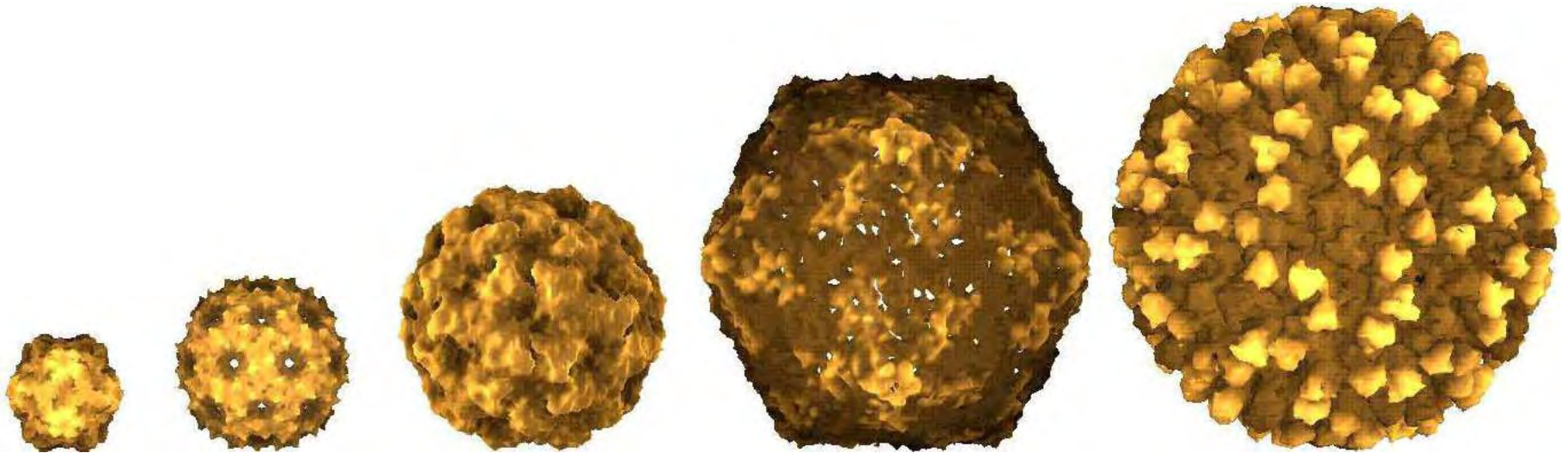
FIGURE 15.3 from Liljas Textbook of Structural Biology



## Just for your personal Triangulation Trials....



## Different Triangulation Numbers



**Virus capsids with triangulation numbers 1, 3, 4, 7 and 13 showing their relative sizes..**

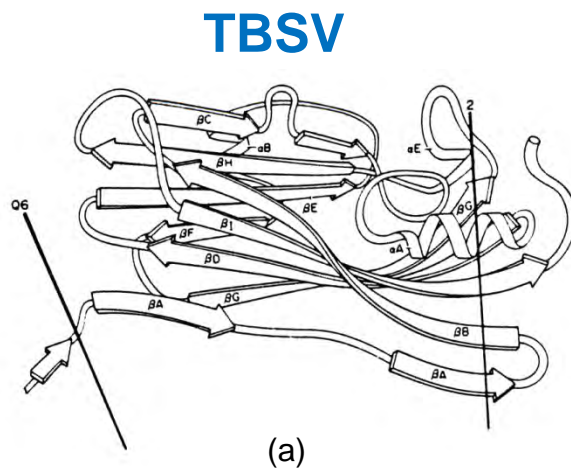
The surface of the virus particles is shaded according to its distance from the center, darker being closer.

Some particles have clearly an icosahedral shape, and some not so clearly, yet these particles all have icosahedral symmetry.

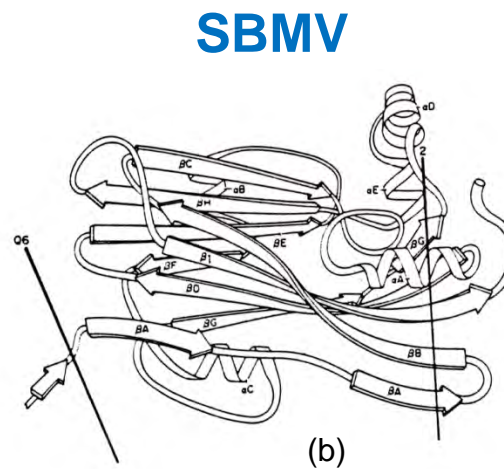
The drawings are based on the crystal structures (from *left to right*) of: satellite tobacco necrosis virus (STNV), phage MS2, *Nudaurelia capensis*  $\omega$  virus, phage HK97 and the bluetongue virus. From VIPER (<http://viperd.b.scripps.edu/>).

# Plant icosahedral virus capsid subunits/domains

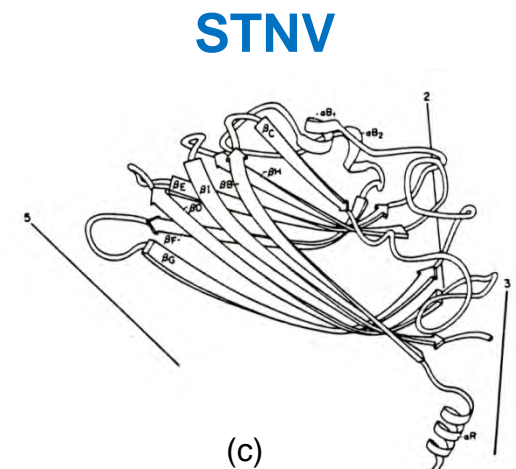
A constant CHEF-BIDG two-sheet framework plus a considerable variation in loops.



**Tomato Bushy Stunt Virus (TBSV)**  
(T=3)  
180 subunits per capsid.  
Each subunit same aa sequence.  
3 x 60 subunits called A, B and C,  
with different conformations.



**Southern Bean Mosaic Virus (SBMV)**  
(T=3)  
180 subunits per capsid.  
Each subunit same aa sequence.  
3 x 60 subunits called A, B and C,  
with different conformations.



**Satellite Tobacco Necrosis Virus (STNV)**  
(T=1)  
60 subunits per capsid.  
Each subunit same aa sequence.

The backbone folding for TBSV, SBMV and STNV subunits shown in roughly comparable orientations.

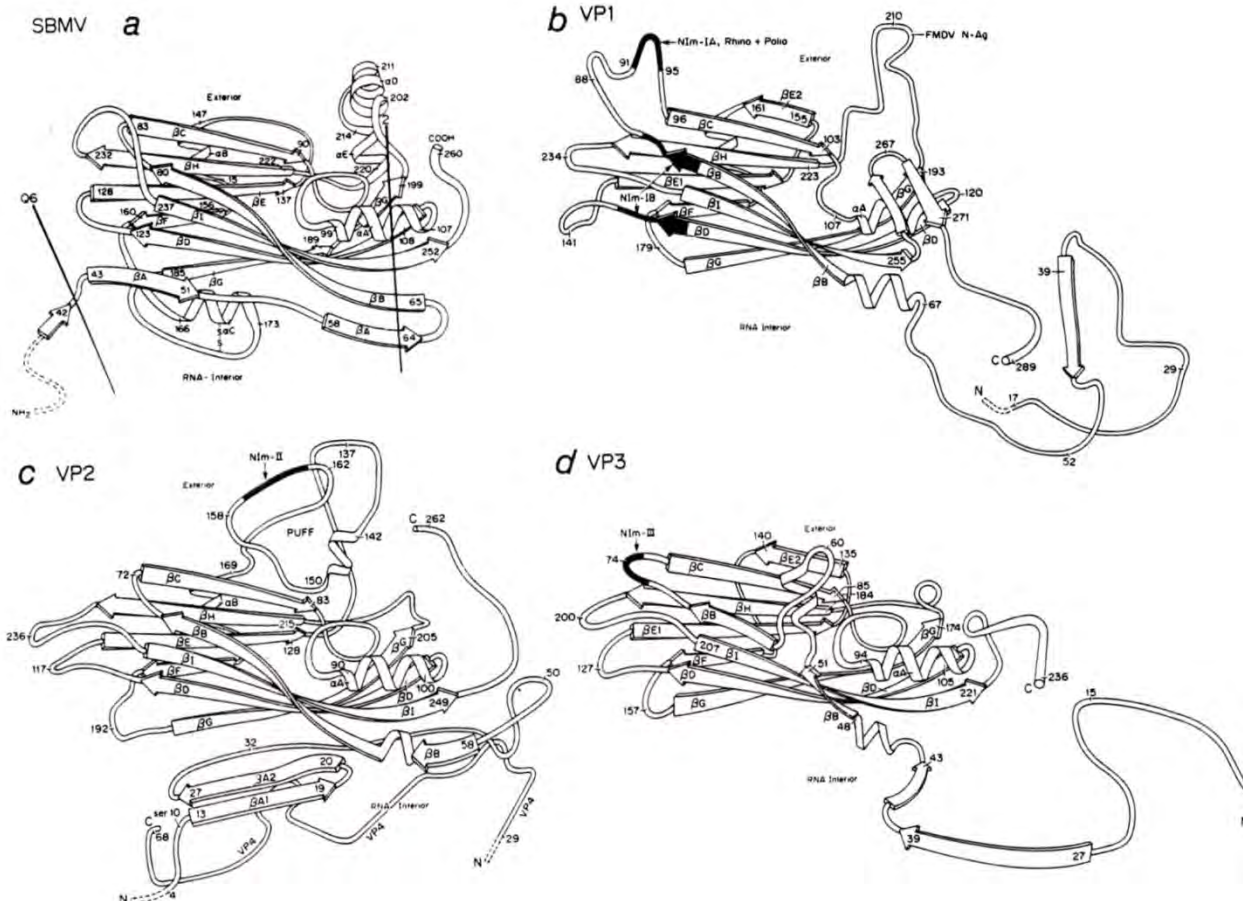
In all three cases:  $\beta$ -sheet 1: strands CHEF, and  $\beta$ -sheet 2: strands BIDG

In all three cases the capsid of these viruses is made up of IDENTICAL subunits.



