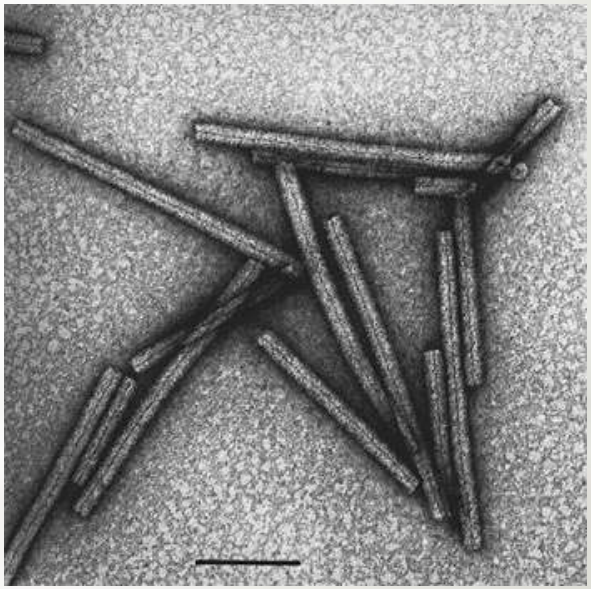
History of viruses

Louis Pasteur speculated about a pathogen “smaller than bacteria”

Chamberland developed a filter to remove bacteria

Dmitri Ivanovsky (1892) isolated an extract from tobacco plants which could infect other plants

Wendell Stanley (1935) visualized and crystallized “Tobacco Mosaic Virus”



Source: ICTVdB

History of viruses

Freidrich Loeffler and Paul Frosch (1898):
Foot-and-mouth disease in cattle caused by virus

Sources: Australian Govt. Dept. of Agriculture
VIPERdB

What are viruses?

Infectious, obligate intracellular parasite

Very small, diameter 20 – 300 nm
Exceptions, pandoravirus

Genome size 2 kb – 2 Mb

Infectious unit "virion"
Components: Nucleic acid, proteins, lipids (sometimes)

What are viruses?

Viruses are everywhere (literally)

Biomass of bacteriophages more than the biomass of elephants!

More viruses in 1 liter of ocean water than people on earth

Not all viruses are disease-causing agents

ICTV classification of viruses

Order (*-virales*)

Family (*-viridae*)

Subfamily (*-virinae*)

Genus (*-virus*)

Species (*-virus*)

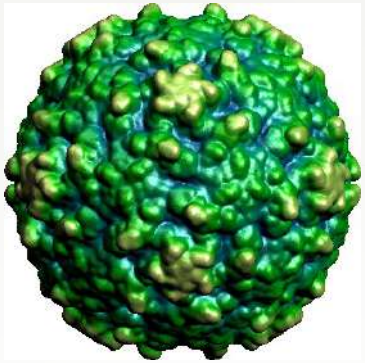
Seven orders - *Caudovirales*, *Herpesvirales*, *Ligamenvirales*, *Mononegavirales*, *Nidovirales*, *Picornavirales*, and *Tymovirales*

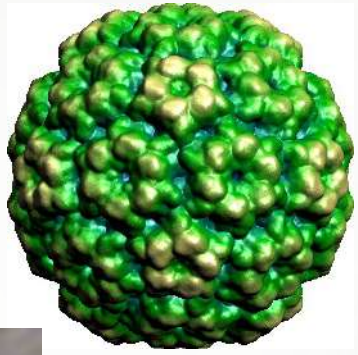
103 families, 22 subfamilies, 455 genera, 2827 species

Virus Types - Broad Classification

1) Host-specificity based

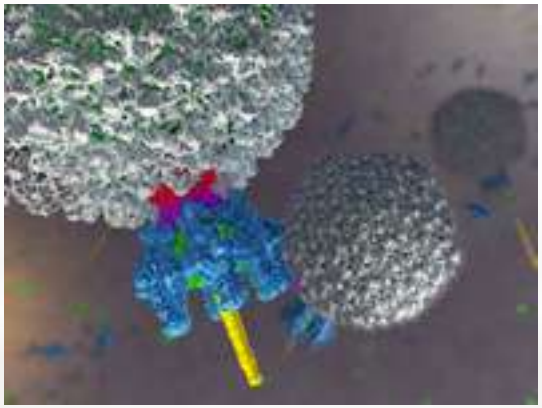
Animal viruses, e.g. poliovirus

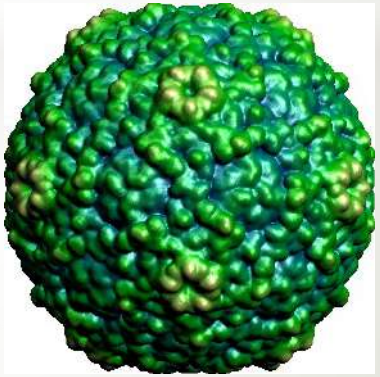




Plant viruses, e.g. cucumber mosaic virus

Bacteriophages, e.g. P22



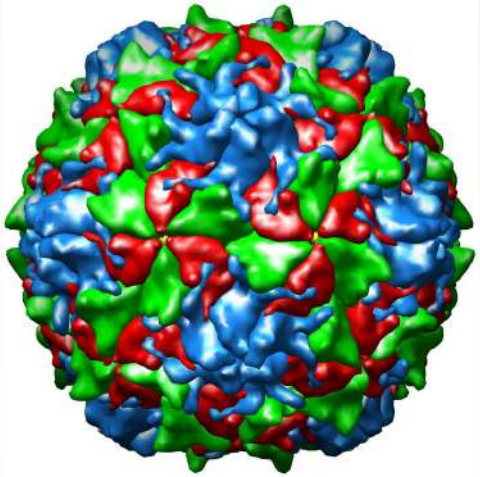


Insect viruses, e.g. Cricket paralysis virus

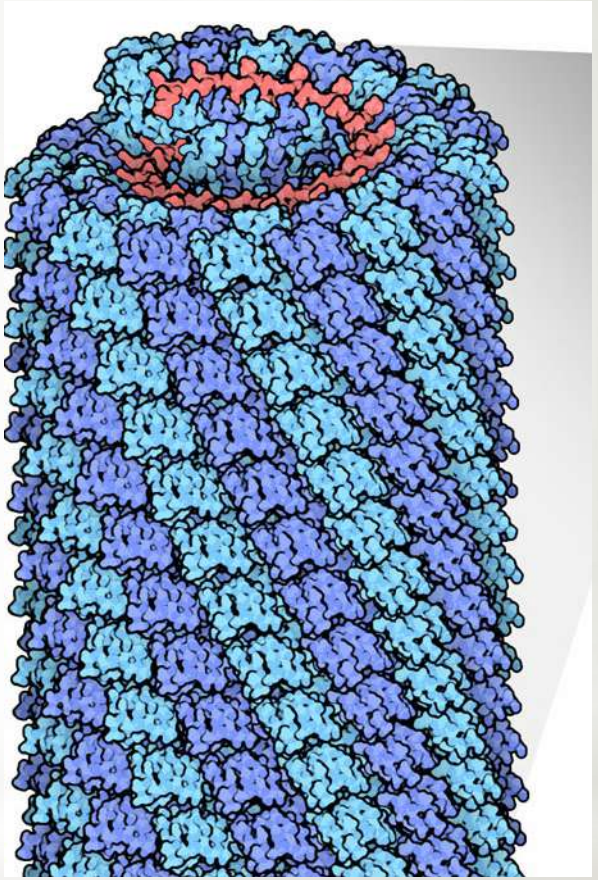
Sources: VIPERdb
Lander laboratory, TSRI

