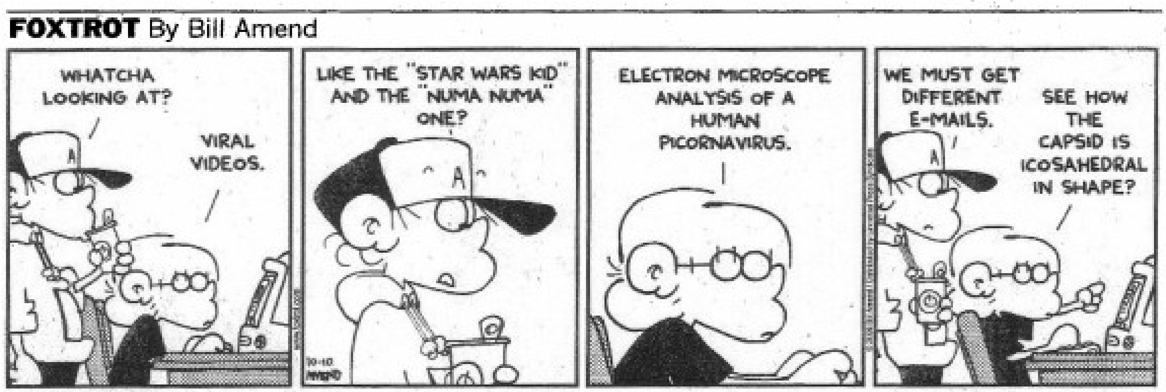
Virus Architecture and Viral Genome Structure



TOPICS COVERED:

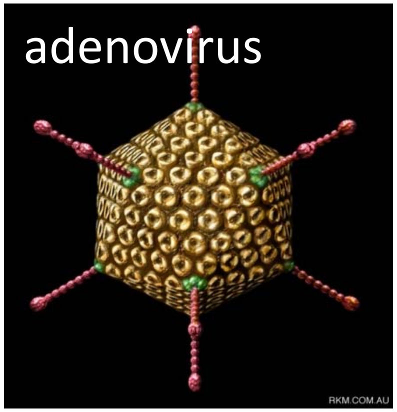
Particle Structure:

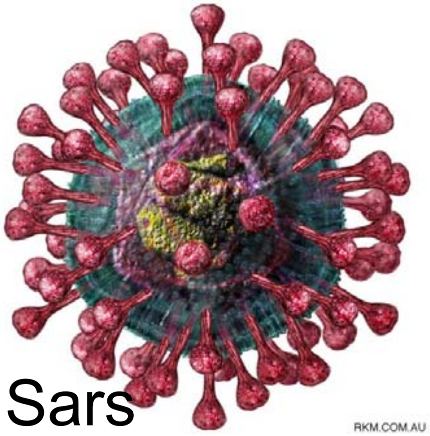
1. Description of the different types of virus architecture
2. Terminology used with virus architecture
3. How virus particles assemble
4. Relevance/importance of virus structure

Genome Structure

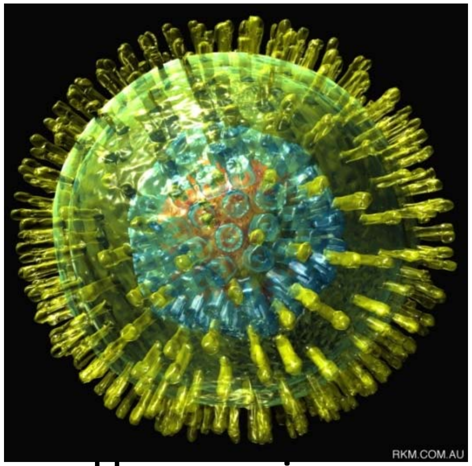
1. Components that can be present on viral genomes
2. Terminology used to describe viral genomes
3. Description of the different types of viral genomes

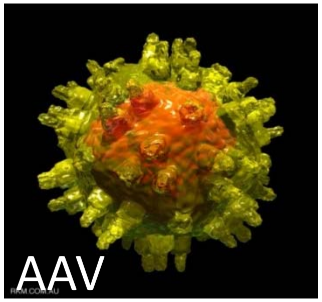
Viruses vary in shape and size

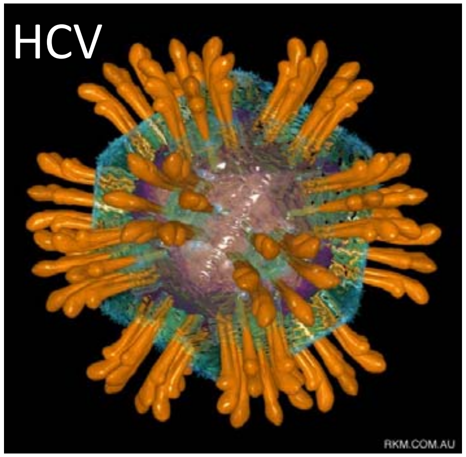

adenovirus


Sars


HIV


Herpes virus


AAV


HCV

PLANT VIRUSES

In general, more stream-lined architecture than viruses of bacteria and animals

Koenig, R., and Lesemann. D.-E,
Plant Virus Identification. www. els.net

TERMINOLOGY OF VIRUS STRUCTURE

1. The CAPSID denotes the protein shell that encloses the nucleic acid. It is composed of structural units.

2. STRUCTURAL UNITS are the smallest functional equivalent building units of the capsid (a molecule of coat protein).

3. CAPSOMERS are morphological units seen on the surface of particles and represent clusters of structure units – pentamers (5 sided) and hexamers (6 sided)

4. The capsid together with its enclosed nucleic acid is called the NUCLEOCAPSID.

5. The nucleocapsid may be invested in an ENVELOPE which may contain material of host cell as well as viral origin.

6. The VIRION is the complete infectious virus particle.

Virus Particle Architecture

Common features and general principles of virus architecture:

- **The capsid is made of identical subunits (most viruses) of molecules of coat protein**
- **Subunits are packaged (symmetry) so that they have an identical environment**
- **Subunits and the viral genome undergo “self-assembly” to form virions.**
- **Virion shape is determined by the outer shell of a virus particle**

Examples of Subunit Construction

R. Buckminster Fuller

BASIC TYPES OF PLANT VIRUS MORPHOLOGIES

I. Non-enveloped Virions

- Helical
- Icosahedral
- Other variations

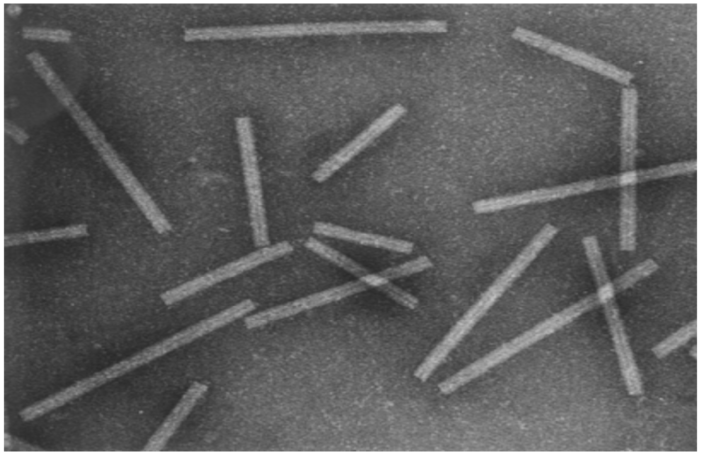
II. Enveloped Virions

- Pleomorphic
- Bacilliform

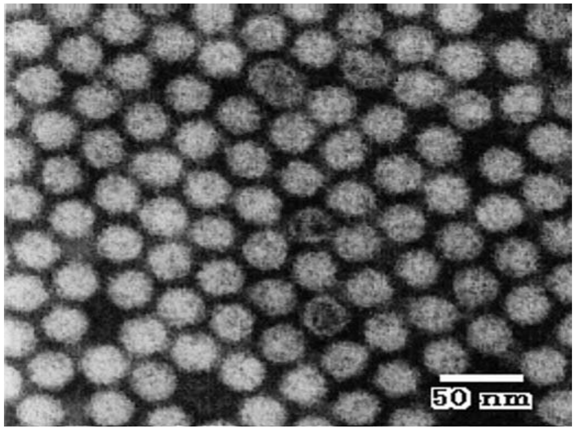
I. Non-enveloped Virions

Three Basic Structures:

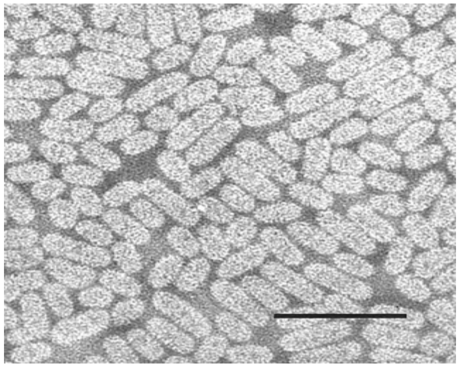
A. Helical (Rod-shaped)



B. Icosahedral (isometric)



C. Other (Bacilliform, Geminate)



I. Non-enveloped Virions A. Helical (Rod-shaped) structure

Subunits are arranged in the form of a helix
Rod-shaped viruses contain ~ 95% protein
All known to contain RNA genomes

3 Types of Helical particles:

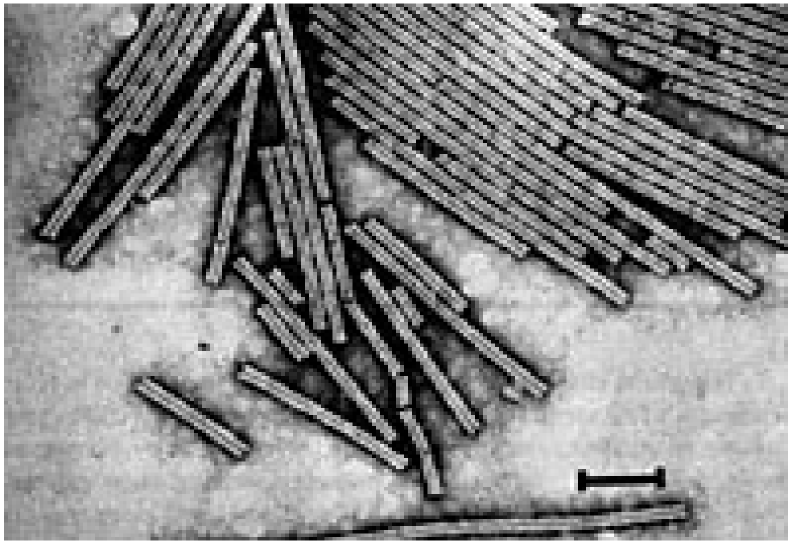
- ► **Rigid**
- ► **Flexuous**
- ► **Filamentous**

Size:

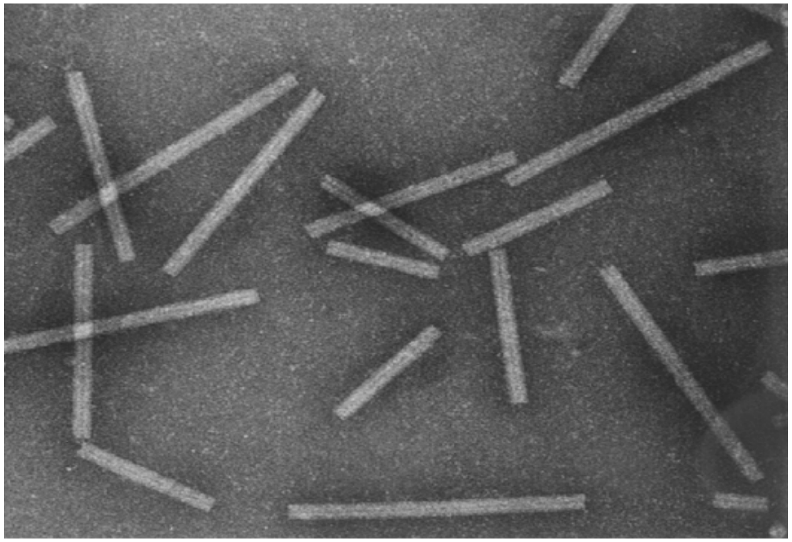
Particles vary in length:

- √ **size (length) varies among different viruses**
  - several hundred nanometers (nm) up to 2000 nm
- √ **size varies among virions of the same virus**