# RHINO VIRUS

Human common cold virus



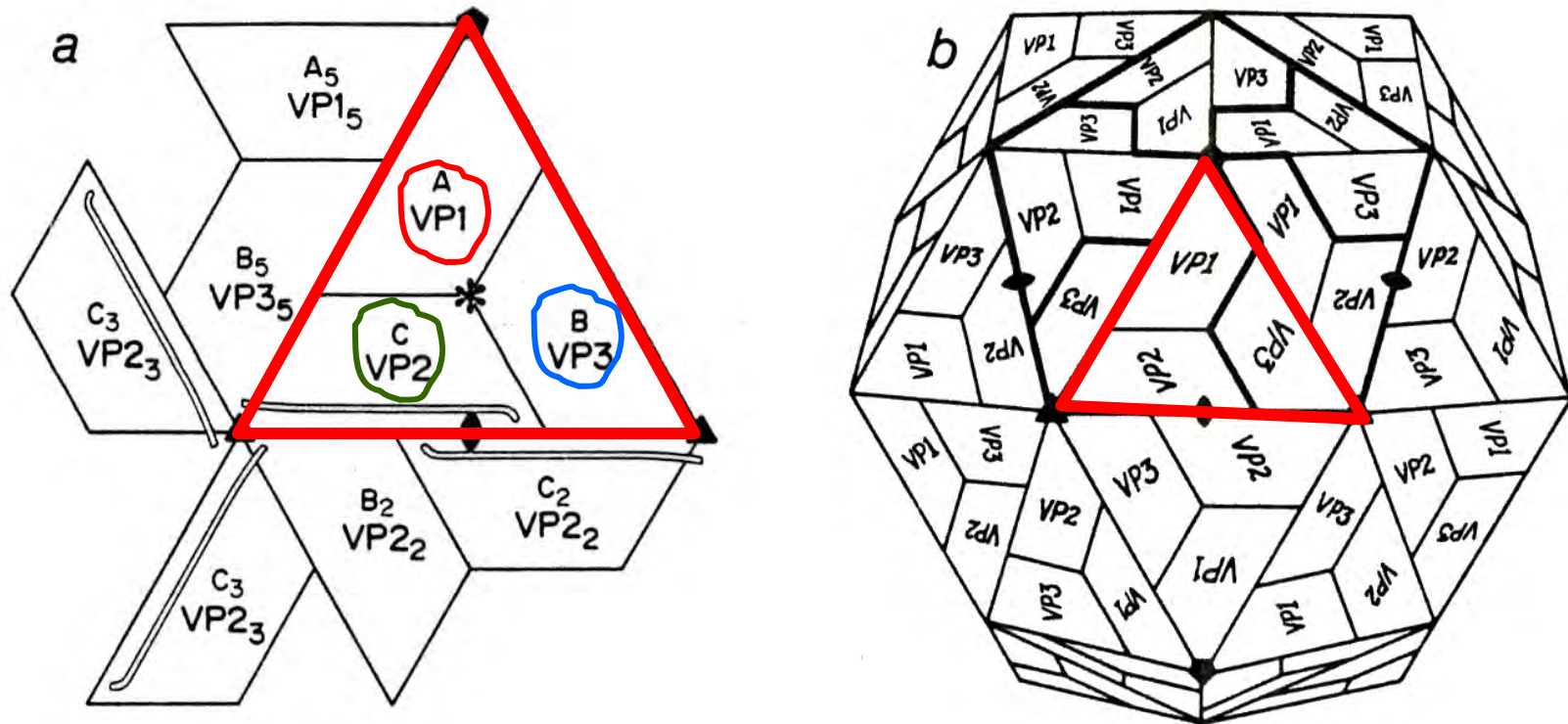
The three **different** subunits (VP1, VP2, VP3) of Rhinovirus are each related to the CHEF-BIDG fold of Plant Viruses (represented by SBMV, upper left).

Rhino virus and related viruses have molecular weights of ~8.5 million daltons of which ~30 is RNA. They form icosahedral particles with diameters of roughly 300 Å.

In size they do not differ too much from the previously discussed plant viruses.

# RHINO VIRUS compared to TBSV and SBMV

## Architecture of the Icosahedron

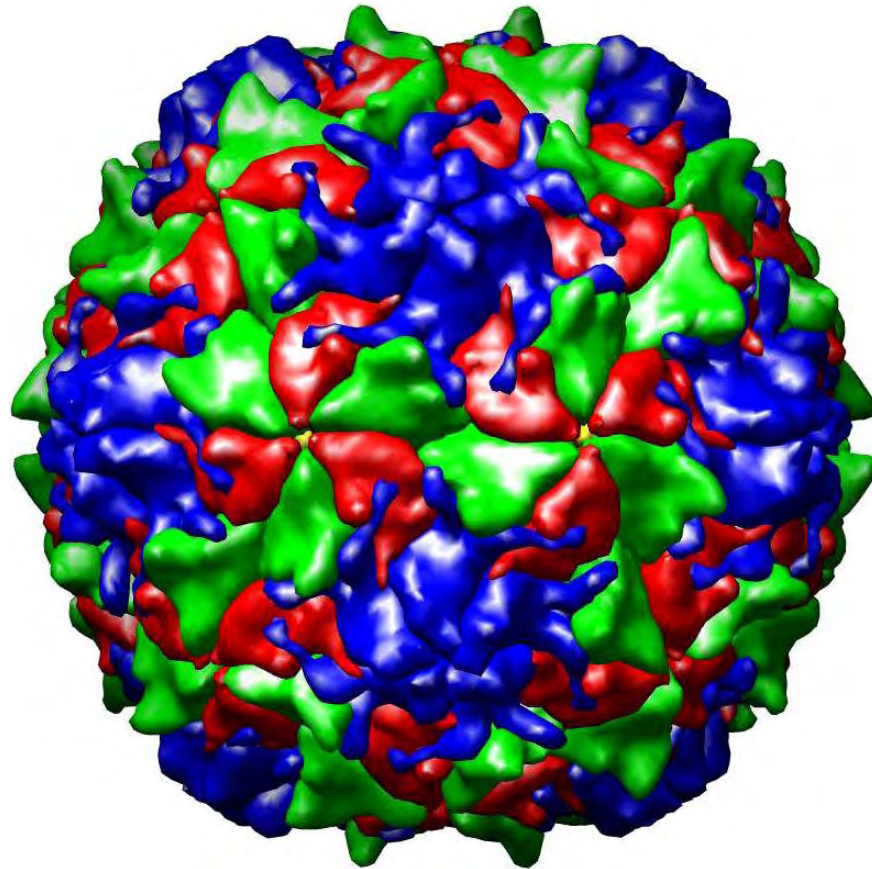


The VP1, VP2 and VP3 subunits of Rhinovirus are pseudo-equivalent to, respectively, the quasi-equivalent subunits A, C and B in TBSV and SBMV.

- Icosahedral asymmetrical unit showing the ordered amino-terminal arm  $\beta A$  (thin wire) present only in the C subunit of the plant viruses and in VP2 of Rhinovirus HRV14. Asterisk indicates the position of the quasi-3-fold axis in SBMV and TBSV analogous to the pseudo-3-fold axis in HRV14. Subscripts designate the symmetry operation required to obtain the given subunit from the basic triangle.
- Icosahedral capsid of rhinovirus.



## Rhinovirus capsid is similar to Poliovirus capsid



Poliovirus, a simple icosahedral virus with a diameter of about 300 Å.  
The blue subunits form a pentamer. The red and green subunits form a hexamer.

There are 60 copies of three different proteins called VP1, VP2 and VP3, i.e. 180 subunits in total per capsid.  
This is a T=3 capsid but with three different subunits with a similar fold per icosahedral asymmetric unit.  
From VIPER (<http://viperdb.scripps.edu/>).

## References spherical viruses – pioneering papers

S.C. Harrison, *Trends in Biochemical Sciences* 9, 345-351 (1984).

Multiple modes of subunit association in the structures of simple spherical viruses.

S.C. Harrison, A.J. Olson, C.E. Schutt and F.K. Winkler, *Nature* 276, 368-373 (1978).

Tomato bushy stunt virus at 2.9 Å resolution A.J. Olson, G. Bricogne and S.C. Harrison, *J. Mol. Biol.* 171, 61-93 (1983).

Structure of tomato bushy stunt virus IV. The virus particle at 2.9 Å resolution.

C. Abad-Zapatero, S.S. Abdel-Meguid, J.E. Johnson, A.G.W. Leslie, I. Rayment, M.G. Rossmann, D. Suck and T. Tsukihara, *Nature* 286, 33-39 (1980).

Structure of southern bean mosaic virus at 2.8 Å resolution.

M.G. Rossmann, E. Arnold, J.W. Erickson, E.A. Frankenberger, J.P. Griffith, H.J. Hecht, J.E. Johnson, G. Kamer, M. Luo, A.G. Mosser, R.R. Ruekert, B. Sherry and G. Vriend, *Nature* 317, 145-153 (1985).

Structure of a human common cold virus and functional relationship to other picornaviruses.

## References Poliovirus

J.M. Hogle, M. Chow and D.J. Filman, *Science* 229, 1358-1365 (1985).

