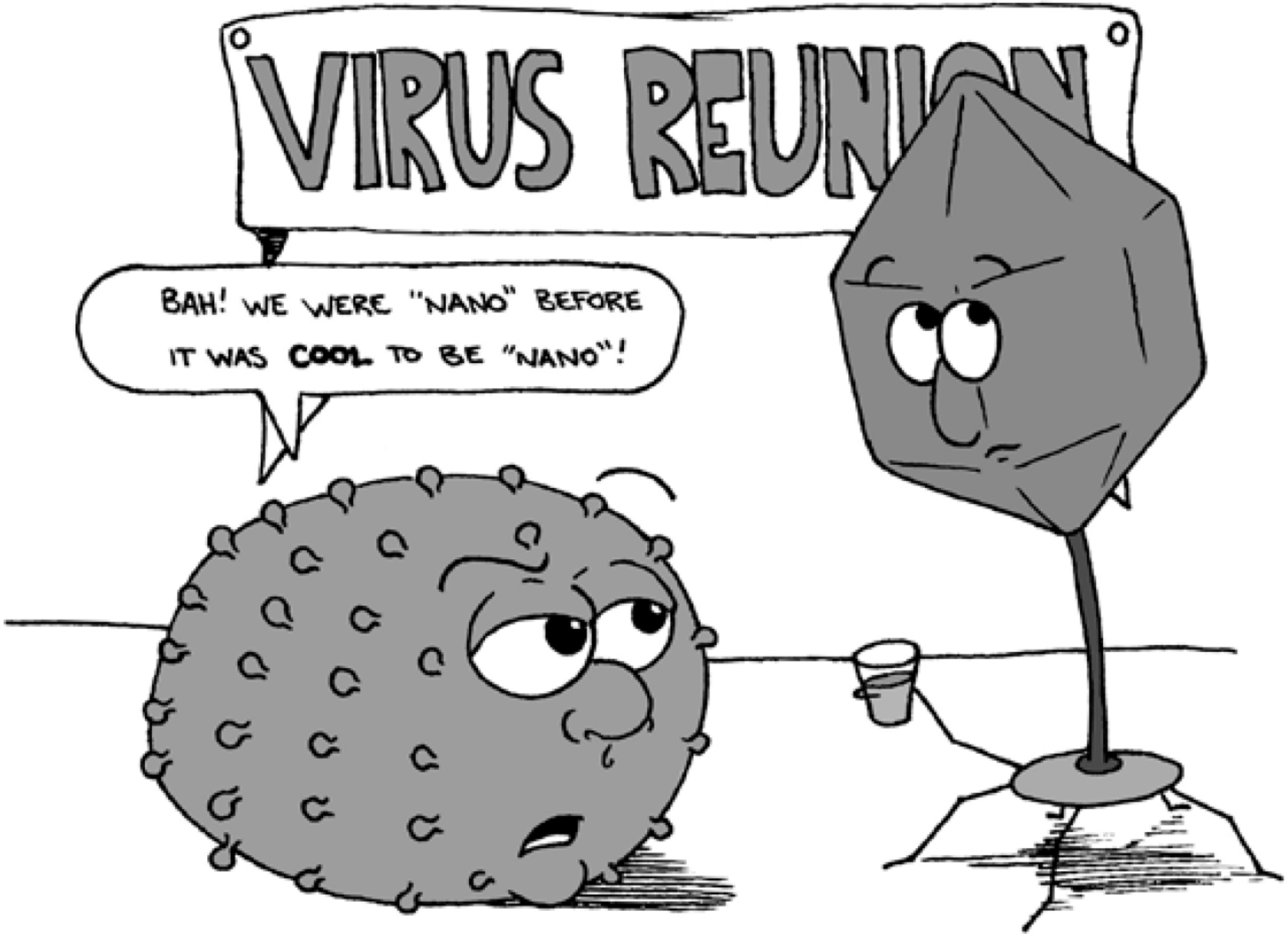

VIRUS REUNION
BAH! WE WERE "NANO" BEFORE IT WAS COOL TO BE "NANO"!

Structural Virology

Spting 2025

Pavel Plevka

Class rules

- Turn off anything that beeps or rings.
- Reading any material that is not related to the class, texting, or checking the internet during the class is discouraged.
- It is ok to eat or drink.
- Ask questions - it will help to clarify the issue not only for you but for your peers as well!
- In class discussions, be respectful of other students' opinions.

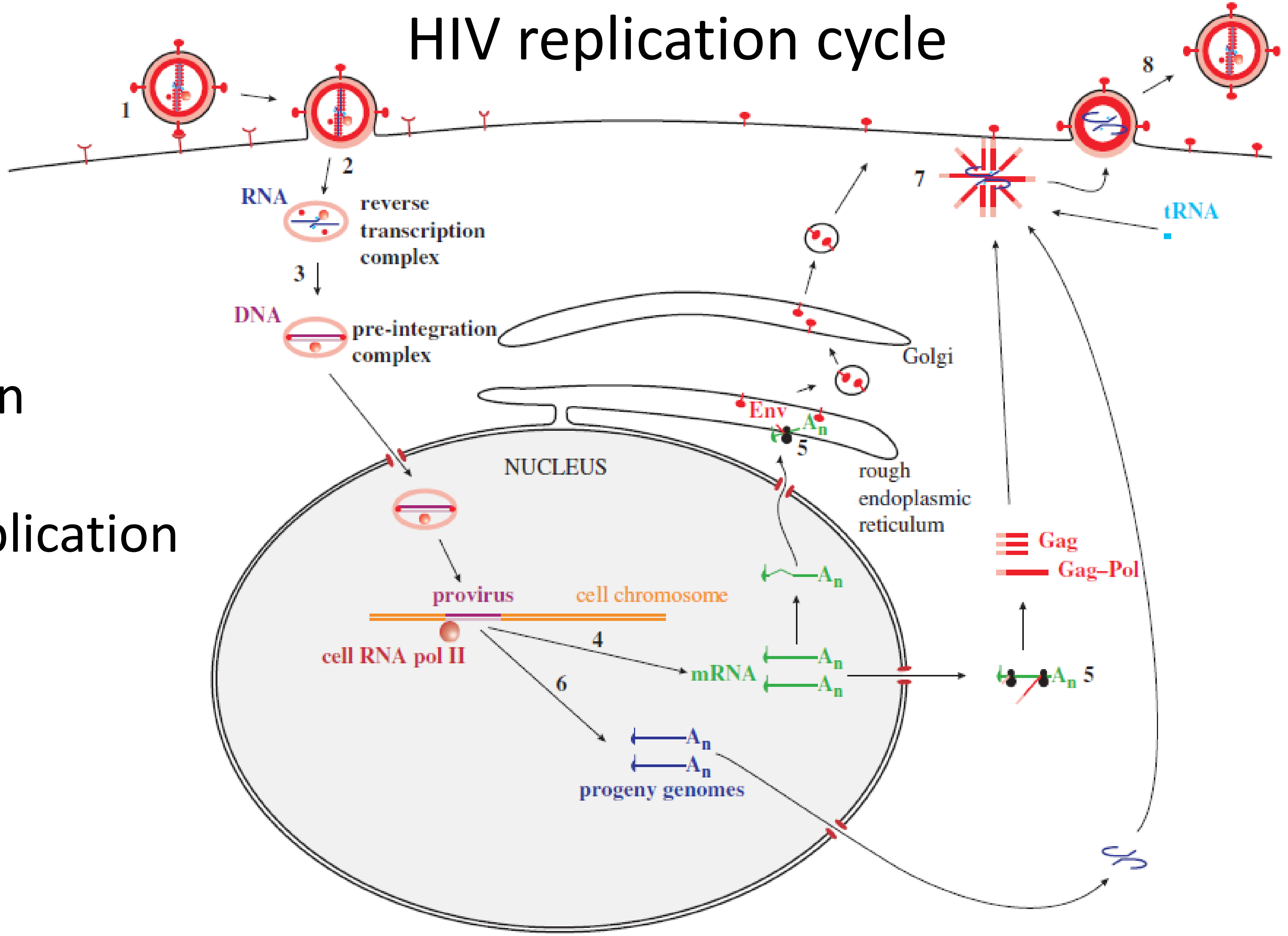
Aims of the course

- learn elementary molecular virology
- appreciate role of structural information in understanding of biological processes
- learn to read and evaluate scientific papers
- learn history of some fundamental discoveries in biology
- be able to evaluate what is an important research question

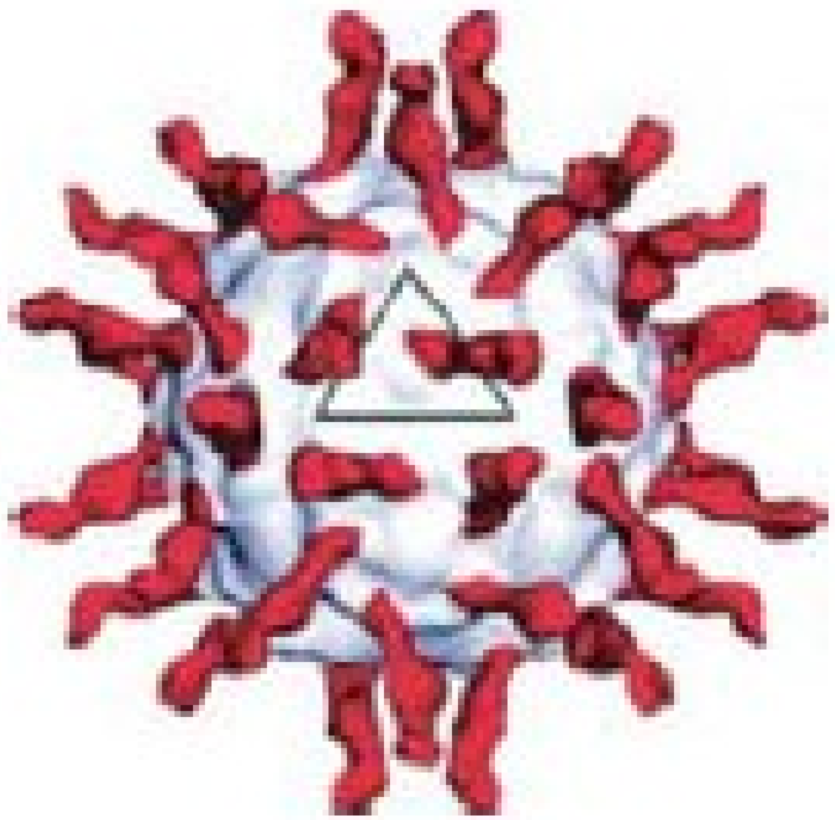
Elementary molecular virology

HIV replication cycle

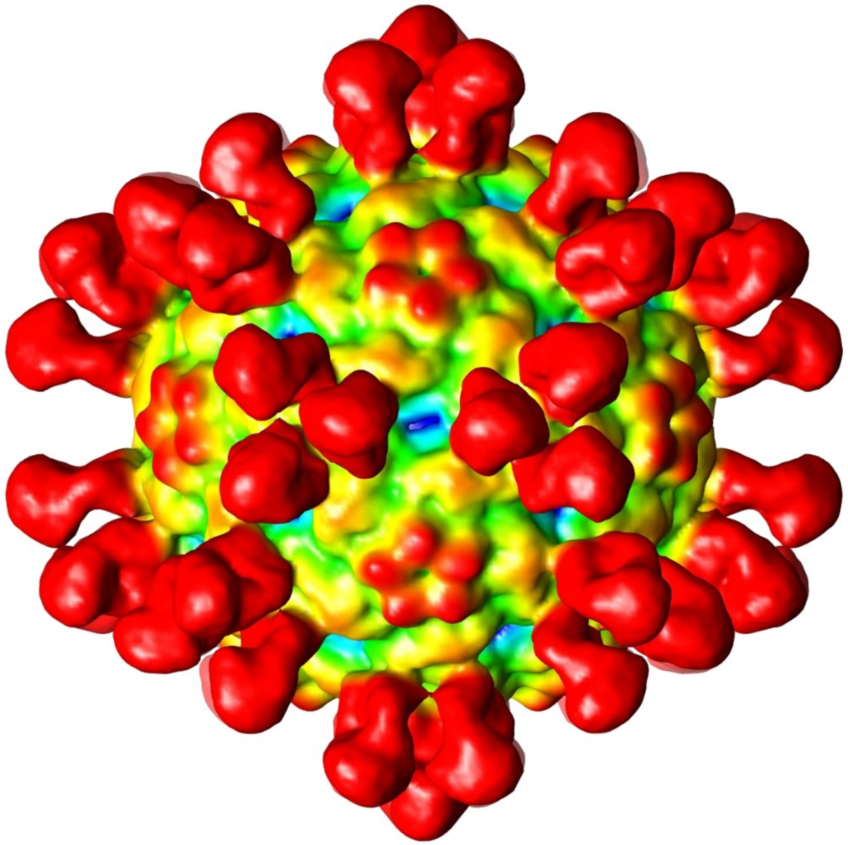
1. **A**ttachment
2. **E**ntry
3. **T**ranscription
4. **T**ranslation
5. **G**enome replication
6. **A**ssembly
7. **E**xit



Structural biology in understanding virus infection



Virus binding to receptor



Binding of antibodies to virus particle

Read and evaluate scientific papers

CHALLENGES IN IRREPRODUCIBLE RESEARCH:
- **more than 50% of drug-related research articles in high profile journals contain irreproducible data**

Reasons for irreproducibility:

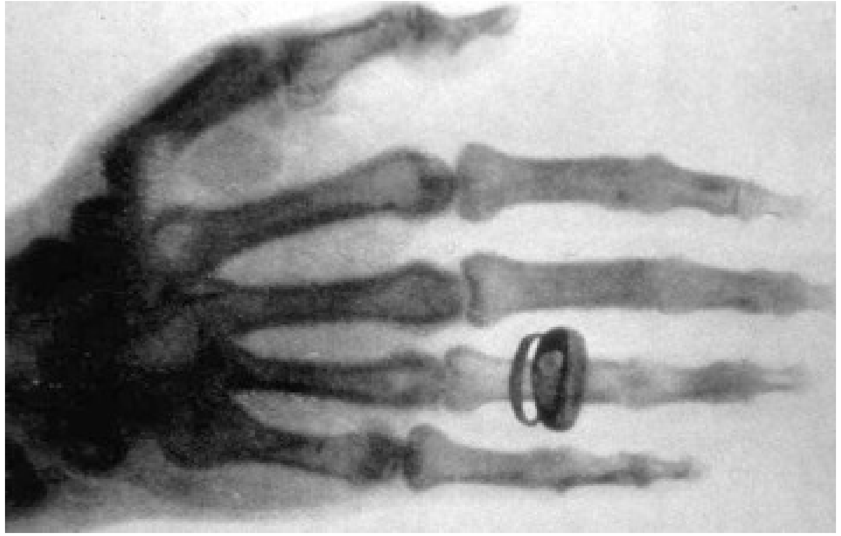
- Under supervised students and post-docs
- “Publish or Perish” threat
- wrong design of experiments – “know your statistics”

History of fundamental discoveries

WILHELM CONRAD RÖNTGEN (1845-1923)

- **1901 Nobel Laureate in Physics**

  discovery of the remarkable rays subsequently named after him



What is important research question?

Interesting x Important

1. *It asks about something other people care about*
2. *It builds on what you and others already know*
3. *It allows you to learn something you don't already know*

Summary – It helps you to predict future in a meaningful way.

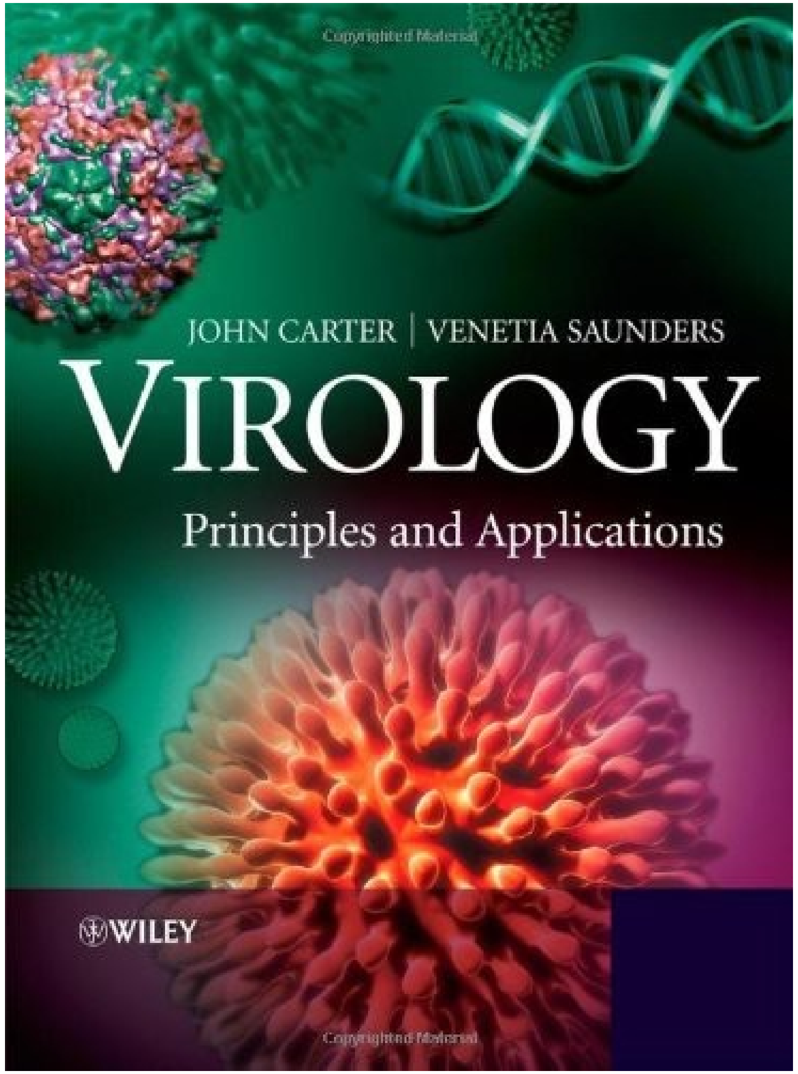
What are evolutionary relationships of anaerobic protists?
What is the probability of Ebola epidemics spreading outside of Africa?

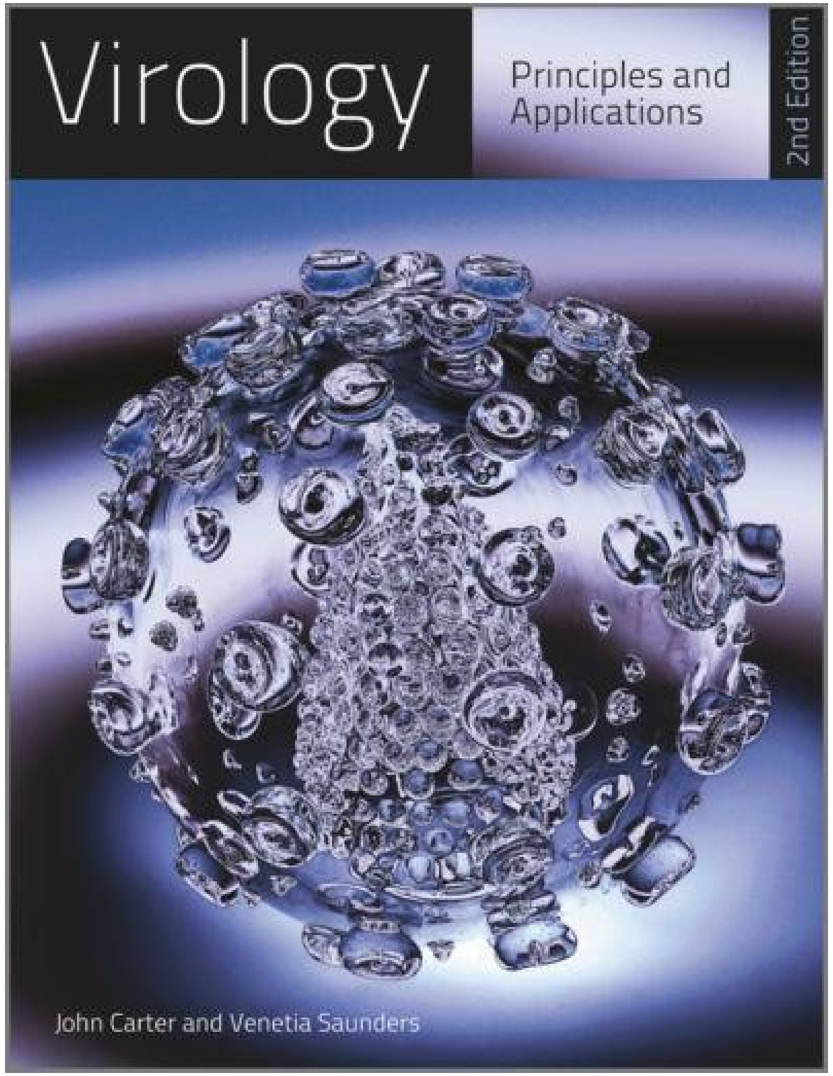
What is asked of you:

- Read assigned readings ***before*** the day for which they are assigned
- Bring five-sentence essay describing the importance of a scientific paper to each lecture
- Participate in discussions
- Submit two mini-assignments
- I am here to help, learning is up to you!