Rigid Helix



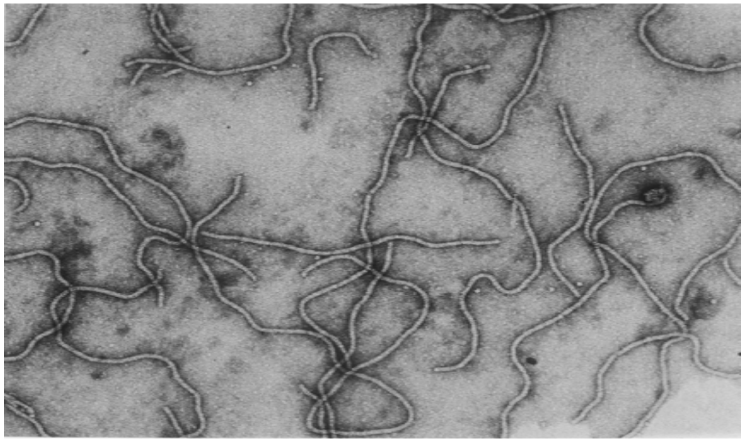
Genus: Tobamovirus



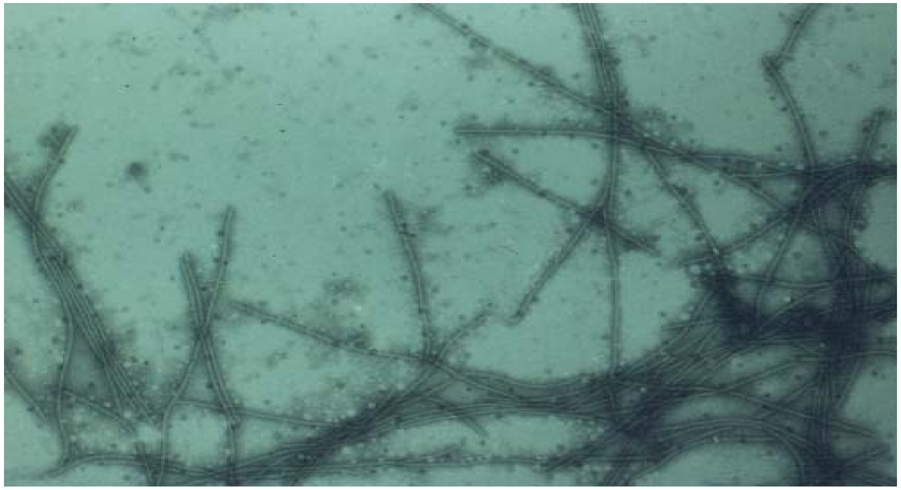
Genus: Furovirus

Flexuous Helix

Genus Potexvirus



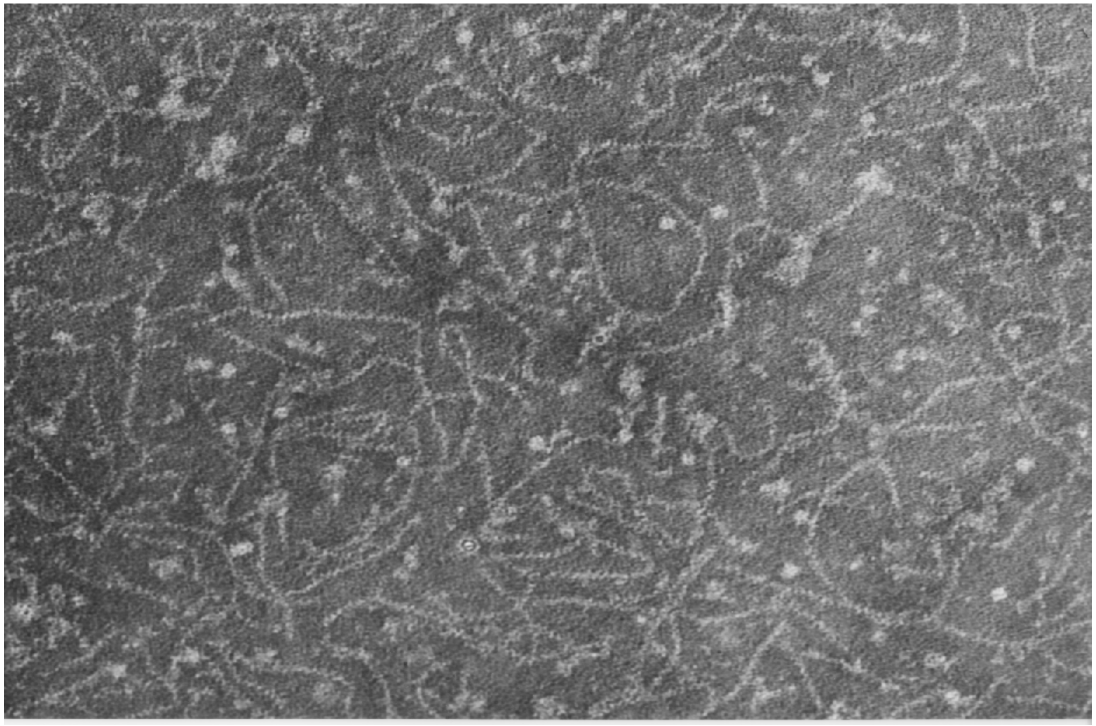
Genus Closterovirus



Genus Potyvirus

Filamentous Helix

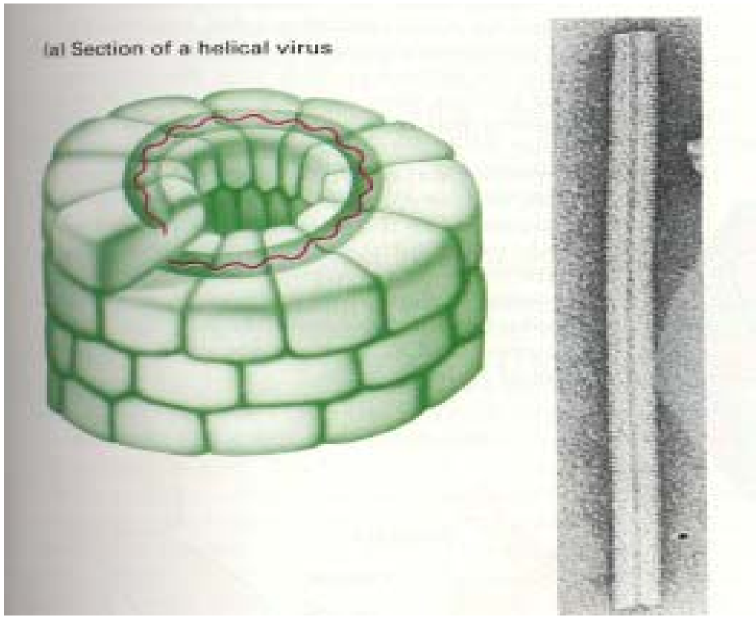
May appear to be spiral, branched or circular



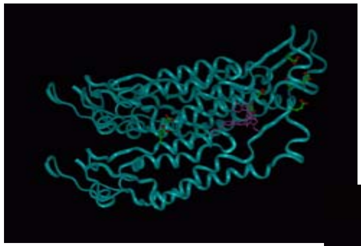
Family: Phenuiviridae
Genus: Tenuivirus

Helical Virus Particles

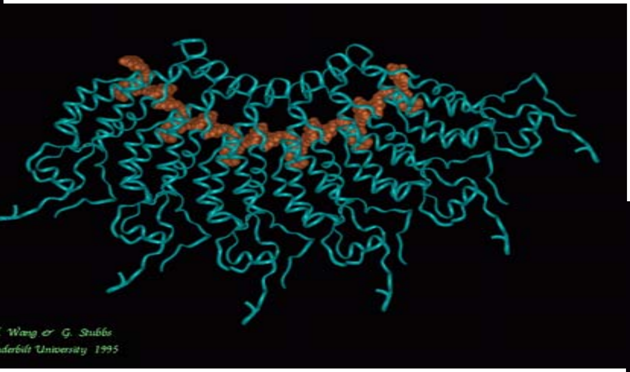
- The virion protein shells or tubes are made up repeating subunits of the capsid protein.
- The capsid protein represents approximately 10% of the coding capacity of the viral genome.
- Capsids of most plant viruses consist of a single type of protein



Assembly of Helical Viruses

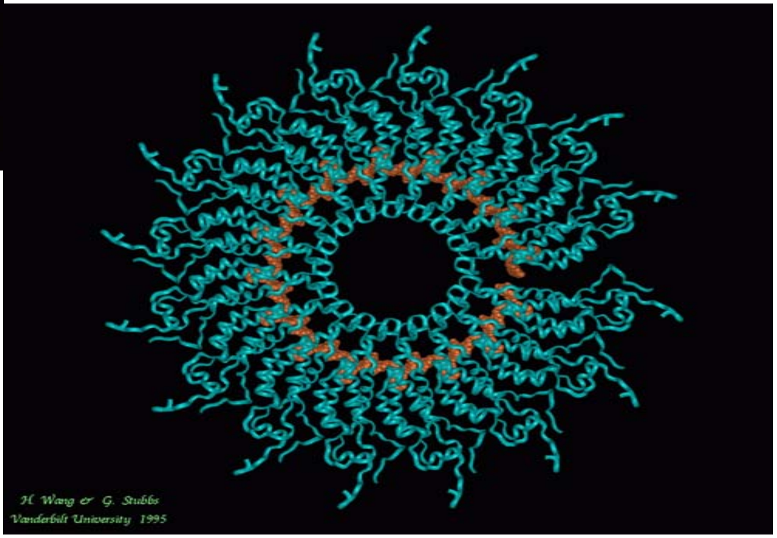


3 subunits of TMV coat protein



H. Wang & G. Stubbs
Vanderbilt University 1995

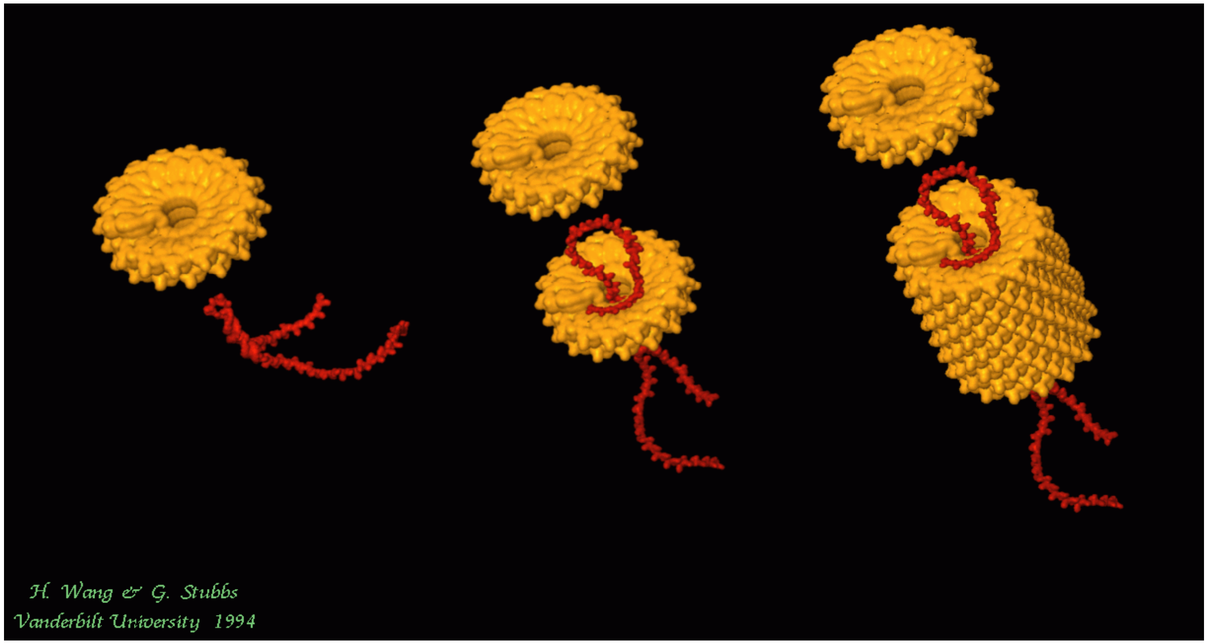
TMV CP assemble into disk-shaped aggregates



H. Wang & G. Stubbs
Vanderbilt University 1995

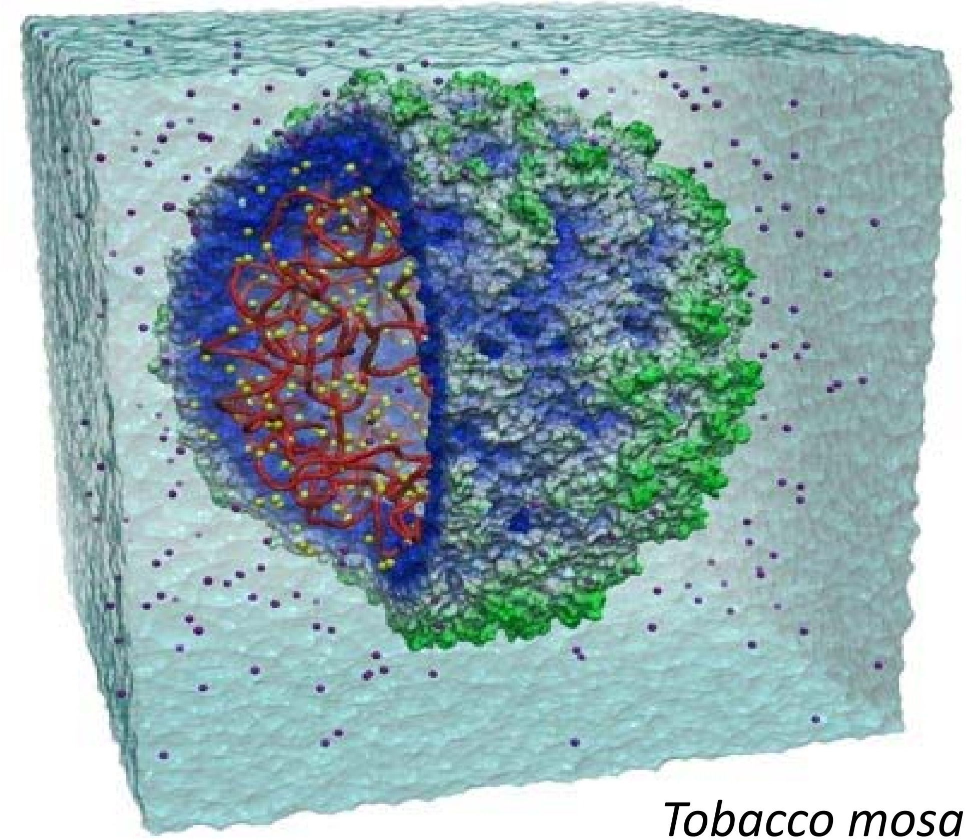
TMV assembly:

A TMV coat protein aggregate binds to the RNA origin-of-assembly loop; additional aggregates then bind to the initial complex, pulling the 5' end of the RNA up through the hole in the middle of the growing virus particle. Assembly terminates when the end of the RNA is reached. (Particle length is determined by the length of the RNA)


H. Wang & G. Stubbs
Vanderbilt University 1994

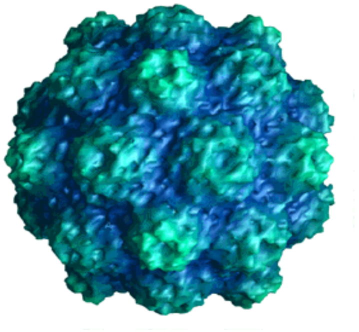
I. Non-enveloped Virions

B. Icosahedral (Isometric) Shaped Plant Viruses



Tobacco mosaic satellite virus

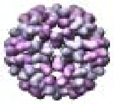
B. Icosahedral (Isometric) Shaped Plant Viruses



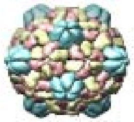
- Viral subunits arranged in the form of a hollow quasi-spherical structure.
- An icosahedron is a minimum free energy structure
- Protein subunits are not spaced independently but cluster because this maximizes intermolecular interactions which stabilize the particle.

Panicum mosaic satellite virus

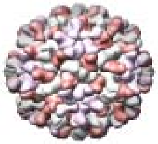
Alfalfa mosaic virus



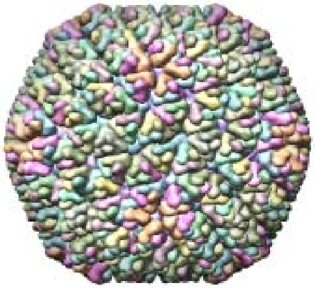
Cowpea chlorotic mottle virus



Tobacco ringspot virus



Tomato bushy stunt virus



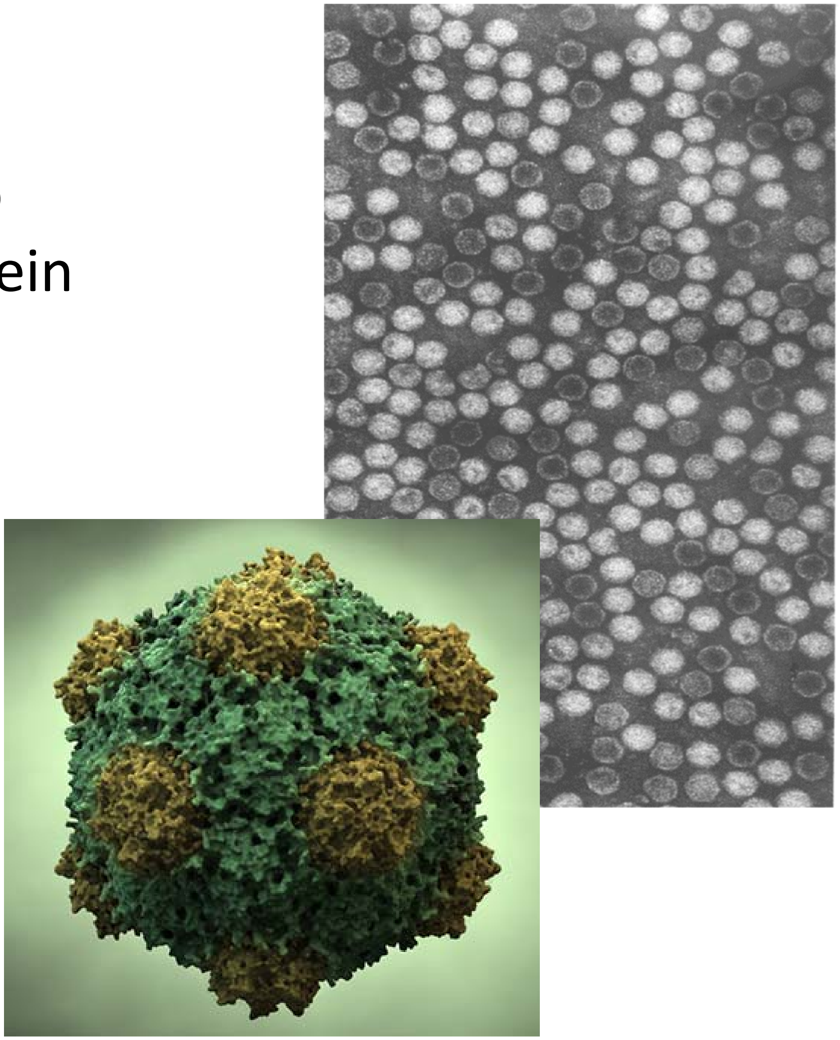
Rice dwarf virus

- Range in size mostly 20-30 nm dia., a few 50-80 nm. in dia.
- Size is dictated by the size of the viral genome.

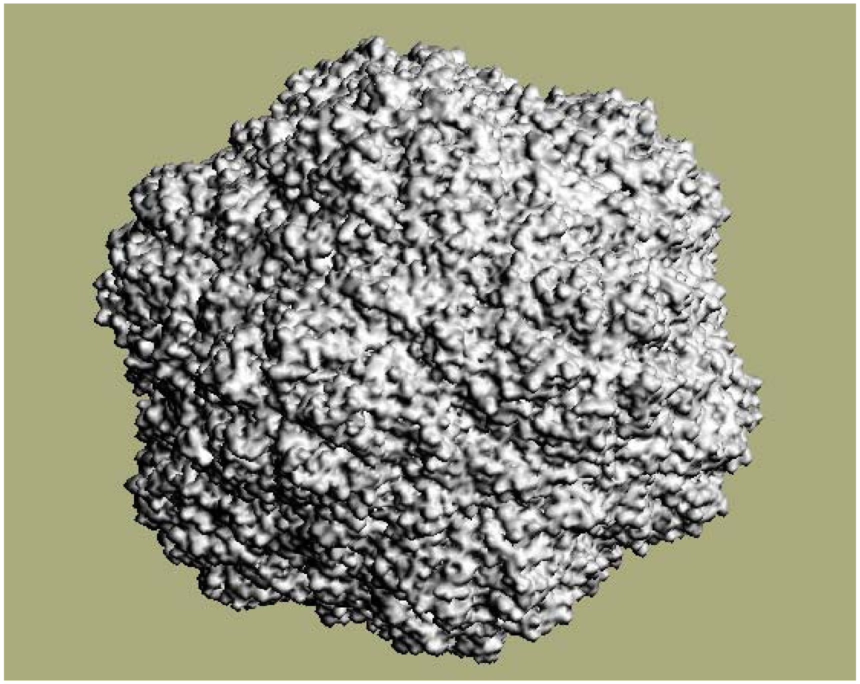
T. Goddard, C. Huang, T. Ferrin Resource for Biocomputing, Visualization and Informatics University of California, San Francisco

Icosahedral Capsids

- The virion protein shells are made up repeating subunits of the capsid protein
- Viruses contain a precise number of subunits per particle
- The number varies among different viruses

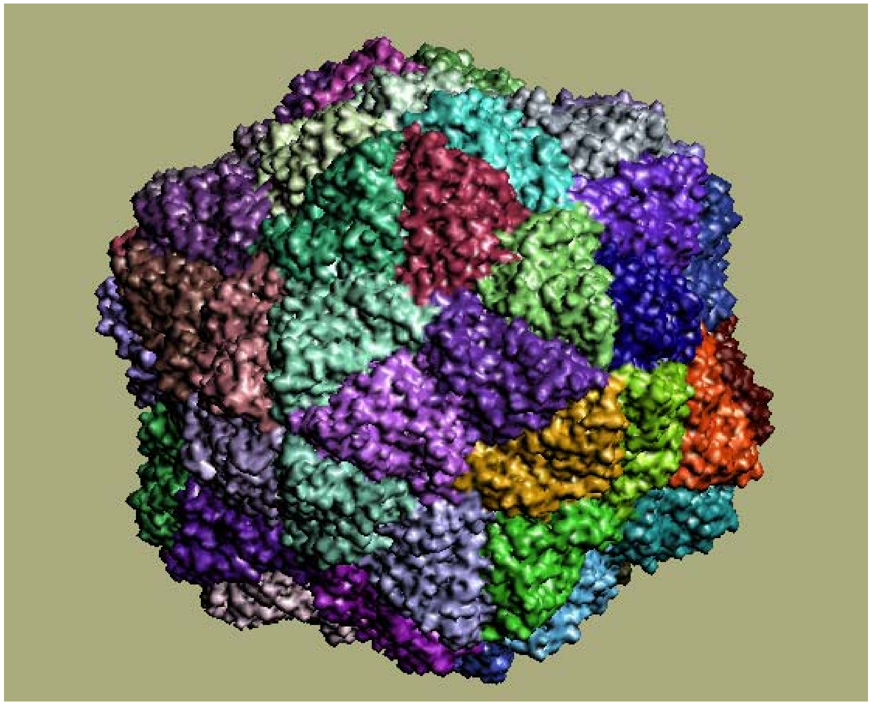


Tobacco necrosis satellite virus

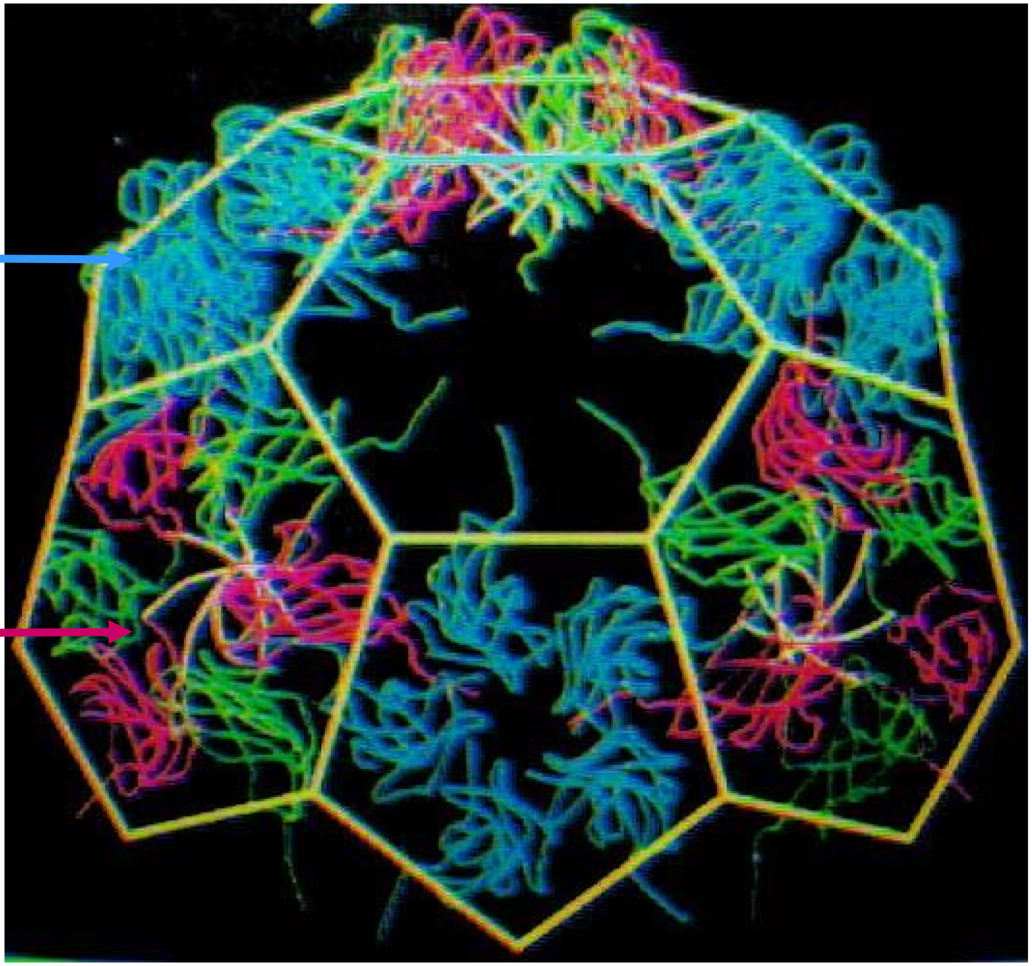


Tobacco necrosis satellite virus

Each coat protein molecule (subunit) is a different color



Protein subunits assemble into 2 types of capsomers:



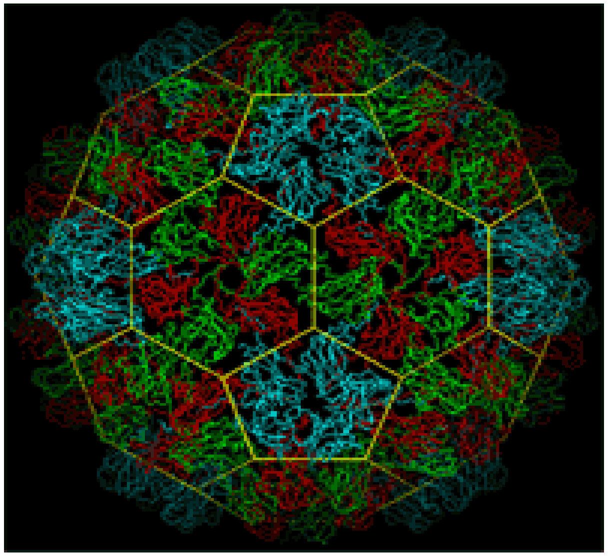
Pentamer

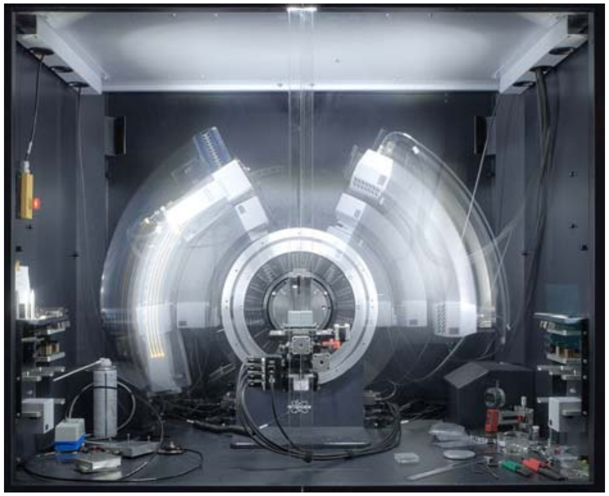
5 molecules of coat protein

Hexamer

6 molecules of coat protein

Cowpea chlorotic mottle virus (CCMV)





- five-sided (turquoise) pentamers and six-sided (red and green) hexamers (viral capsomeres)
  (computer image determined by x-ray crystallography)

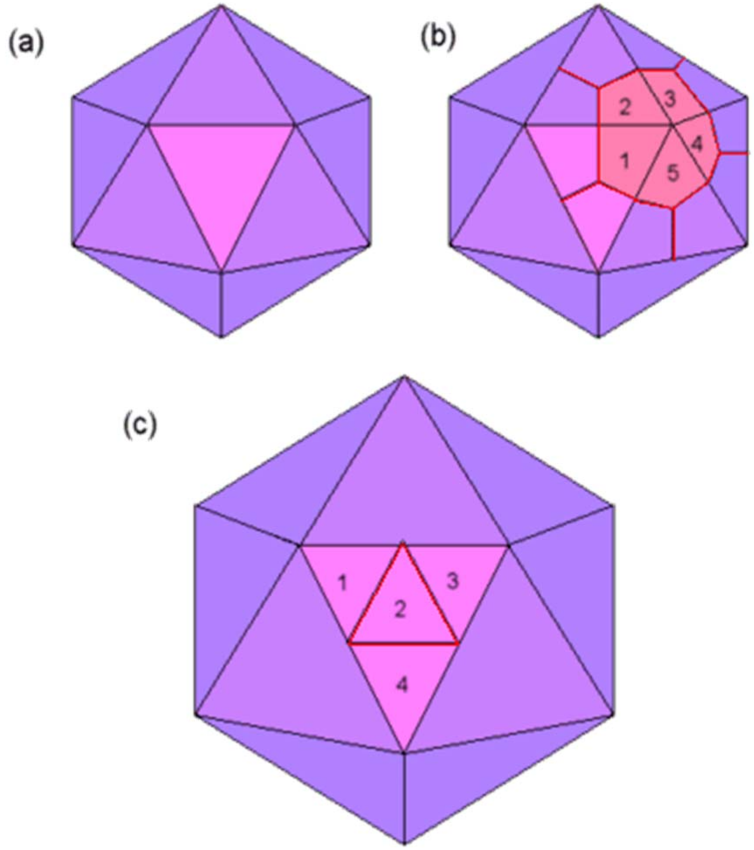
- The capsomeres are composed of protein subunits with identical amino acid chains.

Describing Icosahedral Structure:

All icosahedrons have 20 identical equilateral triangular faces. **(a)**

Simplest Icosahedron:
(b) Each triangular face is made up of three identical protein subunits. 20 faces x 3 proteins per face = 60 coat protein subunits. The five subunits surrounding each vertex are arranged in a five-fold symmetry.

More Complex Icosahedrons:
(c) Larger capsids have more than 60 subunits. Ex. Some of triangular faces of this icosahedron are made up of four subunits.



Nomenclature to describe size/complexity of icosahedral particles: Triangulation numbers

T= 1 Capsomers = 12 x 5 = 60/60 =1

T= 3 Capsomers = 12 x 5 = 60

$$\begin{array}{r} 20 \times 6 = \underline{120}\\ 180 / 60 = 3 \end{array}$$

T= 4

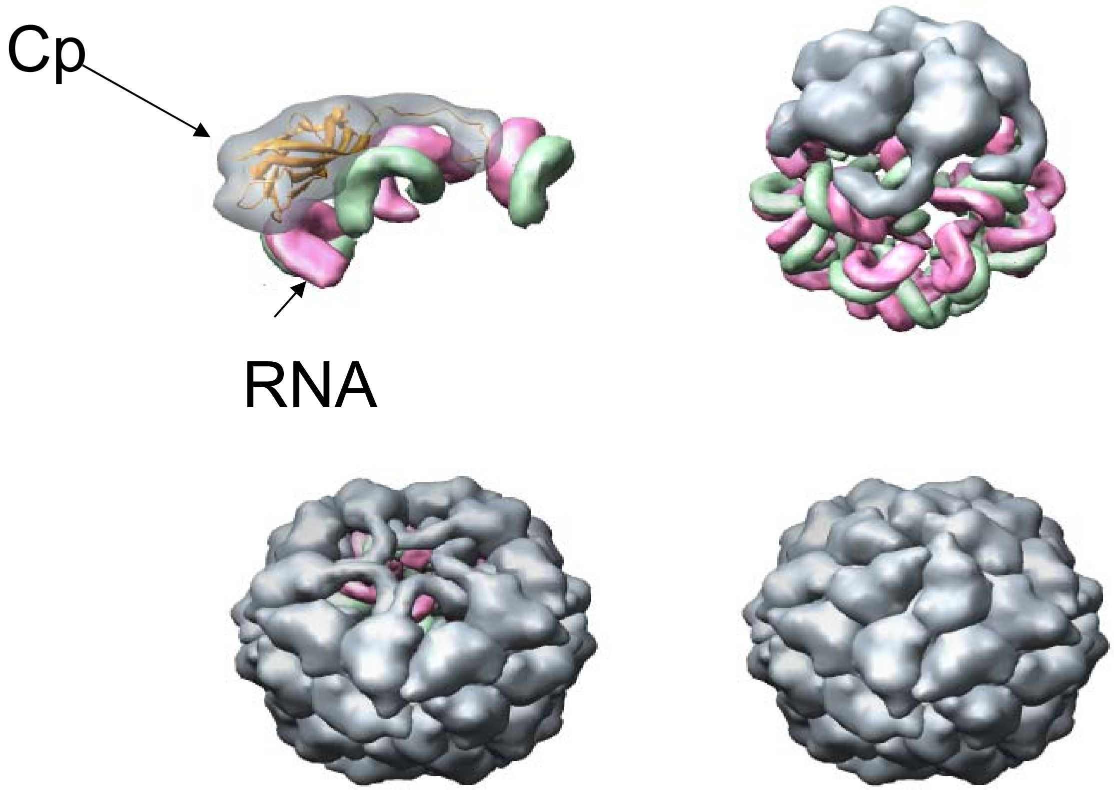
T= 7

T= 9

T= 16

T= 25

Assembly of Icosahedral Viruses



Brome mosaic virus

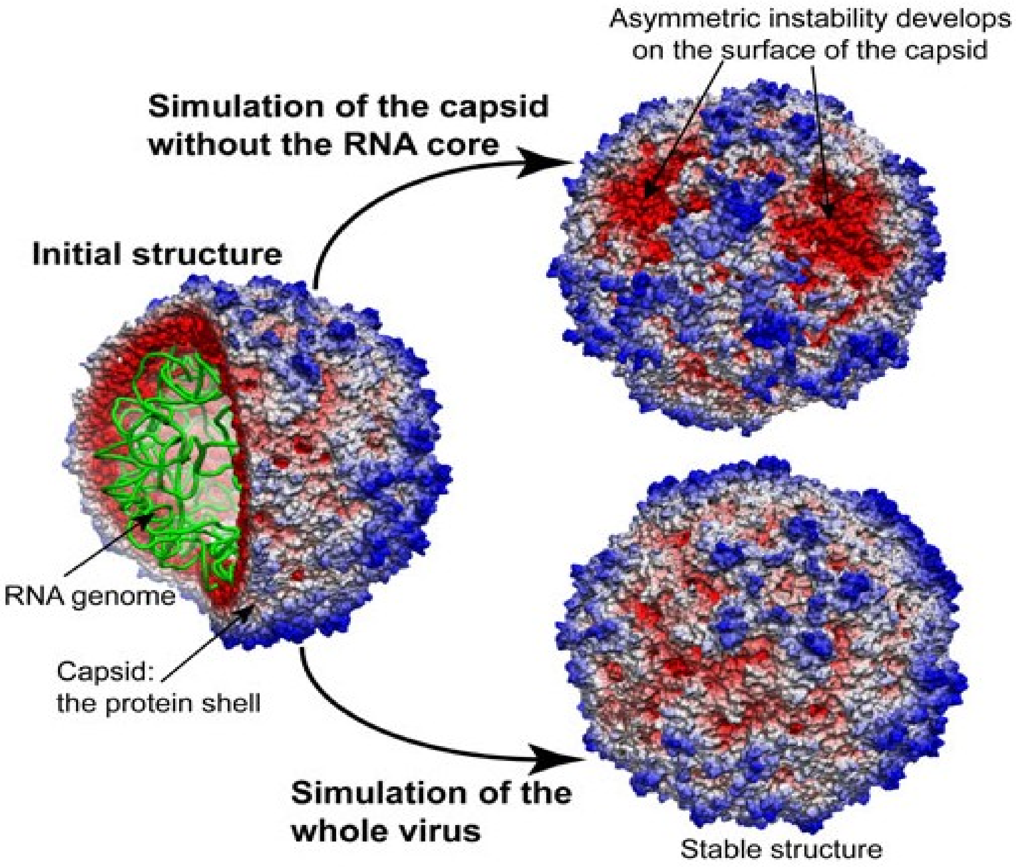
Assembly of some viruses is spontaneous and independent of host factors.

Host factors may help in assembly of other viruses

Assembly of Icosahedral Viruses

Nucleic acid is required by some viruses (not all)
for assembly and stability.

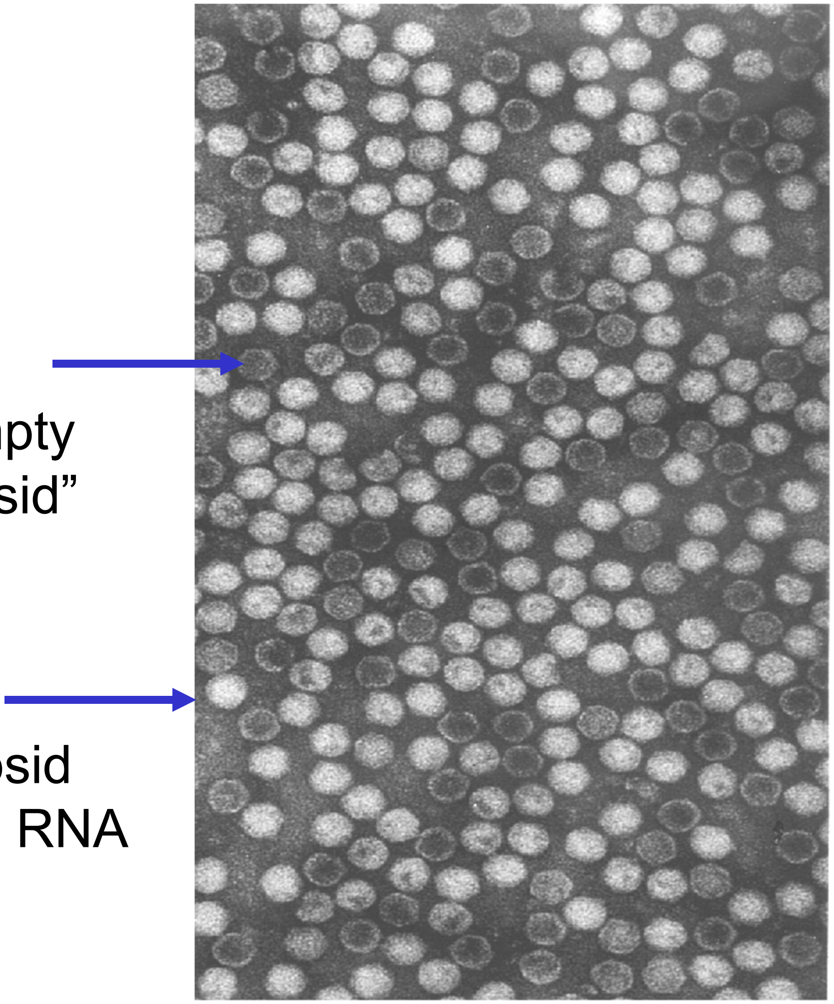
So the genome can be an important element in the structure of the virion



Freddolino PL, Arkhipov AS, Larson SB, McPherson A, Schulten K. Structure 2006: 14 (3): 437-449. Molecular dynamics simulations of the complete satellite tobacco mosaic virus.