Three-dimensional structure of poliovirus at 2.9 Å resolution.

J.M. Hogle, M. Chow and D.J. Filman, *Scientific American* 256, 42-49 (1987).

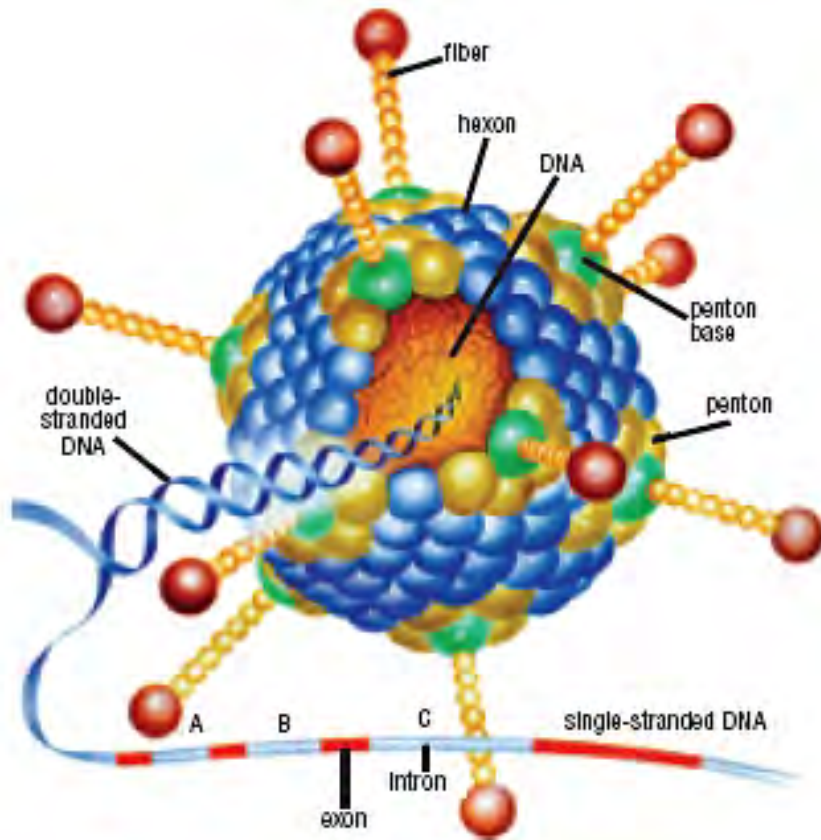
The structure of poliovirus.

# **Human Adeno Virus**

**A BIG icosahedral virus**

**(without an outer lipid bilayer)**

# ADENOVIRUSES



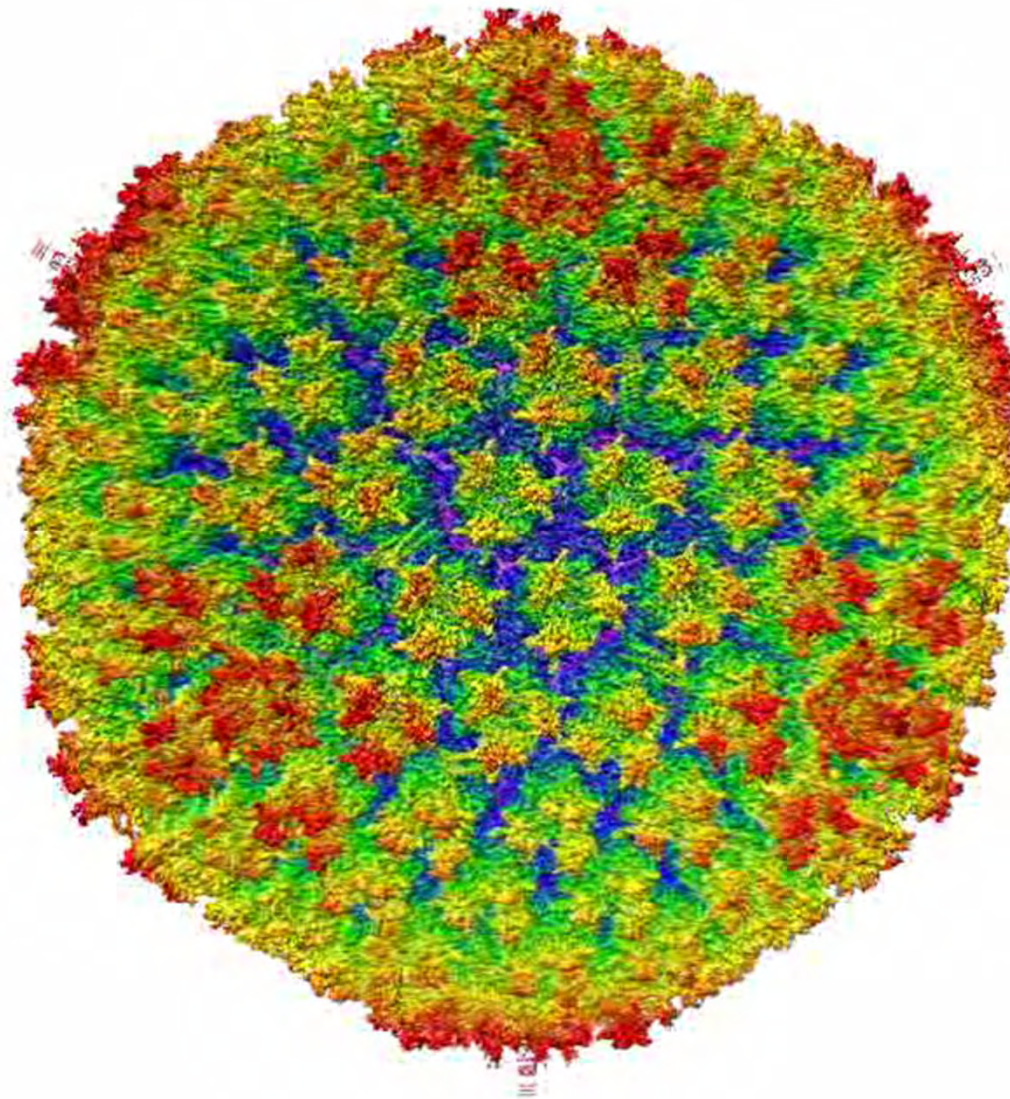
- Non-enveloped, double-stranded DNA viruses
- Some are human pathogens
- Vectors for gene therapy
- Icosahedral symmetry
- Diameter 900 Å (not including spikes)
- 150 MDa

<http://www.iavireport.org>



# Adenovirus

## Cryo-EM structure



Distance from  
Center  
of virus particle:



Liu et al., Science 329: 1038-1043 ( 2010)  
Atomic Structure of Human Adenovirus by Cryo-EM Reveals Interactions Among Protein Networks



# Adenovirus

## Cryo-EM structure

Trimeric fiber:  
Model = fiction!

Penton base

Icosahedral  
asymmetric unit

Peripentonal  
hexons

Cement  
proteins?

Single facet

**Very complex indeed**

Liu et al., Science 329: 1038-1043 ( 2010)  
Atomic Structure of Human Adenovirus by Cryo-EM Reveals Interactions Among Protein Networks

# Functions of Viral Coat Proteins

(Sometimes these functions are performed by one protein, sometimes distributed over several different coat proteins)

1. **Assembly**
  - RNA/DNA recognition
  - Protein-protein recognition
2. **Protection**
  - Sufficient stability
3. **Immune evasion**
4. **Receptor recognition**
5. **Disassembly and delivery of RNA/DNA to other side of target cell membrane**

# **Membrane-enveloped viruses**

Example: Dengue virus

# Dengue Virus

- Dengue virus is a major threat to health in tropical countries.
- It is transmitted by female *Aedes* mosquitoes in the tropics
- 50-100 million people are infected each year.
- Most infected people experience dengue fever, with terrible headaches and fever and rashes that last a week or two.
- In some cases, however, the virus weakens the circulatory system and can lead to deadly hemorrhaging.

## The Dengue Virus Genome

- Dengue virus is a small virus with a single strand of RNA as its genome.
- The genome encodes only ten proteins.
- Seven of these are nonstructural proteins that orchestrate the production of new viruses once the virus gets inside the cell.
- Three of these form the coat of the virus and deliver the RNA to target cells.
- The virus is enveloped with a lipid membrane, and 180 identical copies of the envelope protein are attached to the surface of the membrane by a short transmembrane segment.
- The job of the envelope protein is to attach to a cell surface and begin the process of infection.