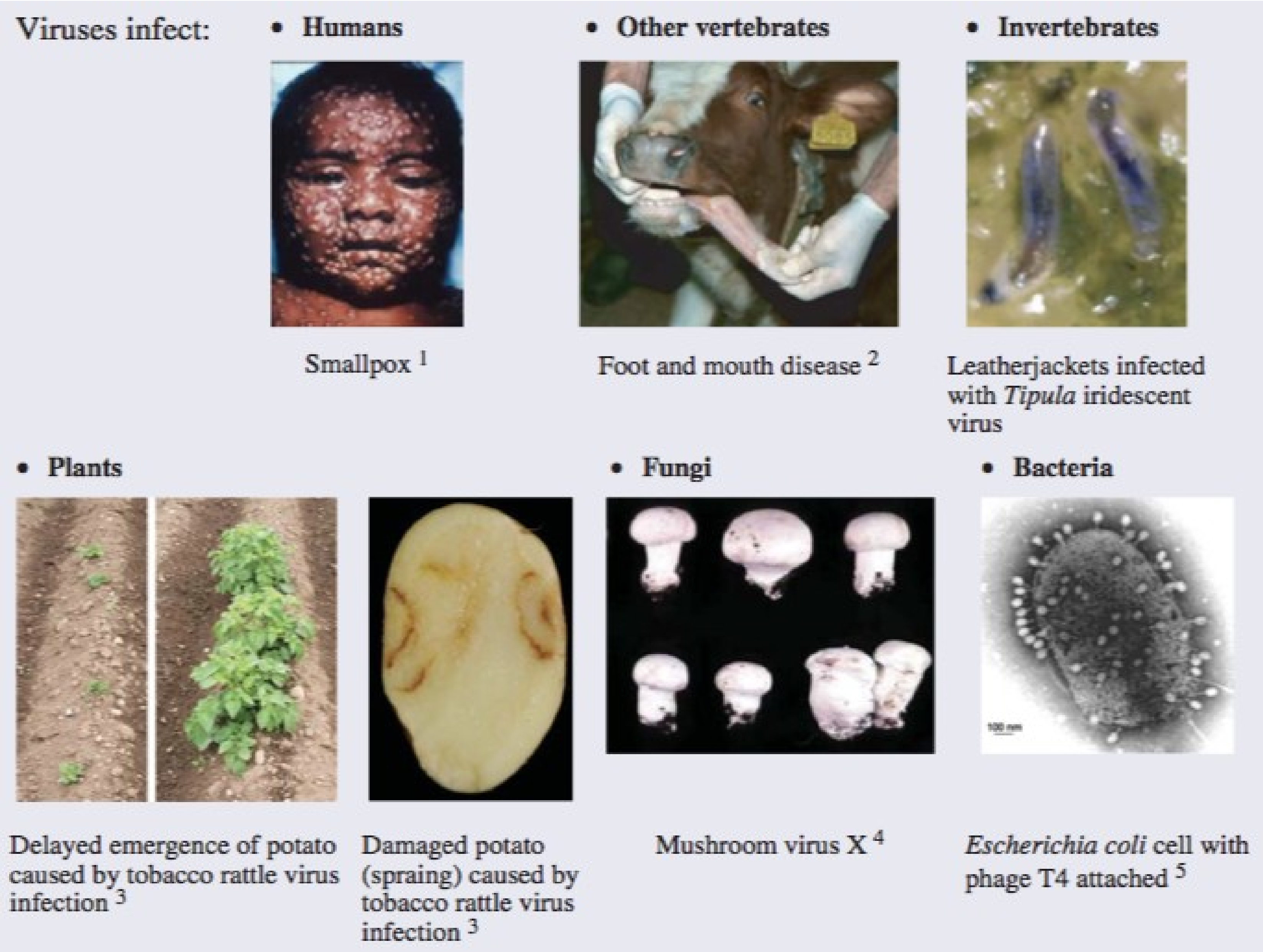
Course textbook:

14 copies are available in campus library

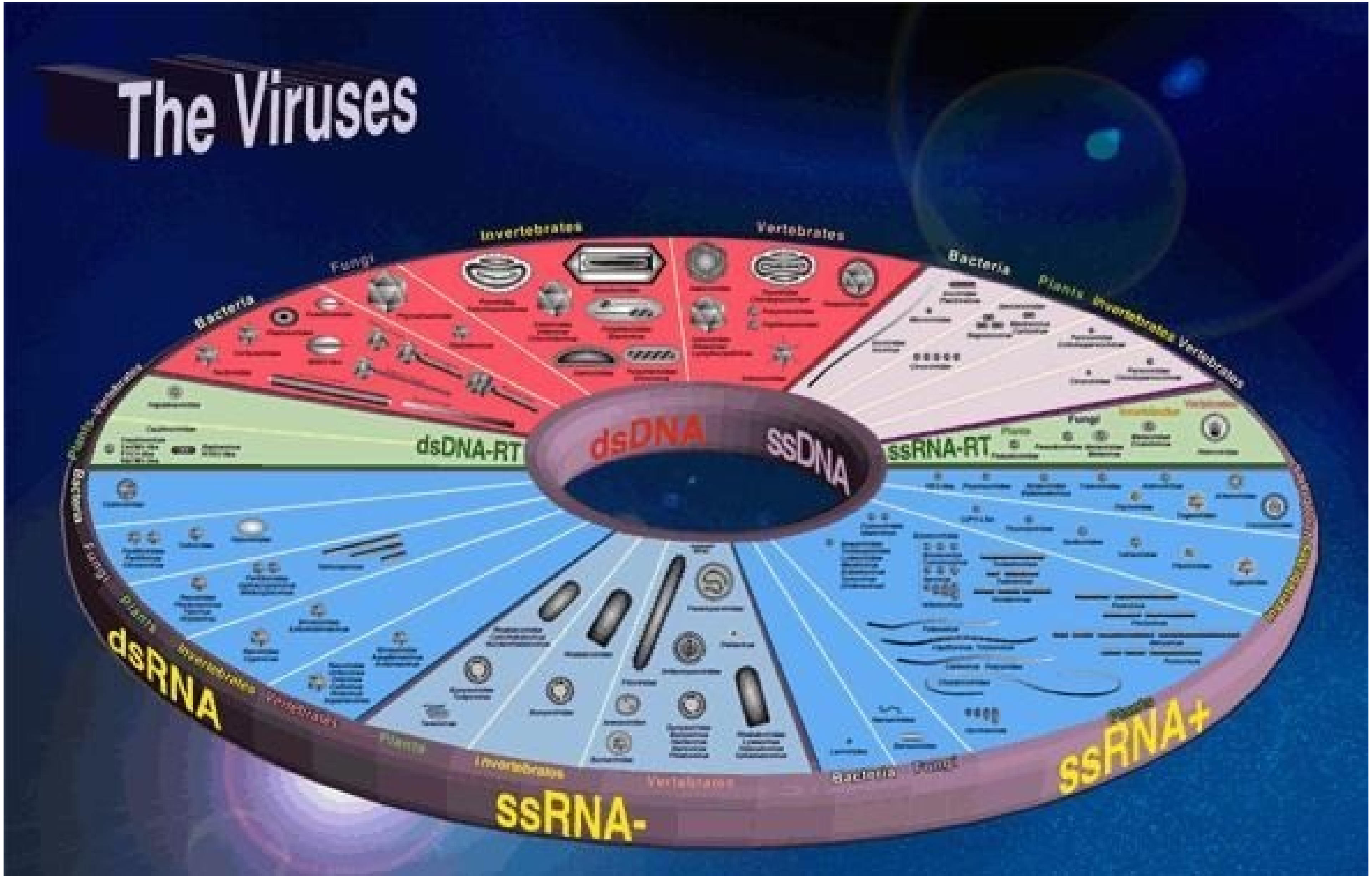




Viruses and their importance



Virosphere!


The Viruses
dsDNA
ssDNA
dsDNA-RT
ssRNA-RT
dsRNA
ssRNA-
ssRNA+
Invertebrates
Vertebrates
Fungi
Bacteria
Plants

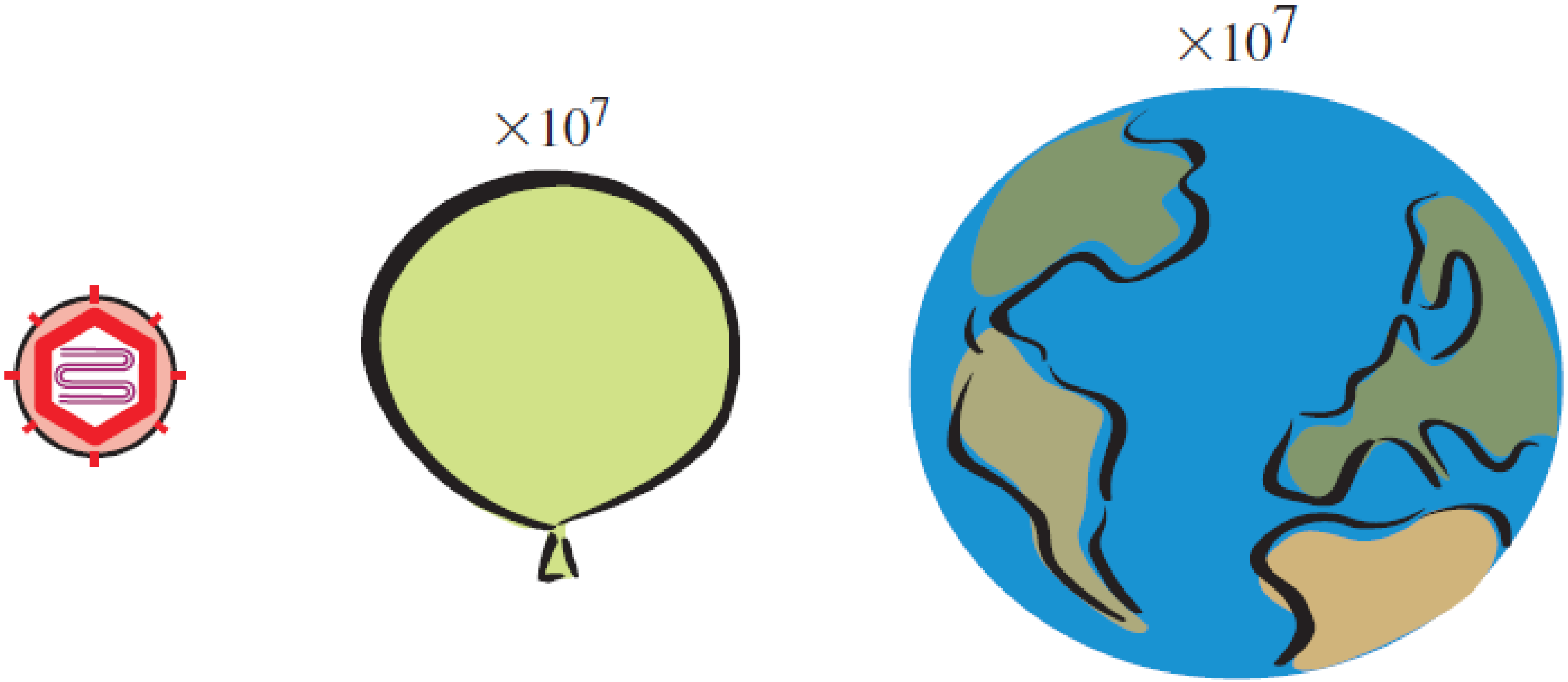
Useful viruses

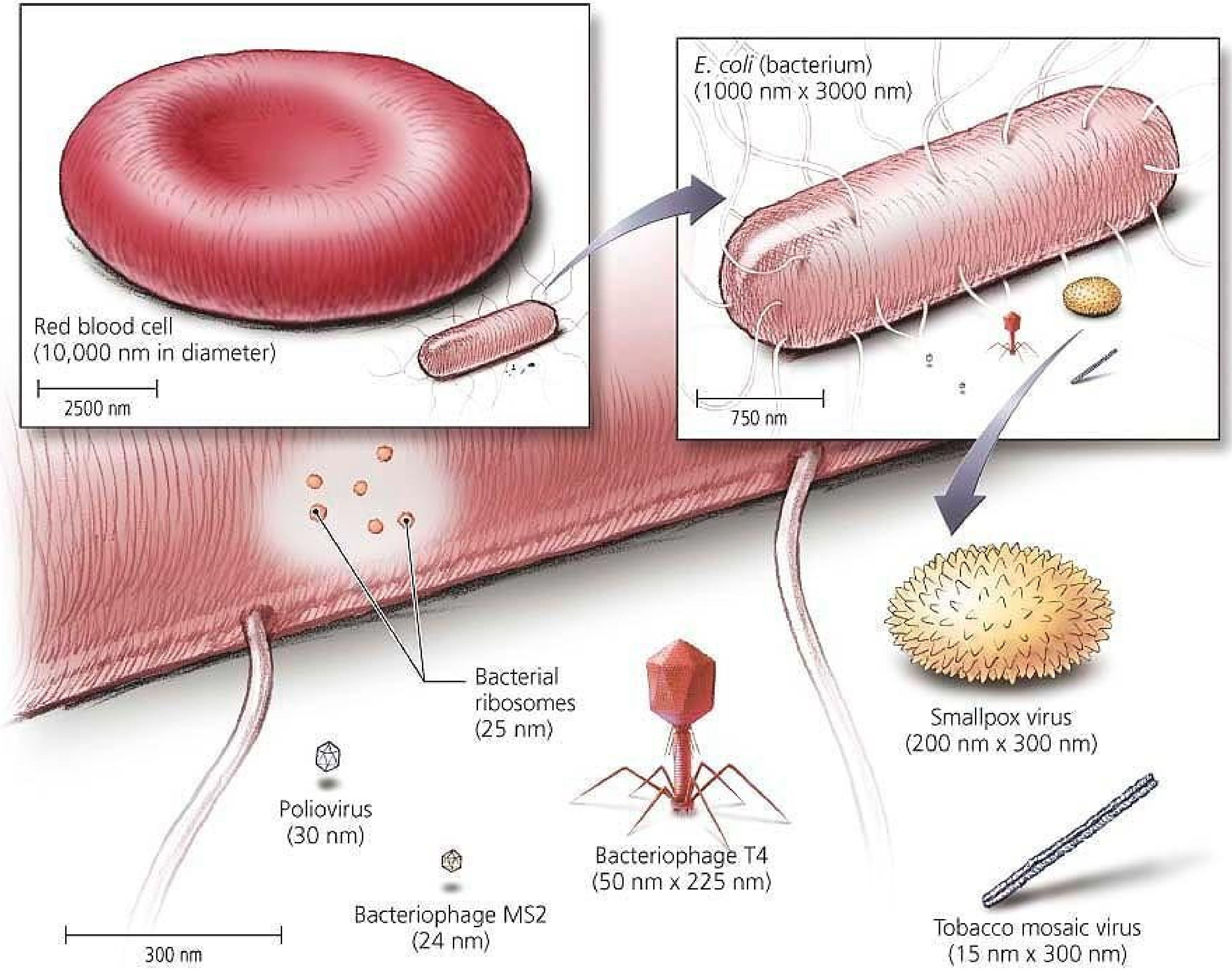
- Phage typing of bacteria
- Sources of enzymes (polymerases, T4 ligase, reverse transcriptase)
- Pesticides (baculoviruses – lepidoptera, hymenoptera, myxoma virus - rabits) SPECIFICITY!
- Anti-bacterial agents (*S. aureus* phage phi812)
- Anti-cancer agents (adeno-associated viruses)
- Gene vectors (capsids used as vehicles for gene delivery)
- Mass protein production

Fundamental discoveries in biology

- Phage T2 and *E. coli* were used to provide evidence that genes are composed of DNA.
- The first enhancers to be characterized were in genes of simian virus 40 (SV40).
- The first transcription factor to be characterized was the T antigen of SV40.
- The first nuclear localization signal of a protein was identified in the T antigen of SV40.
- Introns were discovered during studies of adenovirus transcription.
- The role of the cap structure at the 5’ end of eukaryotic messenger RNA was discovered during studies with vaccinia virus and a reovirus.
- The first internal ribosomal entry site was found in poliovirus.
- The first RNA pseudoknot in the genome of turnip yellow mosaic virus.

Most viruses are 25-400 nm in size

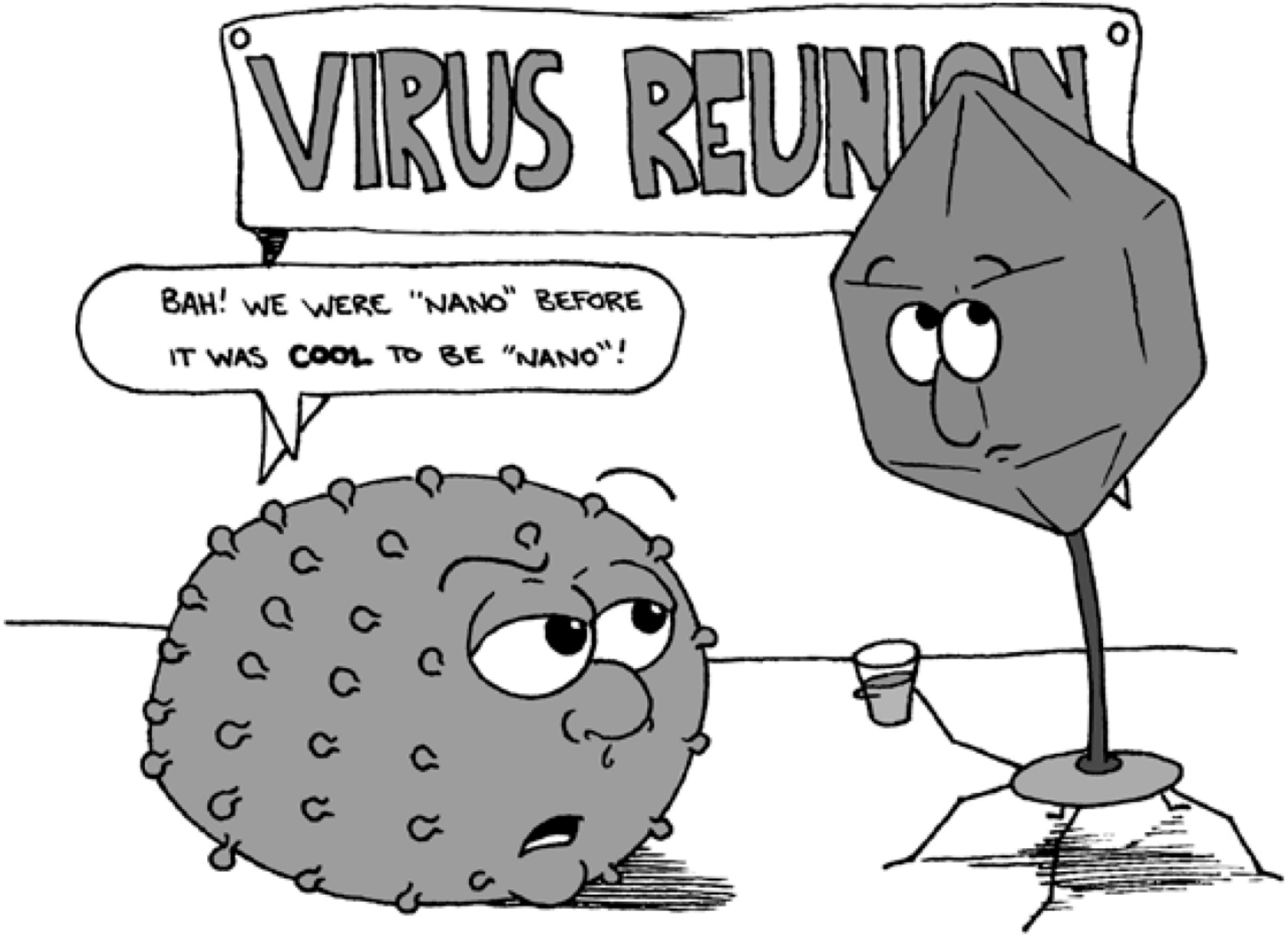



Red blood cell
(10,000 nm in diameter)
2500 nm
E. coli (bacterium)
(1000 nm x 3000 nm)
750 nm
Bacterial
ribosomes
(25 nm)
Smallpox virus
(200 nm x 300 nm)
Poliovirus
(30 nm)
Bacteriophage T4
(50 nm x 225 nm)
Bacteriophage MS2
(24 nm)
Tobacco mosaic virus
(15 nm x 300 nm)
300 nm