2) Shape-based

Icosahedral

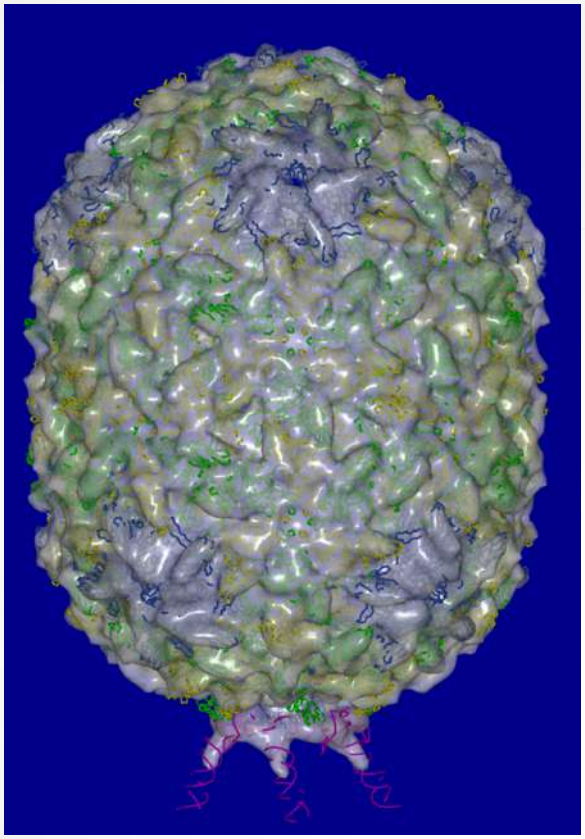


Helical

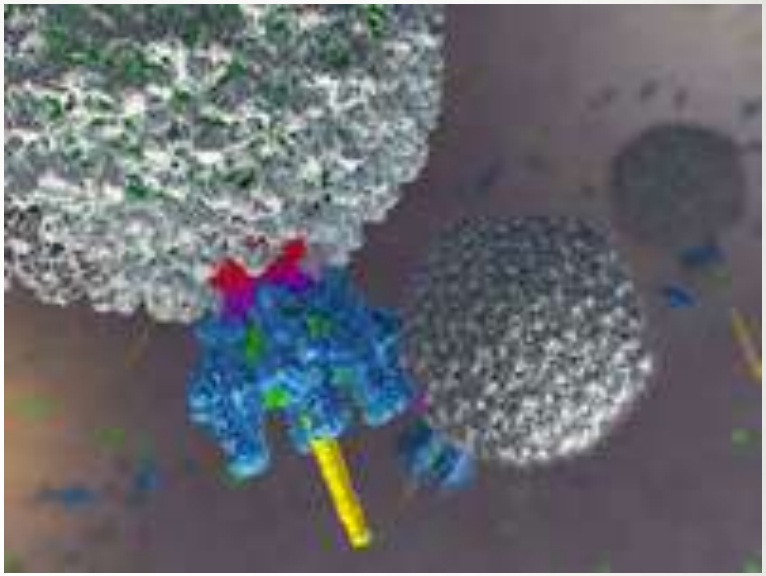


Sources: VIPERdb, PDB

Prolate

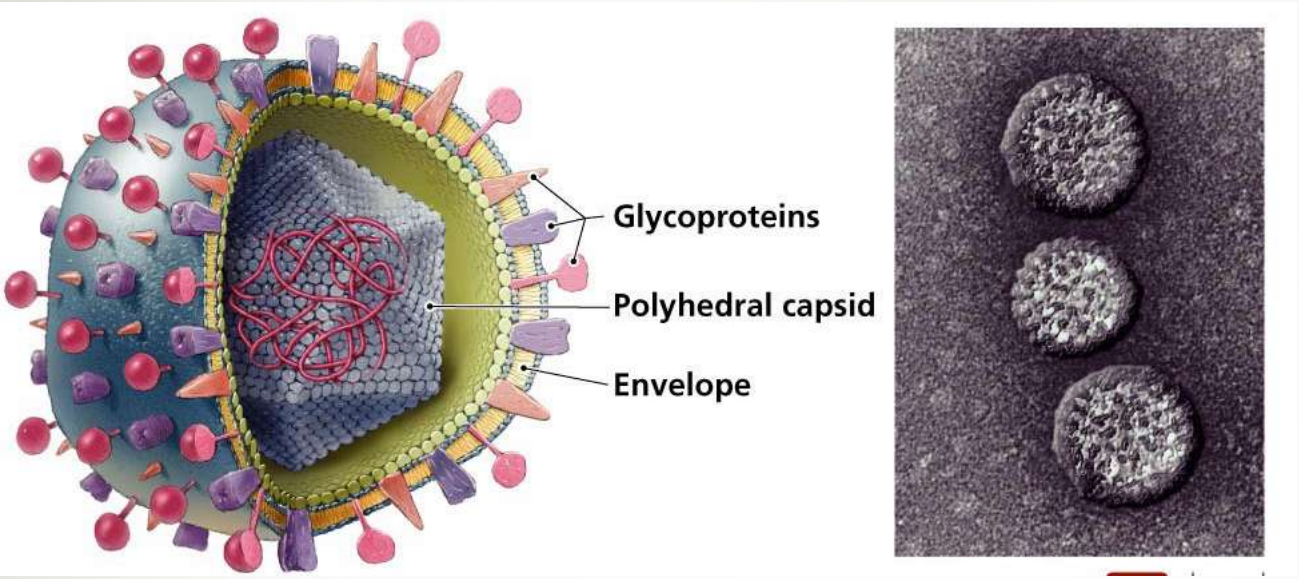


Complex structures

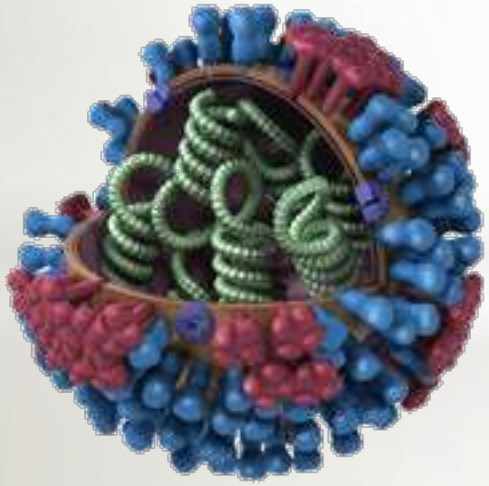


Sources: Morais *et al.*, Molecular Cell, 2005
Lander laboratory, TSRI

3) Lipid content based

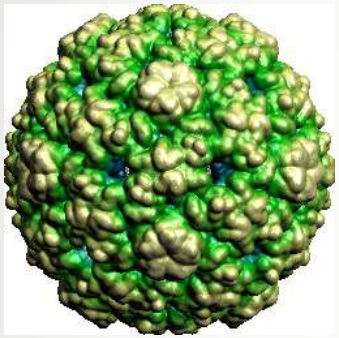


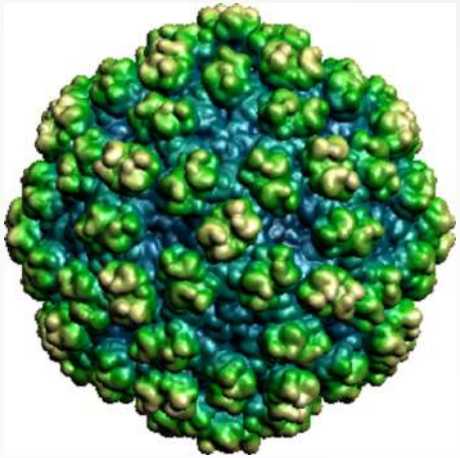
Enveloped

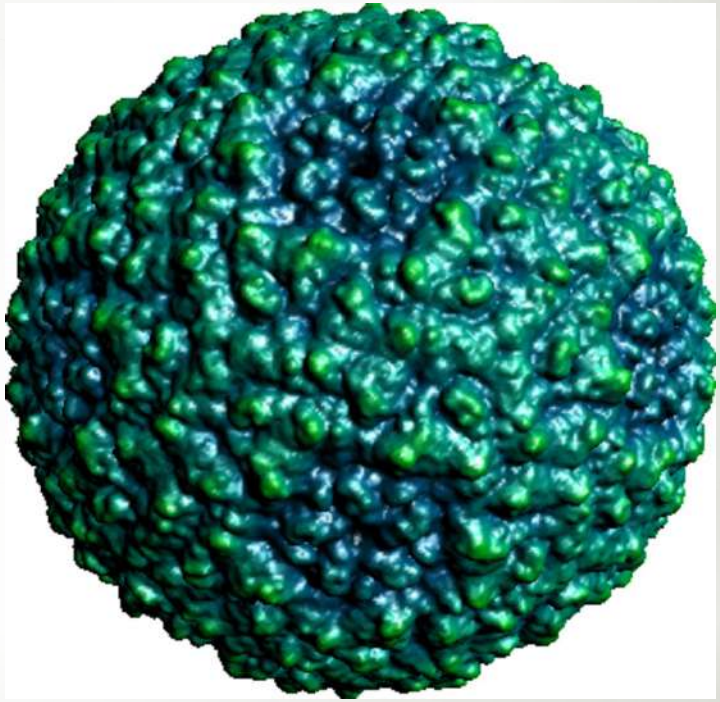


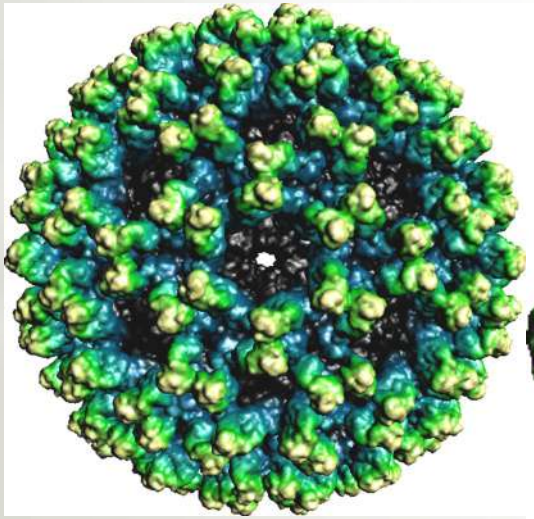
Sourses: CDC
Linda M. Stannard, University of Cape Town
Pearson Education Inc

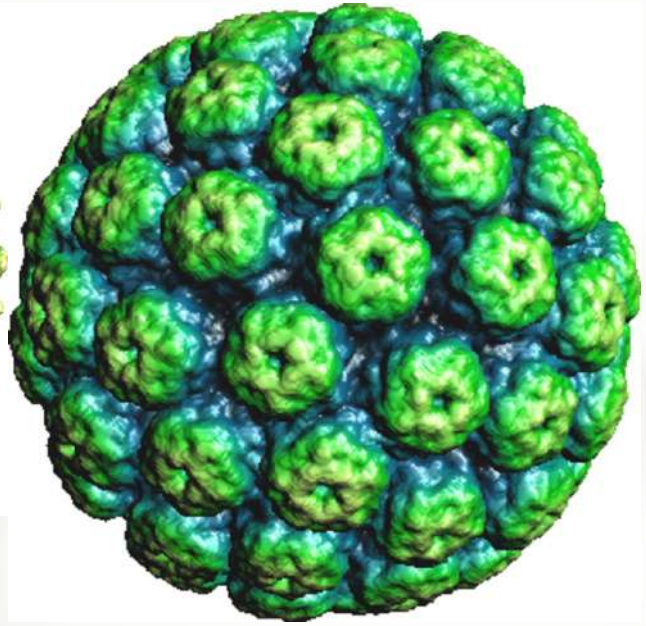
Non-enveloped











Sources: VIPERdb

4) Genome content based

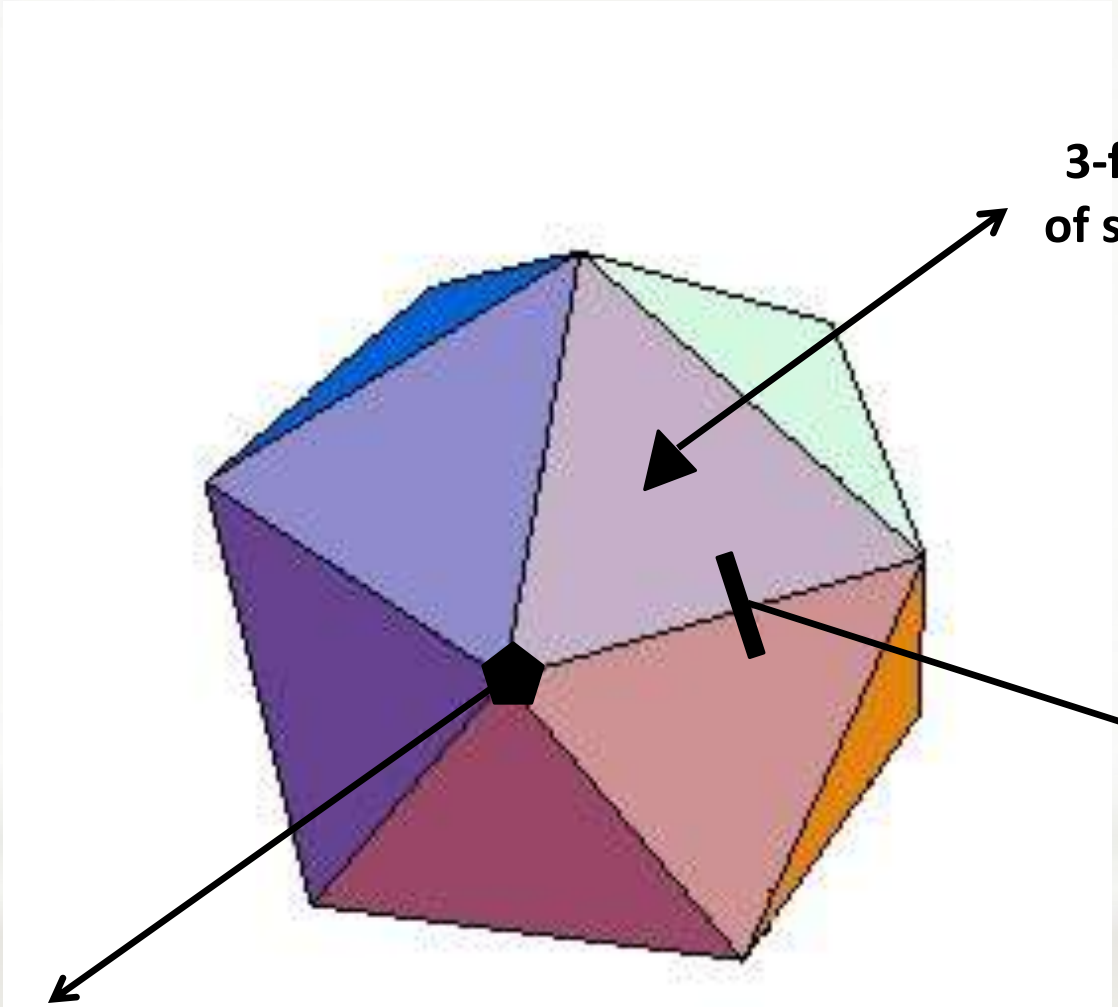
RNA viruses
DNA viruses

Single stranded (positive or negative sense), double stranded, segmented

RNA viruses accumulate more mutations

DNA viruses have larger genomes

Symmetry of an icosahedron



12 5-folds, 20 3-folds and 30 2-folds

Construction of complex viruses

What if we want to build a bigger virus?

How to regularly arrange > 60 subunits??

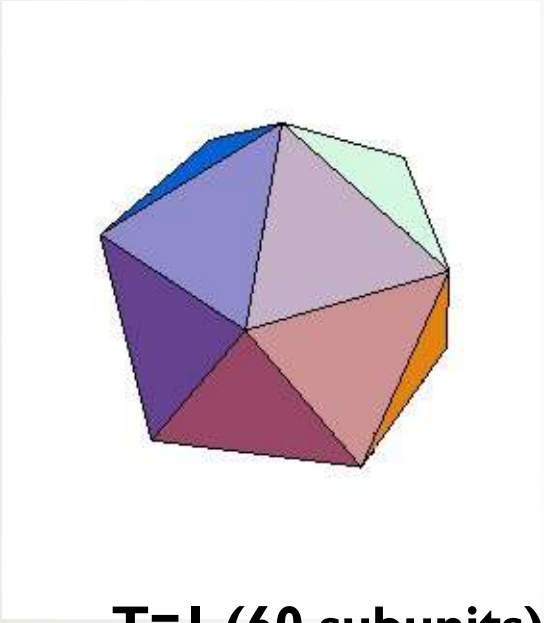
The basic triangular facet of the icosahedron has to be first enlarged and then subdivided into smaller triangles.

Triangulation dictated by the equation:

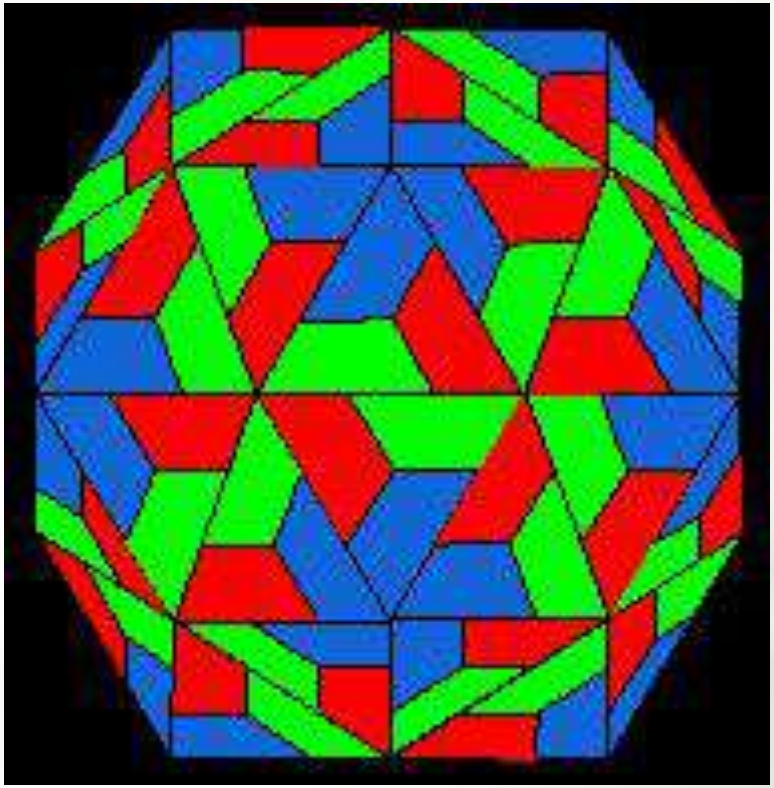
$T=h^2+hk+k^2$

where T is **the triangulation number**, and h and k are 0 or positive integers

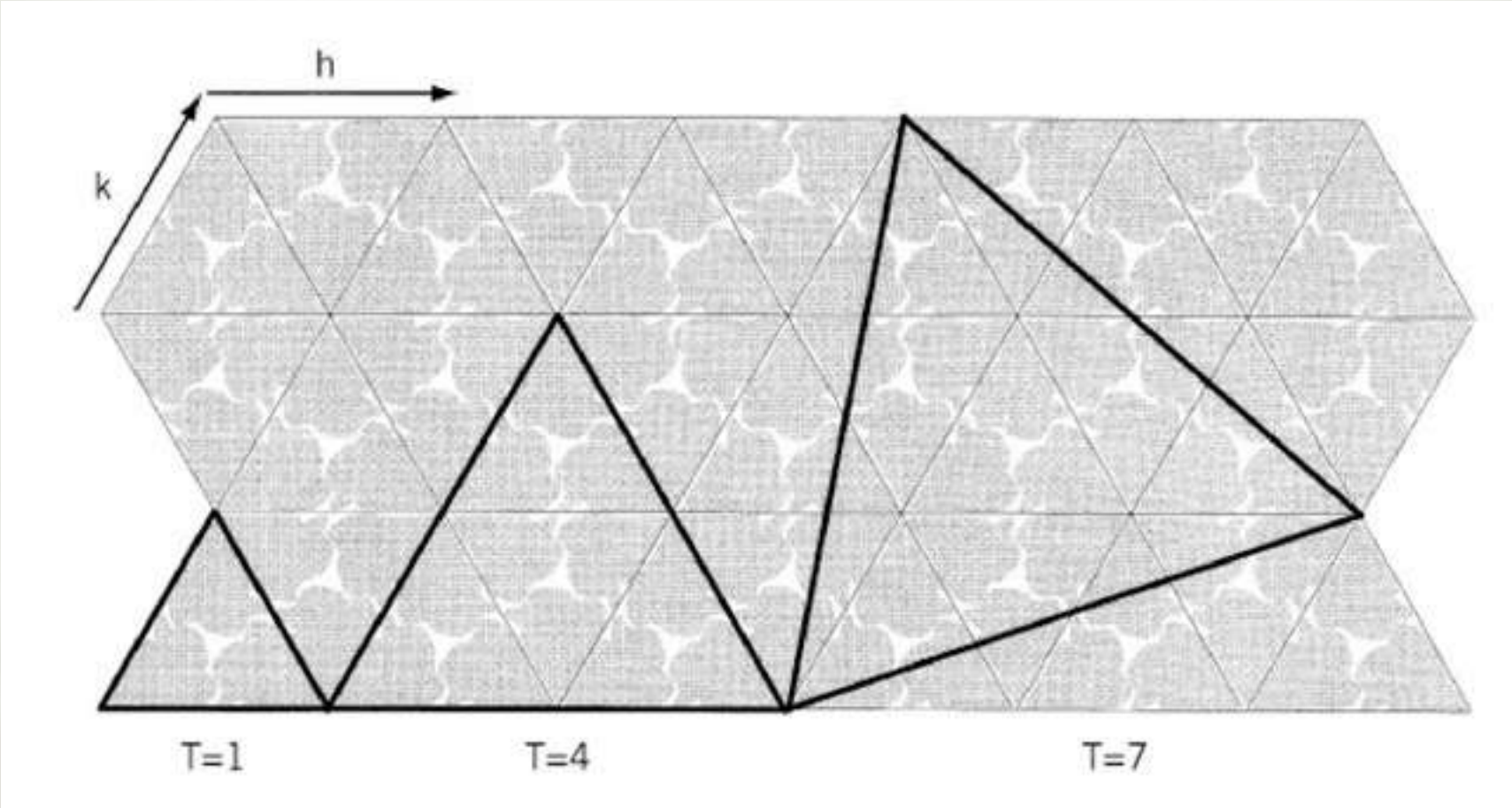
Construction of complex viruses



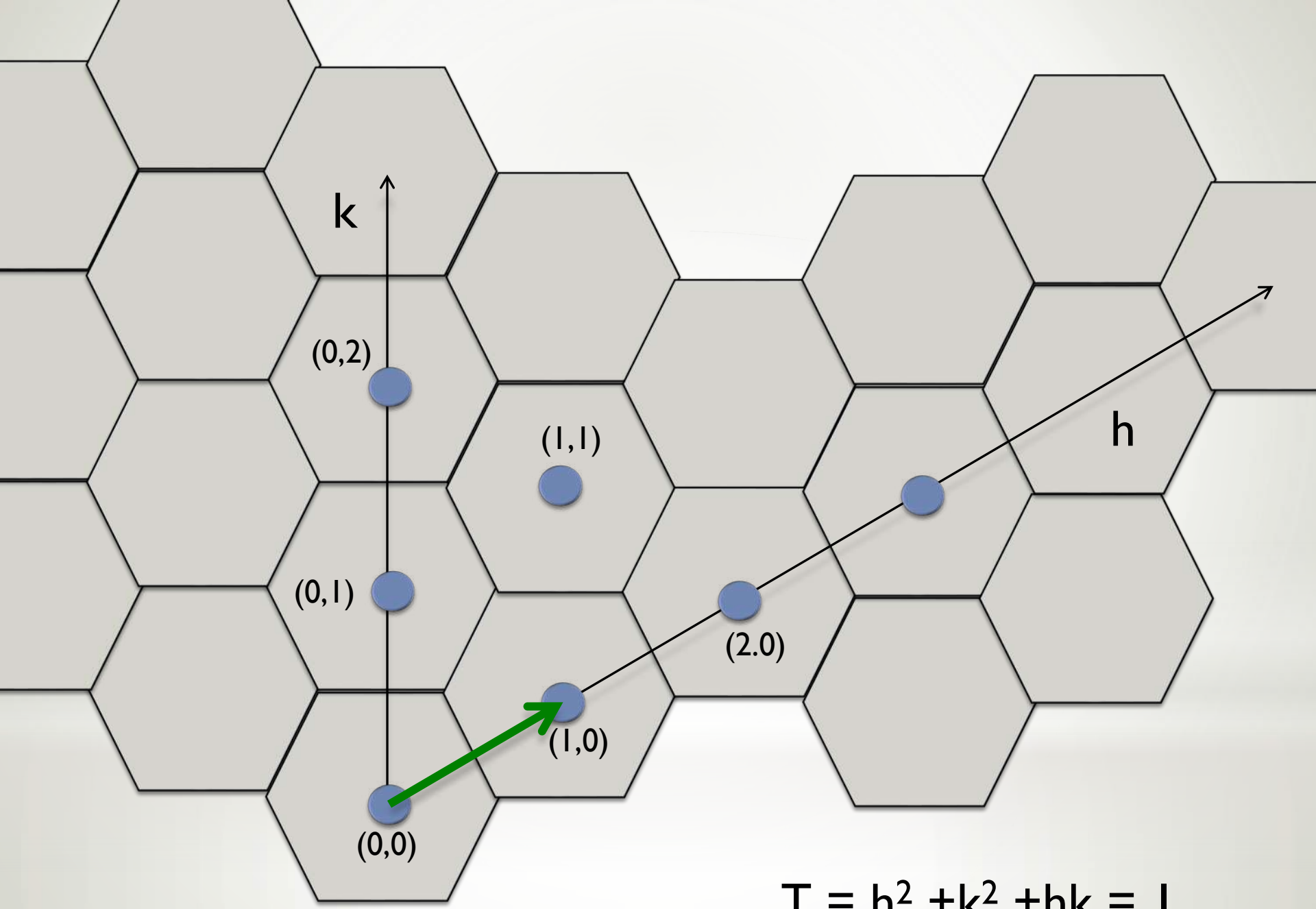
T=1 (60 subunits)



T=3 (180 subunits)