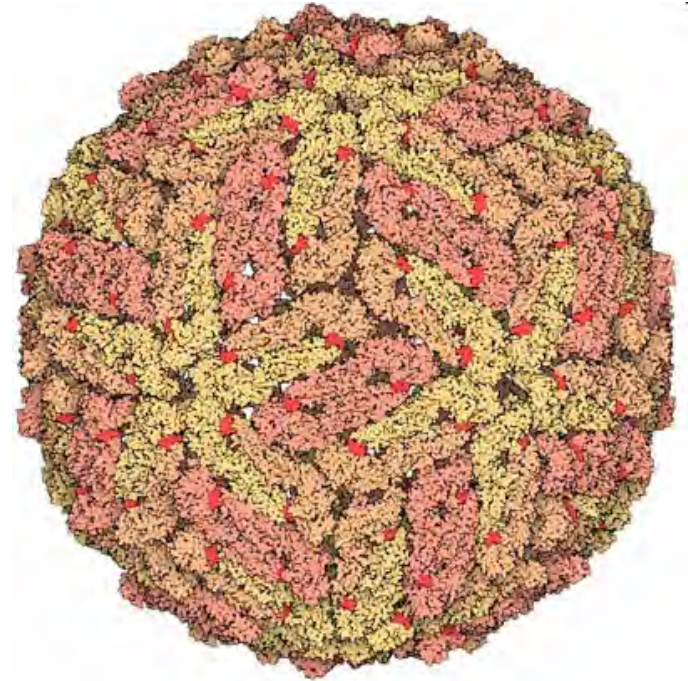
# Dengue Virus : A Surprising capsid

**180 subunits with icosahedral symmetry.  
Yet: no  $T=3$  organisation!**

**A three-dimensional image reconstruction shows that the virion has a well-organized outer protein shell, a lipid bilayer membrane, and a less-well-defined inner nucleocapsid core.**

**The known atomic structure of the homologous E protein dimer of (TBEV) (Rey et al., 1995) has been fitted into the outer layer of density in the cryoelectron microscopy (cryoEM) reconstruction.**

**The icosahedral scaffold consists of 90 such dimers with three monomers in the icosahedral asymmetric unit, **lacking**  $T=3$  quasi-equivalent environments (Caspar and Klug, 1962).**



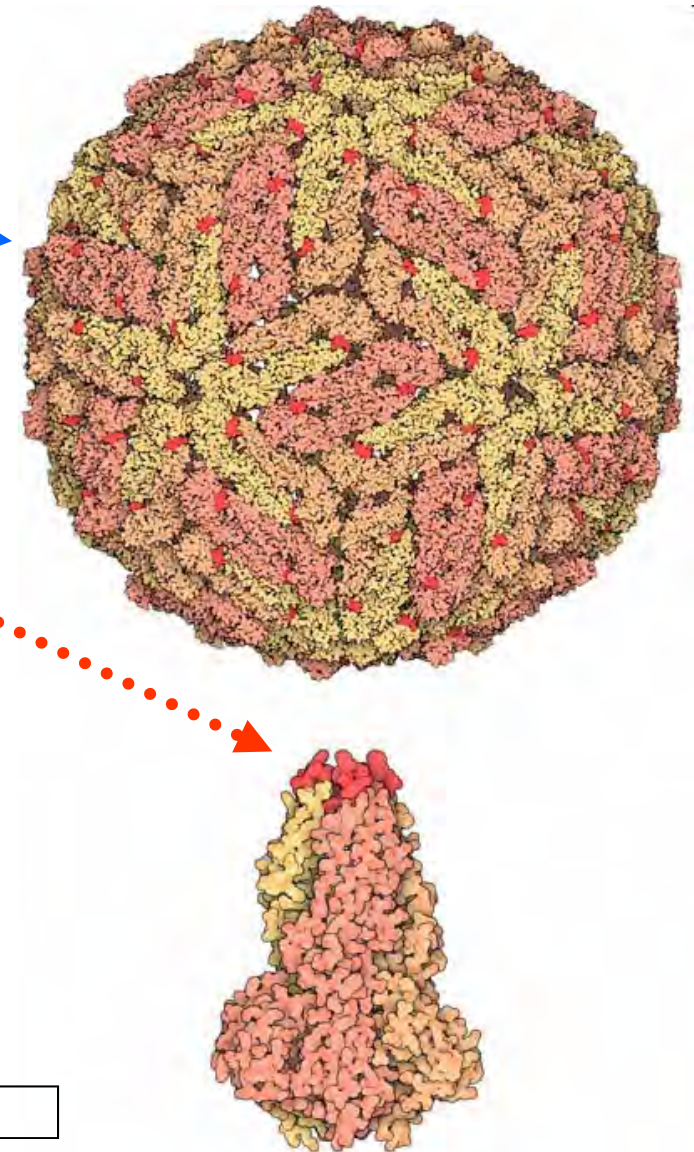
Most surprisingly:  
The icosahedral asymmetric unit  
contains one-and a-half dimer.

# Dengue Virus: A Deadly Switch

In the infectious form of the virus, the envelope protein lays flat on the surface of the virus, forming **a smooth coat** with icosahedral symmetry.

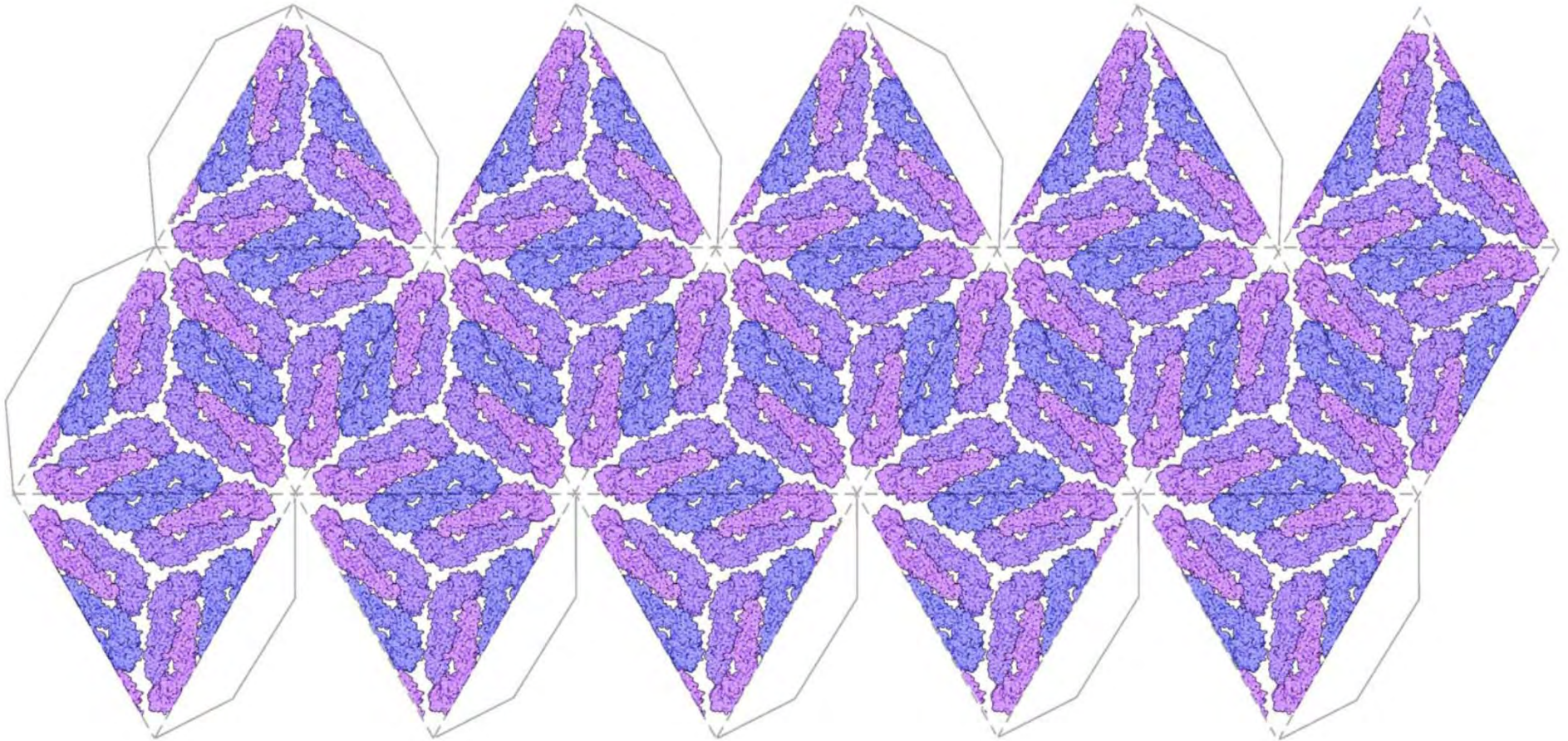
However, when the virus is carried into the cell and into lysosomes, the acidic environment causes the protein to snap into a different shape, **assembling into trimeric spike**, as shown at the right (PDB entry 1ok8).

Several hydrophobic amino acids at the tip of this spike, colored bright red here, insert into the lysosomal membrane and cause the virus membrane to fuse with lysosome. This releases the RNA into the cell and infection starts.