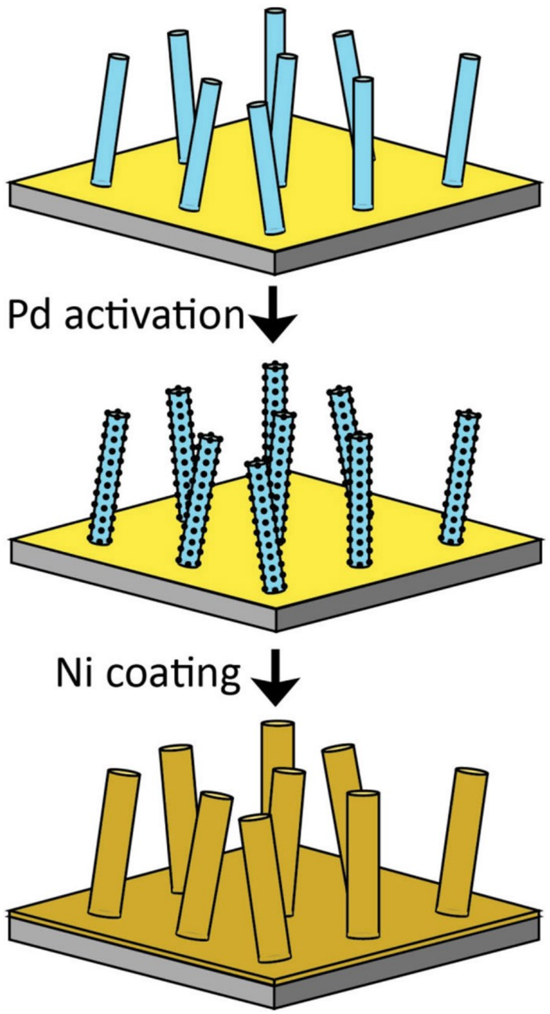
Units of length

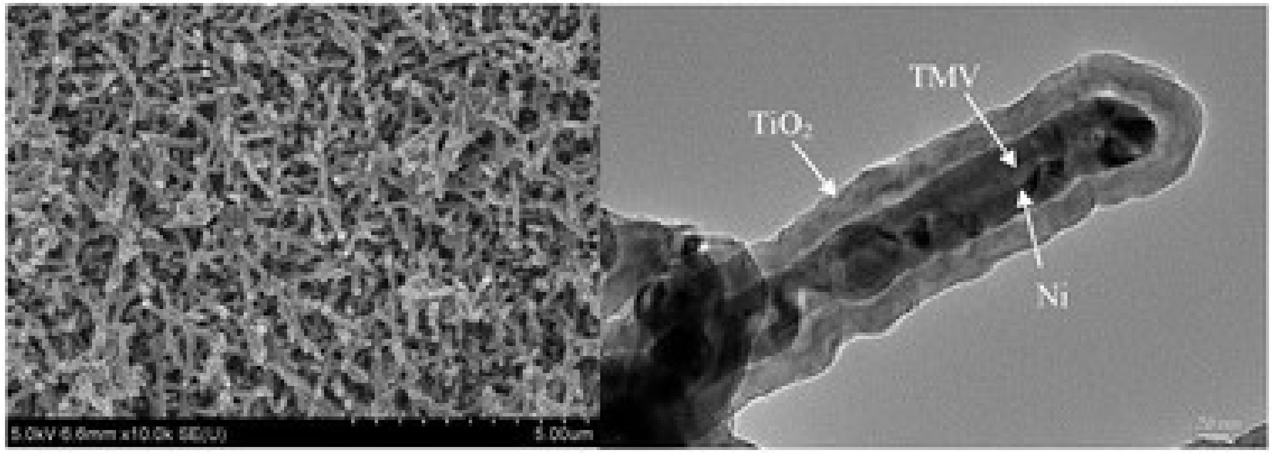
Meter	1				
Centimeter	100	1			
Milimeter	1,000	10	1		
Micron	1,000,000	10,000	1,000	1	
Nanometer	1,000,000,000	10,000,000	1,000,000	1,000	1
Angstrom	10,000,000,000	100,000,000	10,000,000	10,000	10

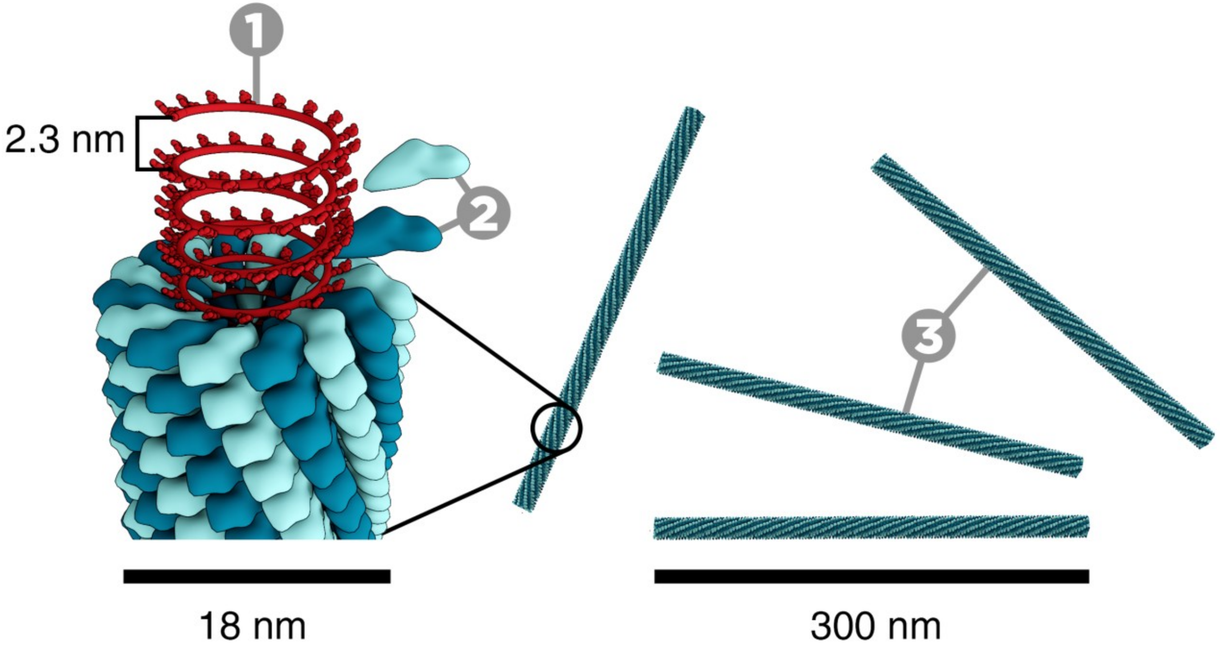
Angstrom	1				
Nanometers	.1	1			
Microns	.0001	.001	1		
Milimeters	.0000001	.000001	.001	1	
Centimeters	.00000001	.0000001	.0001	.1	1
Meters	.0000000001	.000000001	.000001	.001	.01


VIRUS REUNI
BAH! WE WERE "NANO" BEFORE IT WAS COOL TO BE "NANO"!

Using TMV to increase surface of electrodes







Methods to study virus structures

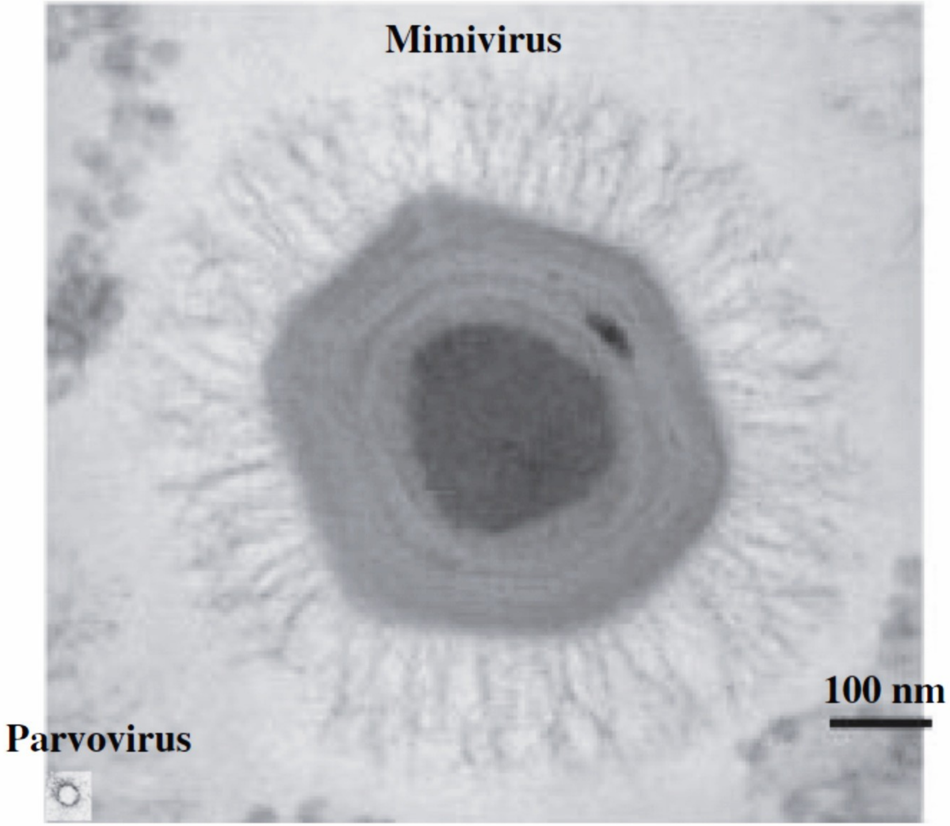
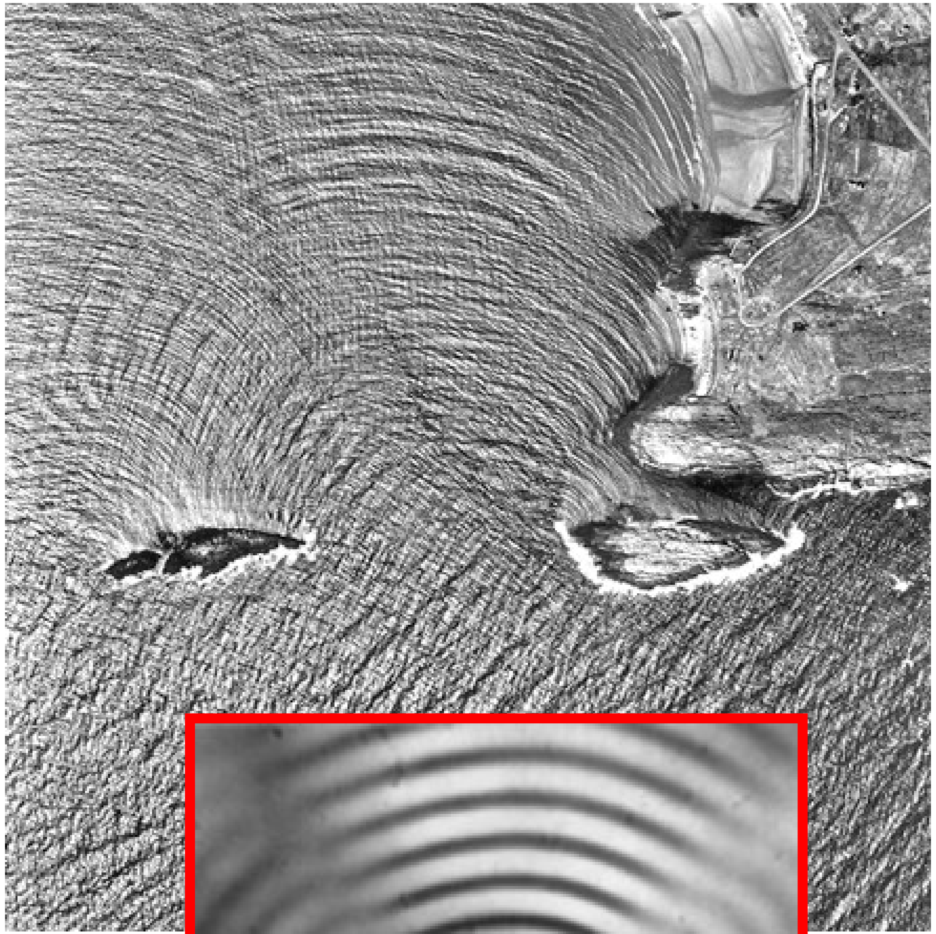
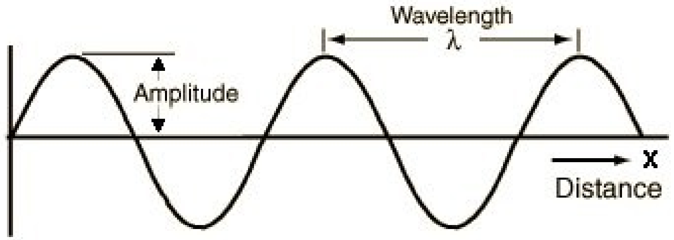


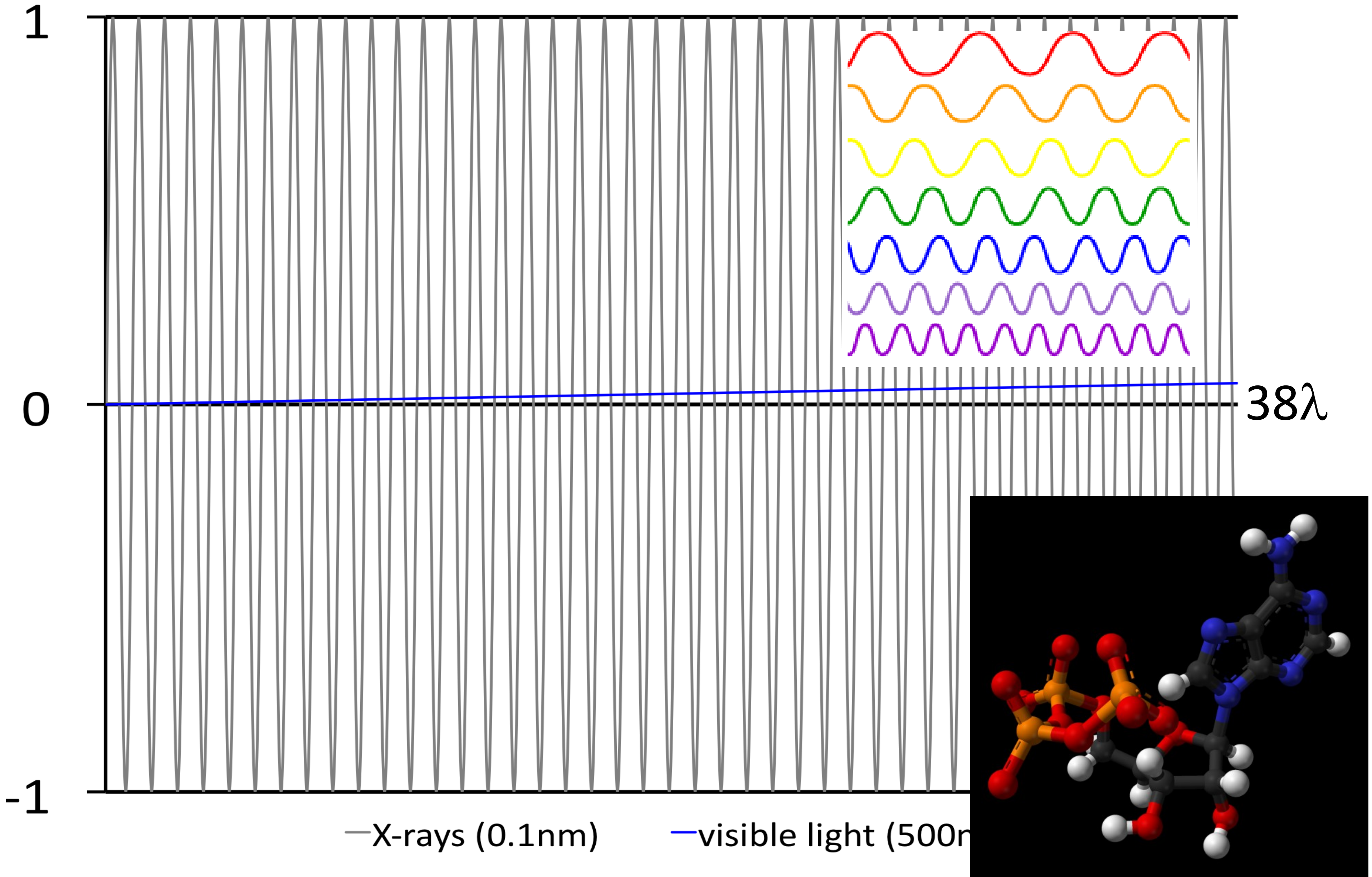
Figure 1.3 Virions of mimivirus, one of the largest viruses, and a parvovirus, one of the smallest viruses.

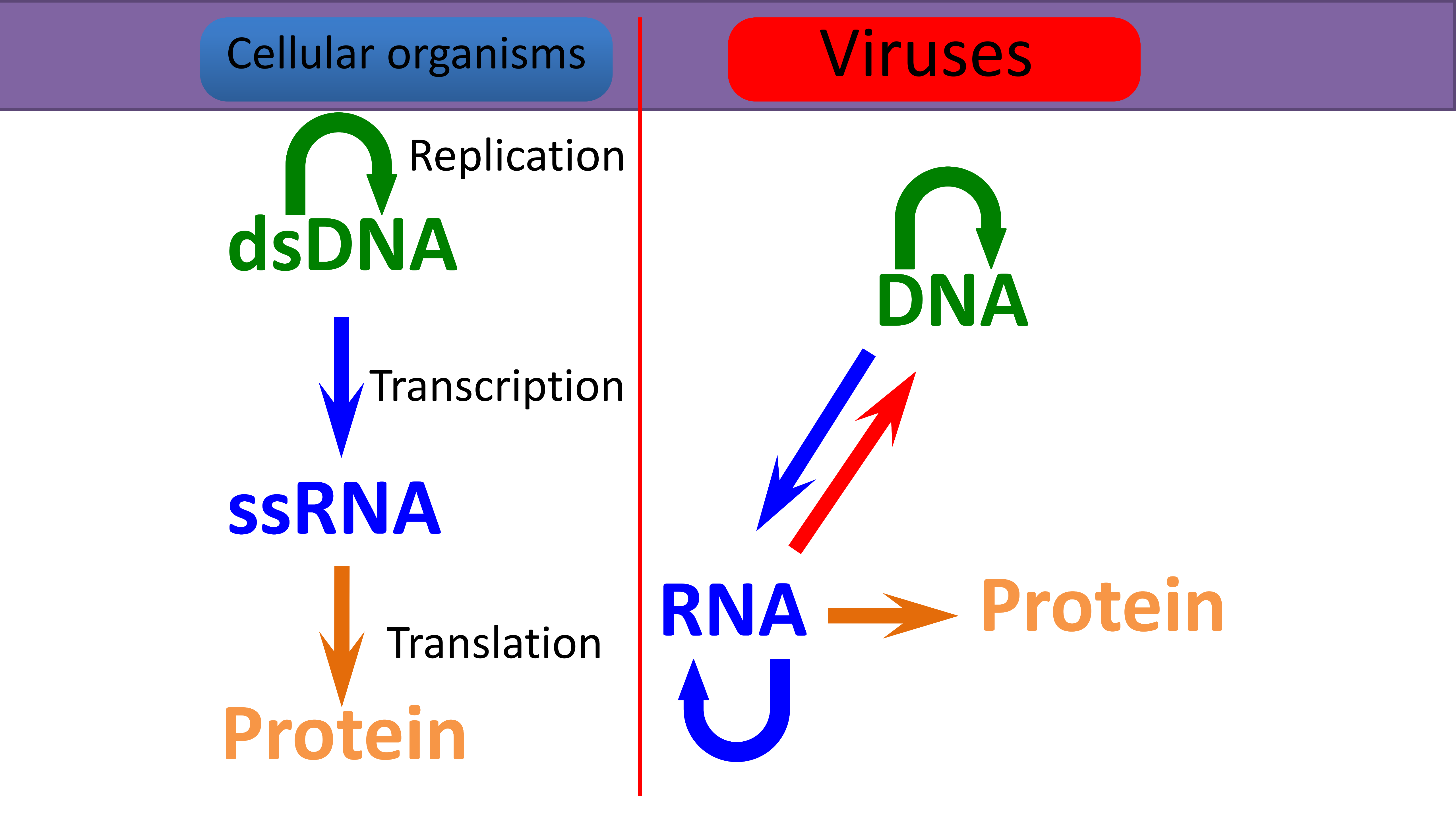
Wavelength and diffraction





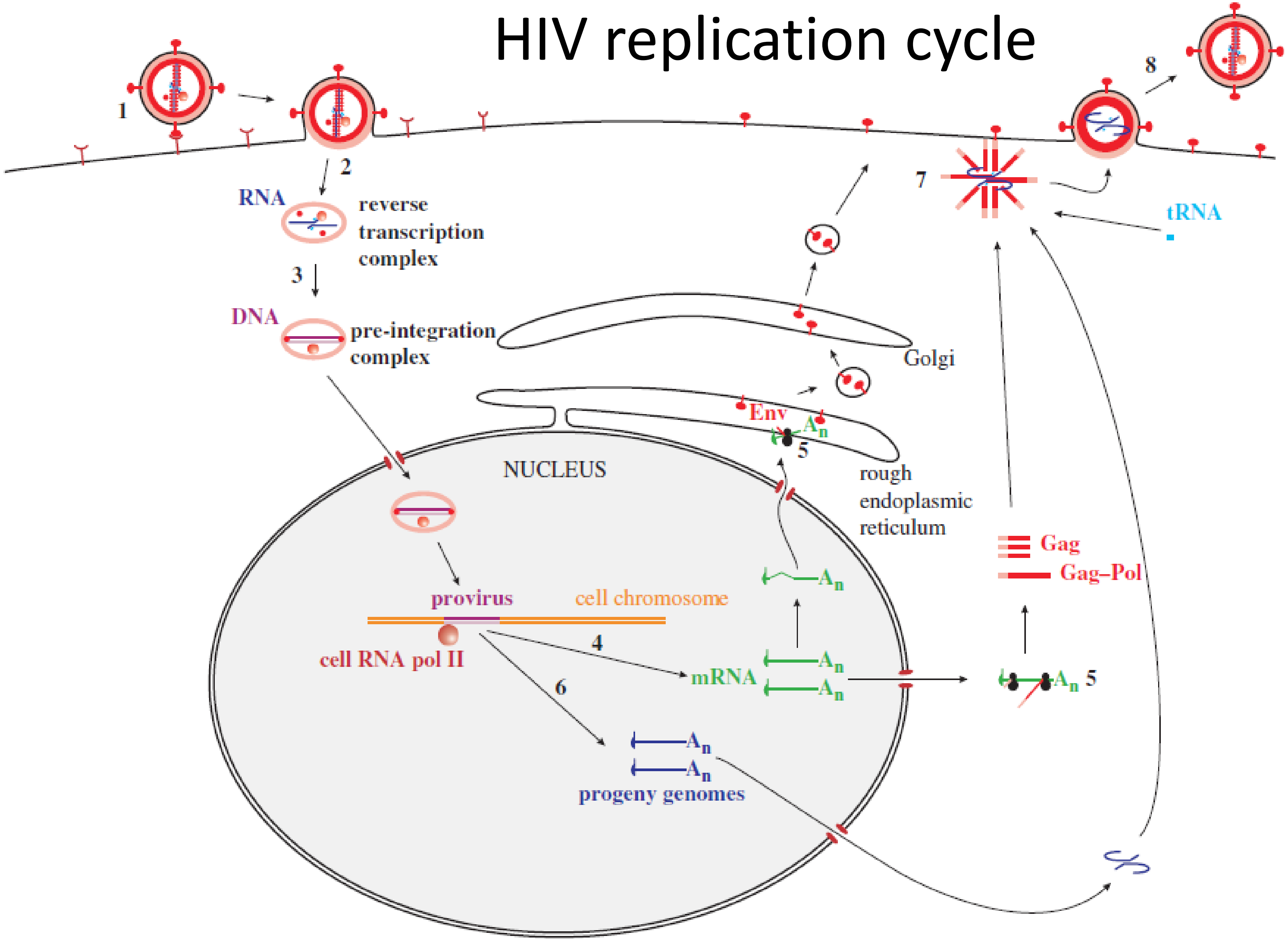
Wavelength comparison of X-rays and visible light