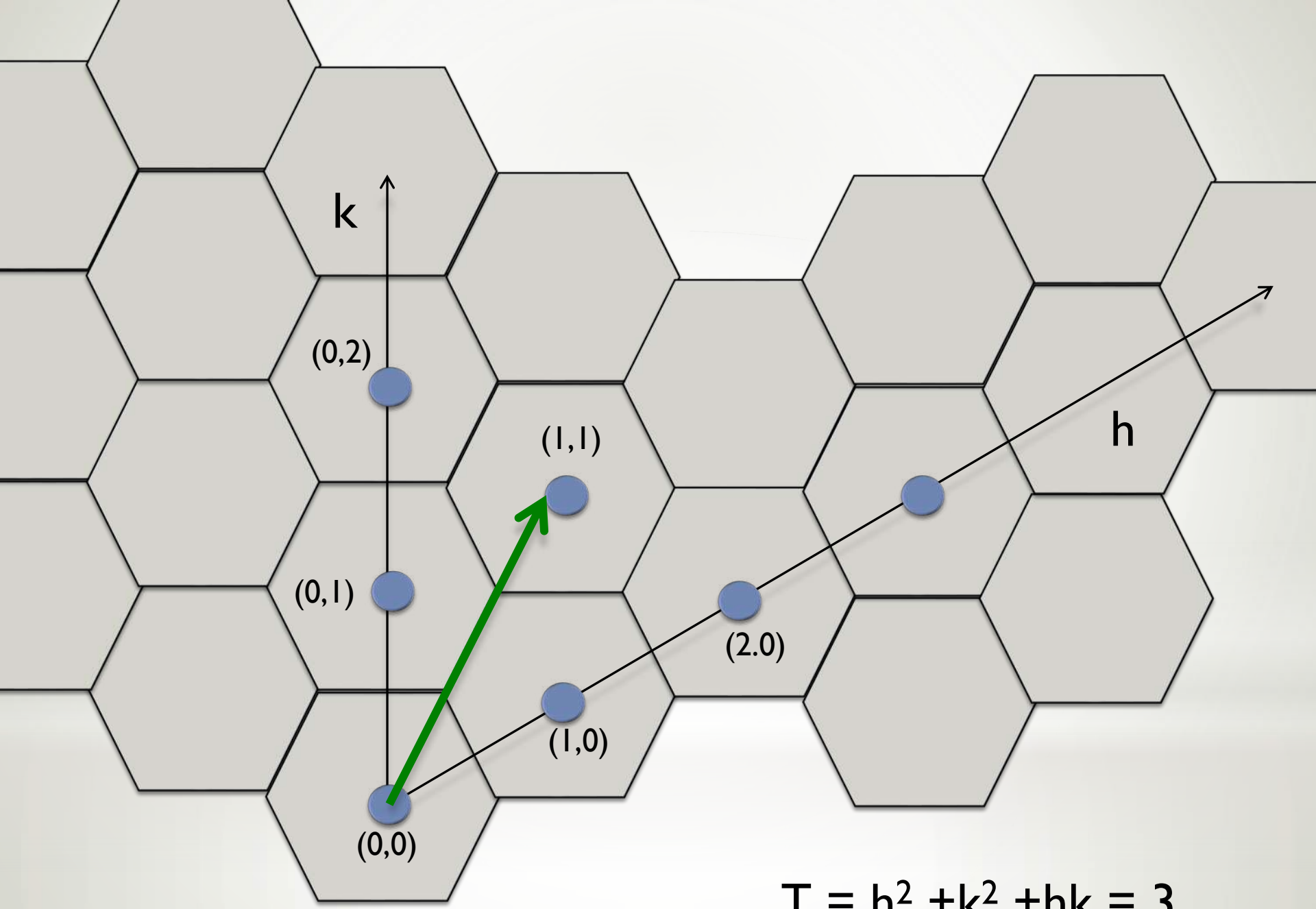


http://what-when-how.com/molecular-biology/capsids-viral-molecular-biology/



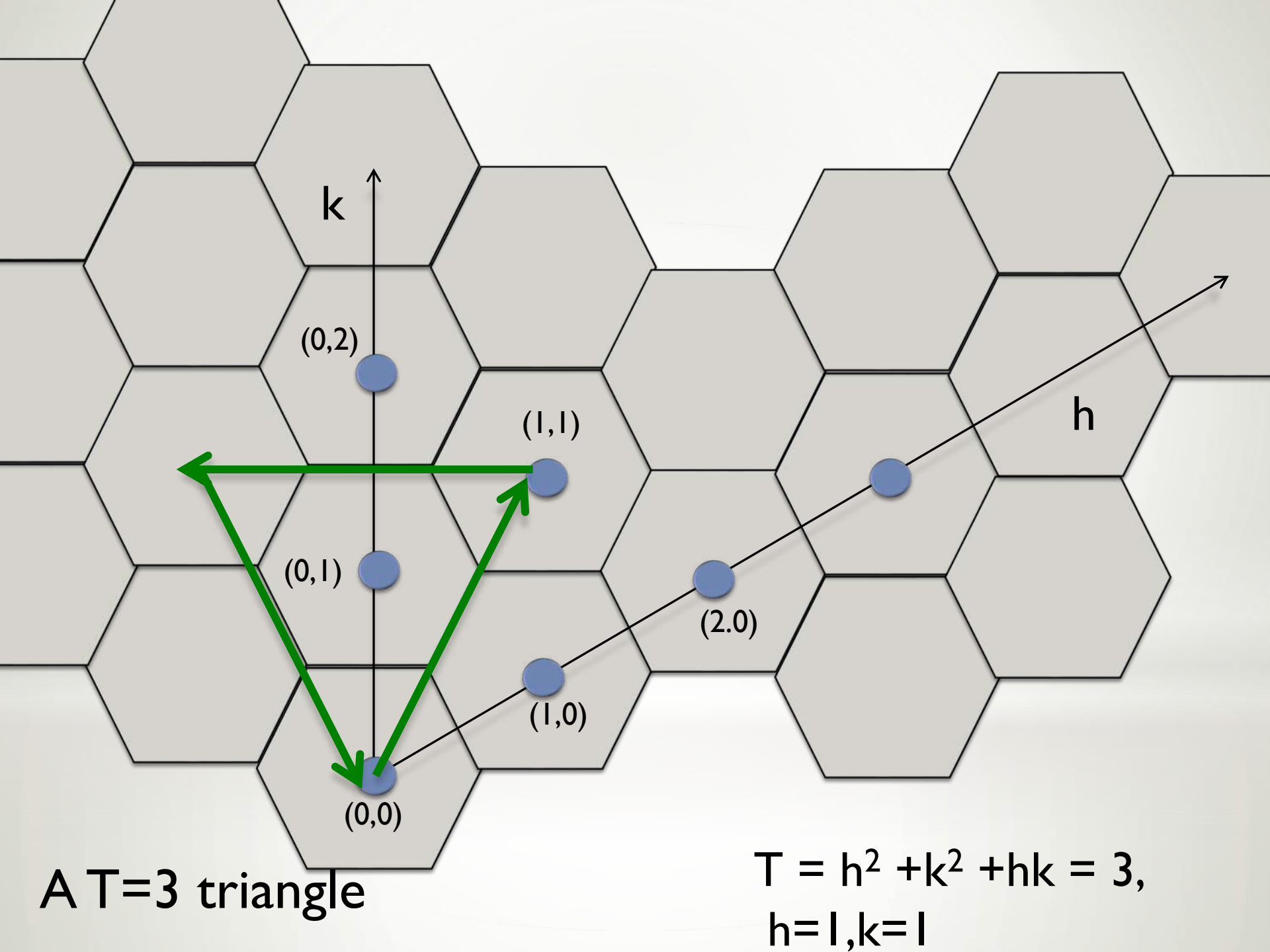
$T = h^2 + k^2 + hk = 1,$

$h=1, k=0$



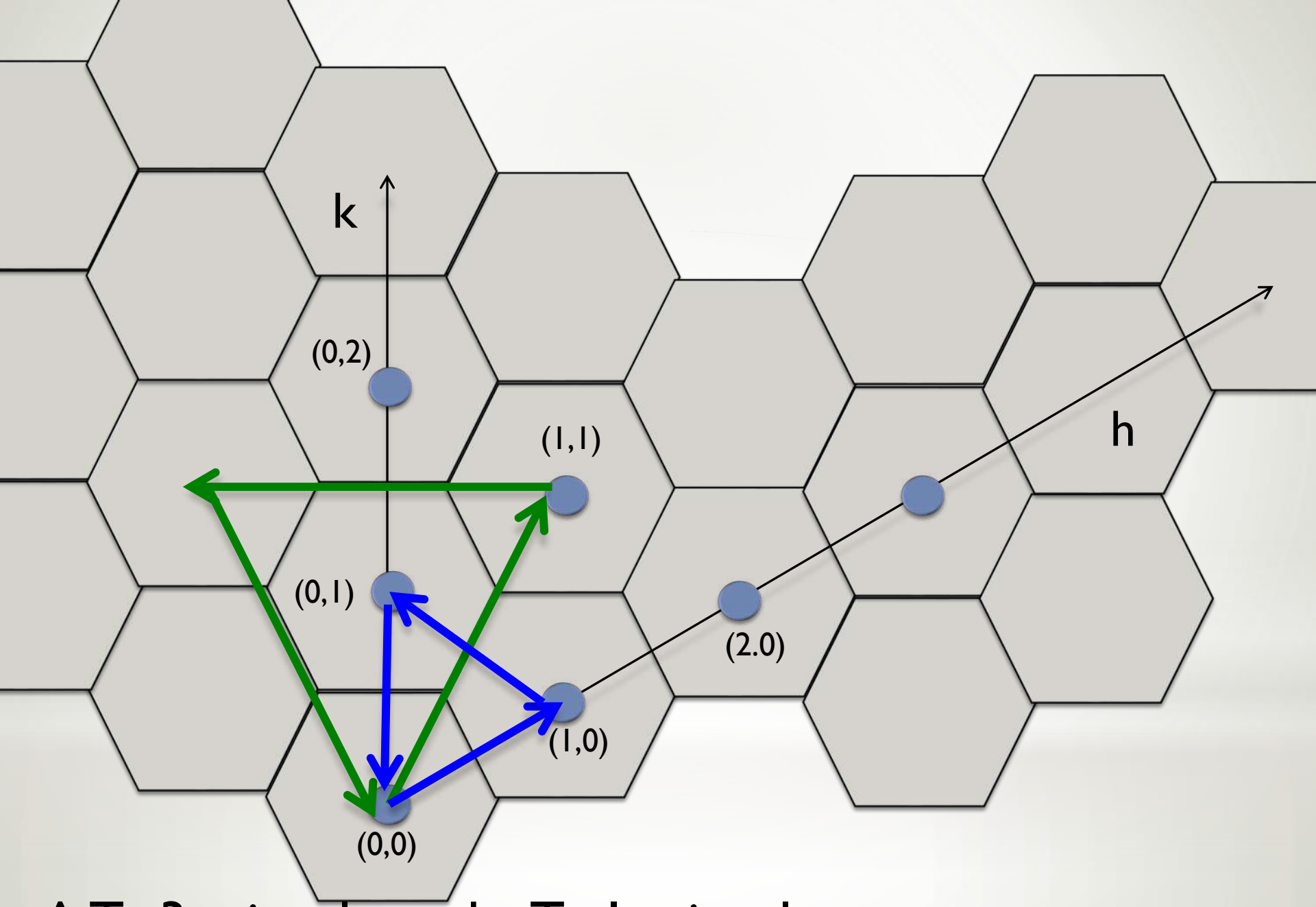
$T = h^2 + k^2 + hk = 3,$

$h=1, k=1$

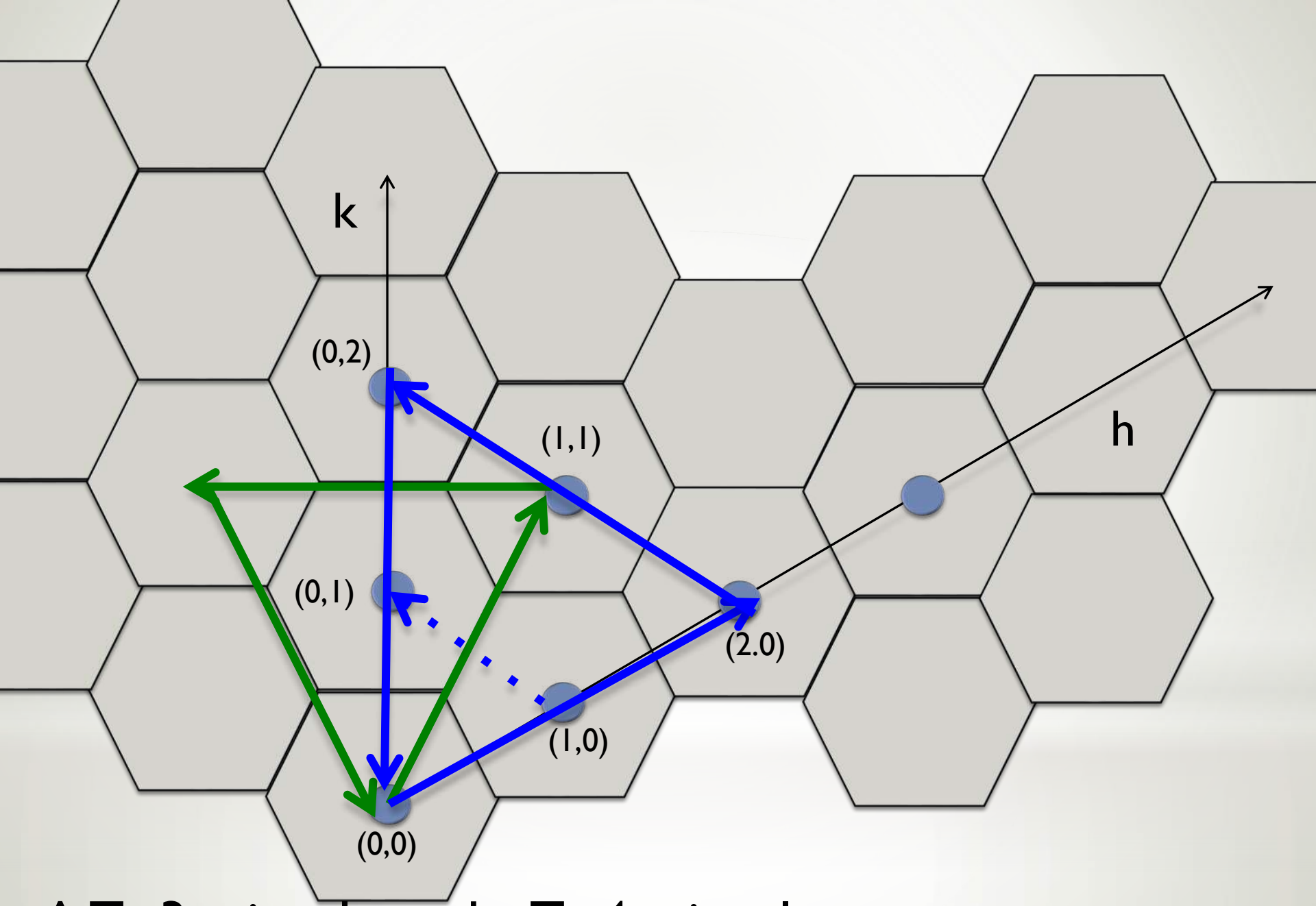


A T=3 triangle

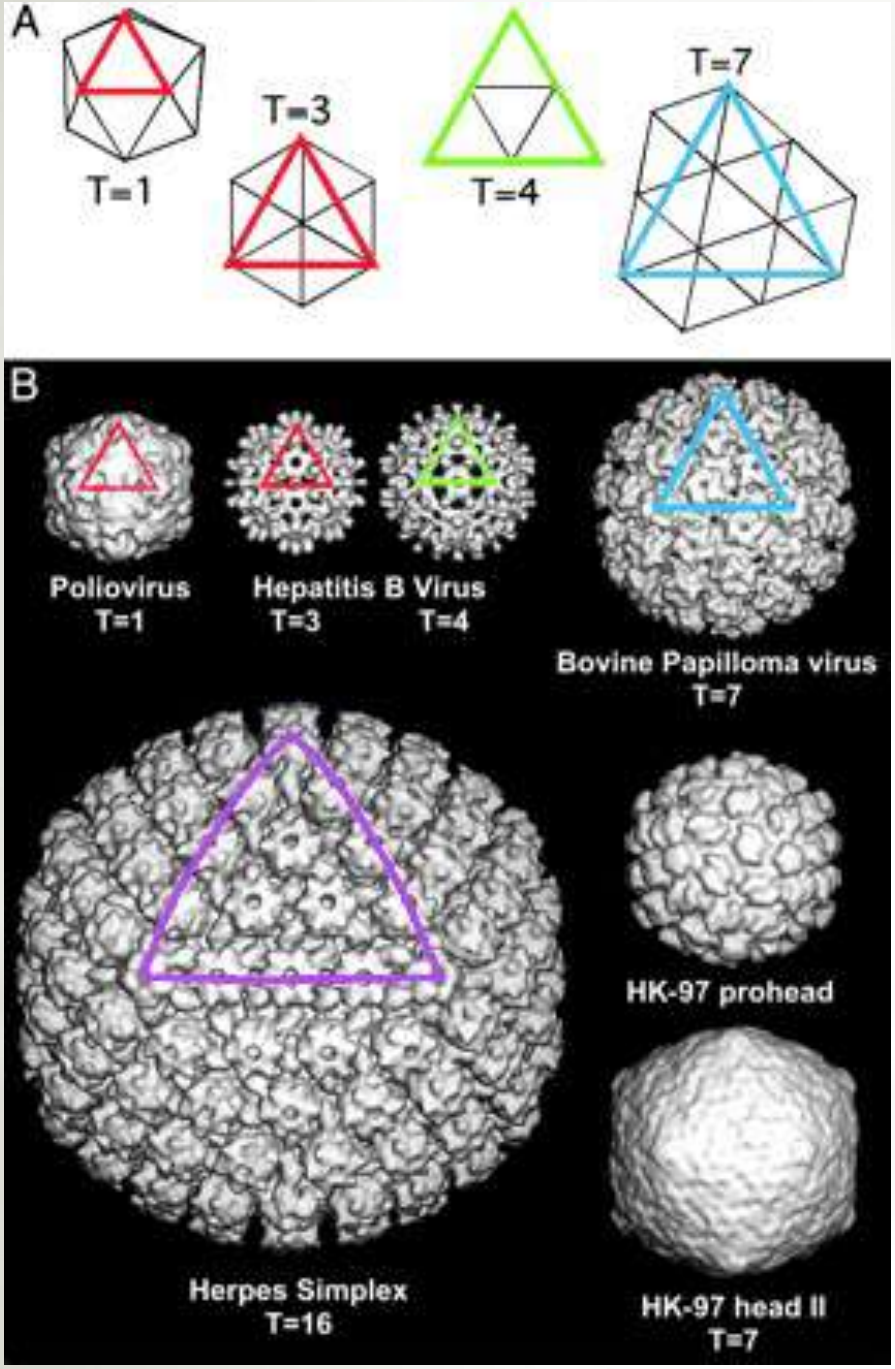
$T = h^2 + k^2 + hk = 3$,
$h=1, k=1$



A T=3 triangle and a T=1 triangle



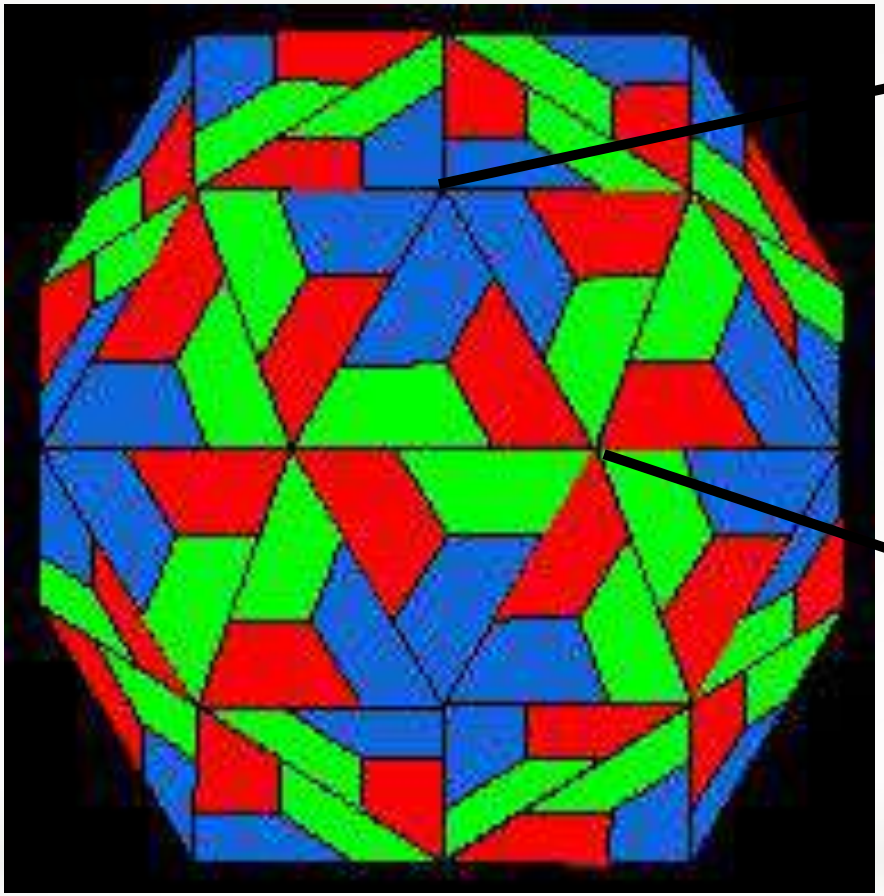
A T=3 triangle and a T=4 triangle



T	No. of subunits (60T)	Example
1	60	Satellite tobacco necrosis virus
3	180	picornavirus
4	240	Sindbis Virus
9	540	Reovirus
16	960	Herpesvirus
25	1500	Adenovirus