# Make a Dengue Virus Structure



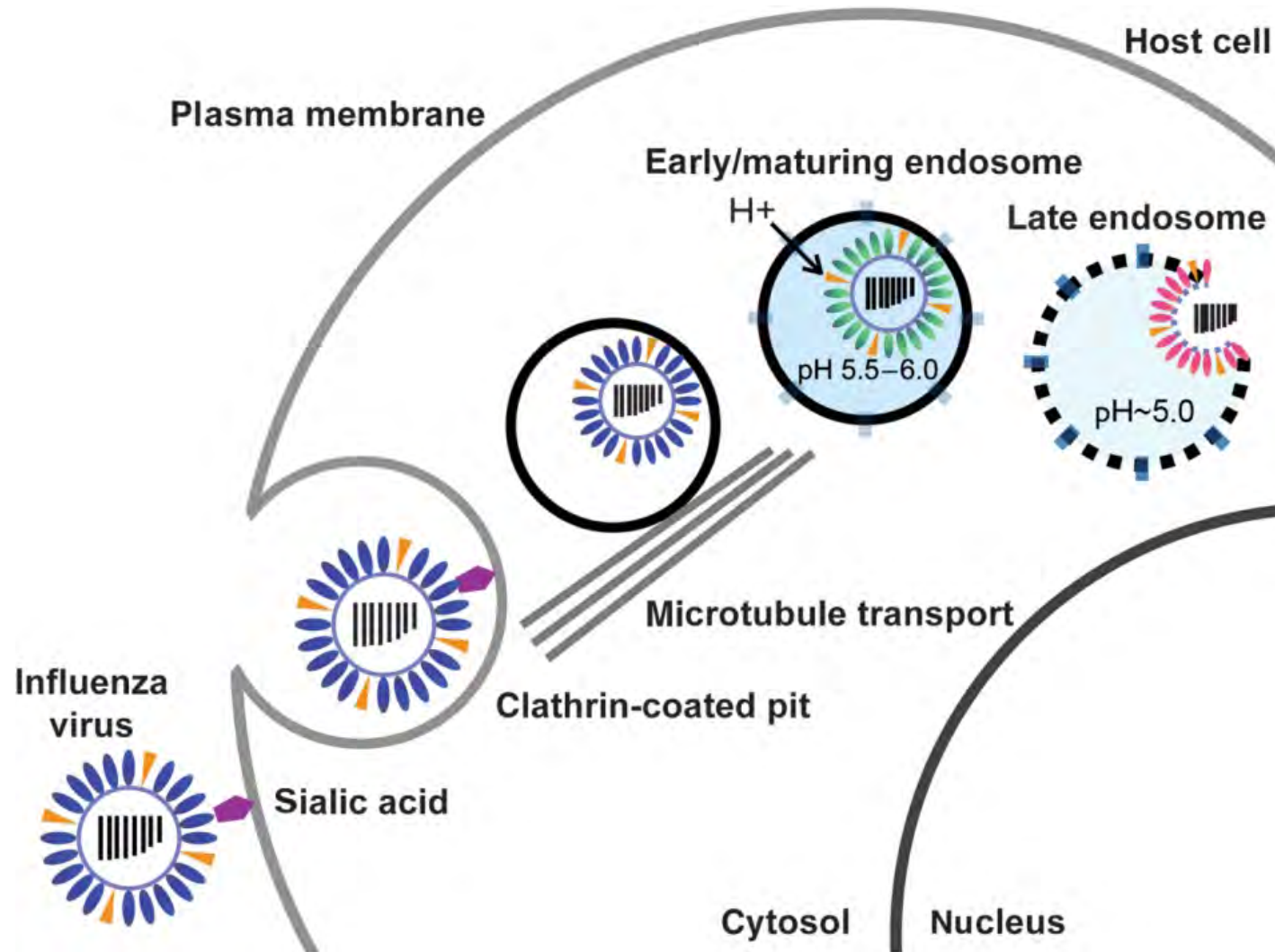
Kuhn et al Cell 108: 717-725 (2002) . Also: doi: 10.2210/rcsb\_pdb/mom\_2008\_7

# Membrane-enveloped viruses

Example: Influenza virus



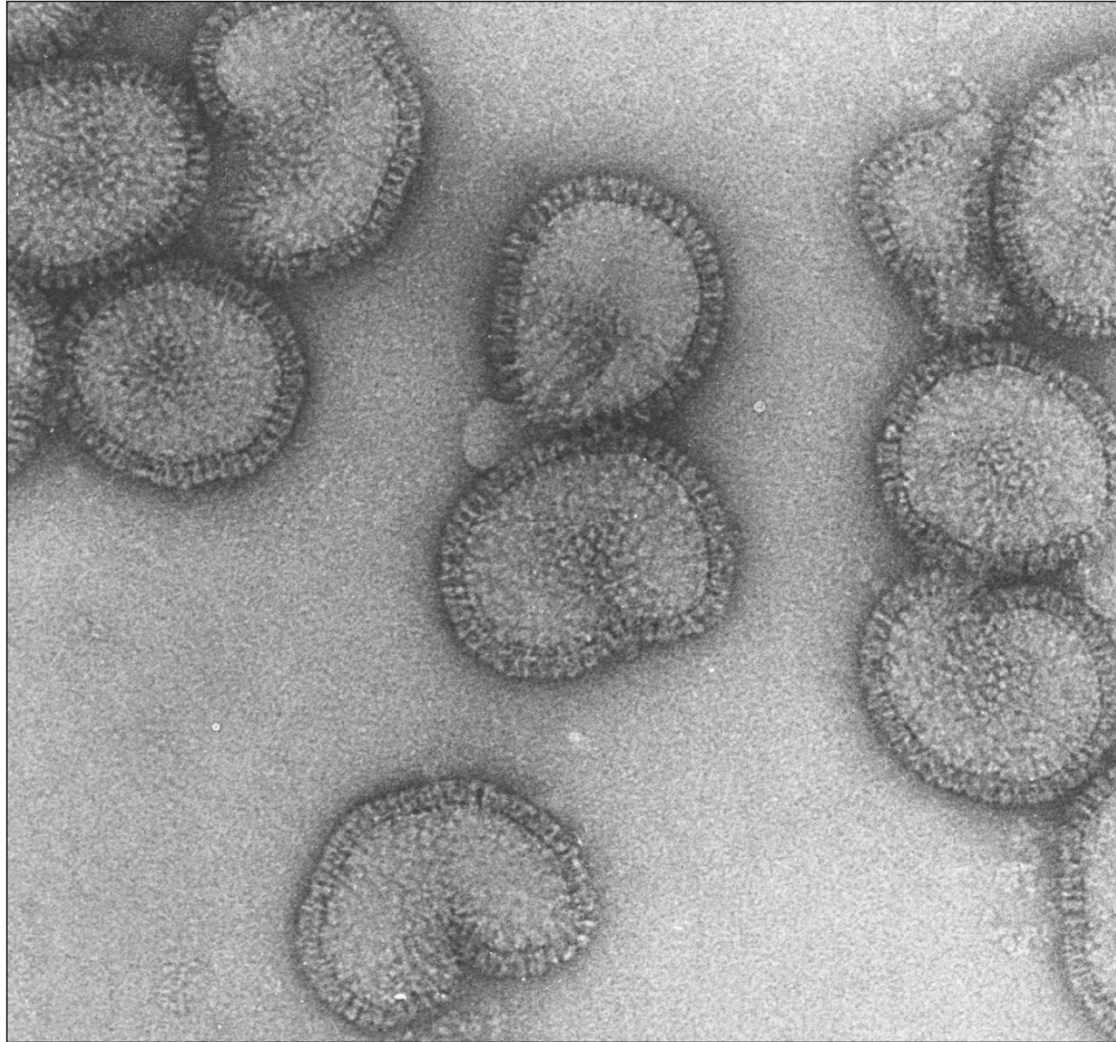
# INFLUENZA VIRUS



Blue = Haemagglutinin (HA)  
Yellow = Neuraminidase

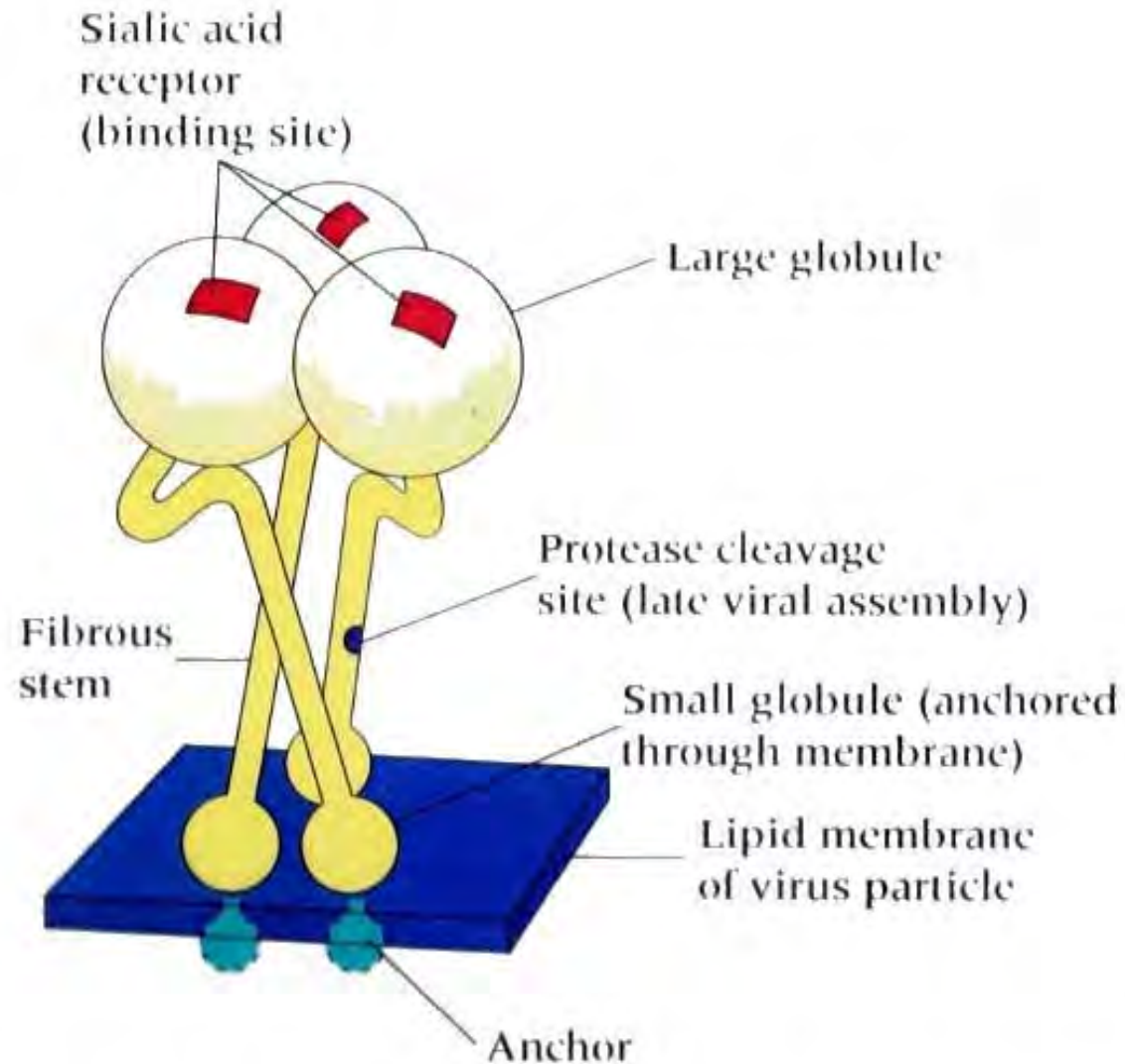
Figure 1 from Kelly K Lee (2010) *EMBO Journal* (2010)

# INFLUENZA VIRUS



Influenza Virus has two main surface proteins:  
haemagglutinin (H) and neuraminidase (N).