Cellular organisms
Viruses
Replication
dsDNA
Transcription
ssRNA
Translation
Protein
DNA
RNA
Protein

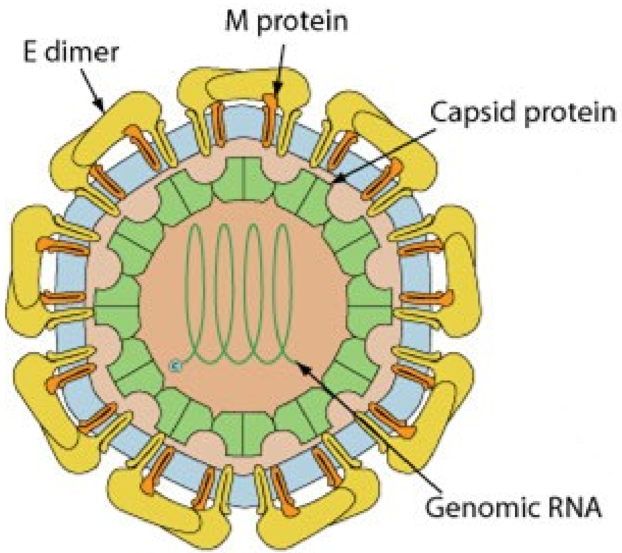
Viruses are intracellular parasites

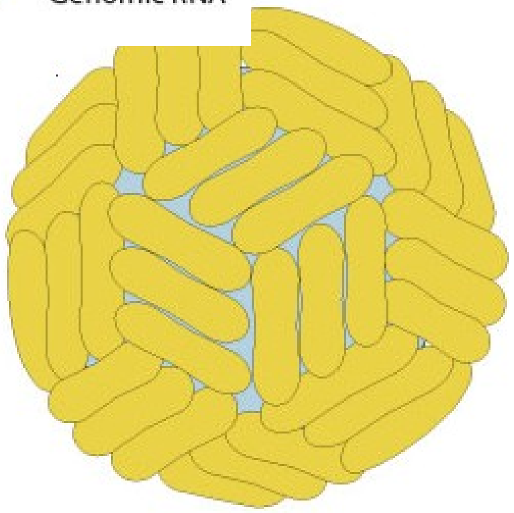
HIV replication cycle



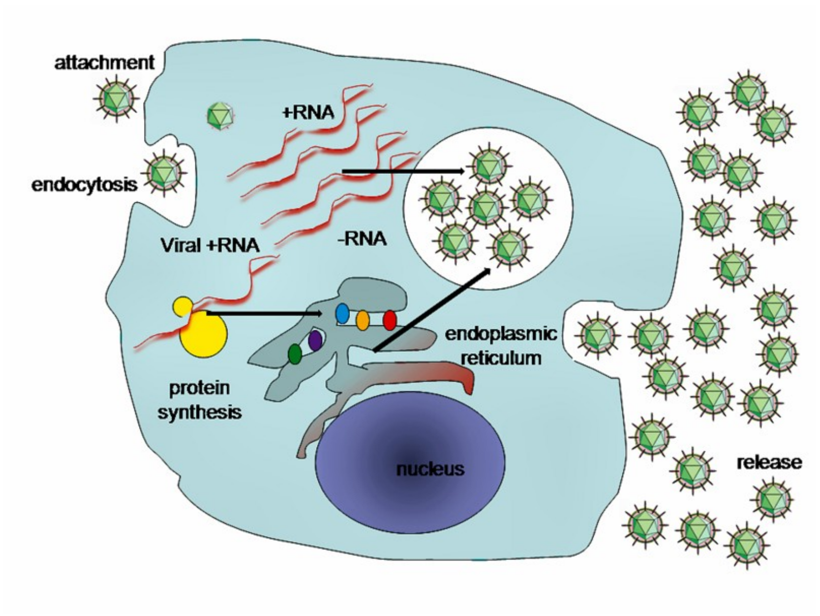
Two forms of virus existence

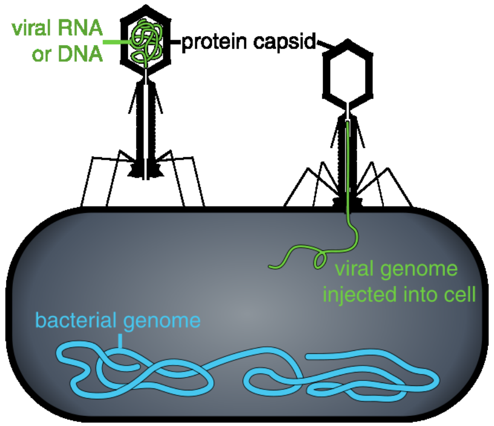
“Virion” – virus particle





Cell infection – Viro-cell





Are viruses alive?

Pro-life	Anti-life
Have genes	Do not have cellular structure
They evolve	No metabolism
Subject to natural selection	Require host cell for pro-creation
Procreate	New viruses assemble from parts

Virus definition

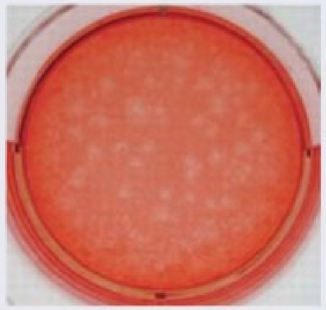
A virus is a very small, non-cellular parasite of cells. Its genome, which is composed of either DNA or RNA, is enclosed in a protein coat.

Learning outcomes

- discuss reasons for studying viruses
- explain how viruses differ from other organisms
- define the term ‘virus’

Methods used in virology

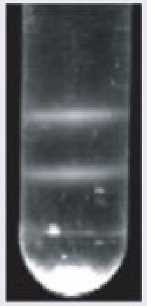
Virus Isolation and Culture



Animal virus plaques in a cell culture [1]

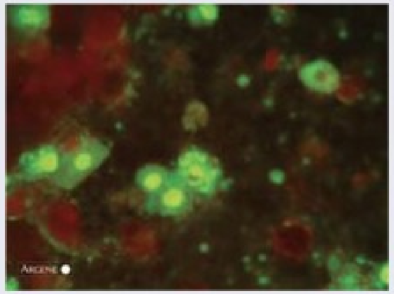
Phage plaques in a lawn of bacterial cells [2]

Density Gradient Centrifugation



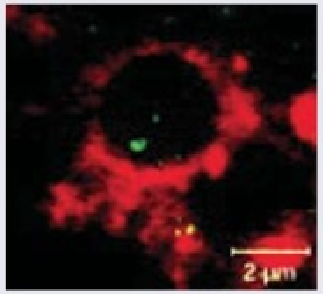
Separation of virus particles in a density gradient [3]

Fluorescence Microscopy



Virus-infected cells detected using a virus-specific antibody labelled with a fluorescent dye [4]

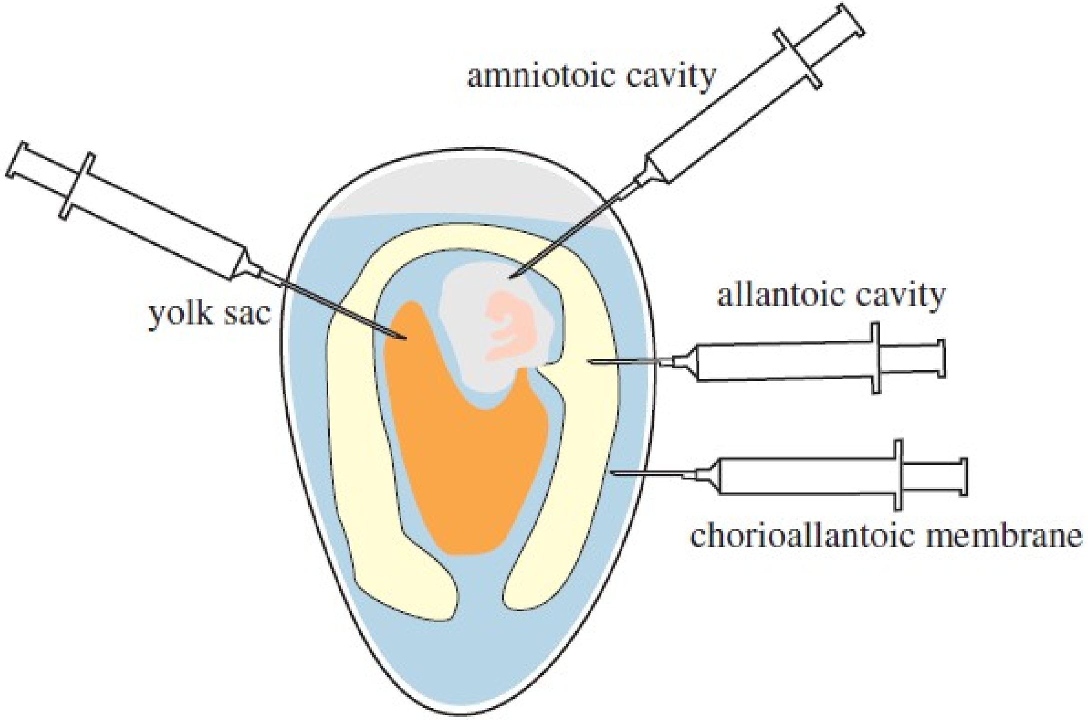
Confocal Microscopy



An endosome (labelled red) containing virus protein (labelled green) in an infected cell [5]

Virus production in eggs

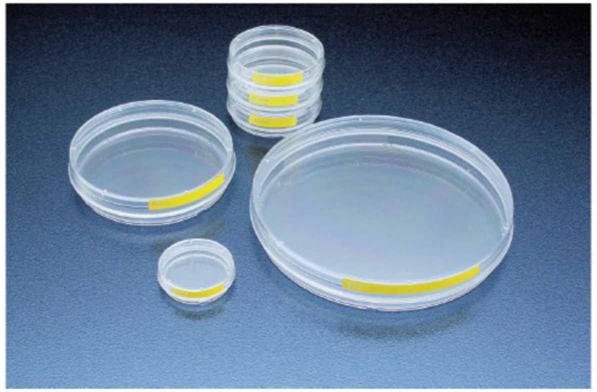
Sites into which viruses can be inoculated

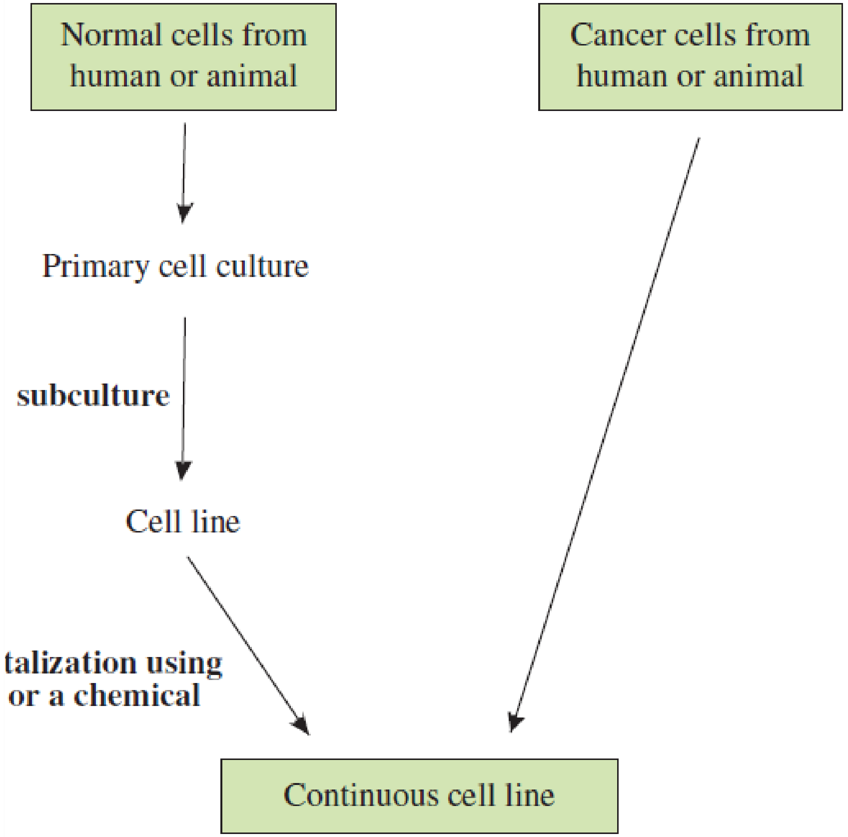


Inoculation of an egg

Virus production honeybee pupae

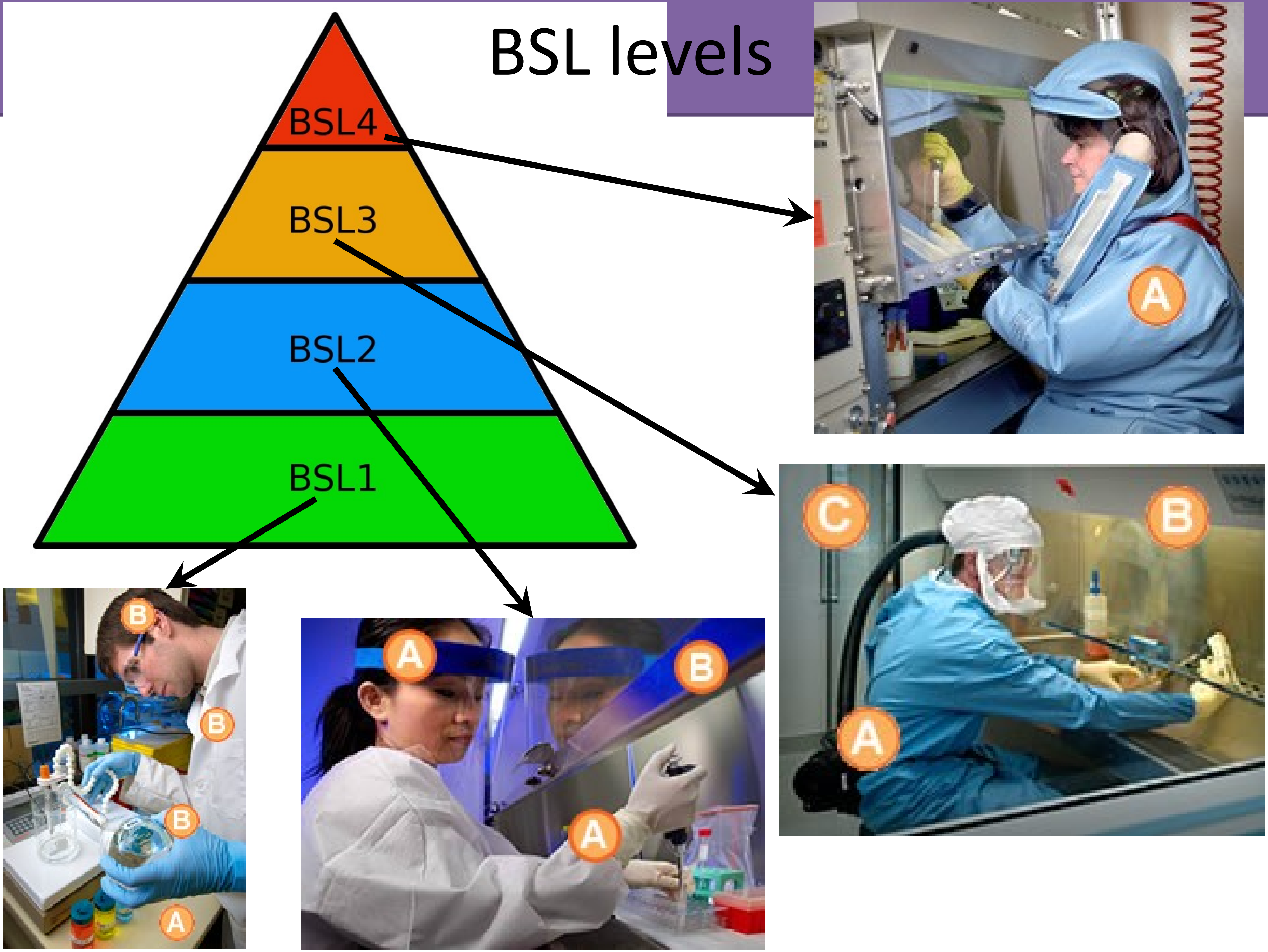
Virus production in vitro in tissue culture





Sterile work with tissue cultures

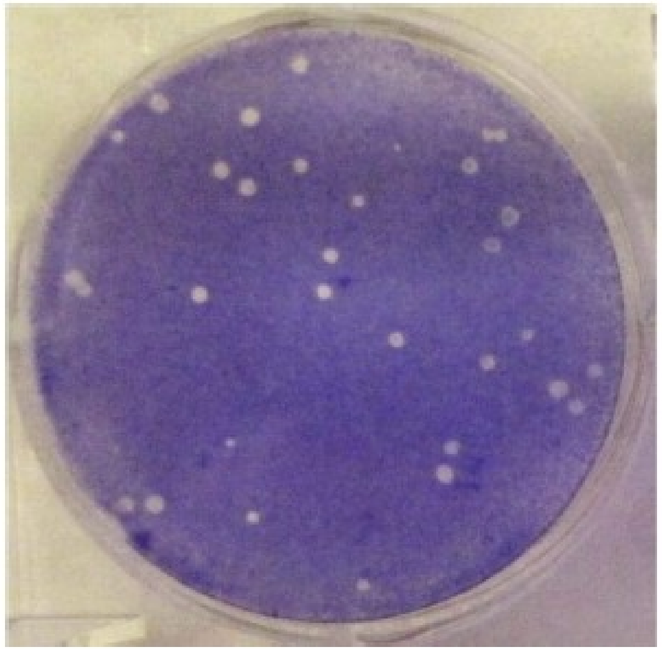
BSL levels



Plaque assay for animal viruses

A cell monolayer is inoculated with virus and overlaid with agarose.