http://www.pnas.org/cgi/content-nw/full/101/44/15549/FIG1

Quasi-equivalence in virus structures



Casper and Klug

The Icosahedral Server

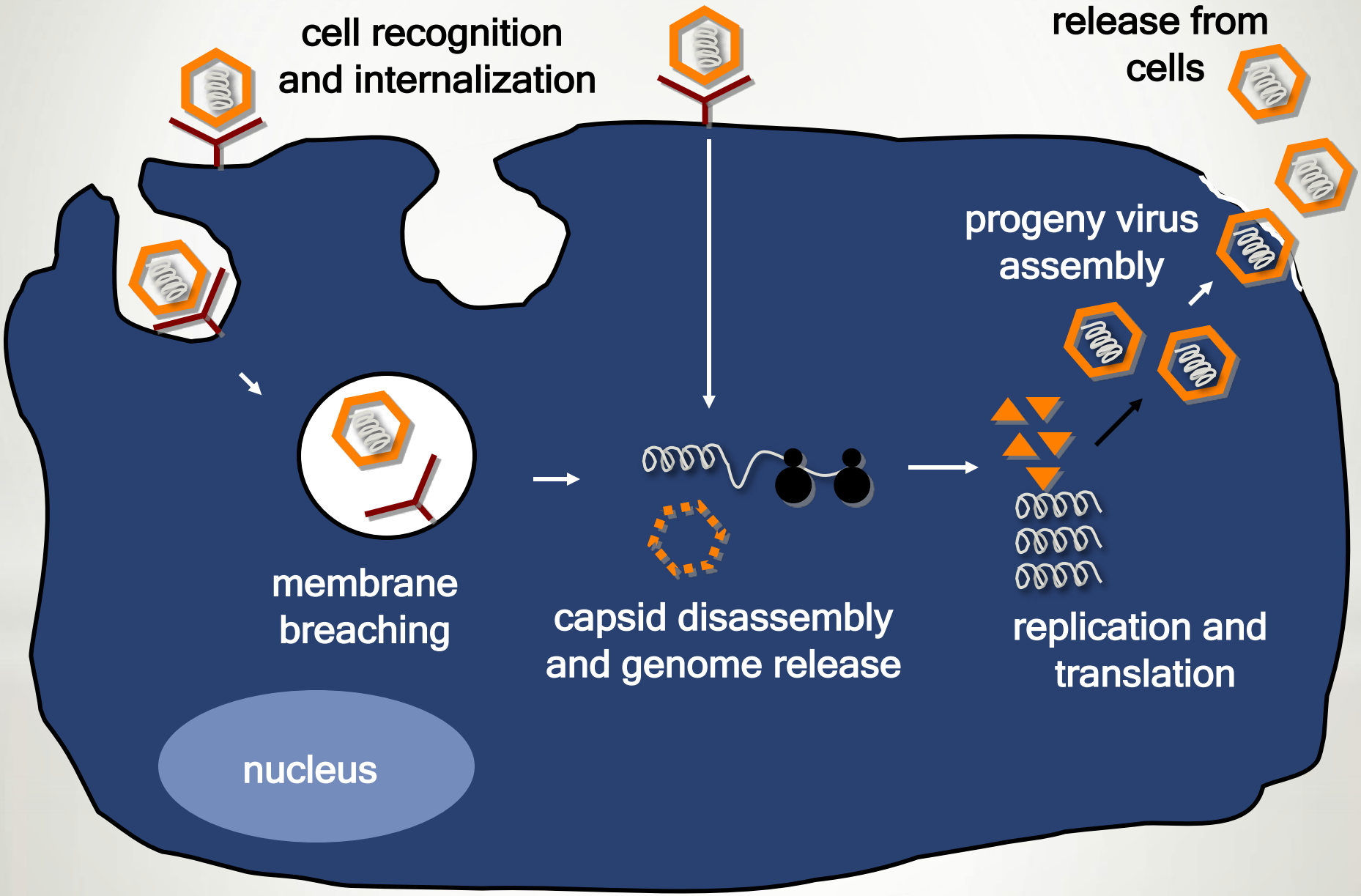
Paper Model Templates

Download the original postscript files

Paper **hexagonal template sheet**

T=1 (h,k) = (1,0)
T=3 (h,k) = (1,1)
T=4 (h,k) = (2,0)
T=7 (h,k) = (2,1) / (h,k) = (1,2)
T=9 (h,k) = (3,0)
T=12 (h,k) = (2,2)
T=13 (h,k) = (3,1) / (h,k) = (1,3)
T=16 (h,k) = (4,0)
T=19 (h,k) = (3,2) / (h,k) = (2,3)
T=21 (h,k) = (4,1) / (h,k) = (1,4)
T=25 (h,k) = (5,0)
T=27 (h,k) = (3,3)
T=28 (h,k) = (4,2) / (h,k) = (2,4)
T=31 (h,k) = (5,1) / (h,k) = (1,5)

Overview of infection cycle



Overview of infection cycle

I. Receptor binding and internalization

II. Membrane breaching

III. Uncoating/disassembly

IV. Replication

V. Translation

VI. Assembly

VII. Egress

Receptor binding and internalization

A receptor is an attachment point on the cell surface

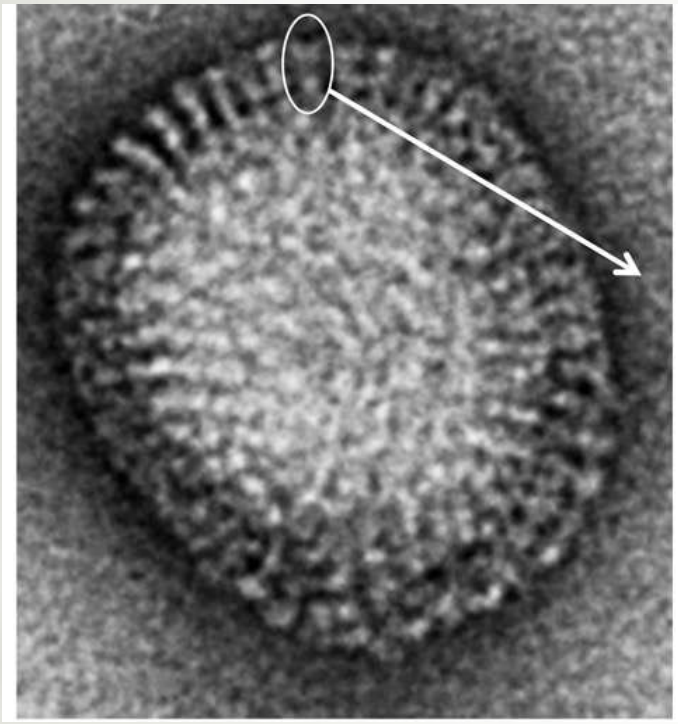
Usually a protein molecule. Exception – sialic acid for Influenza Virus

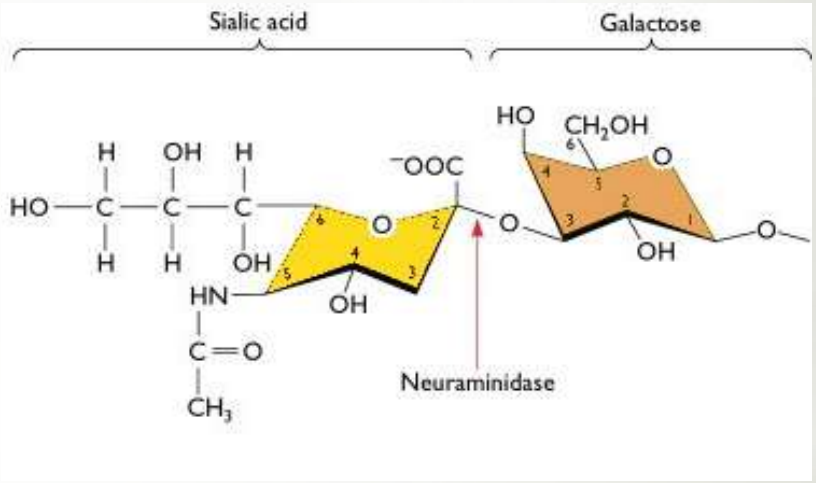
Some viruses need a receptor and a co-receptor. E.g. HIV needs CD4 and CXCR5

Cellular tropism/ species specificity

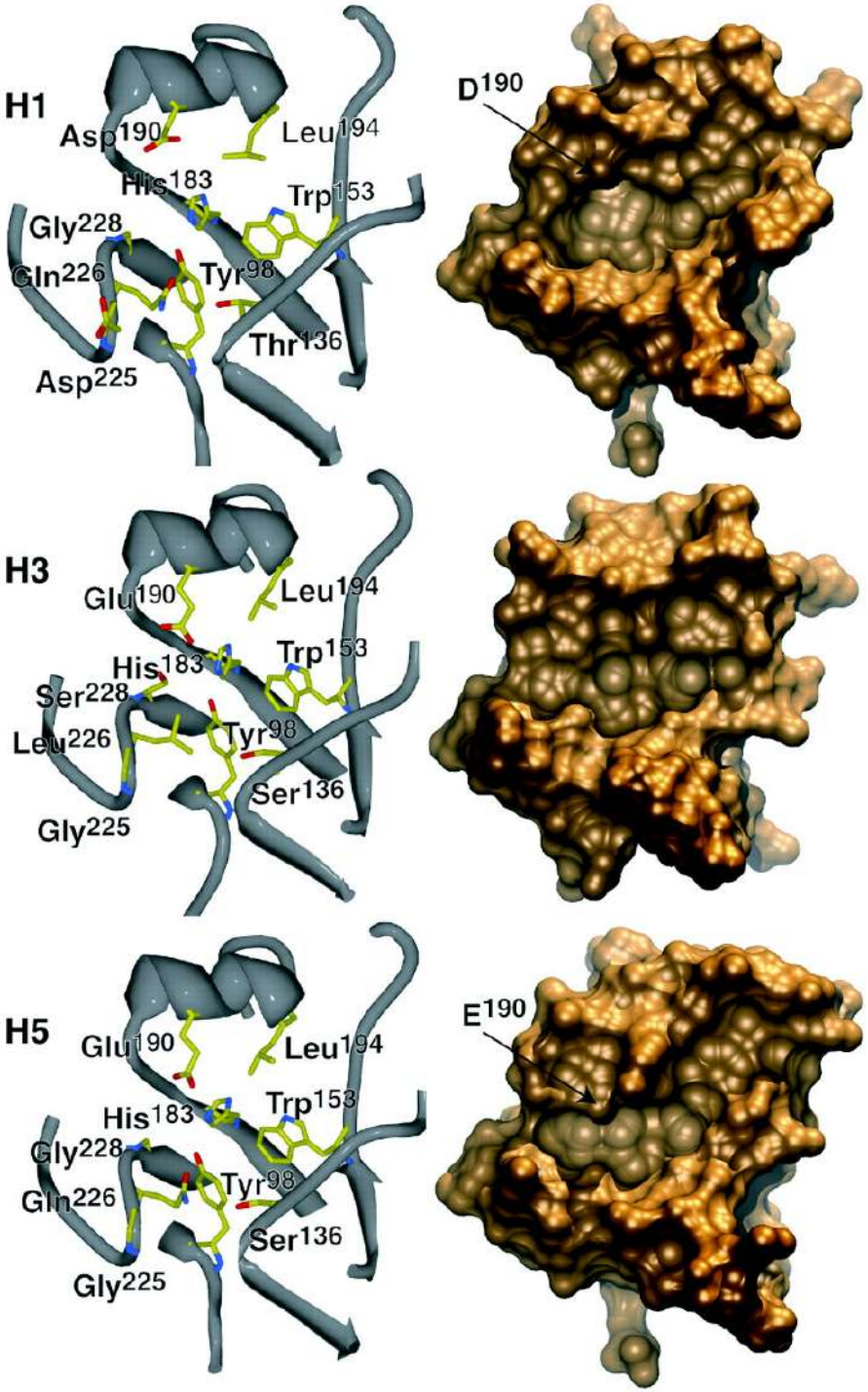
Receptor binding allows internalization of virus through endosomal pathway/direct membrane breaching

Hemagglutinin of influenza virus binds to sialic acid





Receptor binding dictates host adaptation



Hemagglutinin (HA) of human flu virus binds to sialic acid attached to galactose with a 2,6 linkage (SAα2,6GAL)

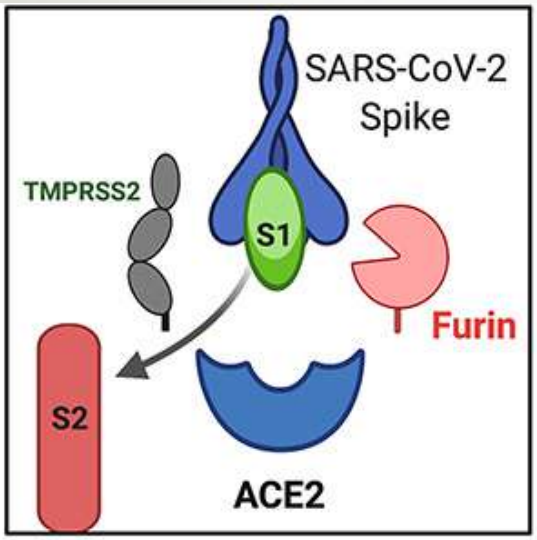
Bird flu HA binds to sialic acid attached to galactose with a 2,3 linkage (SAα2,3GAL)

1918 pandemic strain of flu was of avian origin

Contained a mutated HA with a E190 $\rightarrow$ D190 mutation that allowed binding to SAα2,6GAL

Source: Stevens *et al.*, Science, 2006

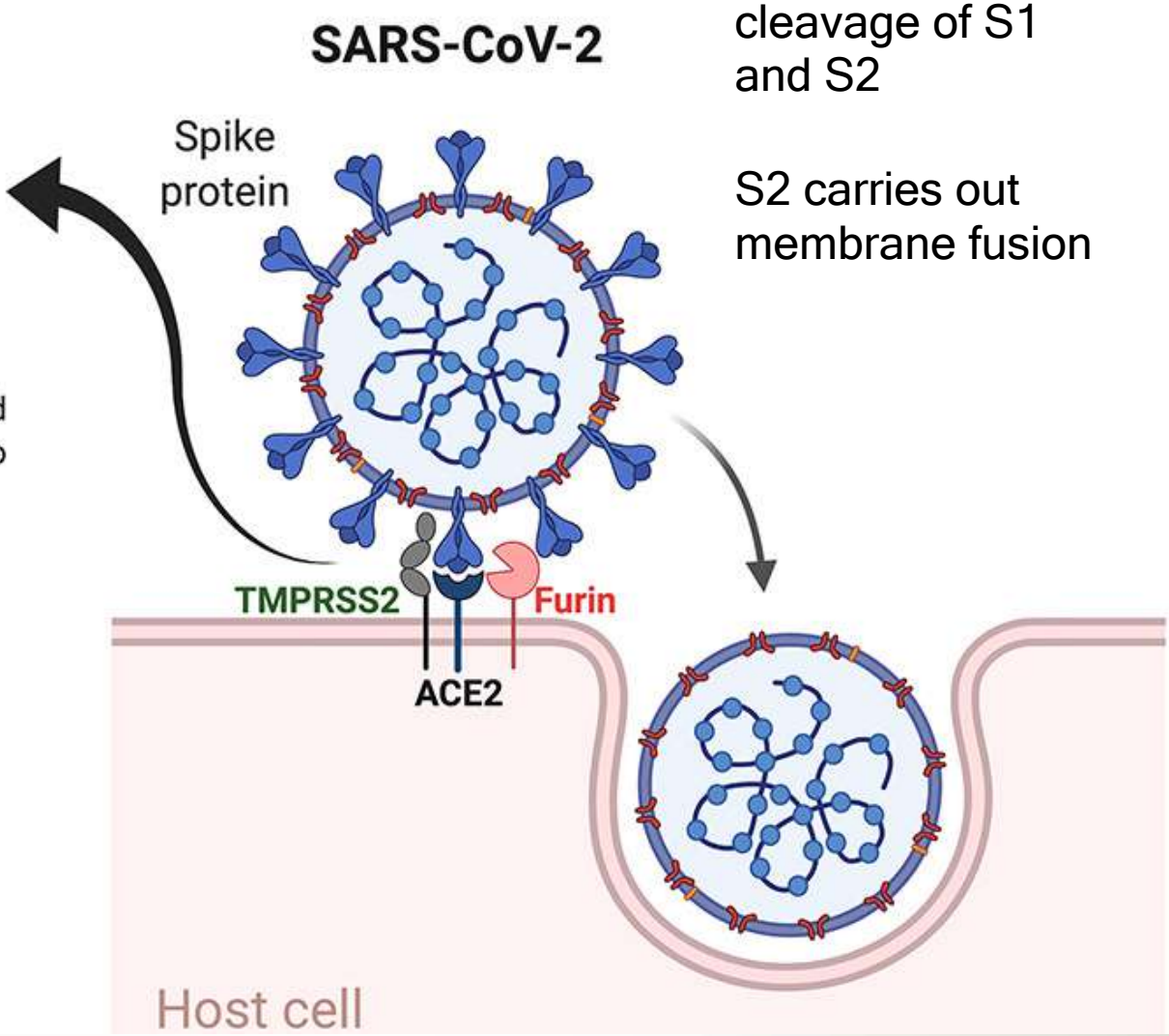
Receptor binding by SARS-CoV-2



S1/S2 subunits cleavage by furin and SARS-CoV-2 genomes penetrate into the host cell