## Influenza Virus Haemagglutinin



Schematic representation of the protein trimer that forms the hemagglutinin antigen on the surface of the influenza A virus: the HA spike.

# **INFLUENZA VIRUS HAEMAGGLUTININ**

**Haemagglutinin (HA) of influenza virus is an integral membrane protein which is a trimer with a molecular weight of 224,640.**

**Each monomer contains two chains, HA1 with 328 amino acids, and HA2 with 221 amino acids. These two chains are obtained by post-translational removal of arginine329.**

**Each monomer contains one small globular domain near the membrane. This domain contains a 5-stranded anti-parallel beta-sheet. Quite remarkably, this sheet is formed by the first few residues of the HA1 chain coming out of the membrane plus four strands from the C-terminus of HA2, which are 350 residues further in the sequence.**

**There is also a large globular domain faraway from the membrane where an 8-stranded anti-parallel beta structure dominates the fold.**

**The most remarkable feature is a 14 turn, 53 amino acids long helix which comes back from the globular “head” to the membrane and spans a distance of 76 Å.**

**In the trimer, this helix twists around two identical helices from other subunits to form the coiled-coiled core of the “fibrous” middle region of the trimer.**

**The total extension from the membrane is ~135 Å, which is the reason why these haemagglutinin molecules appear in electron micrographs of the virus as “spikes”.**

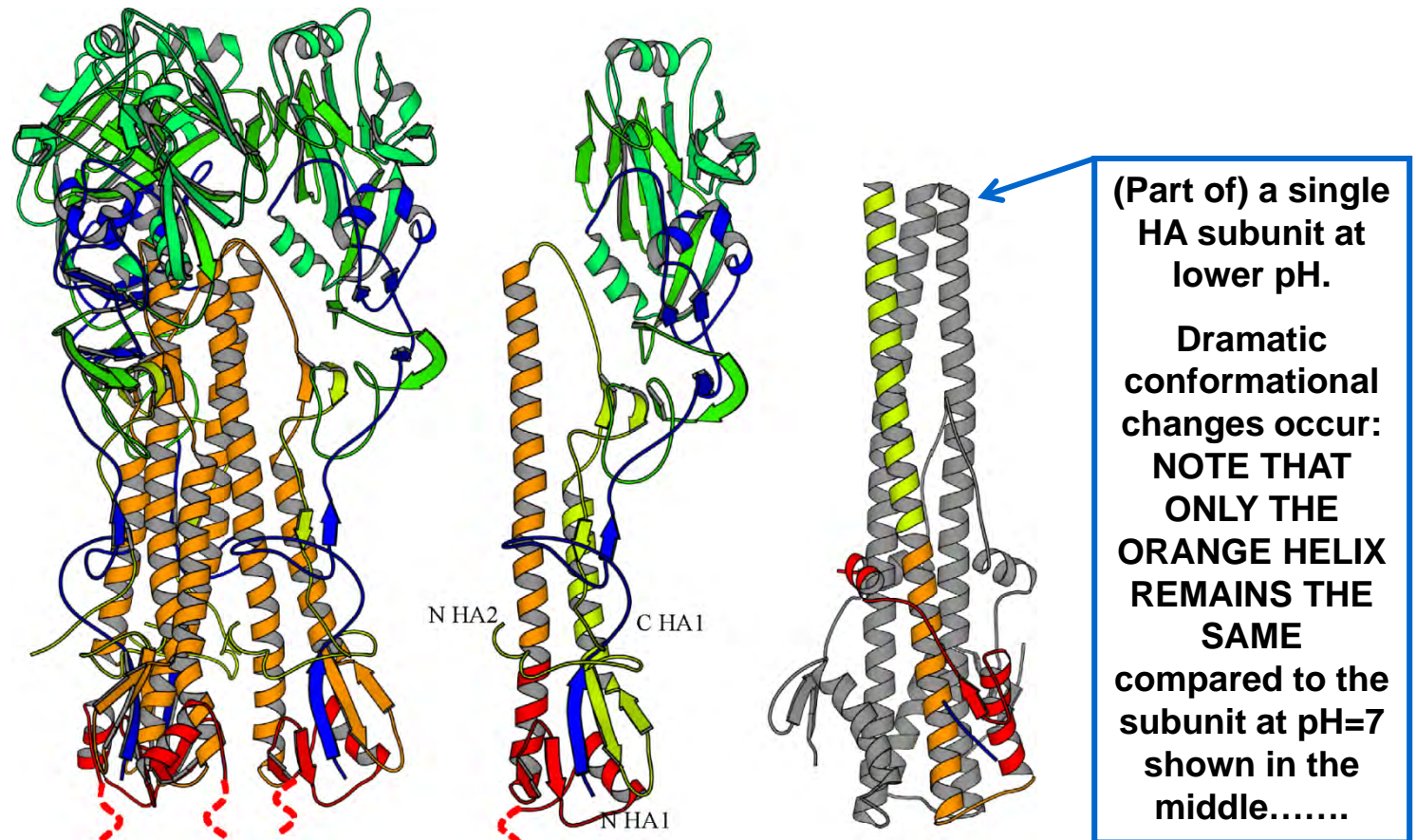


# **INFLUENZA VIRUS HAEMAGGLUTININ**

- 1. There are numerous glycosylation sites on the protein, at seven positions, with a total molecular weight of 13,000 for the carbohydrate chains. These oligosaccharide chains are spread out over the entire molecule.**
- 2. Several antibody binding sites are known from immunological studies, and they appear to map on the part of the molecule which is furthest extended from the surface.**
- 3. Antigenic variation at these sites is responsible for the influenza epidemics which travel across the planet with irregular intervals.**
- 4. The receptor binding site for sialic acid is located in the globular head of the trimer.**
- 5. After binding of the virus to the target cells, the virus is taken up in the endosome where the lower pH causes a major reorganization of the haemagglutinin molecule such that it can cross the membrane.**
- 6. The dramatic conformational change includes a further elongation of the already impressively long helices of the trimer's coiled-coiled region.**

# Influenza Virus Haemagglutinin

## Large Conformational Change in the Endosome with lower pH



**Left: The trimer at neutral pH** after cleavage into the HA1 and HA2 peptides (PDB: 1RU7). The membrane anchor if it were present would be attached to the C-terminus of HA2 (at the end of the dashed red lines).

**Middle: A monomer at neutral pH.** The fusion peptide (N-terminus of HA2) is at the center of the molecule, hidden by interactions with the other proteins in the trimer.

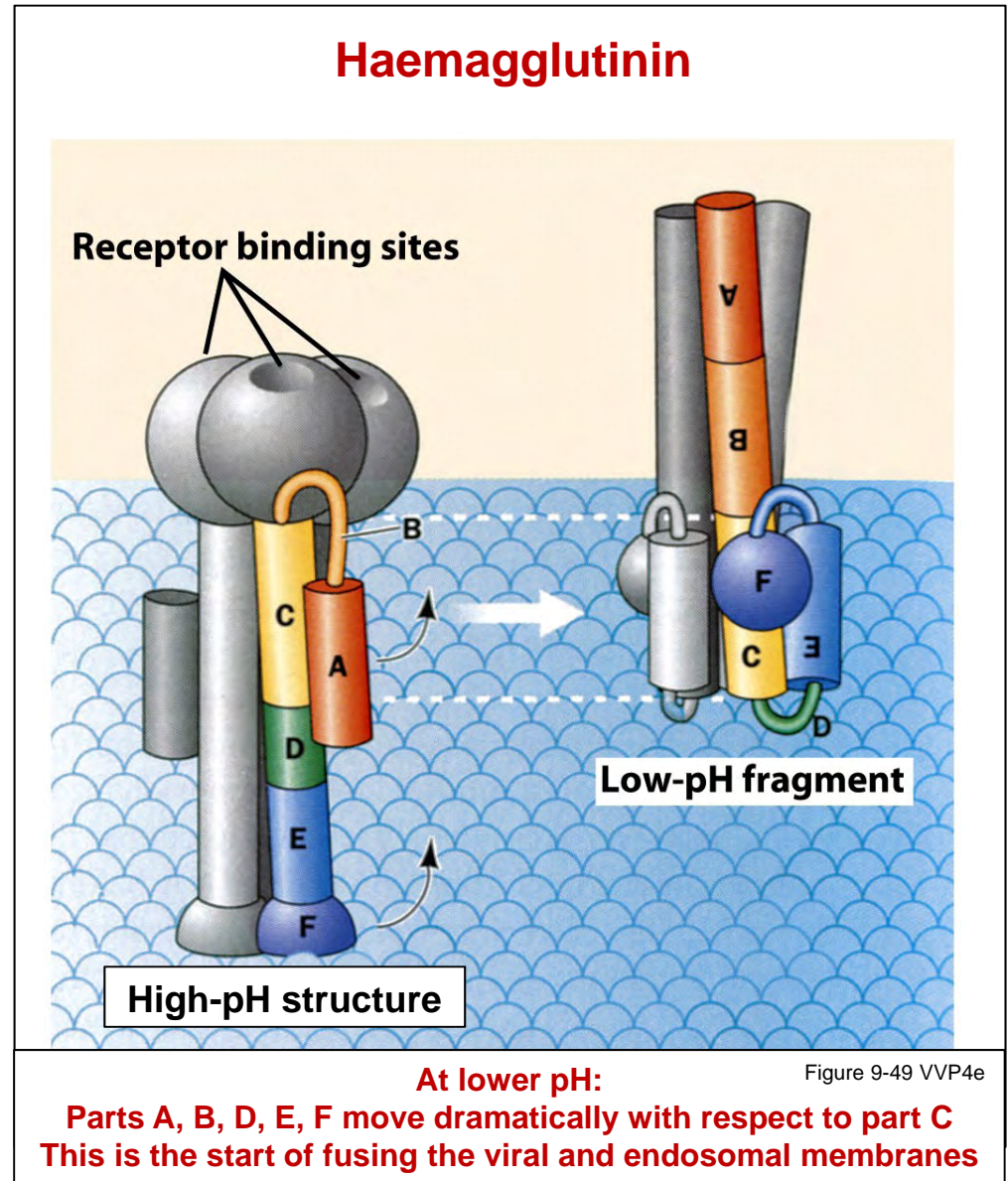
**Right: The low pH form** (PDB: 1HTM). The fusion peptide is disordered, but must be located on top of the three helices. Large conformational changes have occurred. The lower part of the long helix in the neutral pH form (red) is now bent and packed at the side of the long helix. The upper part of the long helix (green-yellow) forms a long loop and a helix in the neutral pH form.