Plaques formed by influenza virus in a cell monolayer



HeLa 300 tis bb./ml, 10.2.14
Fixed after 72h
BEST OF CMC

HRV16 from stock 28.11.13,
PFU 5.10^3

Phage plaque assay

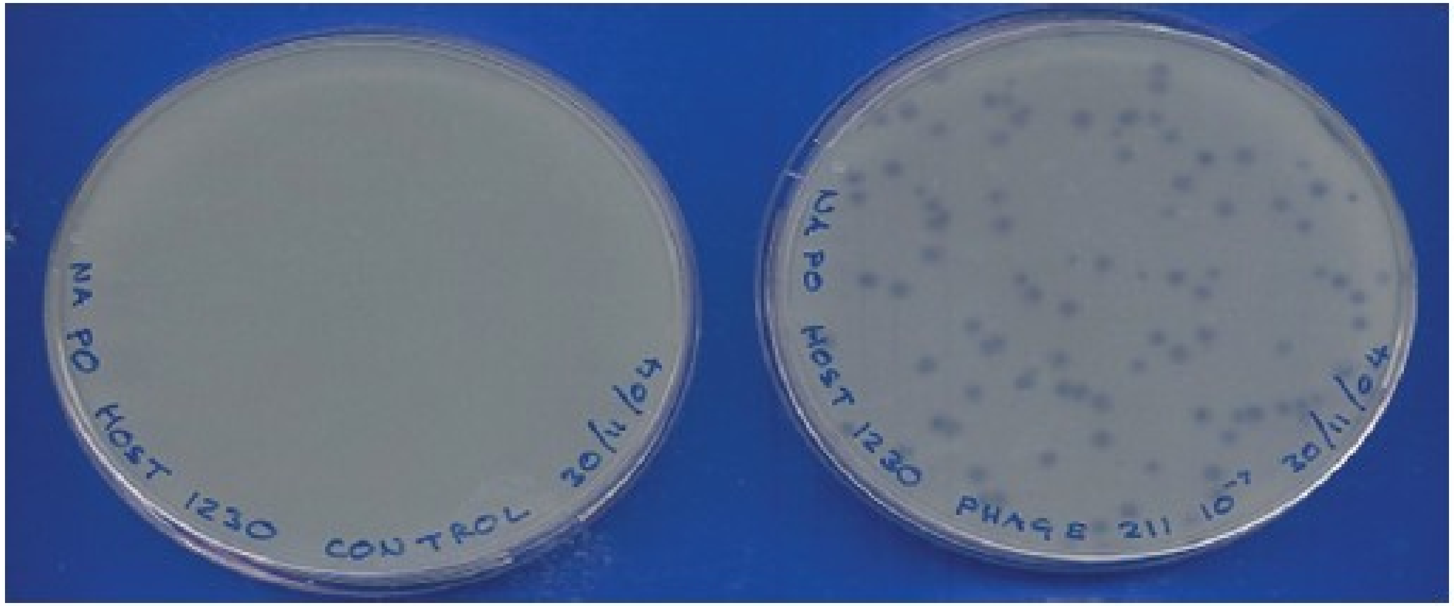
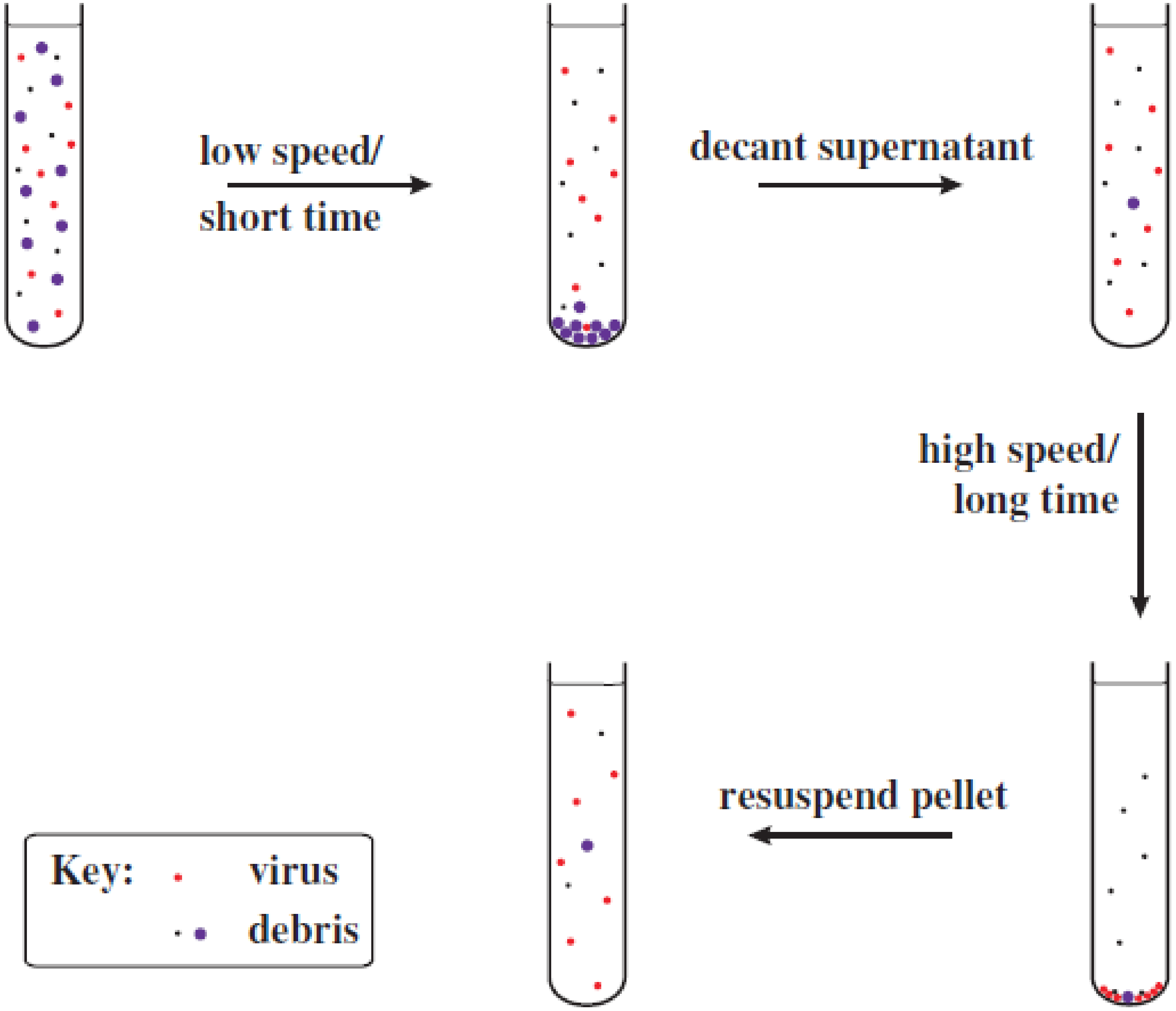
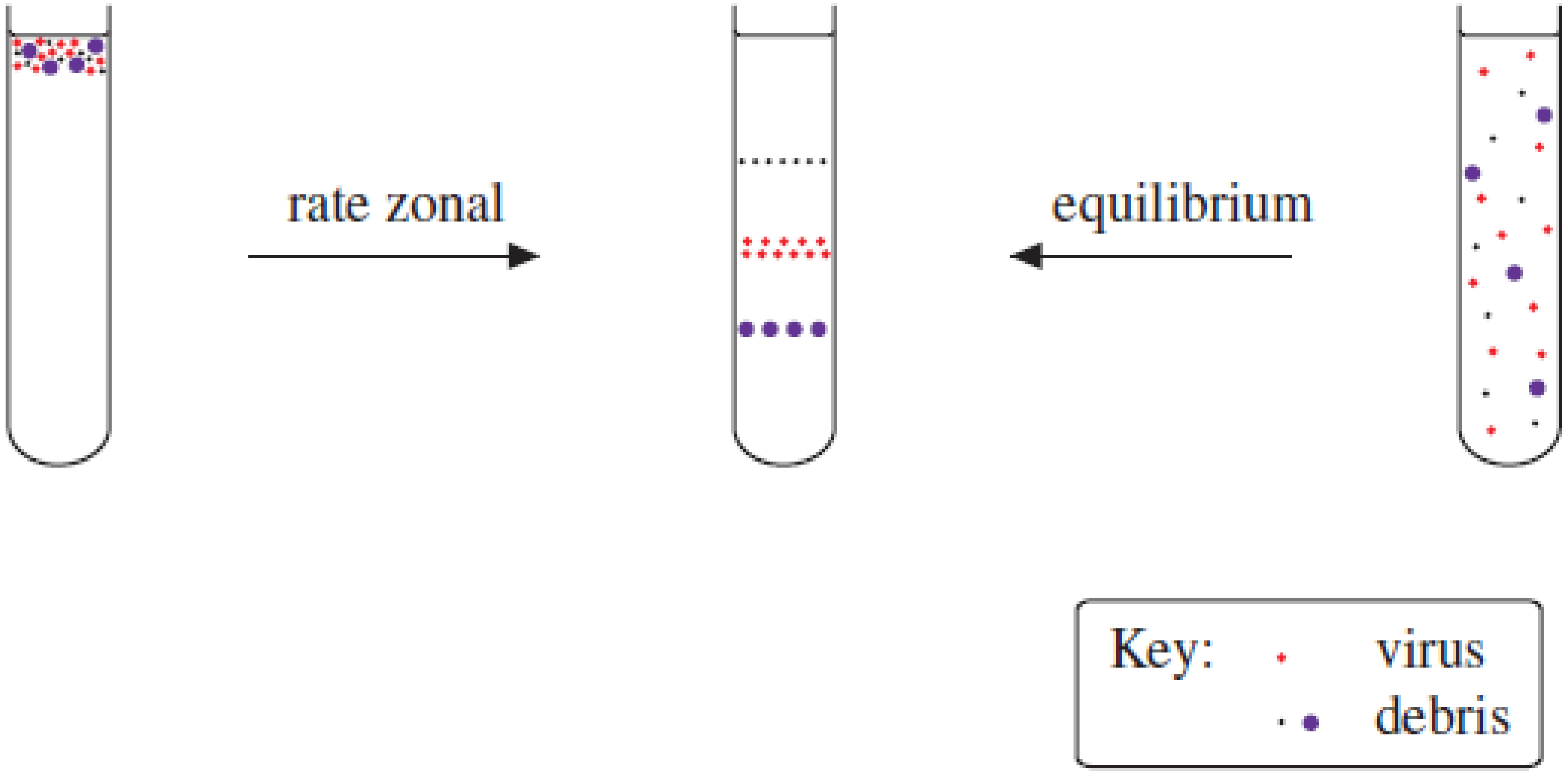


Figure 2.6 Plaques formed by a phage in a bacterial lawn. The control plate on the left was inoculated with only the bacterial host. The plate on the right was inoculated with phage and bacterial host.

Differential centrifugation



Density gradient centrifugation



SDS-PAGE

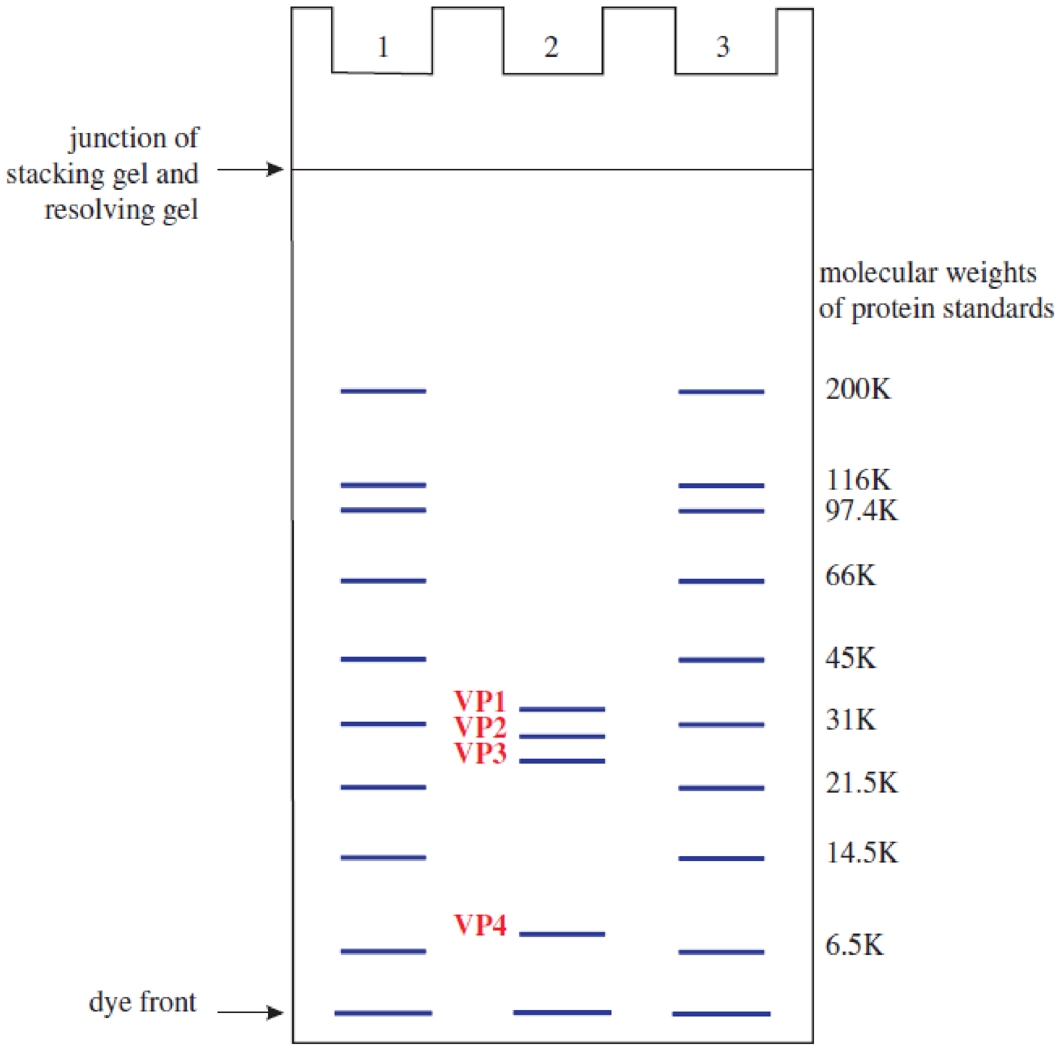
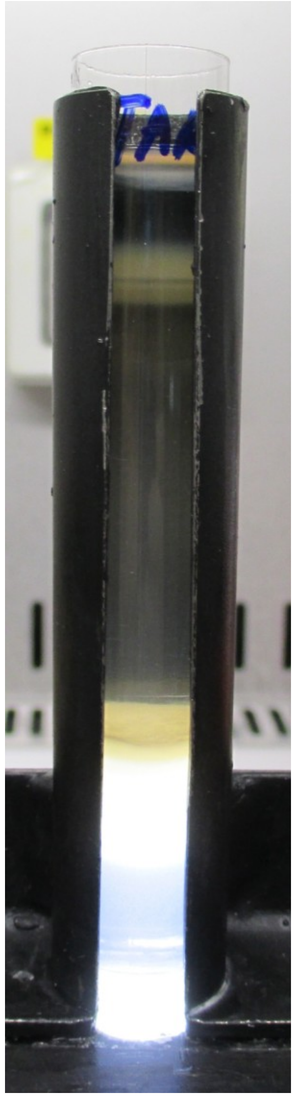
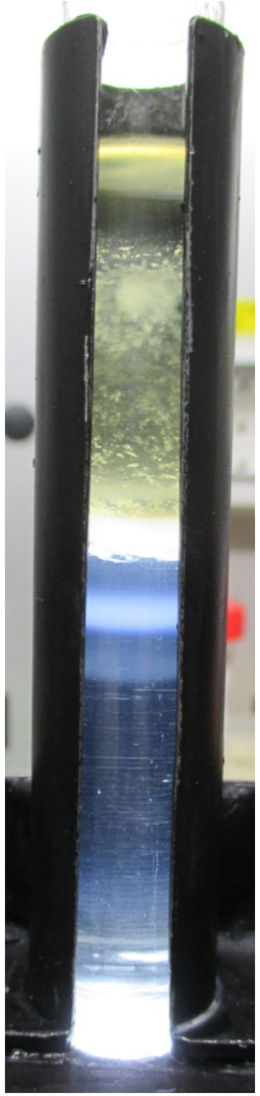


Figure 2.9 Diagram illustrating separation of proteins and estimation of their molecular weights using SDS-PAGE. Lanes 1 and 3 contain standard proteins of known molecular weight. Lane 2 contains the four capsid proteins of a picornavirus.

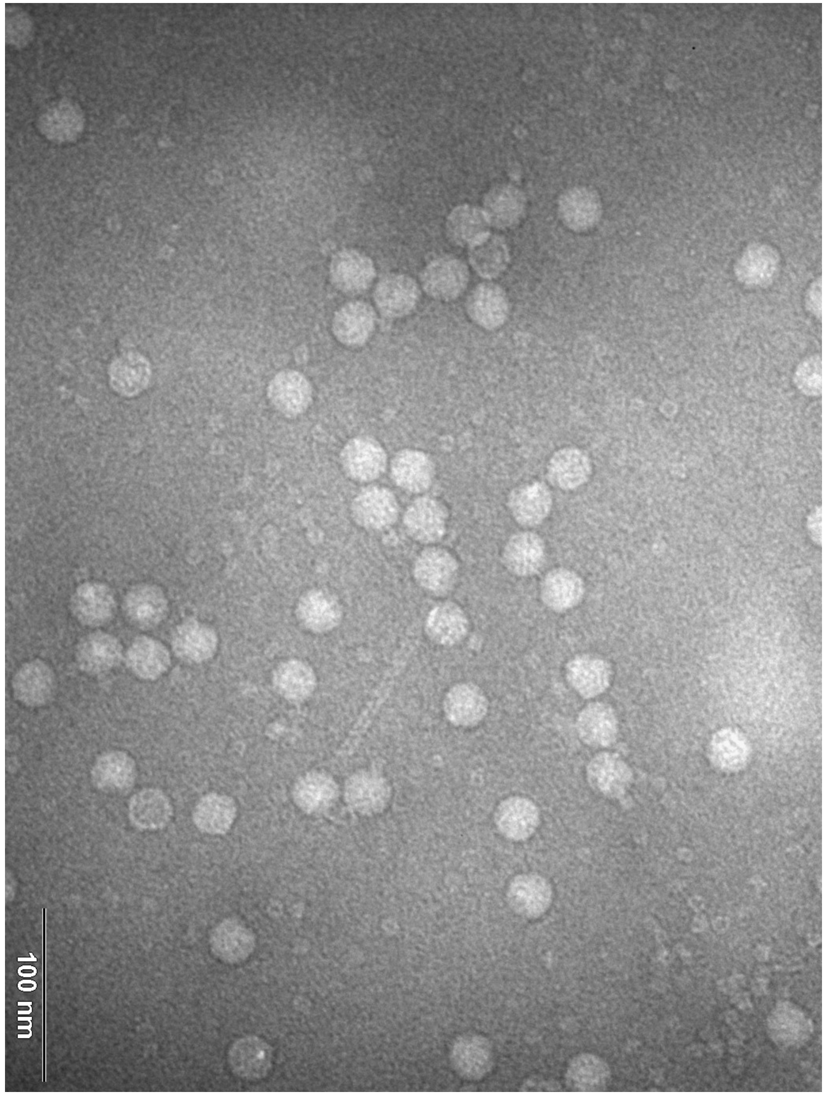
Israeli acute bee paralysis virus



tartrate
gradient

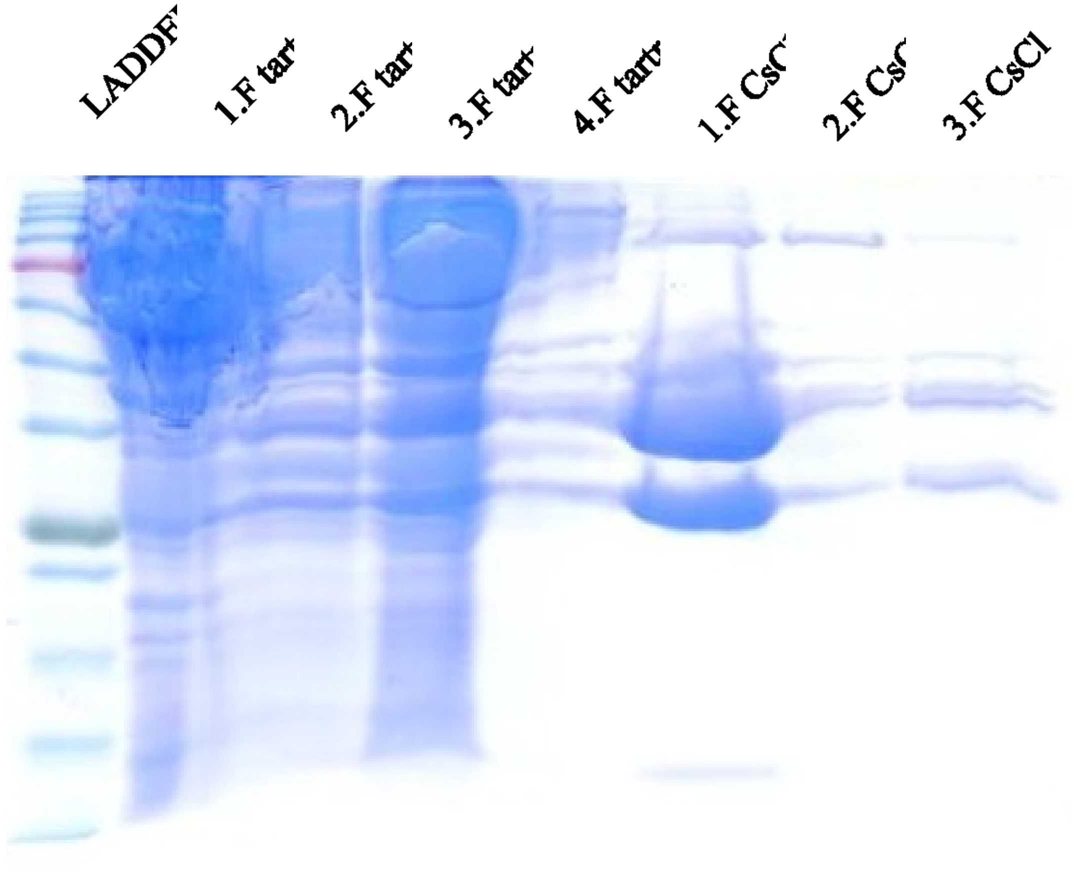


CsCl
gradient



CsCl gradient – middle fraction

SDS-PAGE of IAPV

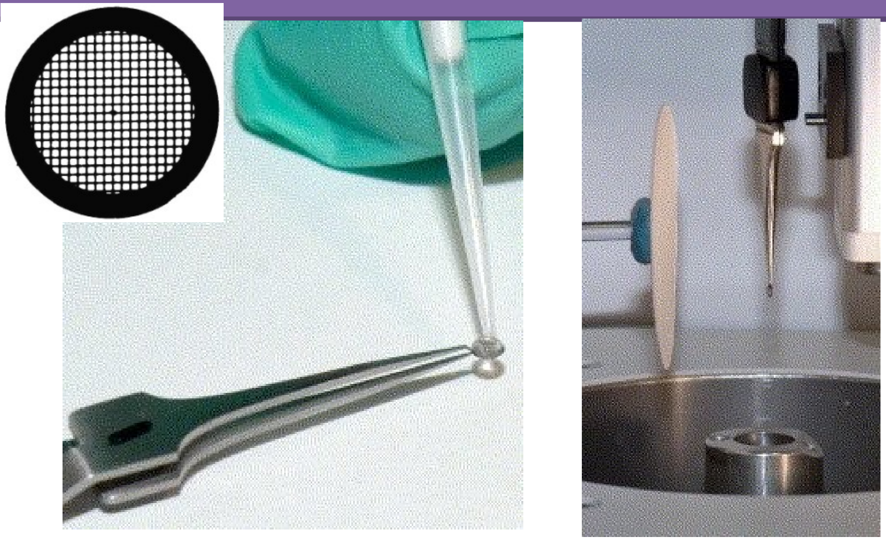


1.F tartrate	A_{260}=133.083, A_{280} = 160.379
2.F tartrate	A_{260}=36.575, A_{280} = 28.762
3.F tartrate	A_{260}=86.795, A_{280} = 70.081
4.F tartrate	A_{260}=10.029, A_{280} = 6.267