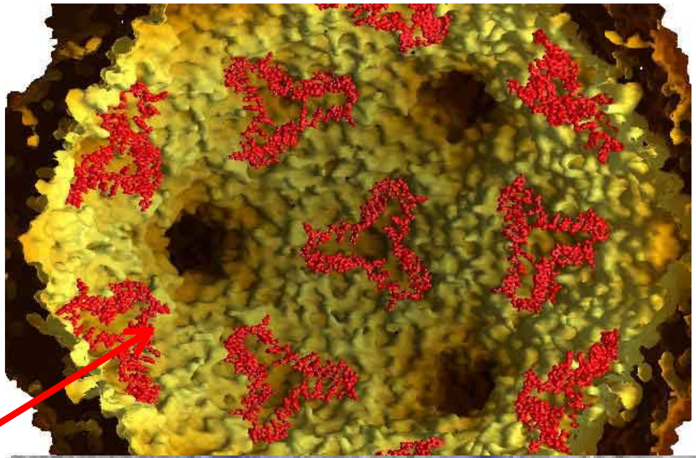
Nucleic Acid and Capsid Stability

If nucleic acid is not required then some viruses will produce “empty capsids”

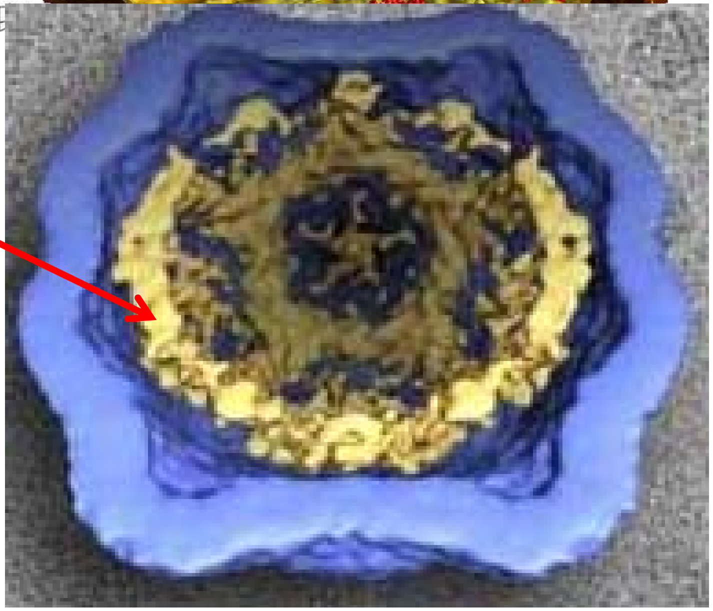


RNA takes a characteristic shape within the virion, a characteristic that varies among viruses

RNA

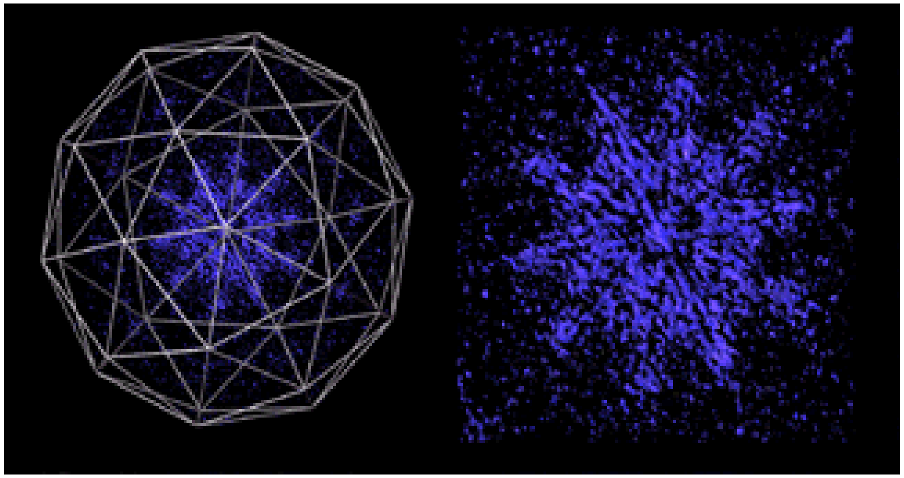


Bean pod mottle virus
Secoviridae, Comovirus



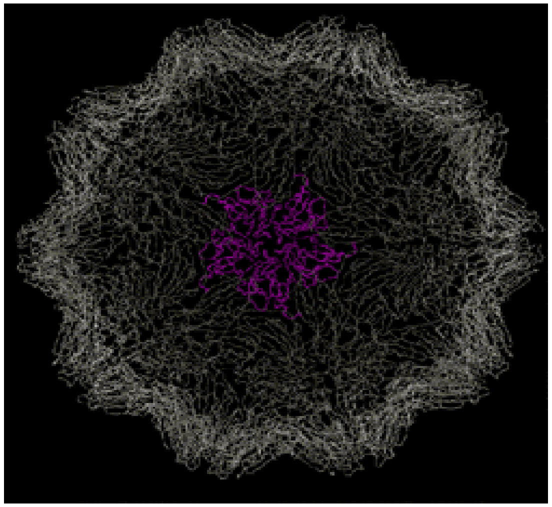
Cowpea mosaic virus
Secoviridae, Comovirus

Disassembly:



Inside Surface of TYMV

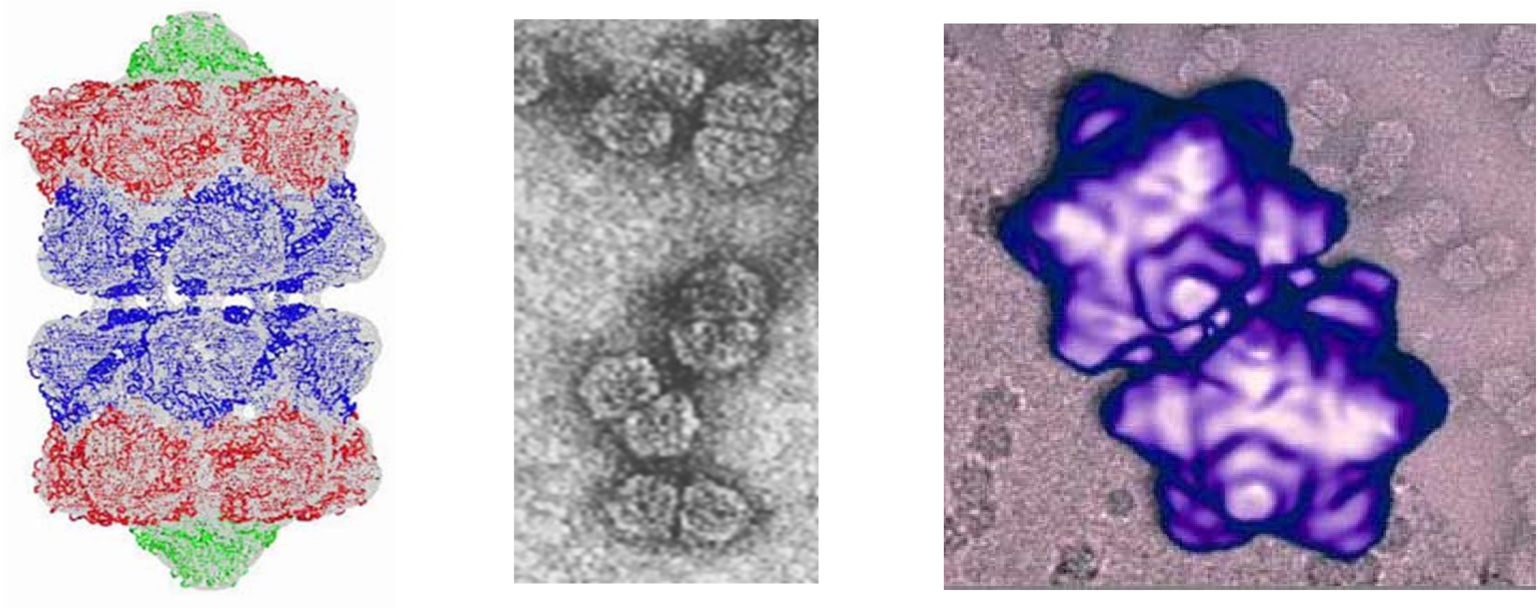
(Obtained by X-ray crystallography difference Fourier techniques and molecular averaging)



- Higher pH conditions trigger the RNA expansion which forces open the protein coat.
- RNA expands, forcing a pentamer out of the capsid. The RNA can then exit the virion and infect the cell.

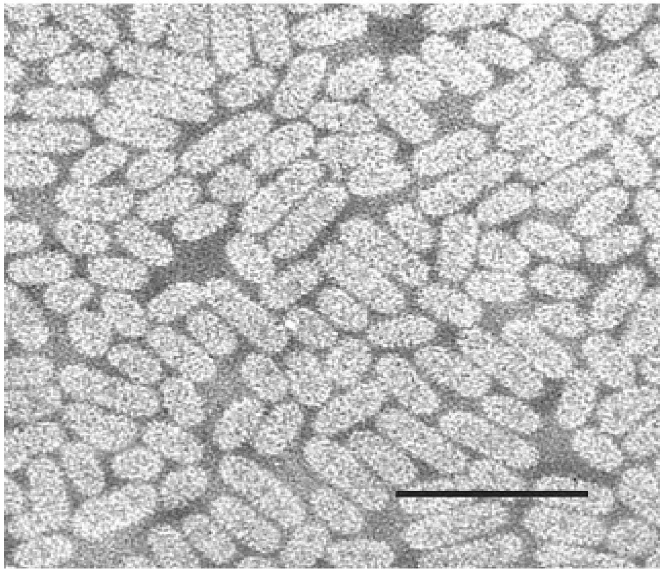
Variations in Icosahedral Morphologies

- *Geminiviridae* members, virions consist of a fused pair of isometric molecules.
- 110 copies of 28 kDa coat protein subunit



Variations in Icosahedral Morphologies

Viruses in the *Bromoviridae* genus *Alfamovirus* multipartite have a range of morphologies from isometric to bacilliform



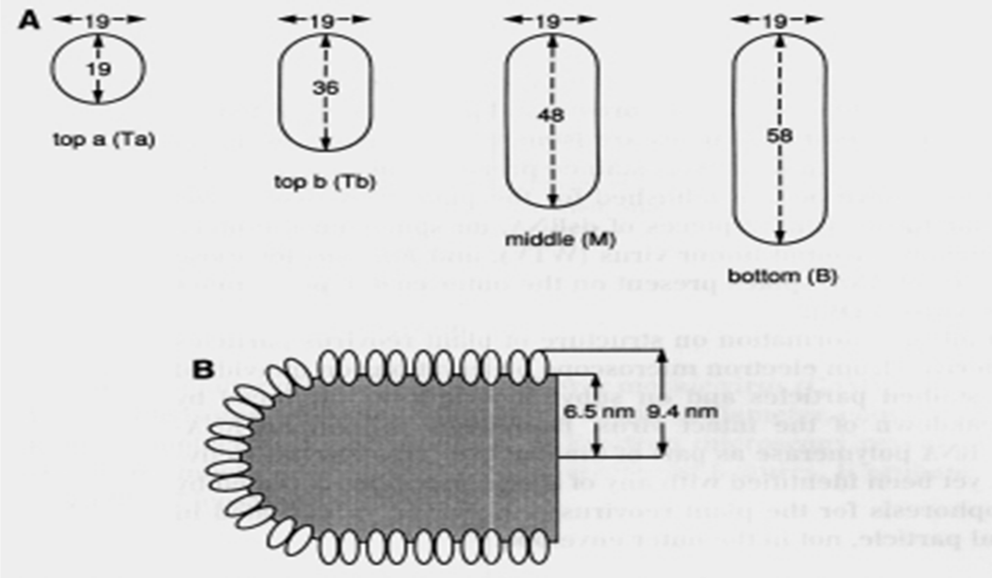
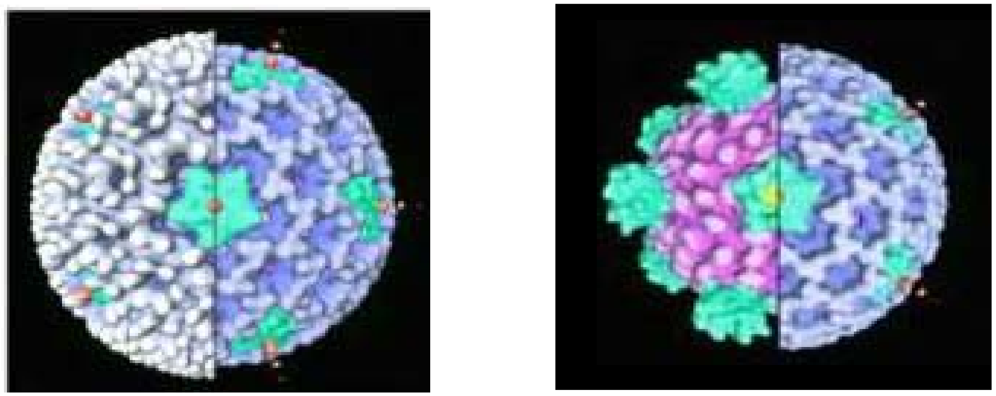


Figure 5.12 AMV particles. (A) Outlines for the four major classes of particle found in AMV preparations. Dimensions in nm. (B) A schematic representation of the distribution of protein and RNA in AMV B component. RNA is indicated by the hatched area; the protein molecules are represented by ellipsoids. The model is derived from an analysis by small-angle neutron scattering. (B) redrawn from Cusack *et al.* (1981).