FIG. 15.8 from Liljas, Textbook of Structural biology.

# Influenza Virus Haemagglutinin

Large Conformational Change in the Endosome with lower pH

- The pH of fluids surrounding cells, and of the cytosol inside the cell, is around 7.
- However, in the organelles called endosomes, the pH is lower.
- This pH difference is exploited by the influenza virus. It enters cells via “endosomes” which have a pH of about 6.
- In this organelle with lower pH, the influenza virus cell surface protein “haemagglutinin” undergoes a spectacular conformational change, enabling the flu virus to infect a cell.



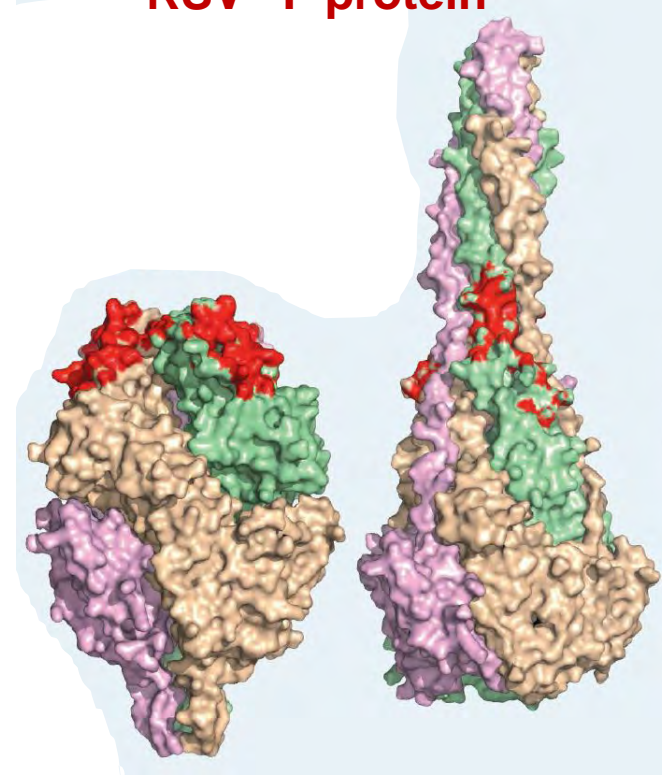


# Respiratory Syncytial Virus (RSV)

## Structure-based vaccine design

- Respiratory syncytial virus (RSV) hospitalizes millions of infants each year with pneumonia and other lung diseases. Severe RSV disease kills worldwide 160,000 kids annually.
- On the surface of RSV is the “F protein”.
- Best neutralizing antibodies bind to the red parts (“epitope”) of the structures at the right.
- The F protein displays lots of *the red surface area needed to trigger potent antibodies* in its **prefusion state** (*left structure in figure at right*)
- Engineering the F protein such that the red epitope remains well accessible, and fixed in the **prefusion state** to generate antibodies, appears a promising way to obtain a protein, which after injection, generates anti-RSV antibodies
- I.e. this is a rational way to obtain a RSV vaccine.

### RSV “F protein”



Left: F protein in the **prefusion conformation**  
Right: F protein in the **postfusion conformation**

From: Science 342:1442 (2013)

McLellan, Science 340: 1113 (2013); McLellan, Science 342:592 (2013)

Another vaccine engineering approach based on the same crystal structures but using scaffolds: Correia, Science 2014 - doi:10.1038/nature12966



# Bacterial viruses

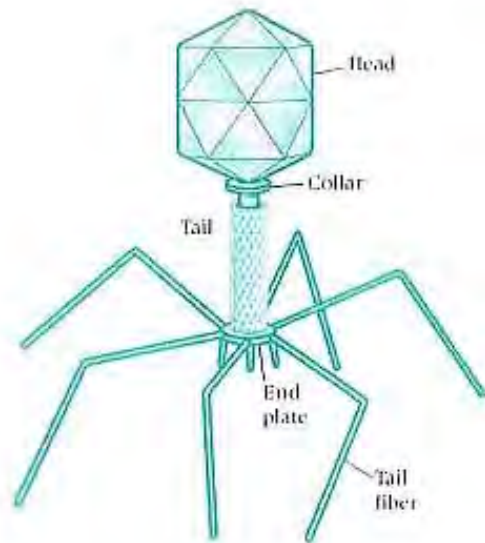
Usually called “bacteriophages”, or “phages”.

Also these are very diverse.

For instance:

- Filamentous phages, like M13.
- Moonlander-like phages, like T4

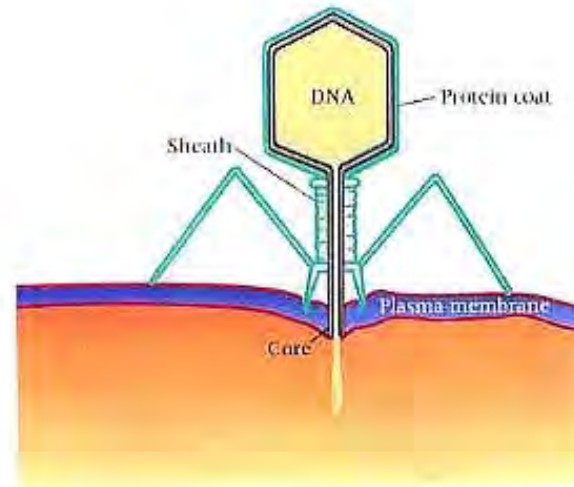
# Bacteriophage T4 – A Fabulous Machine



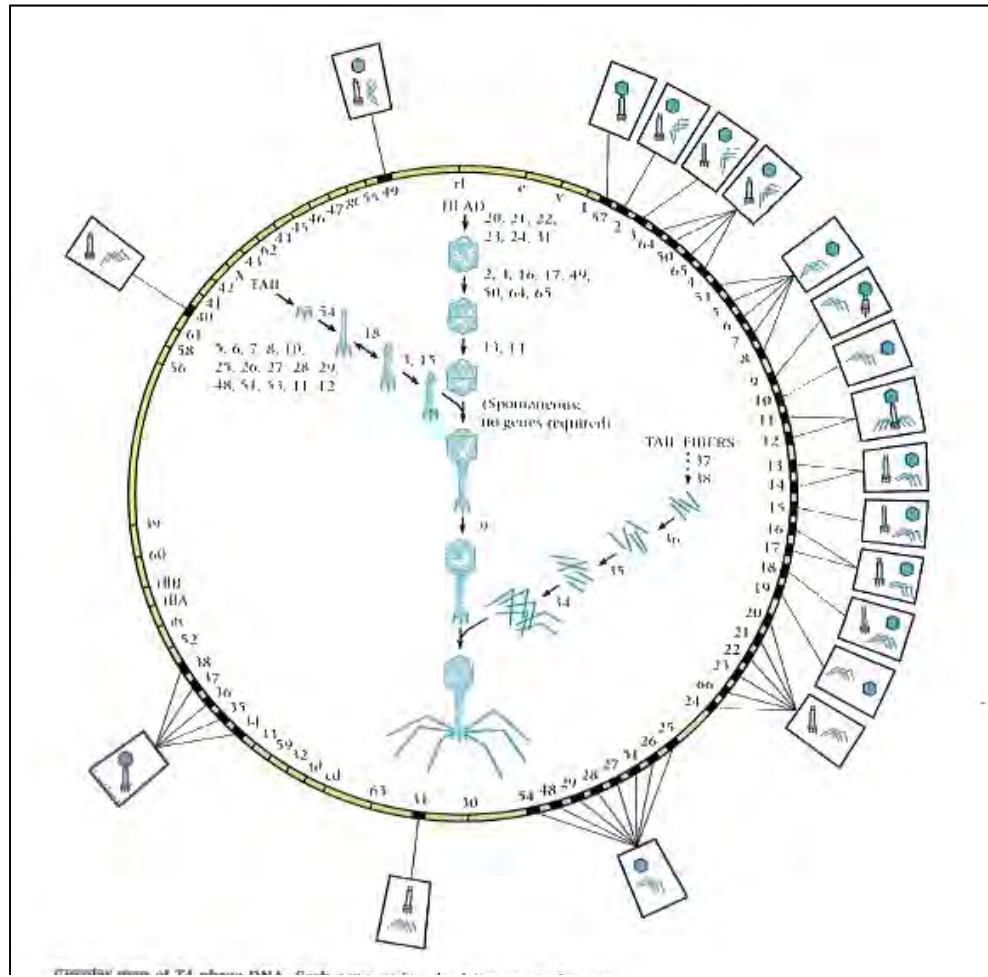
**Upper LEFT: Diagram of bacteriophage T4.**

**Upper RIGHT:** T4 attached to a bacterium and injecting its DNA. The tail contracts, forcing the injection tube through the wall and into the cytoplasm; the viral DNA travels from the head into the cell, while the coat proteins remain outside.