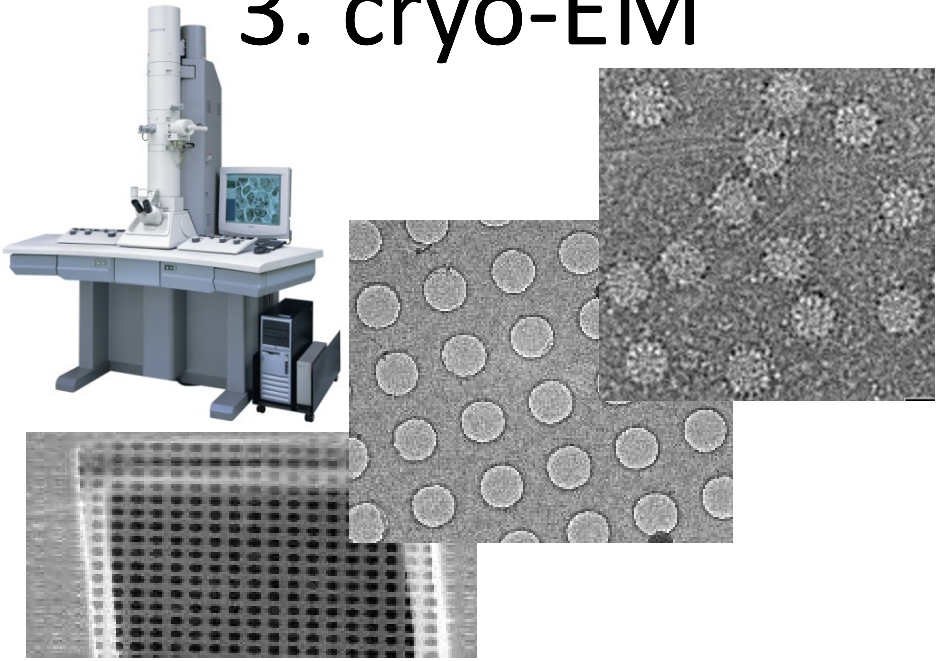
1.F CsCl	A_{260}=111.613, A_{280} = 63.564
1.F CsCl	A_{260}=2.555, A_{280} = 1.556
1.F CsCl	A_{260}=6.836, A_{280} = 3.918

1. Virus purification

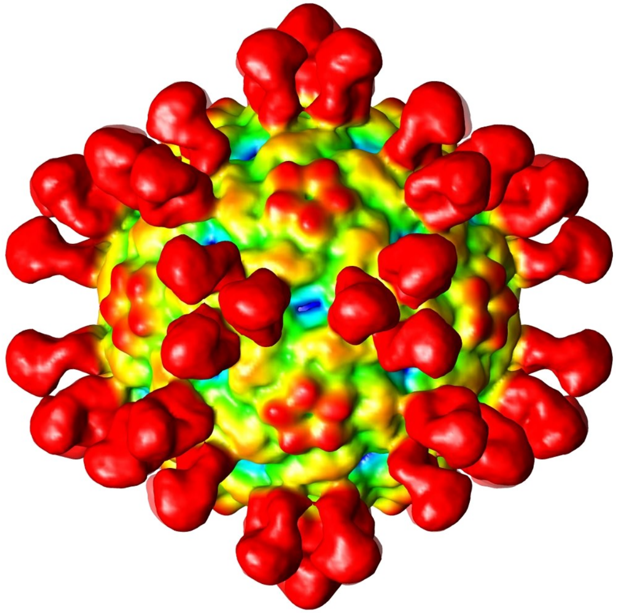
2. Grid preparation



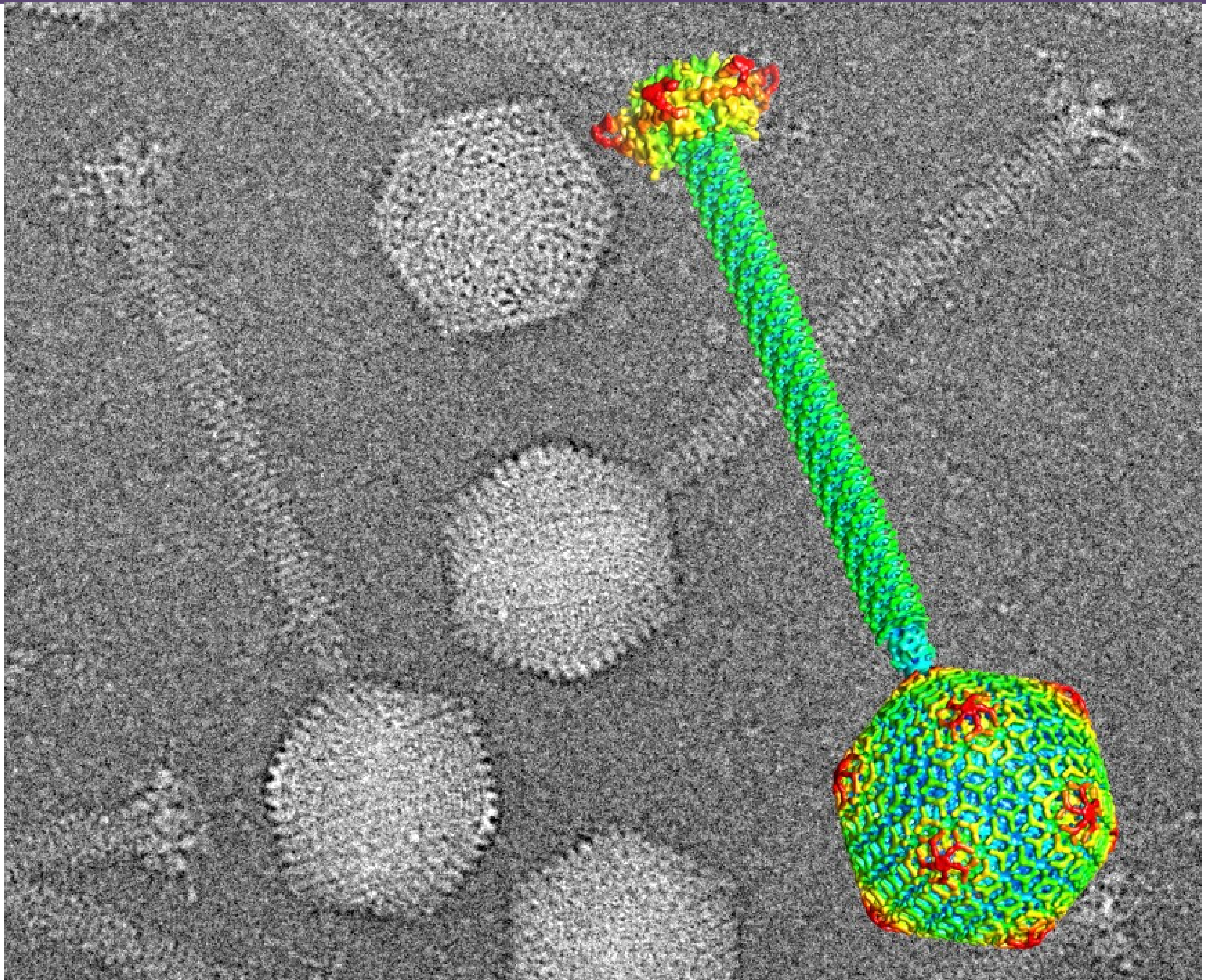
3. cryo-EM



4. Reconstruction

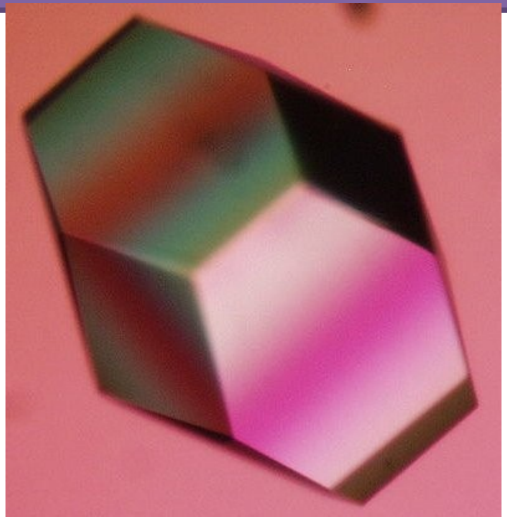


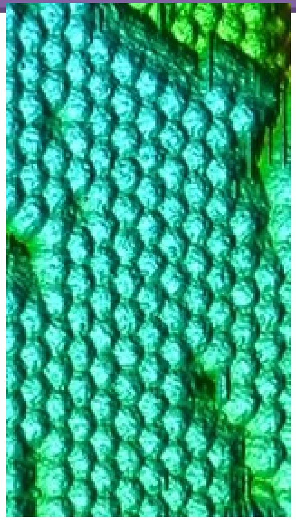
Cryo-EM of phage phi812



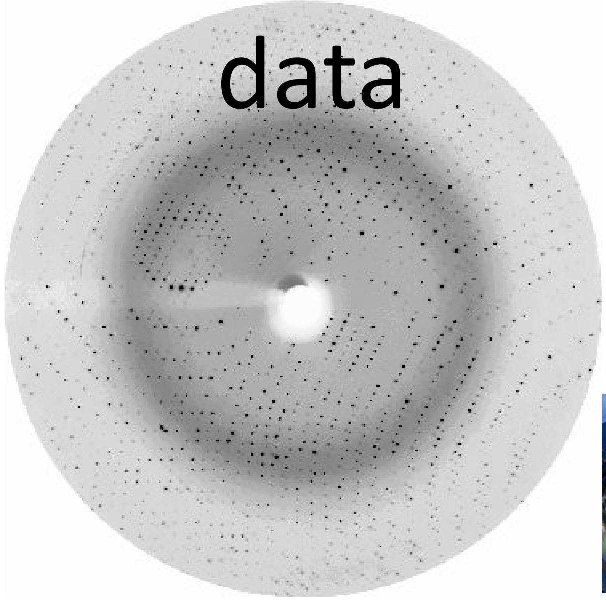
1. Virus purification

2. Crystallization

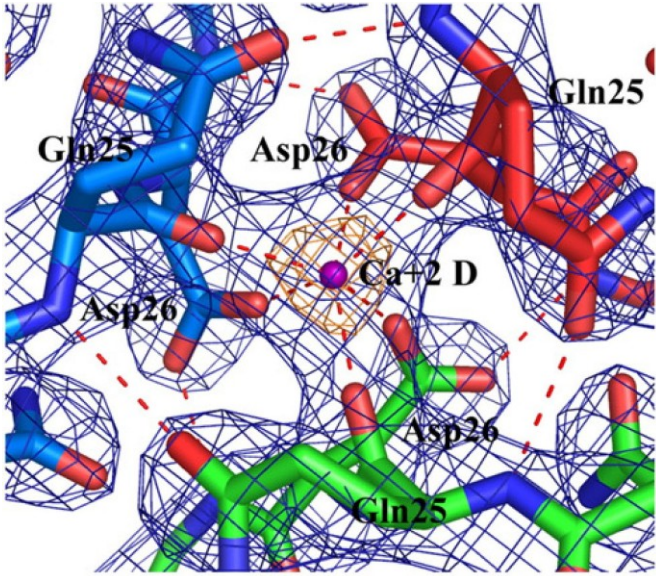




3. Diffraction data

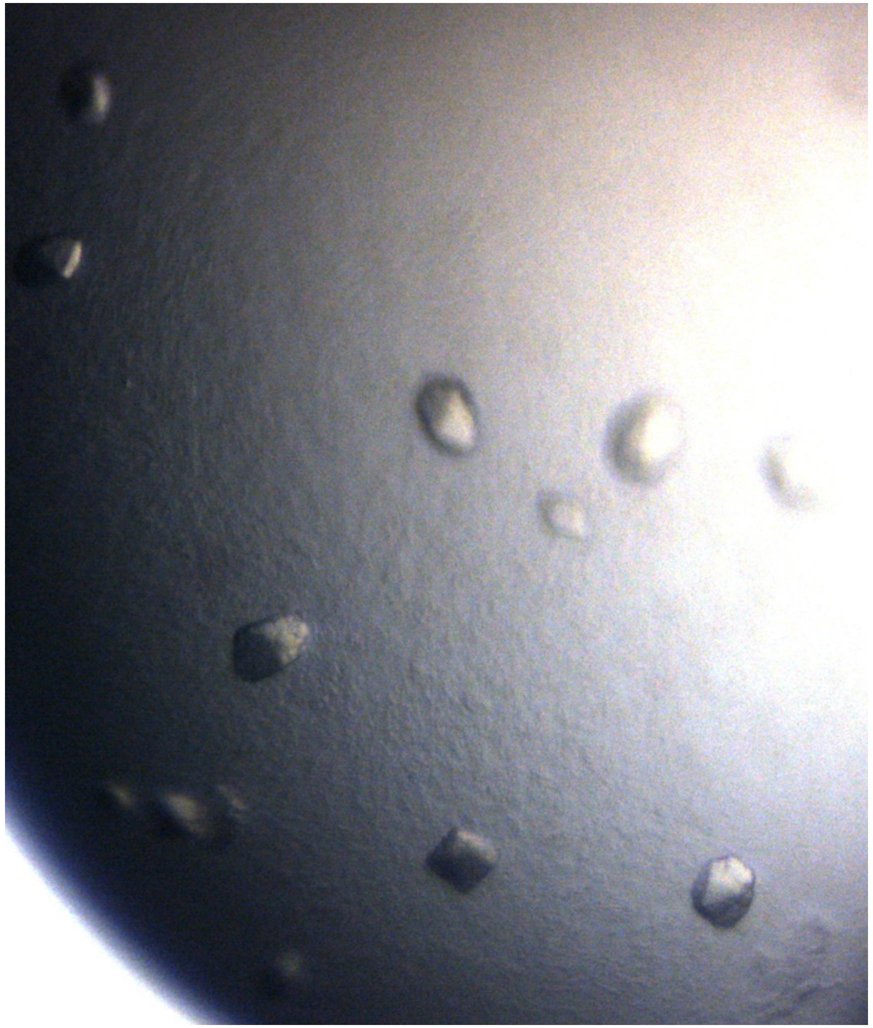


4. Solve structure

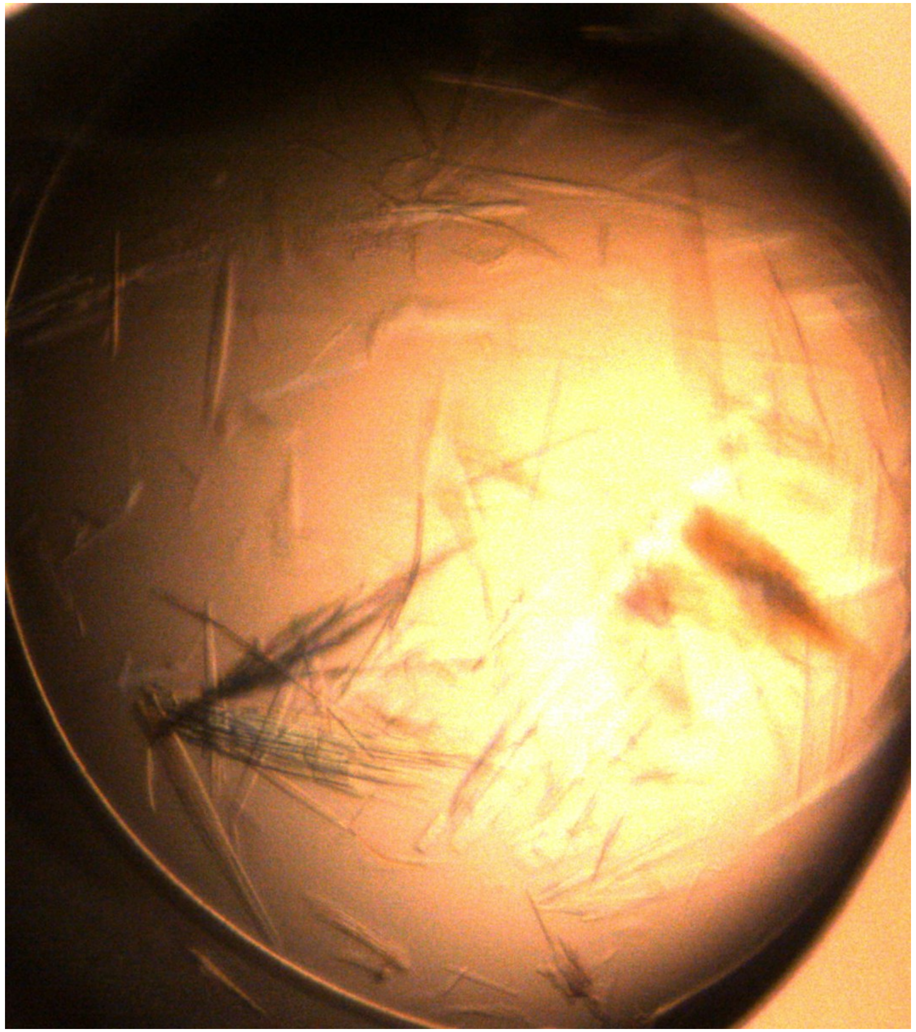


Crystals of IAPV

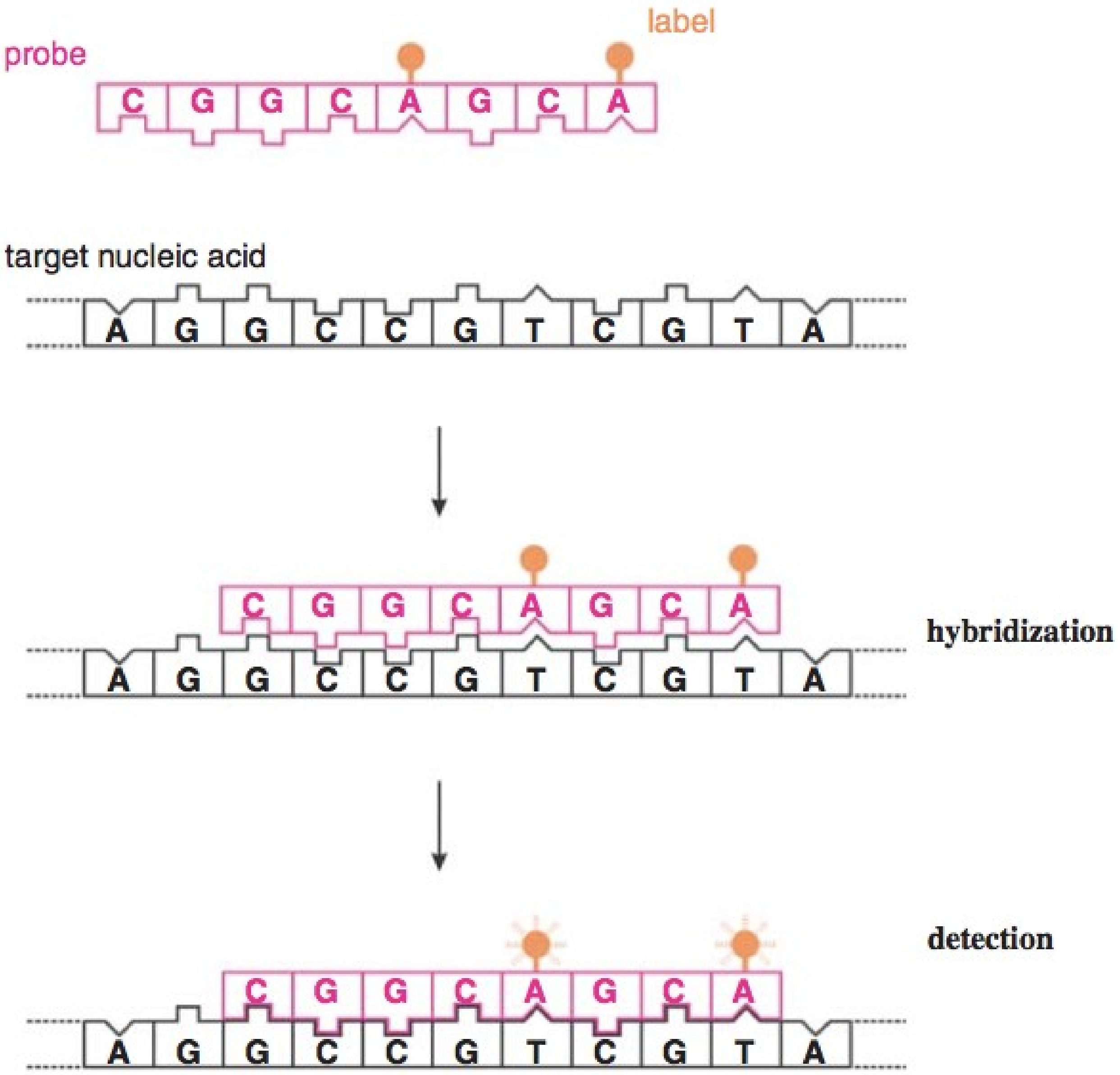
0.1 M Cadmium Chloride
0.1 M Na acetate pH 4.5
15 % PEG 400