Complex Icosahedral Virus Structure: Reovirus Particle Morphology

- Virus particles – have 2 or 3 shells
- Particle comprised of 8 or more coat proteins
- Well-studied T=13 structure
- two genera have ”spikes” at the 12 vertices of the icosahedra.

Example
of virion with
3 shells:



II. Enveloped Viruses:

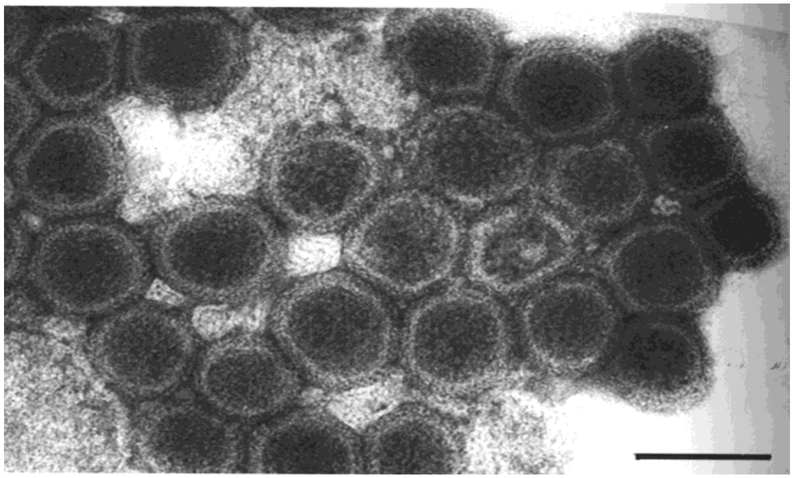
Pleomorphic Virion Morphology

- The envelope is a membrane that surrounds the nucleocapsid
- The envelope derived from the host membrane but may contain viral-coded proteins
- The plant viruses that have an envelope, replicate in insects.
- The envelope is an important part of their replication in insects.
- These viruses are thought to be insect viruses that made a big leap …. And adapted to replication in plants

Enveloped Viruses

Bacilliform

Pleomorphic



II. Enveloped Viruses

Pleomorphic Virion Morphology

Family - *Tospoviridae*

Genus - *Orthotospovirus*

(Ex. Tomato spotted wilt virus)

Envelope + ribonucleoprotein

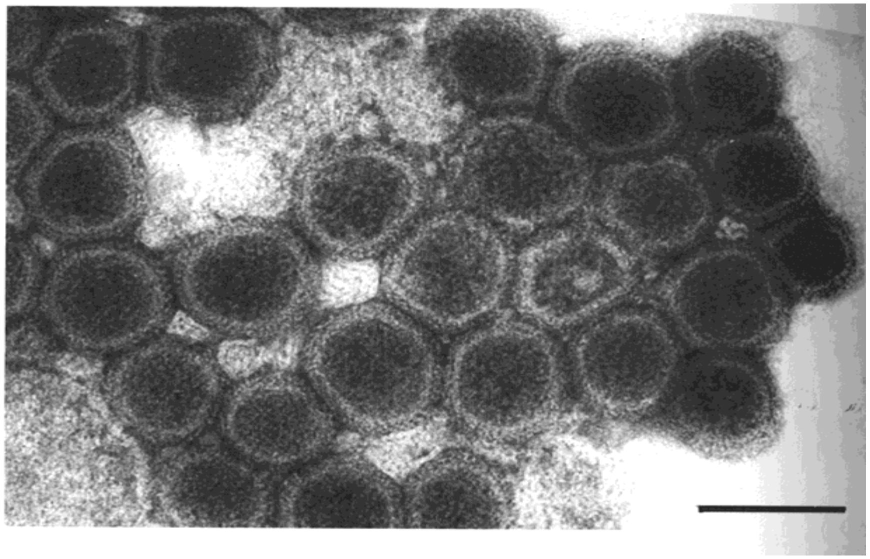


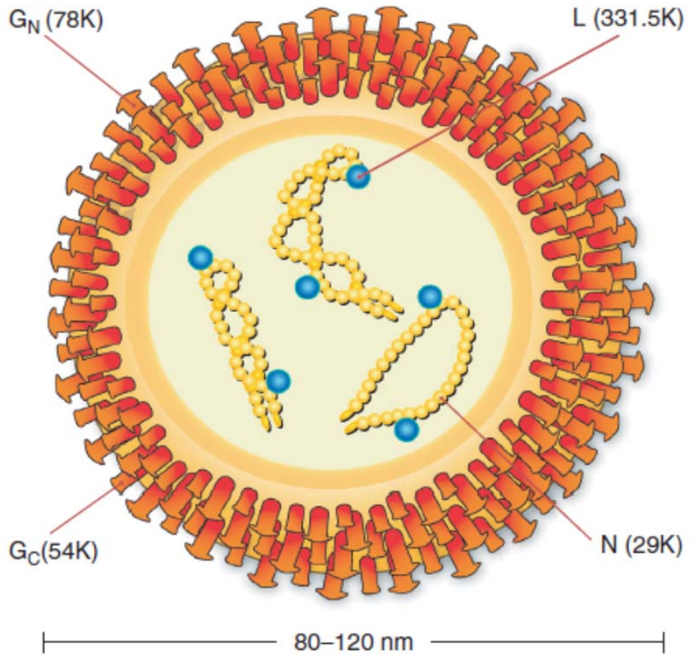
FIGURE 8
Electron micrograph of negatively stained particles of *Tomato spotted wilt virus* (TSWV). The bar represents 100 nm. (Courtesy of J. van Lent.)

The *Bunyaviridae* virus structure

Schematic drawing of a viral particle of TSWV.

A membrane enclosing nucleocapsids

A viral encoded N protein binds to all three segments of viral RNA (S, M, and L) forming ribonucleoproteins which are enclosed by the membrane .

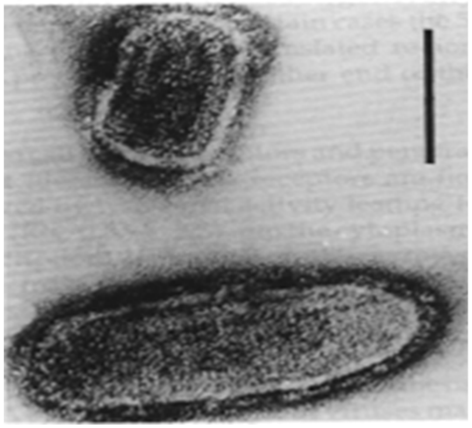


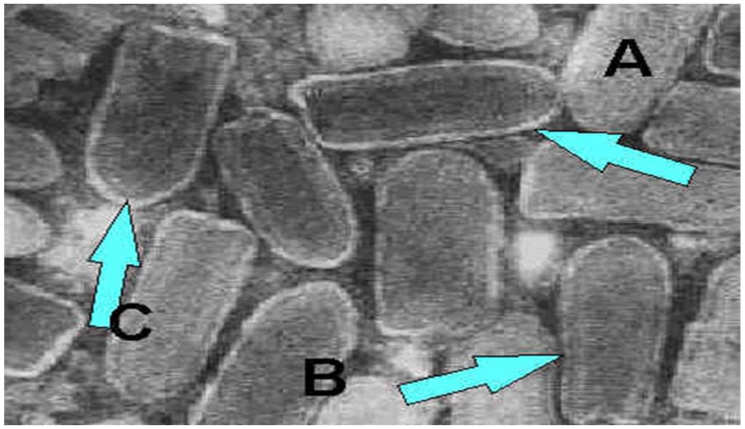
Sergei P. Boudko, Richard J. Kuhn and Michael G. Rossmann . 2007. The Coiled-coil Domain Structure of the Sin Nombre Virus Nucleocapsid Protein Journal of Molecular Biology 366(5): 1538-1544.

II. Enveloped Viruses: Bacilliform Virion Morphology

Family *Rhabdoviridae*, Genus *Cytorhabdovirus* and *Nucleorhabdovirus*

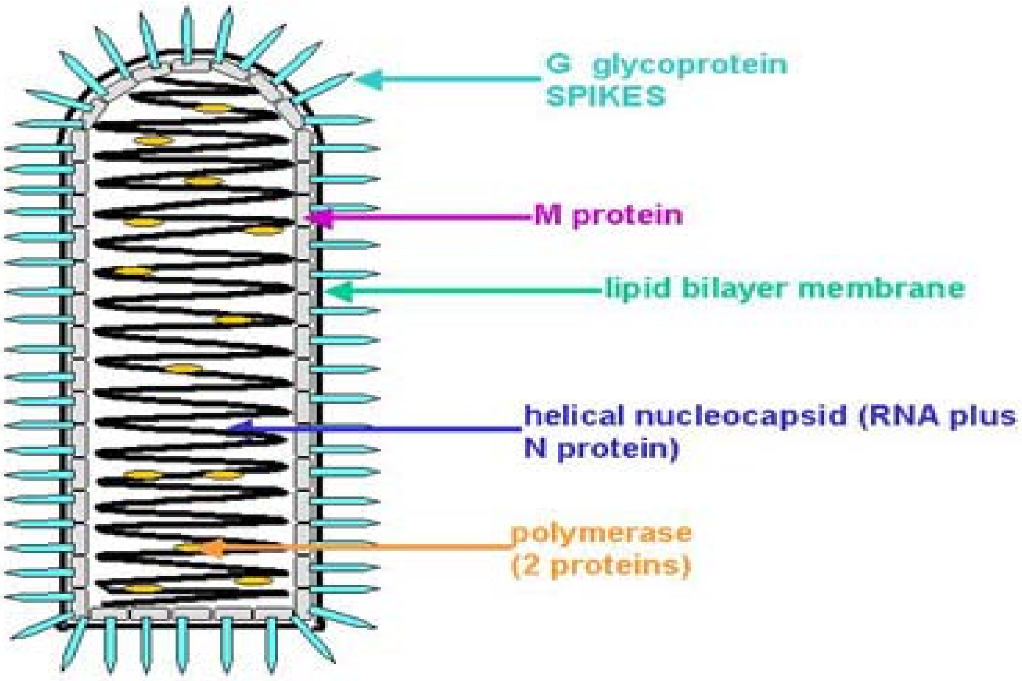
- 45-100 nm in dia. and 100-430 nm in length
- a lipid envelope (20-30% by weight)

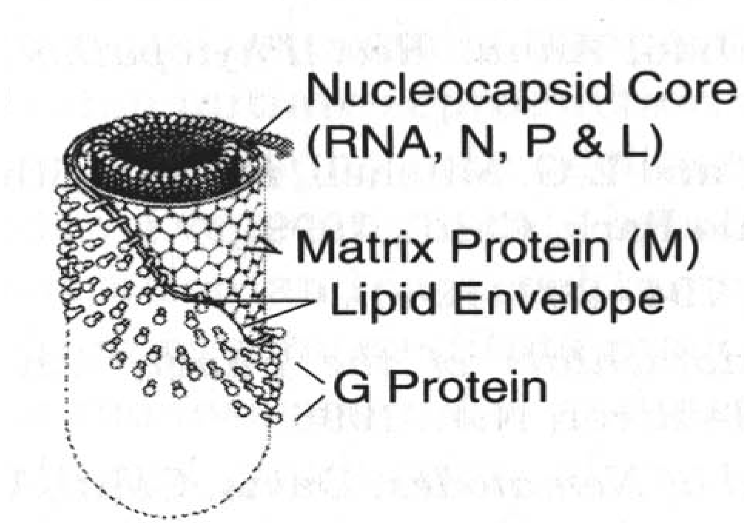




Genus Nucleorhabdovirus,
[*Sonchus yellow net virus*]

II. Enveloped Viruses:
Bacilliform Virion Morphology





Why do Virus Particles Use Subunit Construction?