**Lower Right: EM of bacteriophage T4.** The tail, which has a helical rod symmetry, is used as a syringe to inject the viral DNA contained in the head, which approximates an elongated icosahedron. The fibers that project from the end of the tail are proteins that attach the virus to a bacterial host.



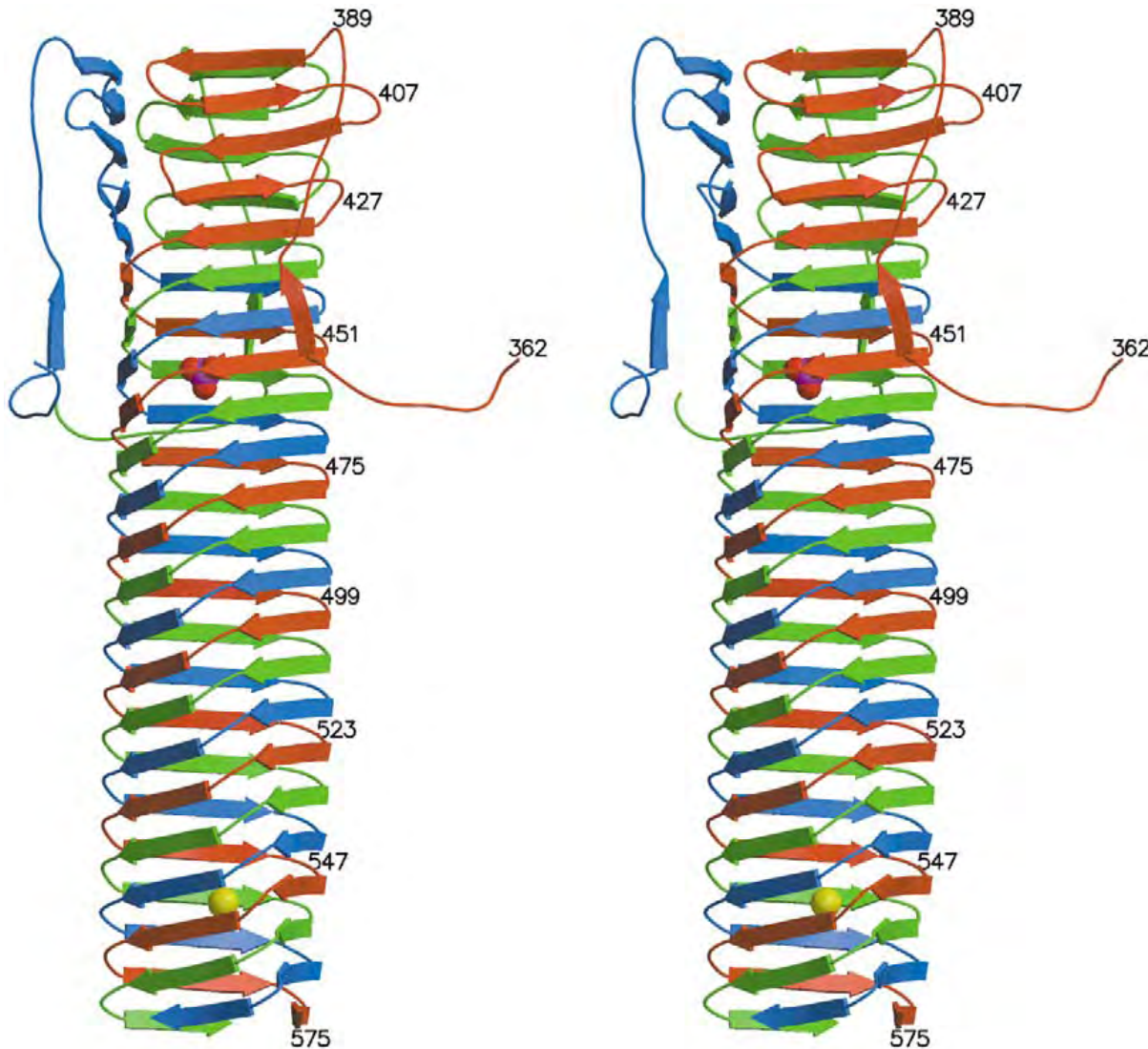
# Circular Map of T4 Phage DNA



- Each gene, assigned a letter or number, encodes one protein.
- Genes for particle assembly – depicted inside the ring – are matched with the faulty structures produced when these genes are defective. For example, genes 34-38 (no tail fibers), gene 49 (no assembly), gene 9 (premature tail contraction).
- Mutations in genes 11-12 generate a complete but fragile bacteriophage.

Numbers next to arrows in the three branches of the assembly line indicate the genes crucial to each step.

## The tail of T4: gp5, a most unusual structure



**Stereo diagram of the gp5 C-terminal domain**

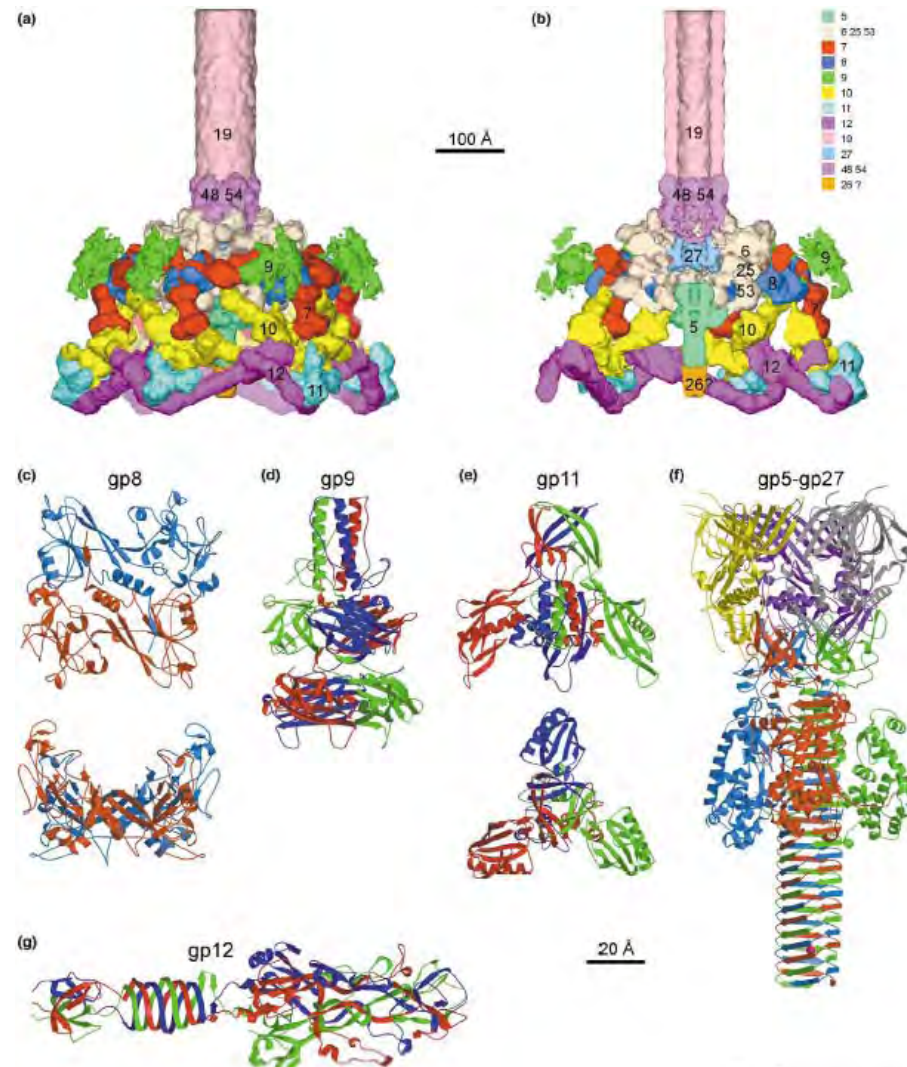
The **three** chains are colored **red**, **green** and **blue**.

The residue numbers are indicated in strategic locations.

The metal and phosphate ions, stabilizing the internal contacts in the beta-helix, are shown as yellow and magenta spheres, respectively.



# The tail of T4



## Combine Electron Microscopy and Crystallography to place Components in the T4 Tail

For a movie from the Rossmann lab about phage T4 entering a bacterial cell see:  
[http://bilbo.bio.purdue.edu/~viruswww/Rossmann\\_home/movies/t4phage\\_final-sorenson3-small.php](http://bilbo.bio.purdue.edu/~viruswww/Rossmann_home/movies/t4phage_final-sorenson3-small.php)

# CONCLUSION

**Viruses  
are  
fascinating,  
but can also be devastating**