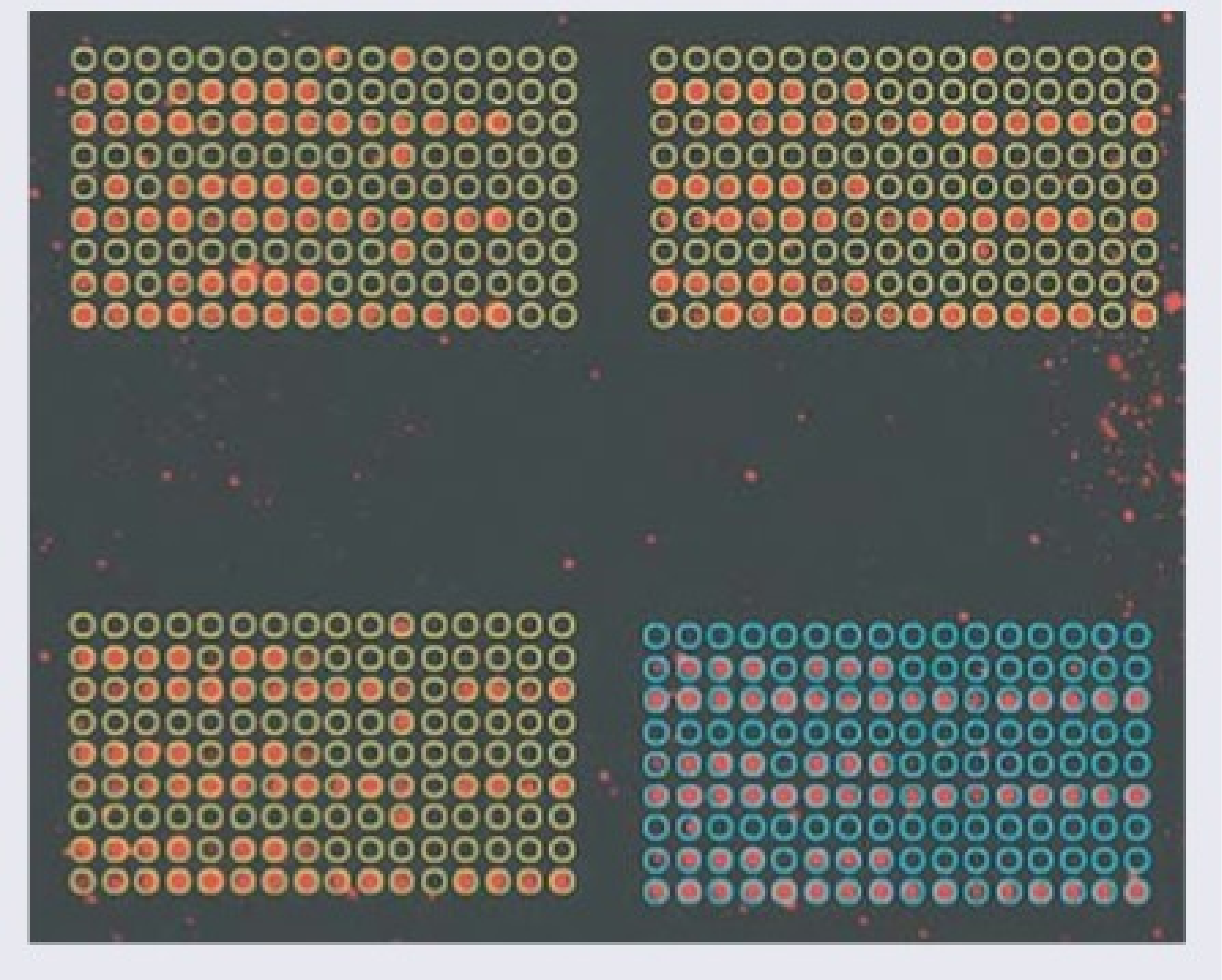
0.2 M Na/K Phosphate
0.1 M BisTris Propane pH 7.5
20 % PEG (w/v)3350



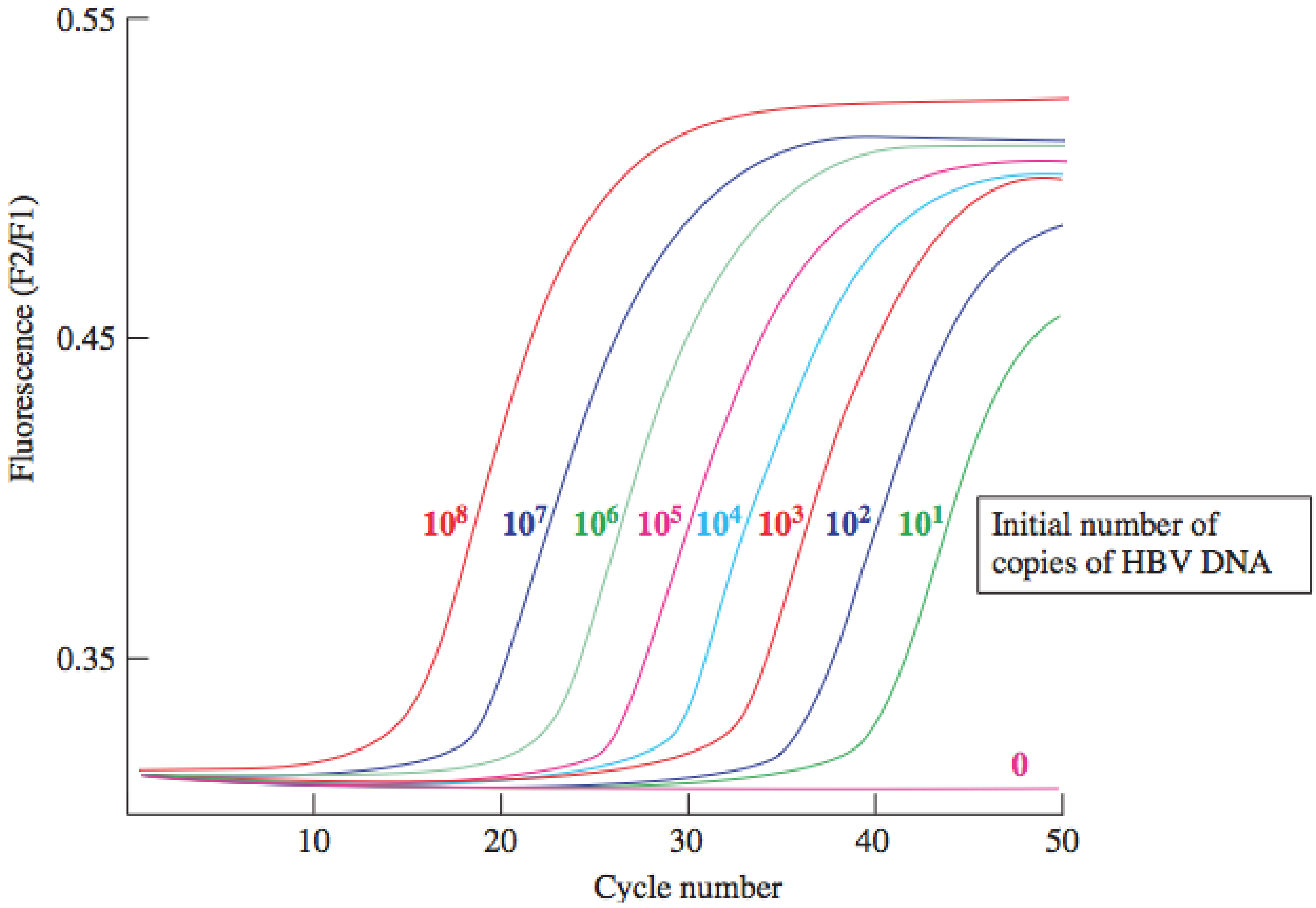
Hybridization detection of nucleic acids



Microarrays

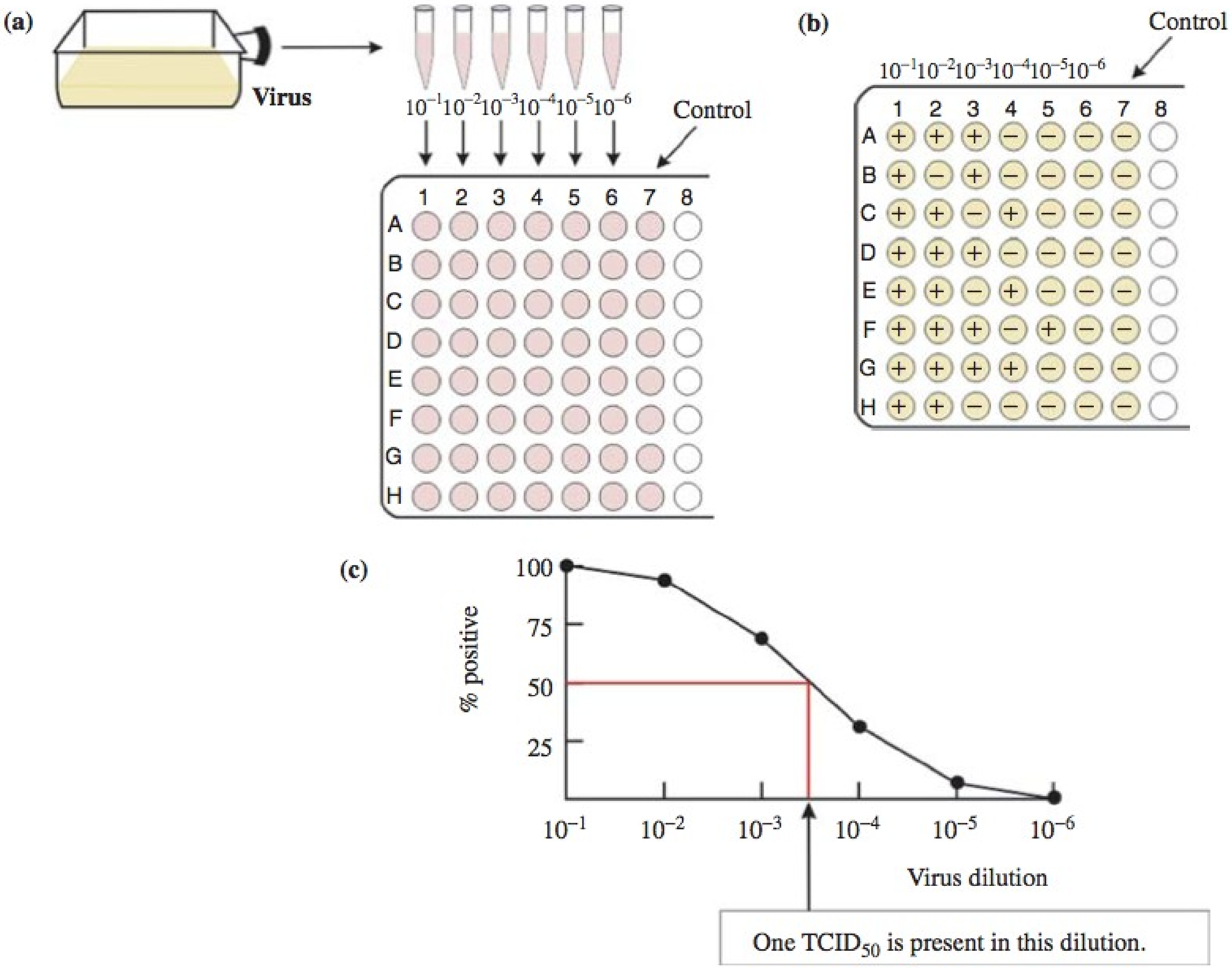


rtPCR assay

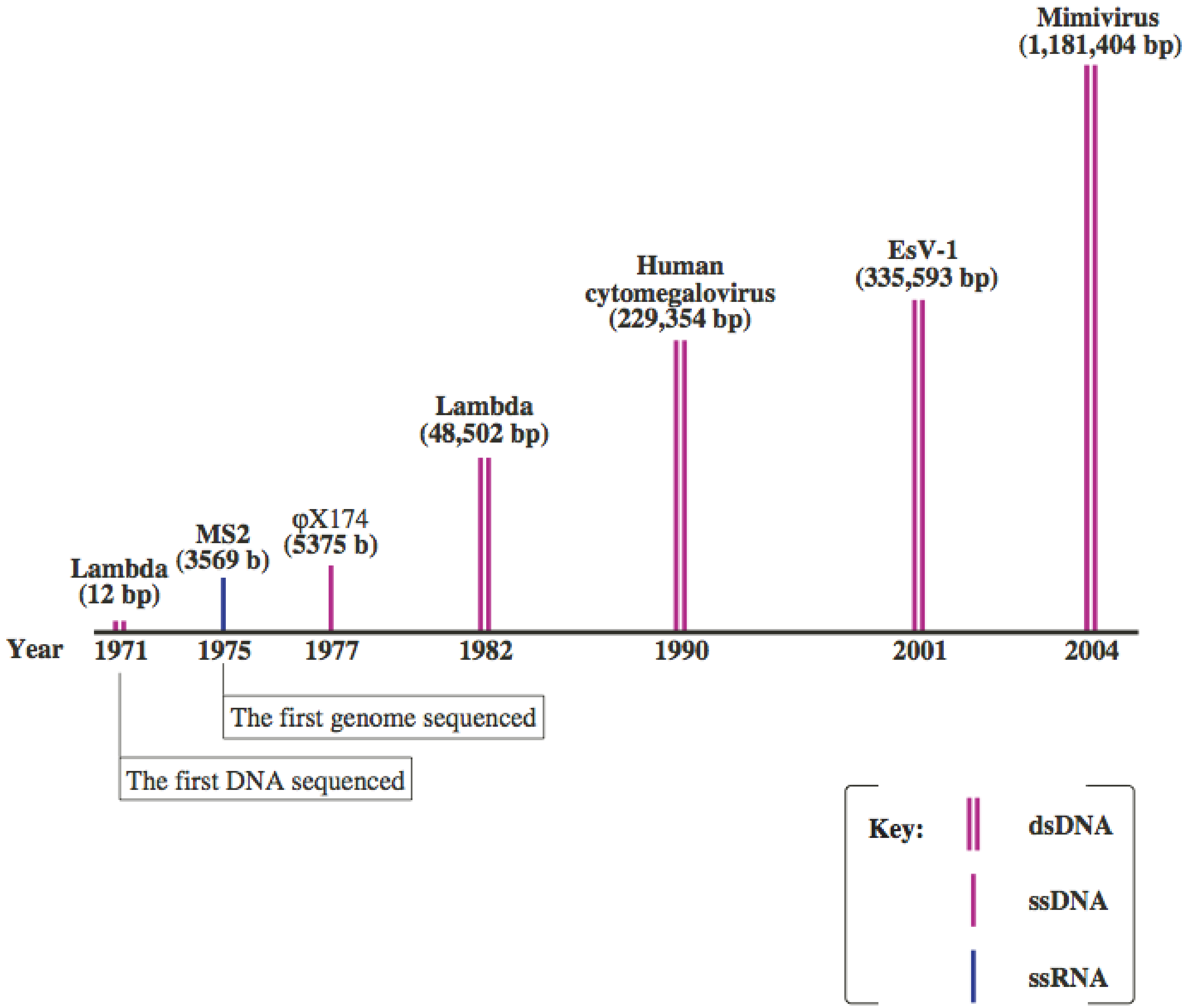


0.55
0.45
0.35
Fluorescence (F2/F1)
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
0
Initial number of copies of HBV DNA
10
20
30
40
50
Cycle number

$TCID_{50}$ assay



Sequencing of virus genomes

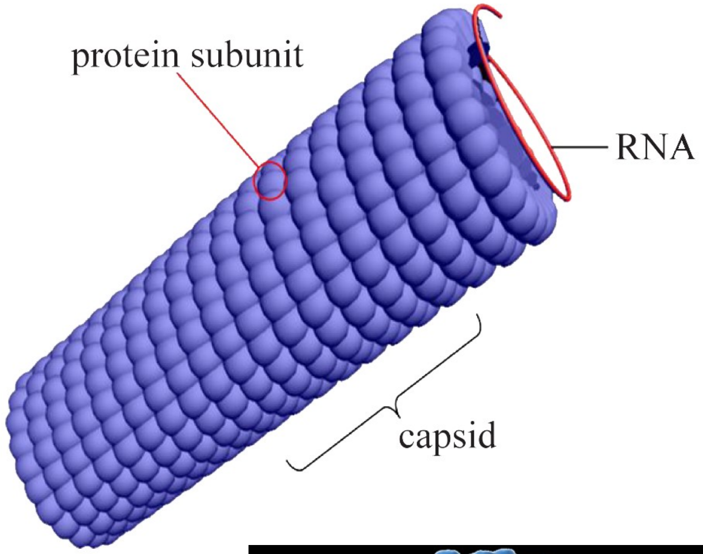


Learning outcomes

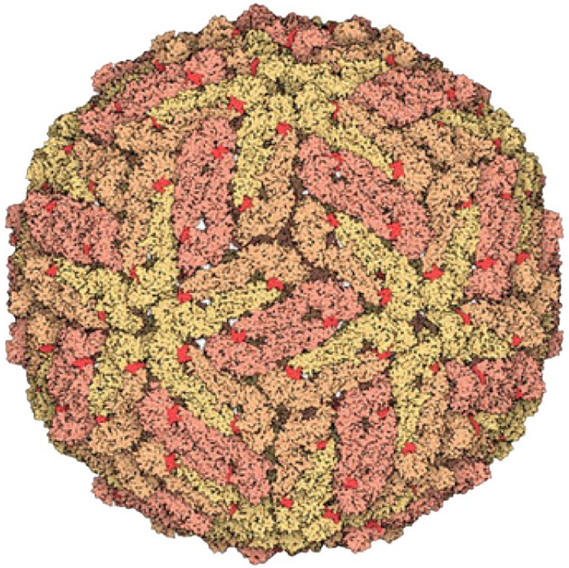
- outline methods for
  - cultivation of viruses;
  - purification of viruses;
  - detection of viruses and their components;
  - assay of virus infectivity;
  - investigation of virus gene function;
- assess the value of virus genome sequencing.