Spike protein contains 2 domains – S1 and S2

Receptor binding domain (RBD) of S1 binds to the Angiotensin converting enzyme (ACE2)

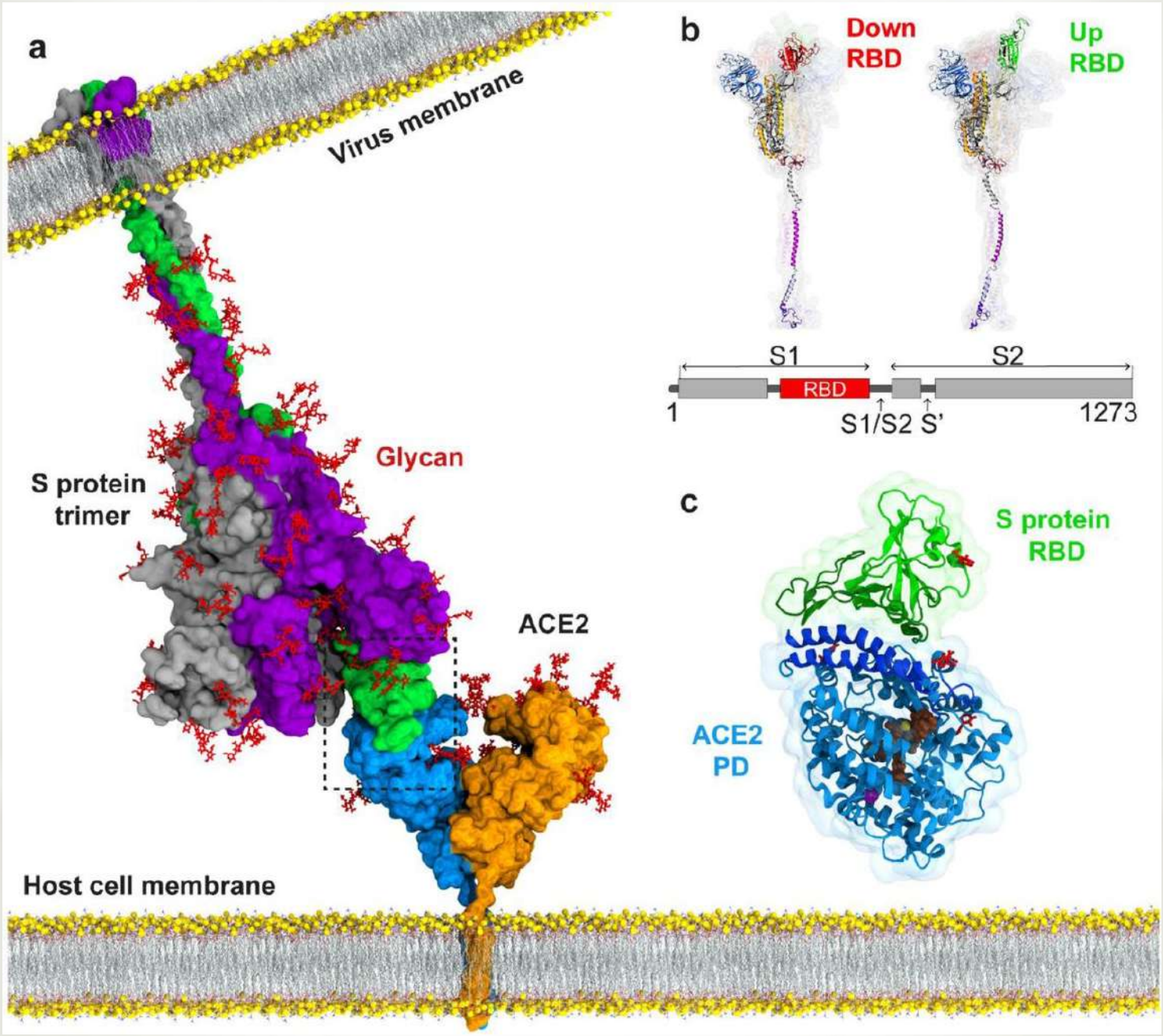


Receptor binding is followed by cleavage of S1 and S2

S2 carries out membrane fusion

Lippi *et al.,* Clin Chem Lab Med 2020; 58(9): 1415-1422

Receptor binding by SARS-CoV-2



Taka E *et al.*, bioRXiV

Notable features of SARS-CoV-2:

Mutations in receptor-binding region of Spike:

Amino acids from SARS-CoV that bind ACE2: Y442, L472, N479, D480, T487, Y491
Amino acids from SARS-CoV-2 that bind ACE2: L455, F486, Q493, S494, N501,Y505

Introduction of a polybasic cleavage site (RRAR) between S1 and S2
Presence of a proline “bend” allows attachment of glycans
Protection from the immune system
Also present in Influenza Hemagglutinin

Andersen *et al.,* Nat Med, 2020

Theories for origin

1. Natural selection in an animal host before zoonotic transfer

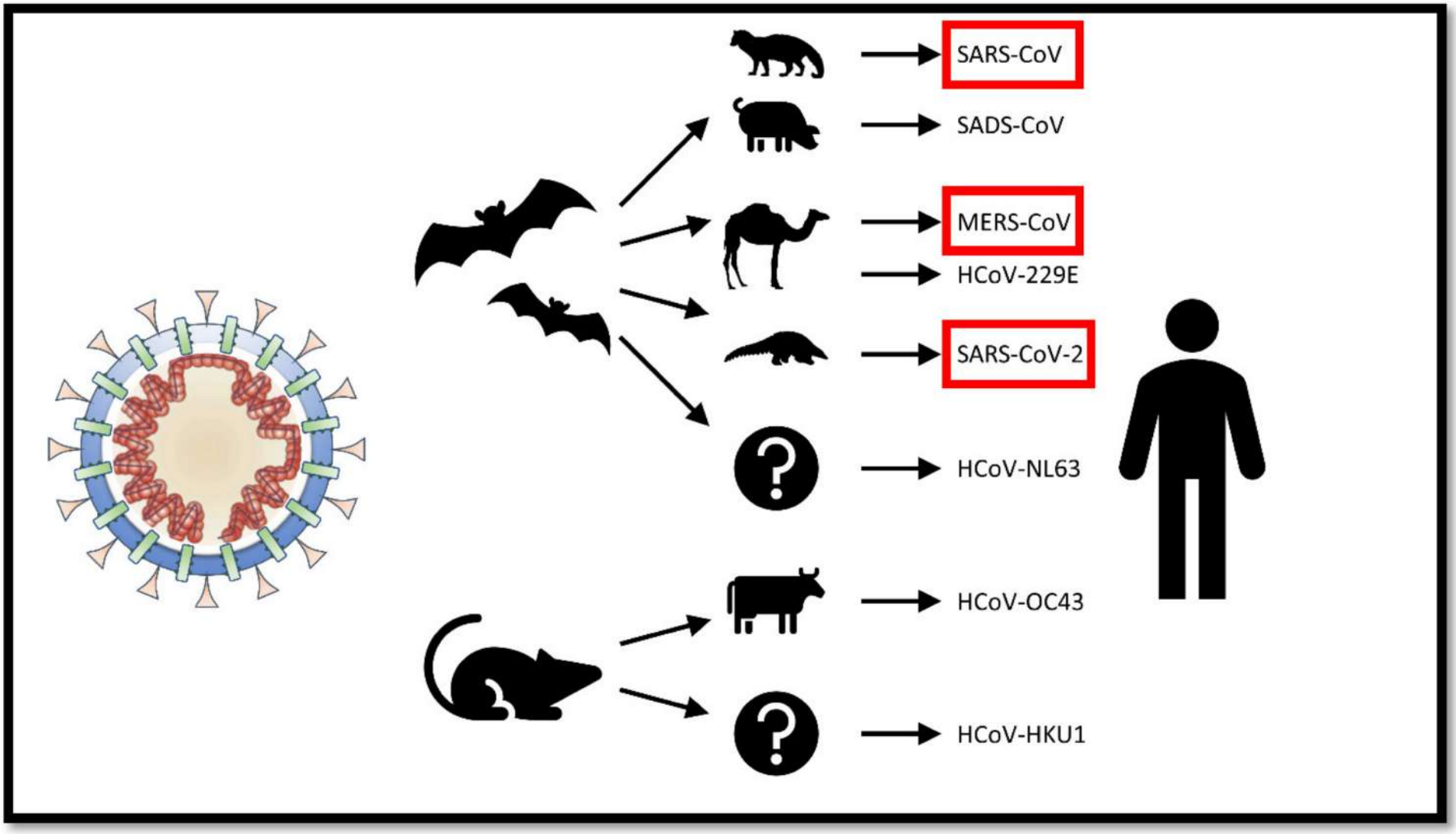
Bat virus RaTG13 96% identical to SARS-CoV-2, RBD differs
Pangolin coronaviruses contain all 6 key RBD residues of SARS-CoV-2