♦ 1. Necessity

One triplet codon has a MW of ~1,000 and codes for an amino acid of average MW 150. So at most a nucleic acid can only code for 15% of its weight as a protein. As viruses are composed of 50-90% by weight protein there must be more than one protein molecule and subunit construction is essential.

◆ 2. Allows Self Assembly

For many viruses, the virion spontaneously forms when mixtures of purified coat protein and genomic RNA/DNA are incubated together.

◆ 3. Fidelity

DNA, RNA and protein synthesis are subject to error. By using a smaller protein (smaller gene), there is less chance of a fatal error occurring.

Why Subunit Construction? Con’t:

- ◆ **4. Economy**

The correct structure can be formed with a minimum of waste (if a subunit is synthesized or folded incorrectly, only a small unit has to be discarded)

- ◆ **5. Complexity**

The use of subunits allows for increases in size without compromising stability (due to multiple bonding sites between subunits).

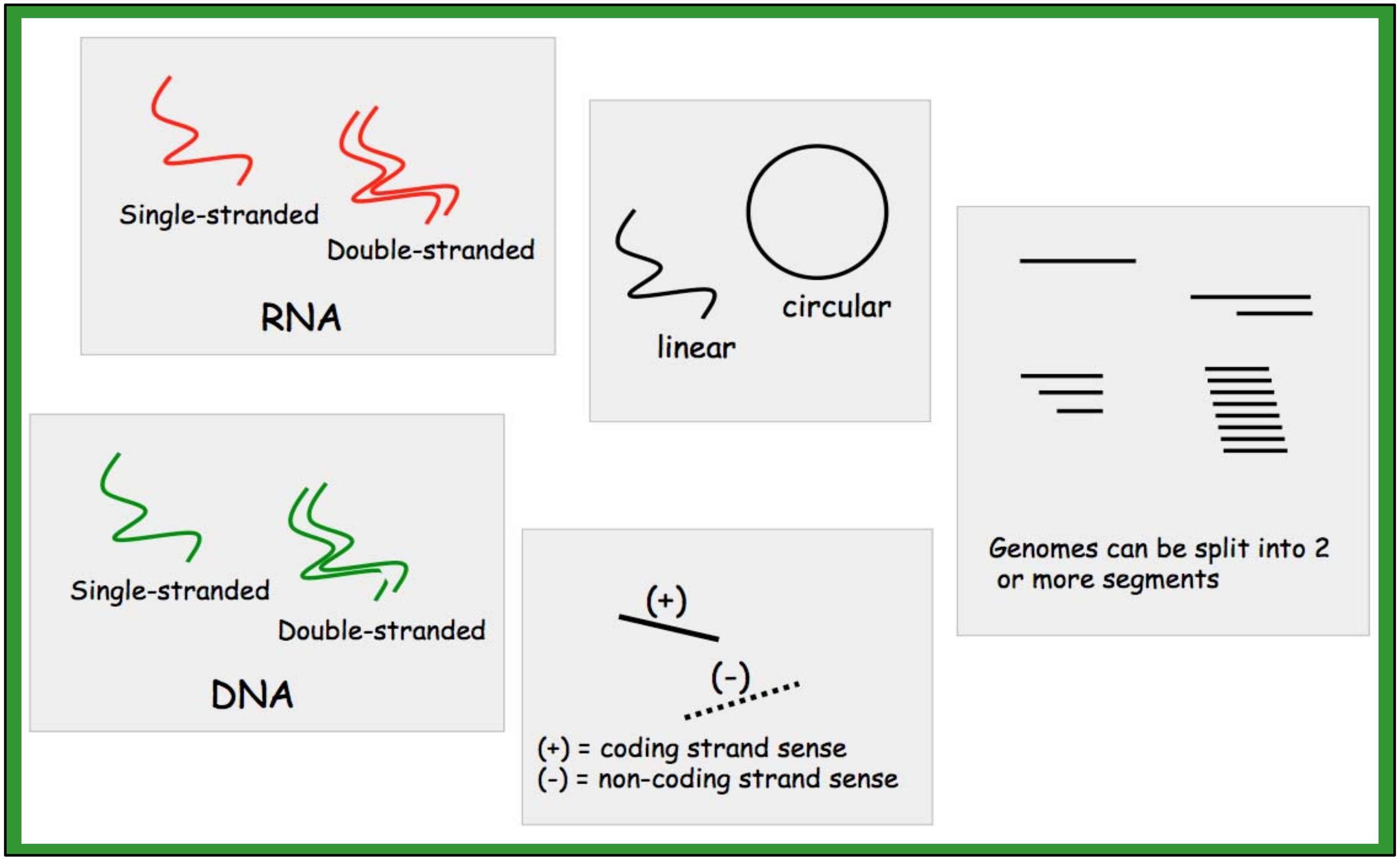
In general –
The larger the number of subunits - the more stable the virus.
The larger the virus particle - the bigger and more complex its genome.

STRUCTURE OF VIRIONS

↕

STRUCTURE OF VIRAL GENOMES

Genomes: Types of Nucleic Acid and Strandedness



Single-stranded

Double-stranded

RNA

linear

circular

Genomes can be split into 2 or more segments

Single-stranded

Double-stranded

DNA

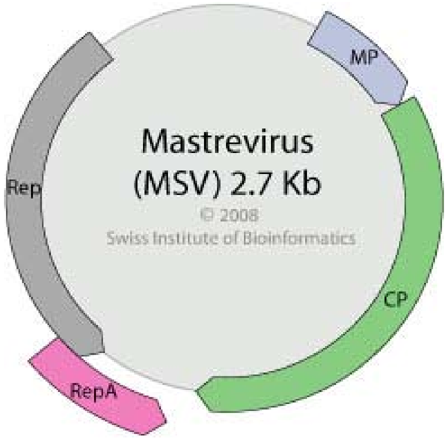
(+)

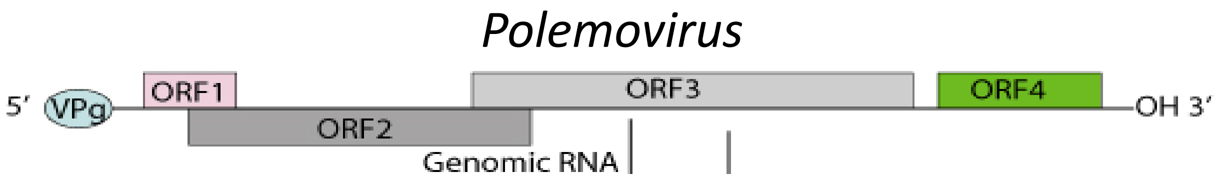
(-)

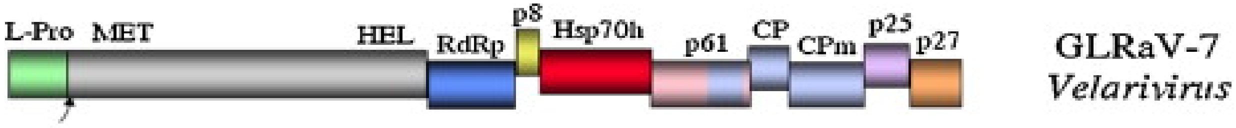
(+) = coding strand sense
(-) = non-coding strand sense

Properties of Plant Virus Virions
Types of Viral Genomes

Monopartite genome - viral genome contained in a single nucleic molecule.

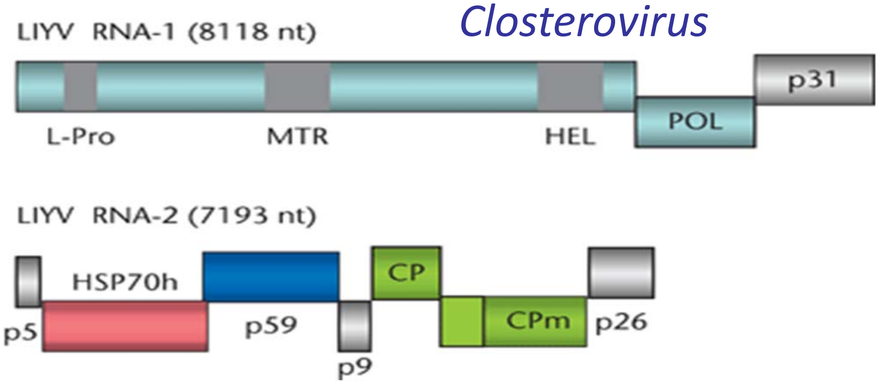


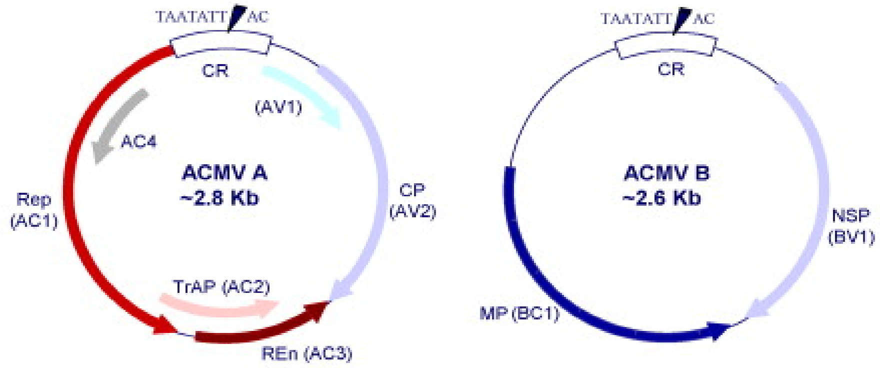




Multipartite genome - viral genome distributed over more than one molecule of nucleic acid

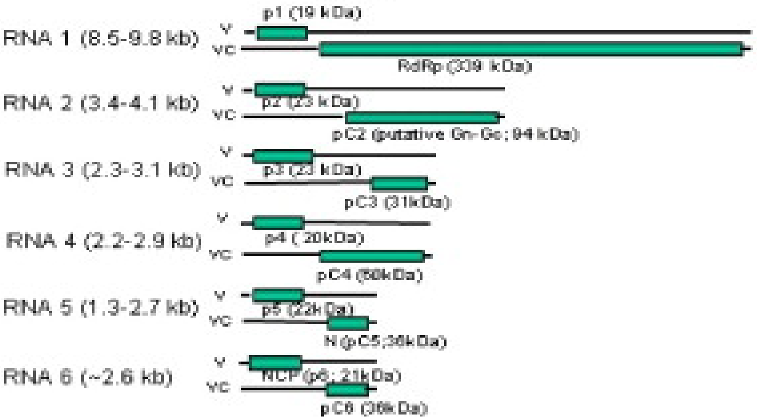
Closterovirus





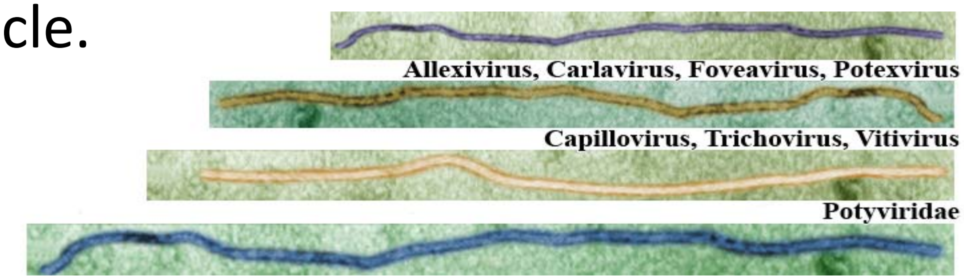
Bipartite Begomovirus

Tenuivirus
RGSV

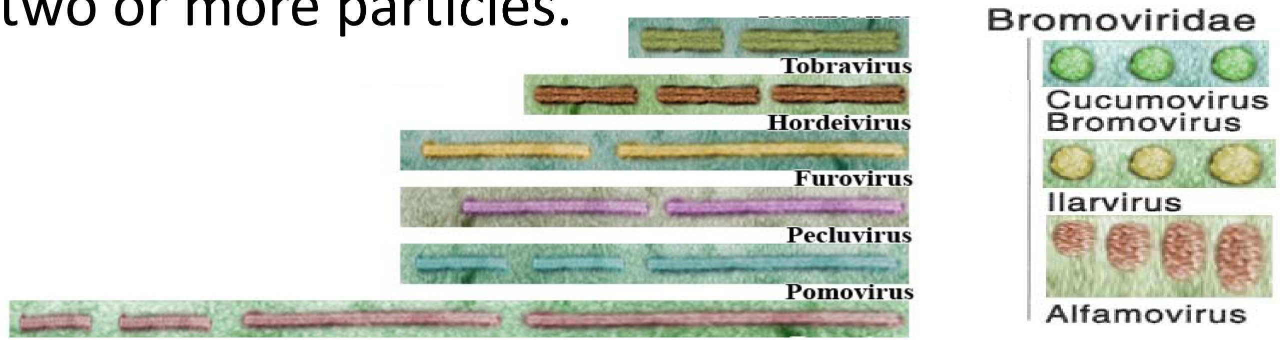


Properties of Plant Virus Virions

Monopartite virus - virus which has its genomic information within a single virus particle.