II. Natural selection in humans following zoonotic transfer

Acquisition of the polybasic cleavage site

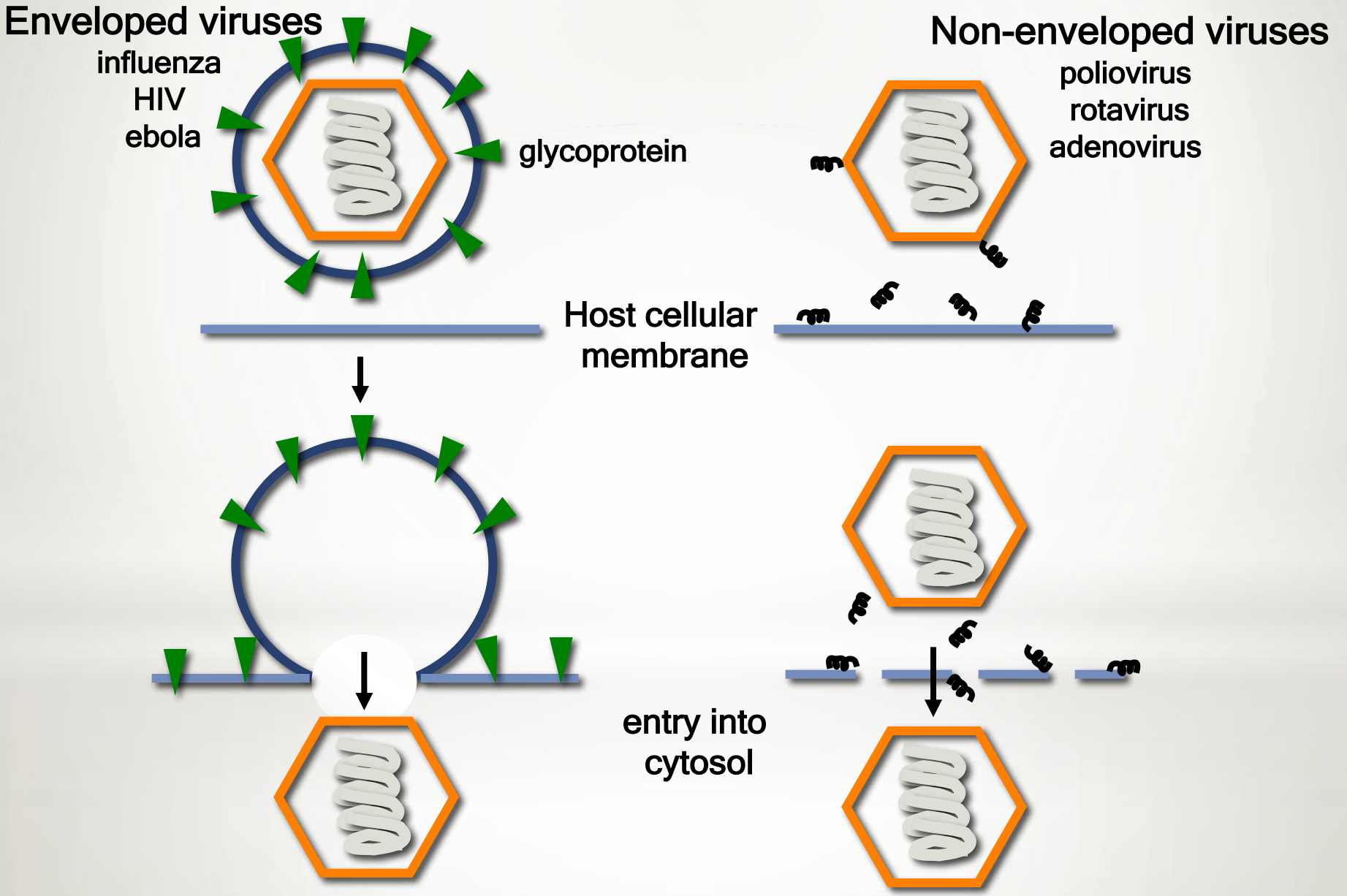
Andersen *et al.,* Nat Med, 2020

Origin and emergence of SARS-CoV-2



Rabi *et al.,* Pathogens, 2020

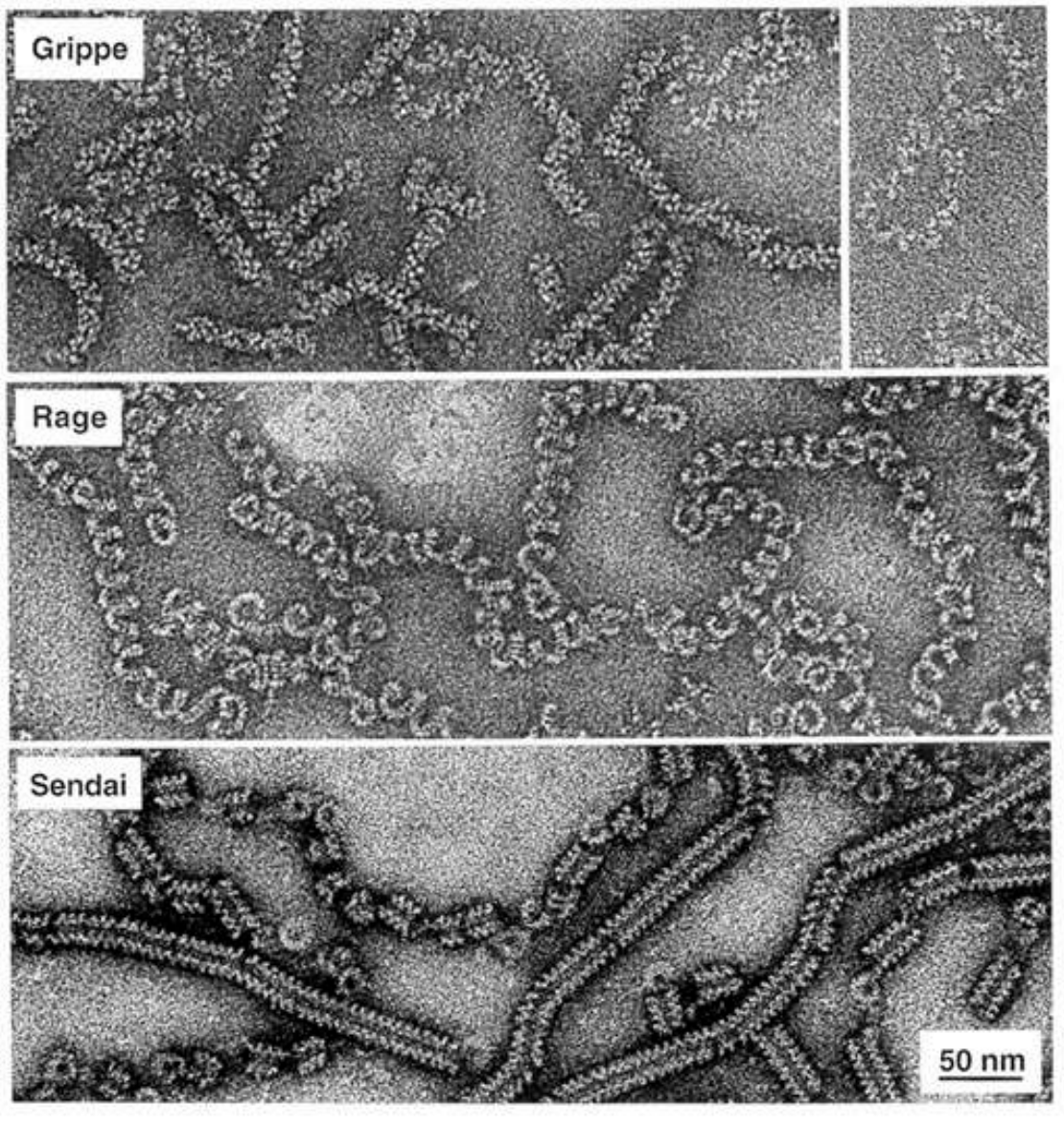
Membrane breaching



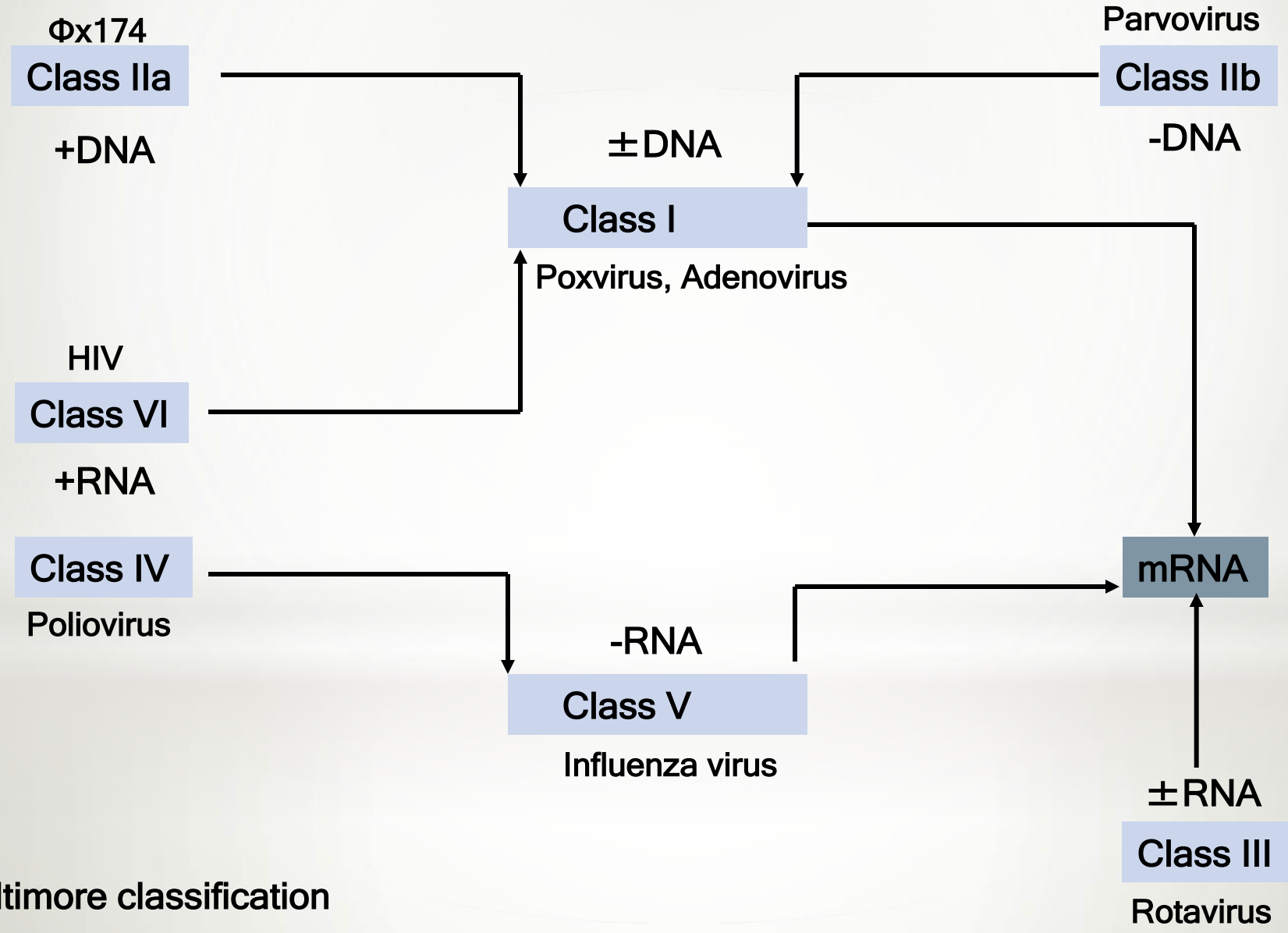
Uncoating/Disassembly

Separation of nucleic acid from structural proteins
(capsid proteins, ribonucleoproteins etc)

Trafficking to the site of replication



Replication scheme



Baltimore classification

Requirements for replication/transcription

Polymerases – DNA dependent DNA polymerase, RNA dependent RNA polymerase, RNA dependent DNA polymerase, DNA dependent RNA polymerase

Other associated enzymes, packaging proteins

Borrowed from the host cell, or carried/coded by the virus

Requirement for host factors higher for viruses with smaller genomes

Replication sites may be cytosol, organelles, nucleus

Suppression of host functions – Influenza polymerase “steals” mRNA caps

Translation of viral proteins

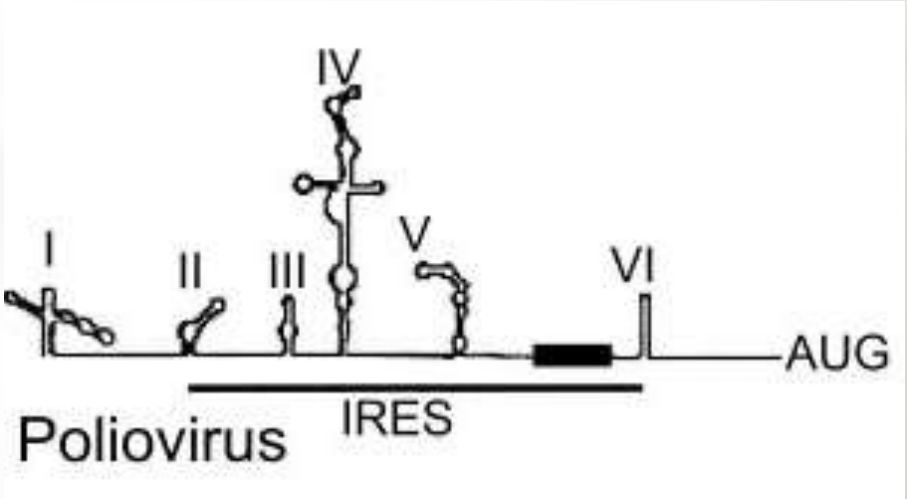
Viruses may or may not utilize host machinery for replication and transcription

Host machinery is always utilized for viral protein synthesis

Viral genome size is limited, translation machinery contains upto ~ 30 factors

Viruses have several mechanisms to promote synthesis of their proteins, and prevent synthesis of host proteins
"Host shutoff"

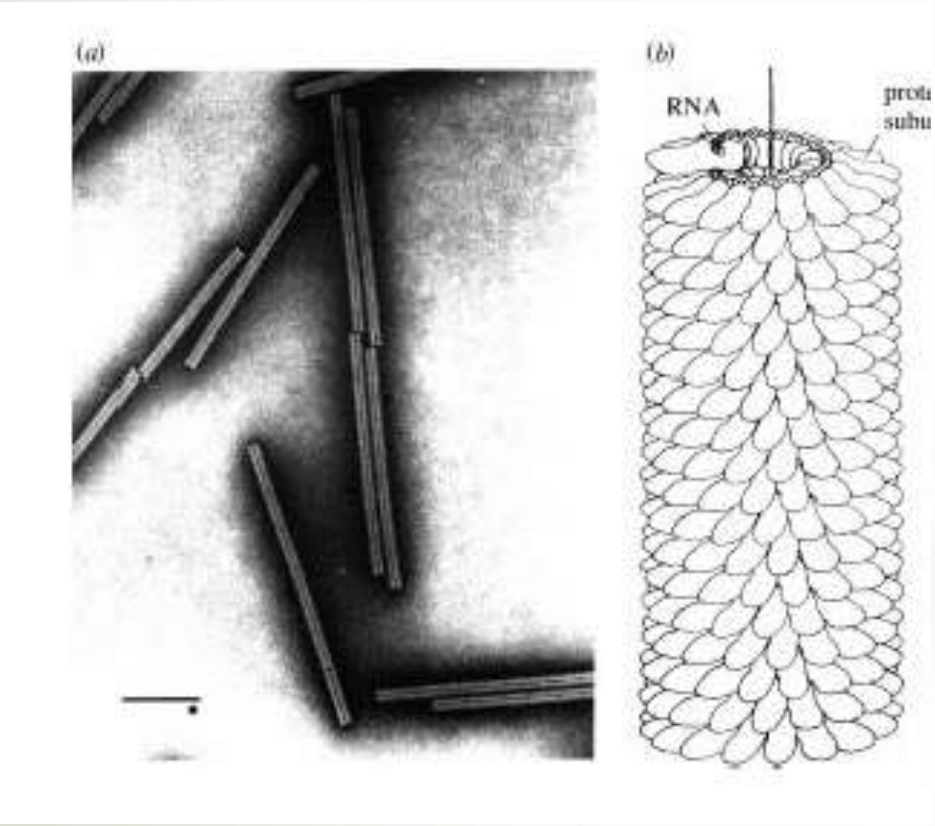
Diverting ribosomes through formation of specific structures in viral RNA
Disrupting host mRNA



Virus assembly

Viral proteins and nucleic acid are generated separately, sometimes in separate compartments

Must be brought together, in correct manner, to form infectious particles

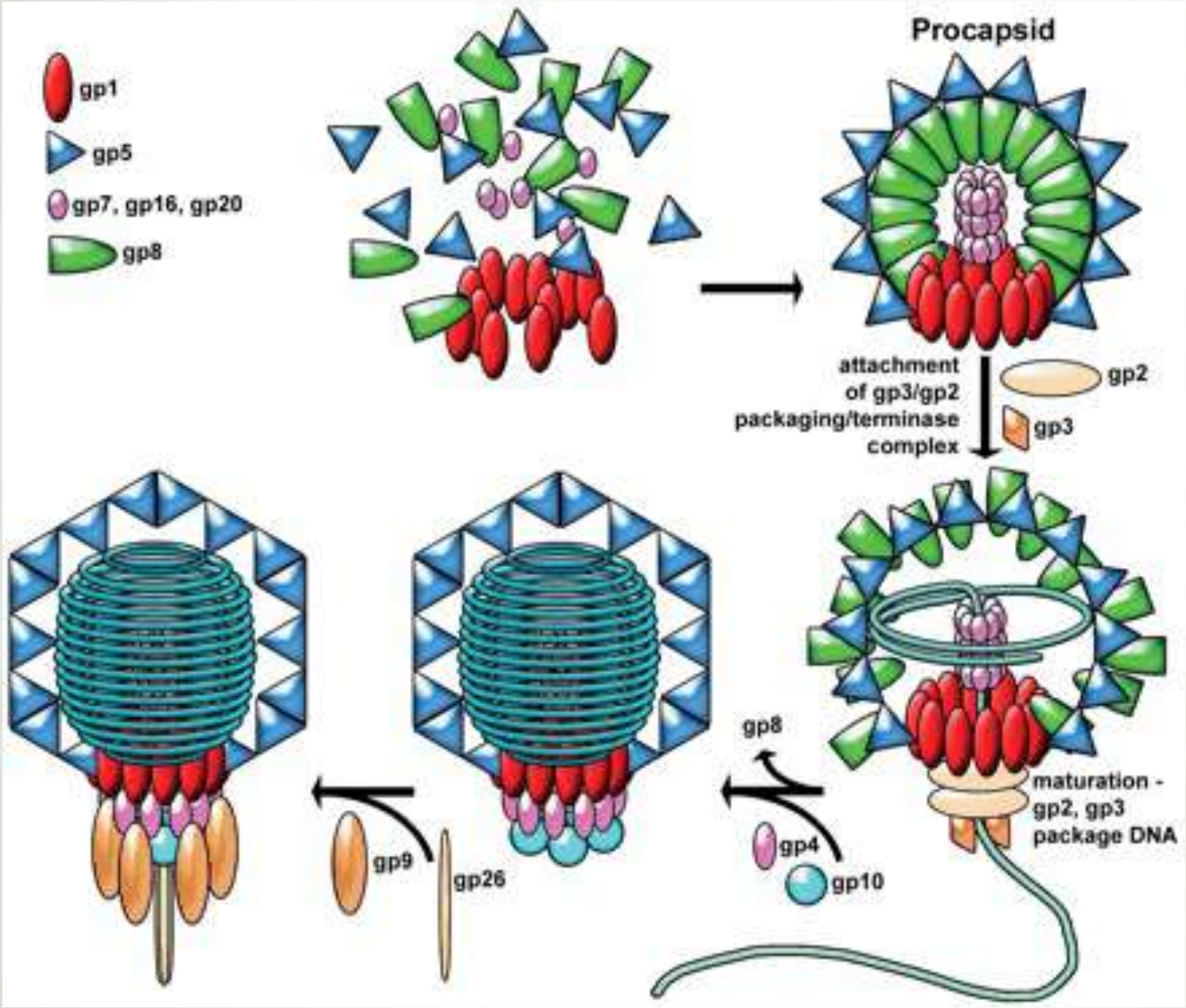


One long, helically wound molecule of RNA

2130 molecules of coat protein arranged helically

Source: A Klug, Phil. Trans. R. Soc. Lond. B., 1999

Complex assembly process

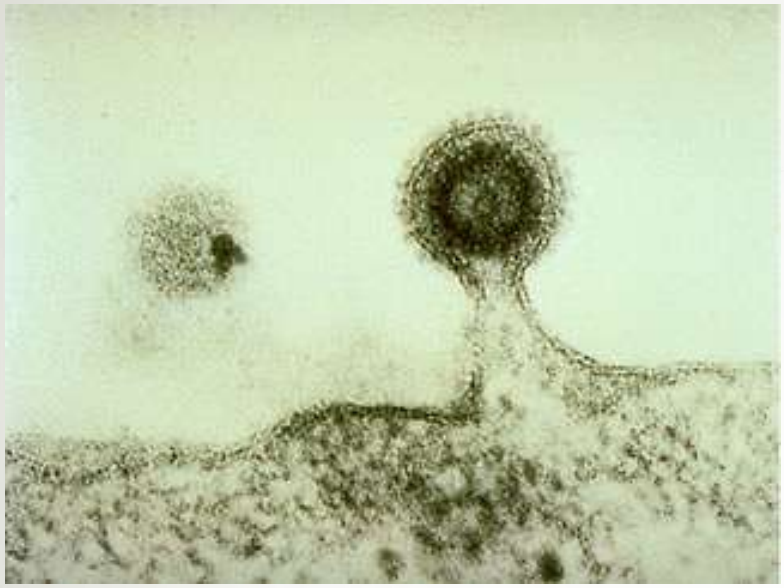


415 copies of gp5

250 copies of gp8

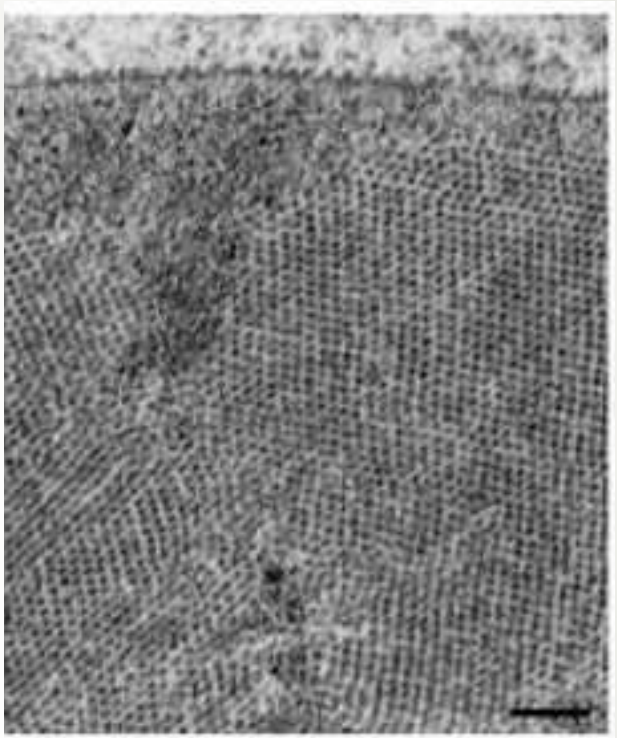
Source: Lander *et al.*, Science, 2006

Virus egress



"Budding out" of enveloped viruses

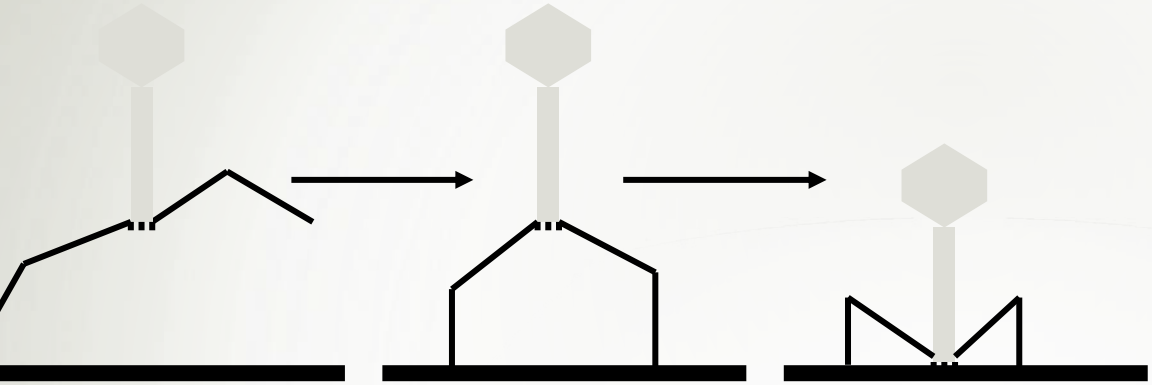
Lipids incorporated from host cell membranes

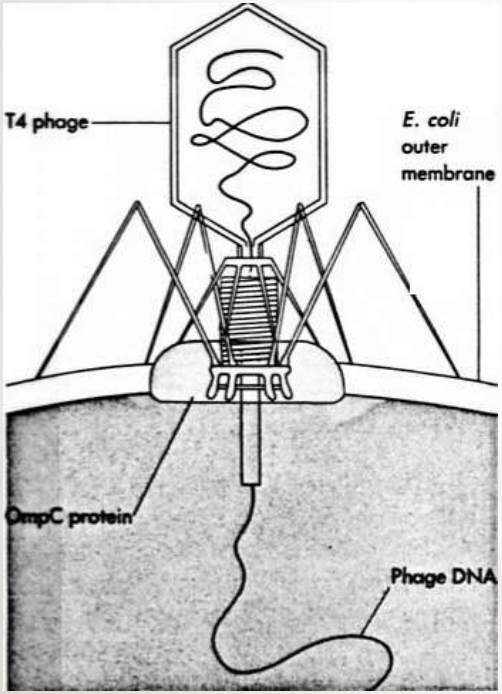


Non-enveloped viruses

Excess virus load causes membrane rupture

Attachment and entry of bacteriophages





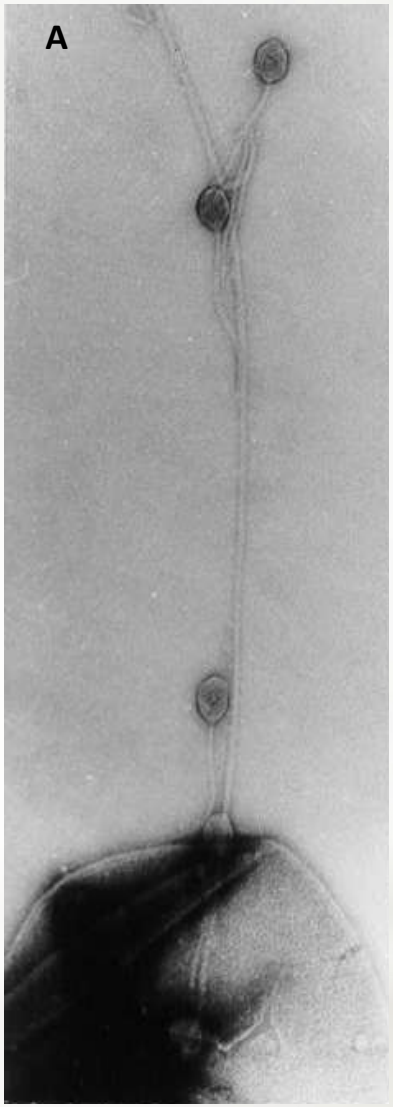
DNA packed under pressure in head of bacteriophage

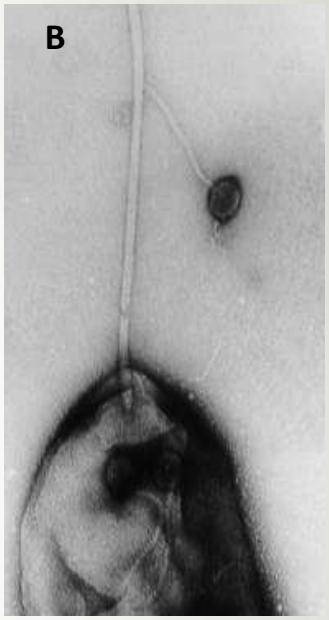
Tail fibers make first contact

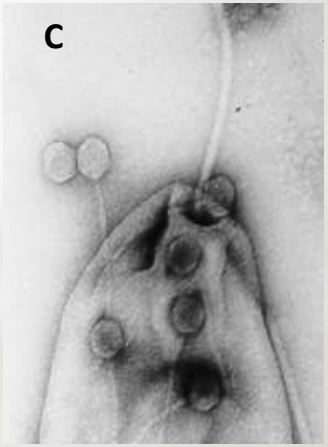
Tail contains lysozyme

Contractile sheath injects DNA

Lytic/lysogenic life cycle

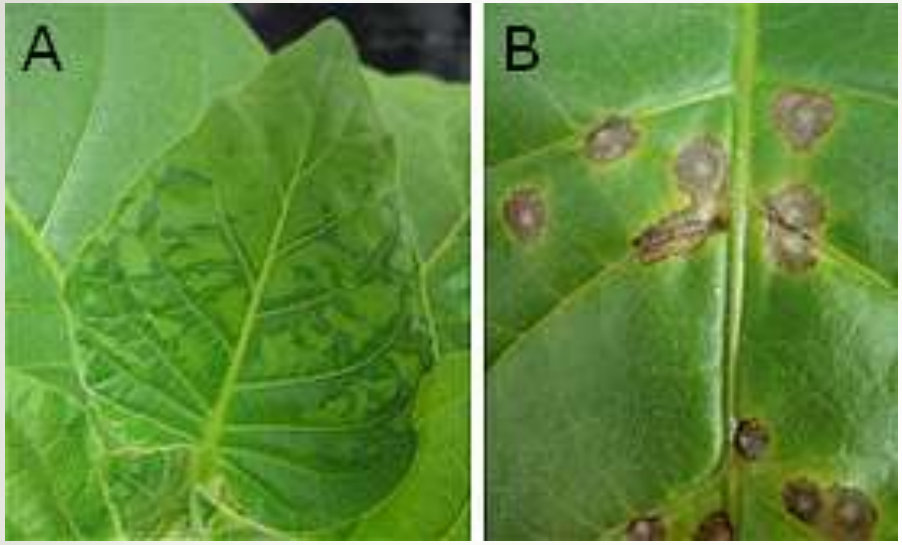






Source: Zhilenkov *et al.*, Virology J, 2006
textbookofbacteriology.net

Plant Viruses



Tobacco Mosaic Virus

Tomato Bushy Stunt Virus

Cucumber Mosaic Virus

Source: www.dpvweb.net, www.apsnet.org/

Entry of Plant Viruses

<u>Modes of “active” entry:</u>

Interaction with plasmodesmata machinery to increase pore size

Enzymes to destroy plant cell walls

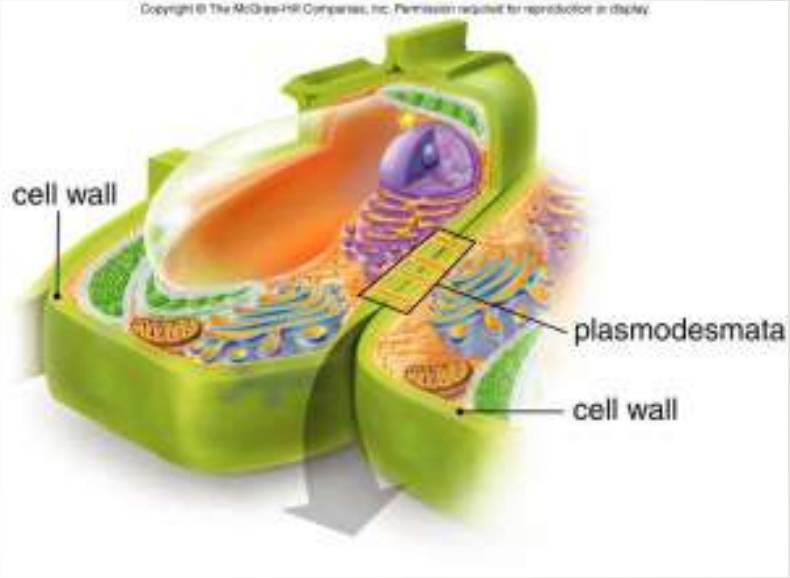
<u>Modes of “passive” entry:</u>

Mechanical injury to breach plant cell walls

Similar injuries due to the actions of arthropods or nematodes

Transfer through fungal parasites

Transmission through infected seed/pollen



Source: lanadayaram.wordpress.com

Viruses in biomedical applications

Gene therapy: Introduction of functioning genes
Treatment of individuals with genetic disorders (Cystic Fibrosis, SCID)
Treatment of cancer

Viruses being tried: Adenovirus, adeno-associated virus, poliovirus, poxvirus, lentivirus
Side-effects, mutagenesis

Viral vaccines:
Prophylactic vaccine, therapeutic vaccine, enhanced killing of cancer cells
Viruses being tried: Adenovirus, papillomavirus
Side-effects, mutagenesis

Biological pest control: Baculovirus insecticides, bacteriophages