Multipartite virus - a virus with a multipartite genome which is divided between two or more particles.



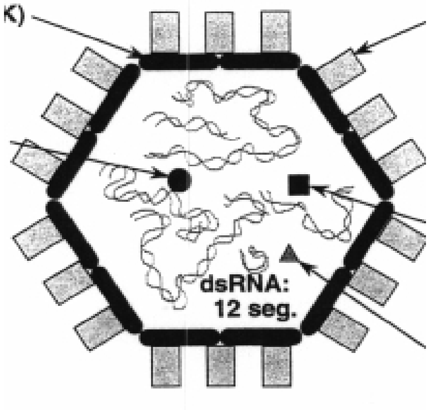
VIRAL GENOMES

Monopartite virus can have a multipartite genome

Reoviridae, Ex. Wound tumor virus: 10-12 strands of genomic RNA are encapsidated within a single virion. [Multipartite genome contained in a monopartite virion.]

Tospoviridae – 3 strands of genomic RNA are encapsidated within a single virion.

Tospoviridae

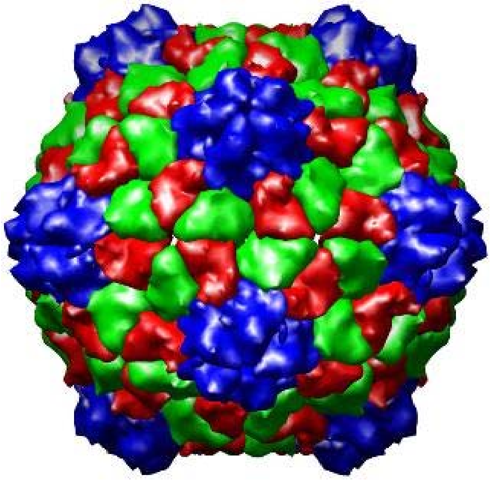


Phytoreovirus, Reoviridae

Why do we have such detailed images of virus structure?

Many applications of plant viruses in Nanotechnology

Viruses are Models and
Scaffolds for Nanotechnology



Nanotechnology:

Example:

Isolate a virus which has a high affinity for gold. Taking this virus and growing gold nanoparticles around it results in the gold nanoparticles being incorporated into the virus coat, resulting in a gold wire of precise length and shape with biological origins.

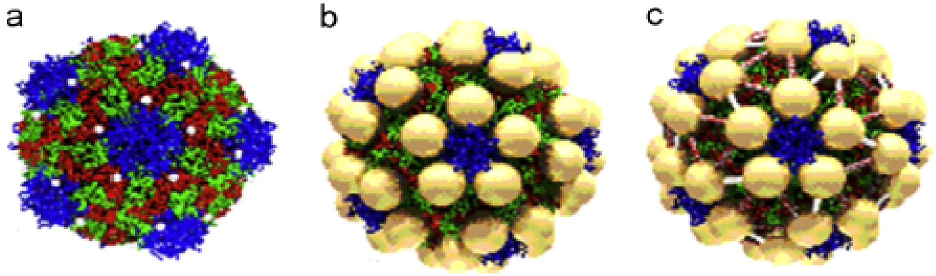


Fig. 1. CPMV genetically modified to surface display cysteine residues (shown in white) at defined sites (a). (b) Binding of gold nanoparticles to cysteine residues. (c) Networking of gold nanoparticles with di-thiolated Pt linkers. Adapted and reproduced in part from Blum et al. (2011); with permission from Elsevier.

Using this approach…

Using bacterial virus, M13, Scientists at MIT have created metallic wires, with the potential to be used for binding to exotic materials, self-assembly, liquid crystals, solar cells, batteries, fuel cells, and many other interesting areas.

https://www.youtube.com/watch?v=pUVrUIV4xu4

Virus-Enabled Synthesis and Assembly of Nanowires for Lithium Ion Battery Electrodes
Ki Tae Nam, *et al.*
Science **312**, 885 (2006);
DOI: 10.1126/science.1122716

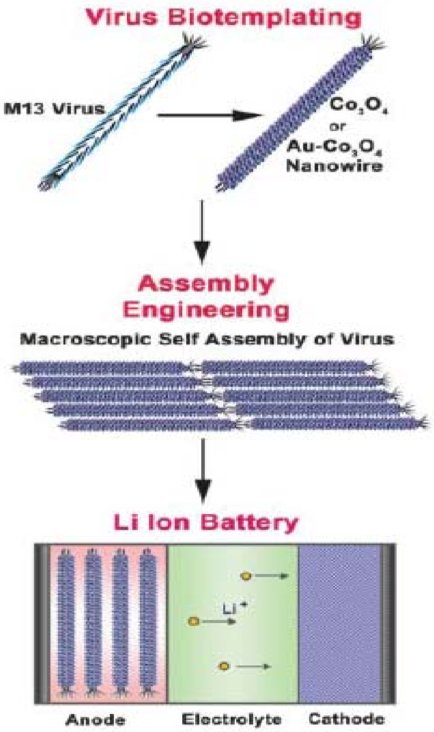


Fig. 1. Schematic diagram of the virus-enabled synthesis and assembly of nanowires as negative electrode materials for Li ion batteries. Rationally designed peptide and/or materials-specific peptides identified by biopanning were expressed on the major coat p8 proteins of M13 viruses to grow Co_3O_4 and $Au\text{-}Co_3O_4$ nanowires. Macroscopic ordering of the engineered viruses was used to fabricate an assembled monolayer of Co_3O_4 nanowires for flexible, lightweight Li ion batteries.

Nanotechnology:

Example.

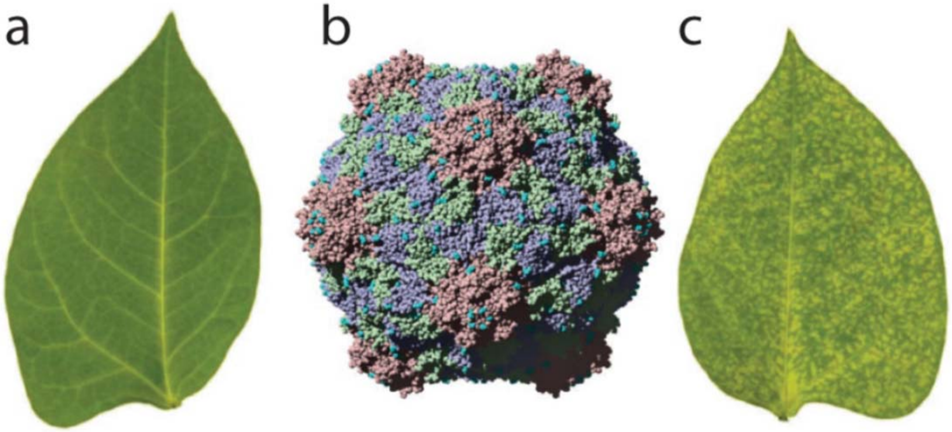
Select a virus with a known protein on its surface. The location of the code for this protein is in a known location in the DNA, and by randomizing that sequence it can create a phage library of millions of different viruses, each with a different protein expressed on its surface. By using natural selection, one can then find a particular strain of this virus which has a binding affinity for a given material.

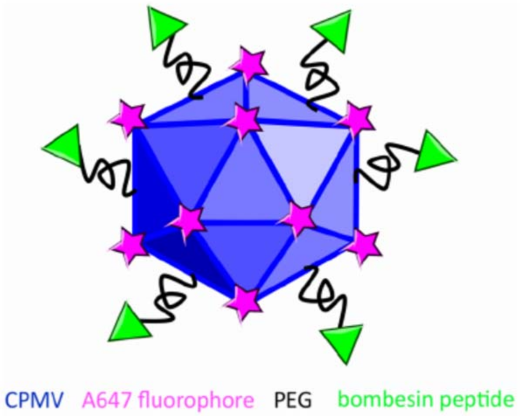
National Institute of Biomedical Imaging and Bioengineering

Creating Biomedical Technologies to Improve Health

Science Highlight: February 29, 2012

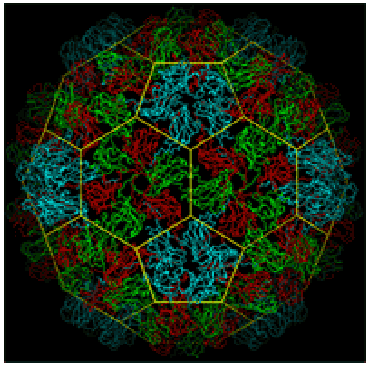
Harnessing Plant Viruses for Cancer Imaging and Therapy





To turn the cowpea mosaic virus (blue) into a nanoparticle for prostate cancer imaging, researchers attach different molecules—the fluorescent dye (pink stars) for visualization, polyethylene glycol (black squiggly lines) for stability, and bombesin (green triangle) for latching onto tumor cells.

VIRAL WORKHORSES



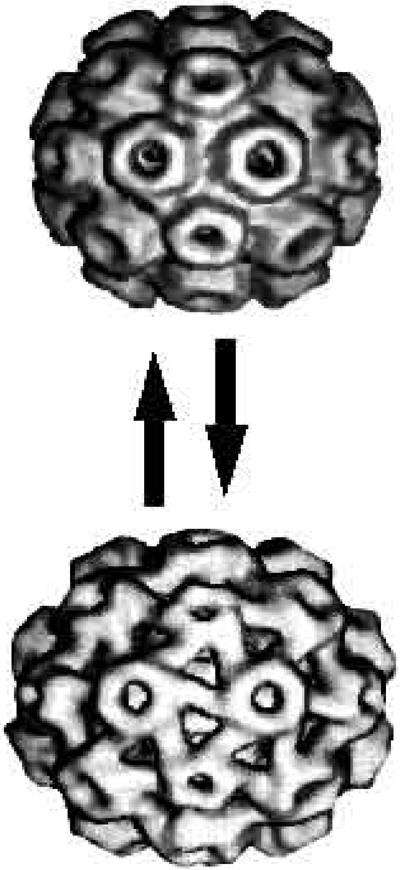
Emptied of their infectious nucleic acids, viruses make surprisingly adaptable tools for nanoengineers

By Anne M. Rosenthal

Scientific American Sept 02, 2002

A virus, essentially nucleic acid clothed in a protein coat, or capsid, is well designed for its lifestyle as a cellular parasite. Targeting, packaging and delivery have all been optimized over billions of years of evolution.

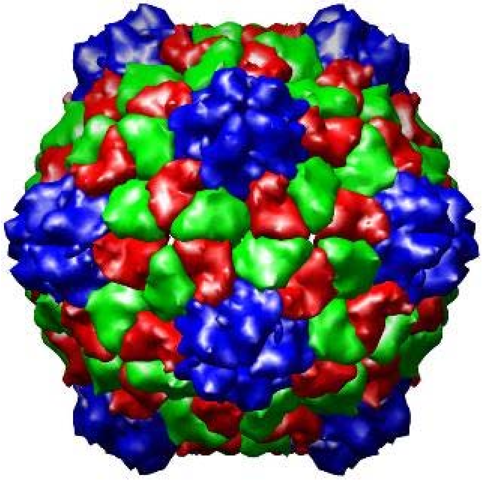
To search out target cells, the viral coat incorporates recognition and docking sites for specific cell types. To stabilize its negatively charged genetic package, a virus may carry a remarkably high positive charge on the capsid interior. And once it arrives at its destination, a virus delivers its genes into the interior of the targeted cell, where it usurps cellular machinery for viral purposes. Now researchers are taking advantage of these viral systems to develop clever nanotechnology applications in medical imaging and drug delivery, as well as new approaches to building electronic devices (see sidebar: "Viral Nanoassemblers for Electronics").



RECONSTRUCTED IMAGE from multiple electron micrographs shows the CCMV virus with pores shut (*top*) and open (*bottom*). Researchers can place materials inside the capsid, as well as remove them, through these pores.

Image: TIM BAKER, Purdue University, and MARK YOUNG, Montana State University, Bozeman

“Below is a picture of this *Cowpea mosaic virus* which has all the characteristics of an ideal nanoscaffold/building block. "It has a sphere-like structure of 28 nm diameter and its properties are defined. The virus particles can be obtained in gram scale from 1 kg of infected plant leaves. Amino acids on the exterior surface of the virus particle provide sites of attachment for a range of chemicals." (Credit for caption: JIC; credit for picture: UCSF Computer Graphics Laboratory)



“The virus … was employed as a "scaffold" on to which other chemicals were attached. By linking iron-containing compounds to the virus's surface, the John Innes Centre team was able to create electronically active nanoparticles. The researchers tell the journal *Small* that their work could be used in the future to make tiny electrical devices.”