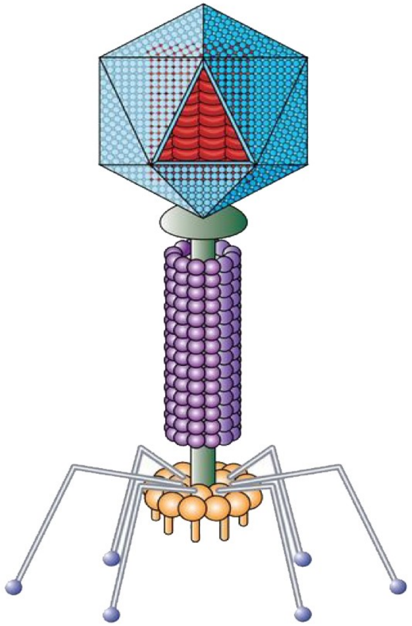
Virus structures

Helical

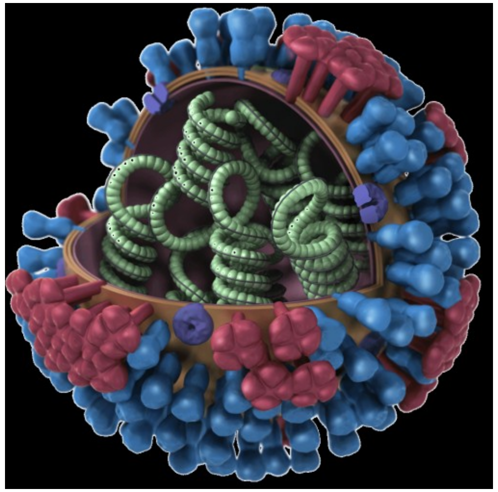


Icosahedral

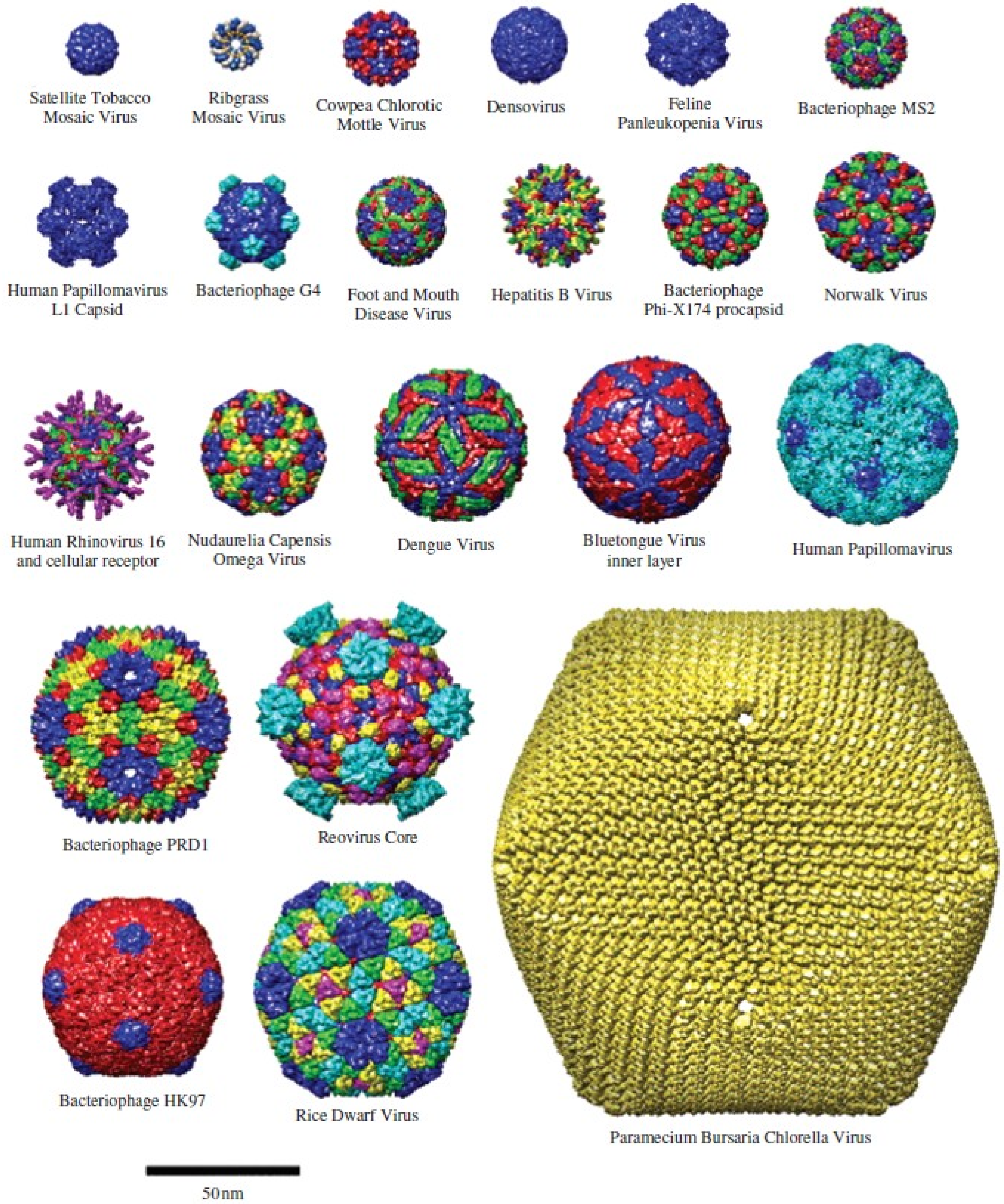




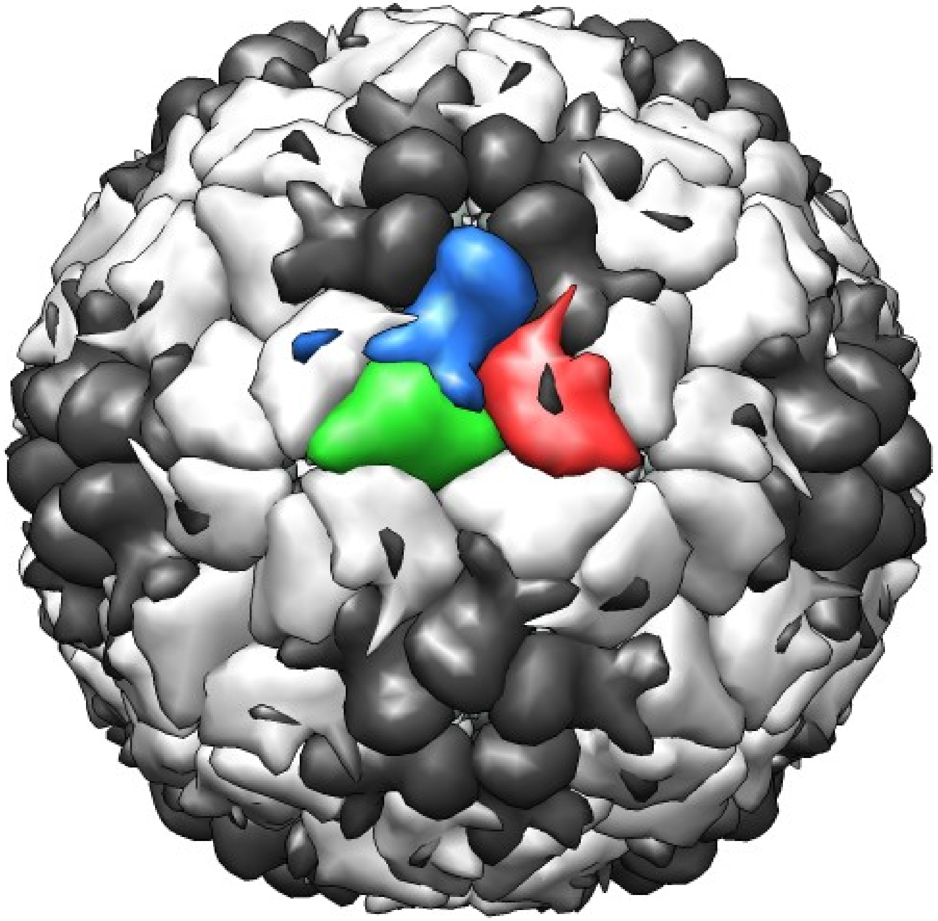
Complex



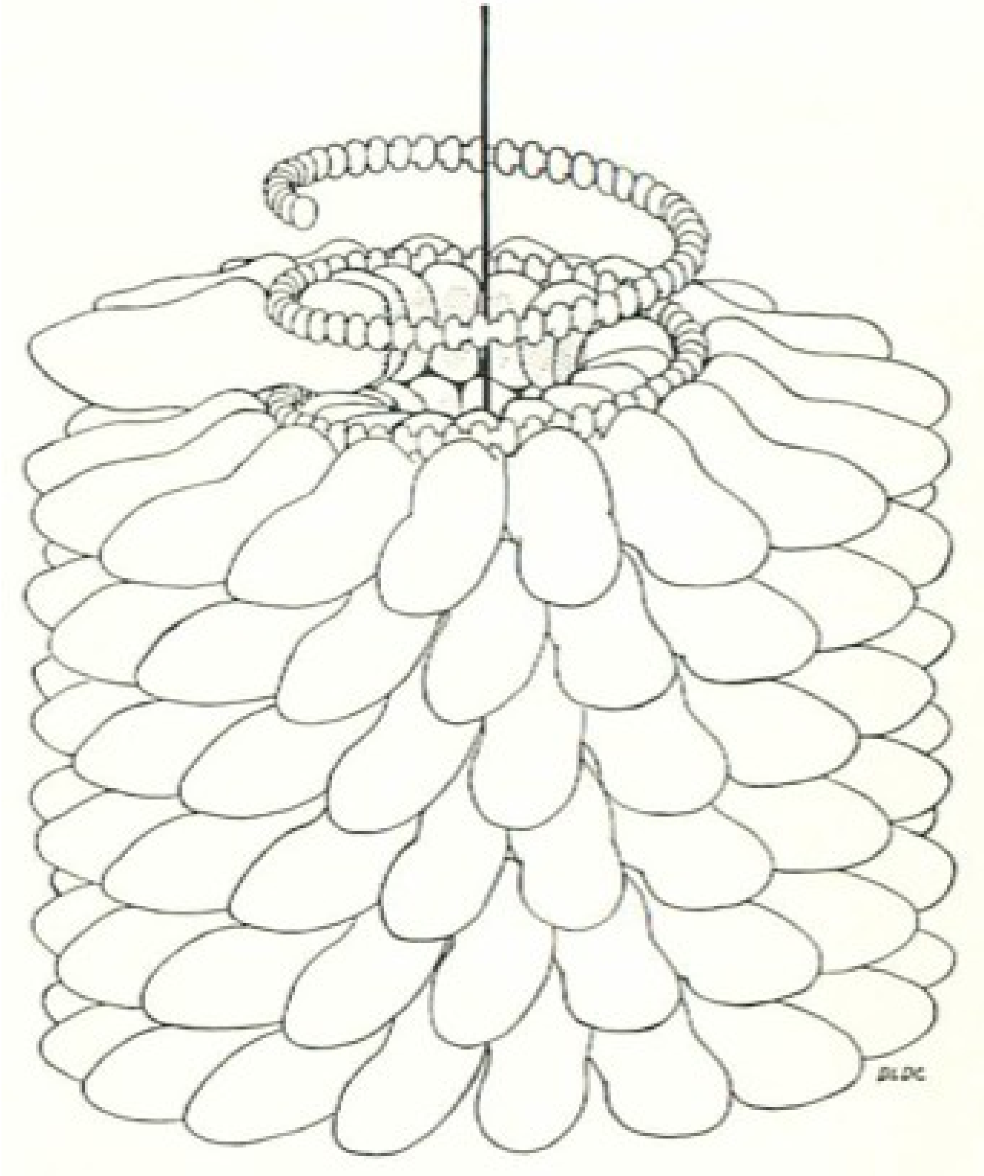
Irregular

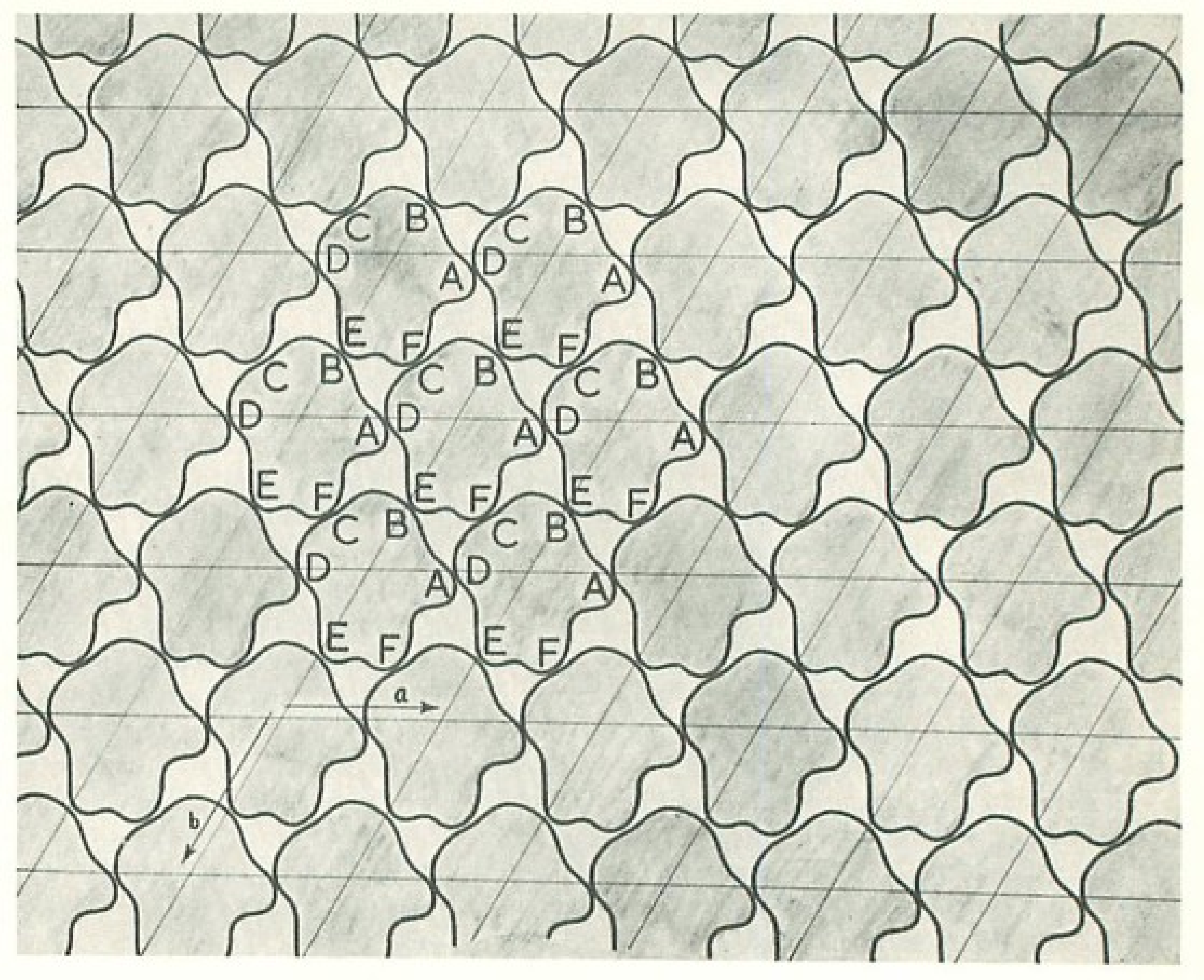

Satellite Tobacco Mosaic Virus
Ribgrass Mosaic Virus
Cowpea Chlorotic Mottle Virus
Densovirus
Feline Panleukopenia Virus
Bacteriophage MS2
Human Papillomavirus L1 Capsid
Bacteriophage G4
Foot and Mouth Disease Virus
Hepatitis B Virus
Bacteriophage Phi-X174 procapsid
Norwalk Virus
Human Rhinovirus 16 and cellular receptor
Nudaurelia Capensis Omega Virus
Dengue Virus
Bluetongue Virus inner layer
Human Papillomavirus
Bacteriophage PRD1
Reovirus Core
Bacteriophage HK97
Rice Dwarf Virus
Paramecium Bursaria Chlorella Virus
50nm

Picornavirus virion

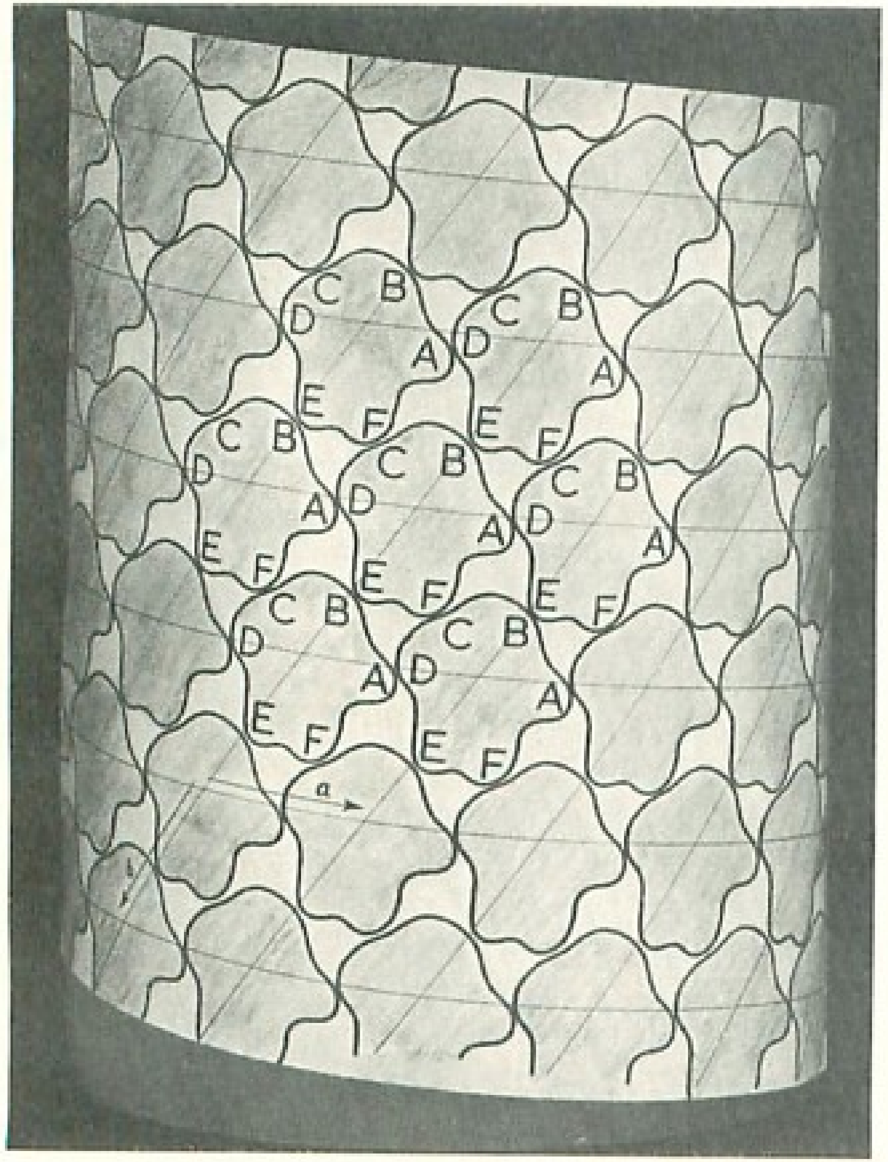


Icosahedron

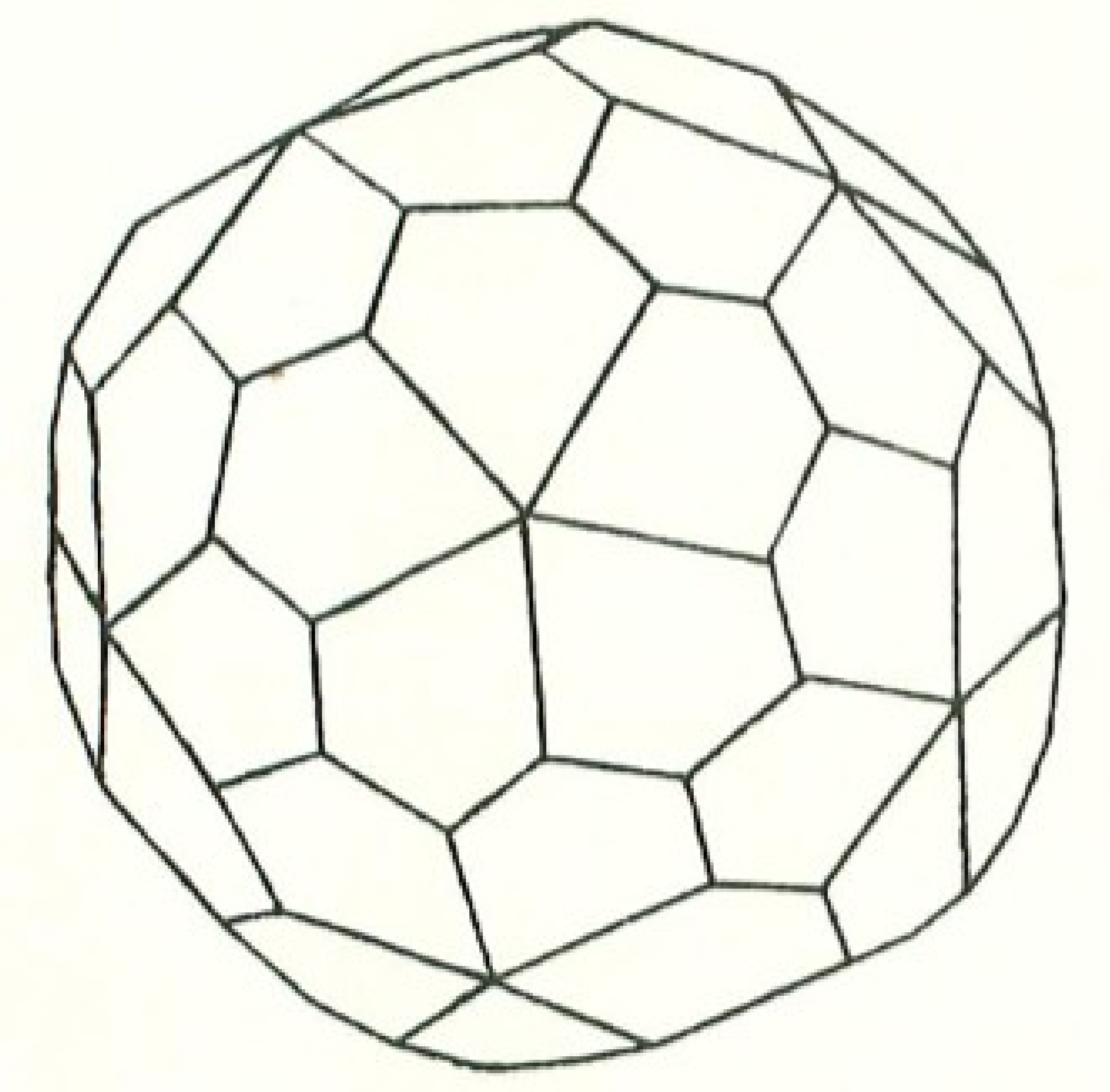


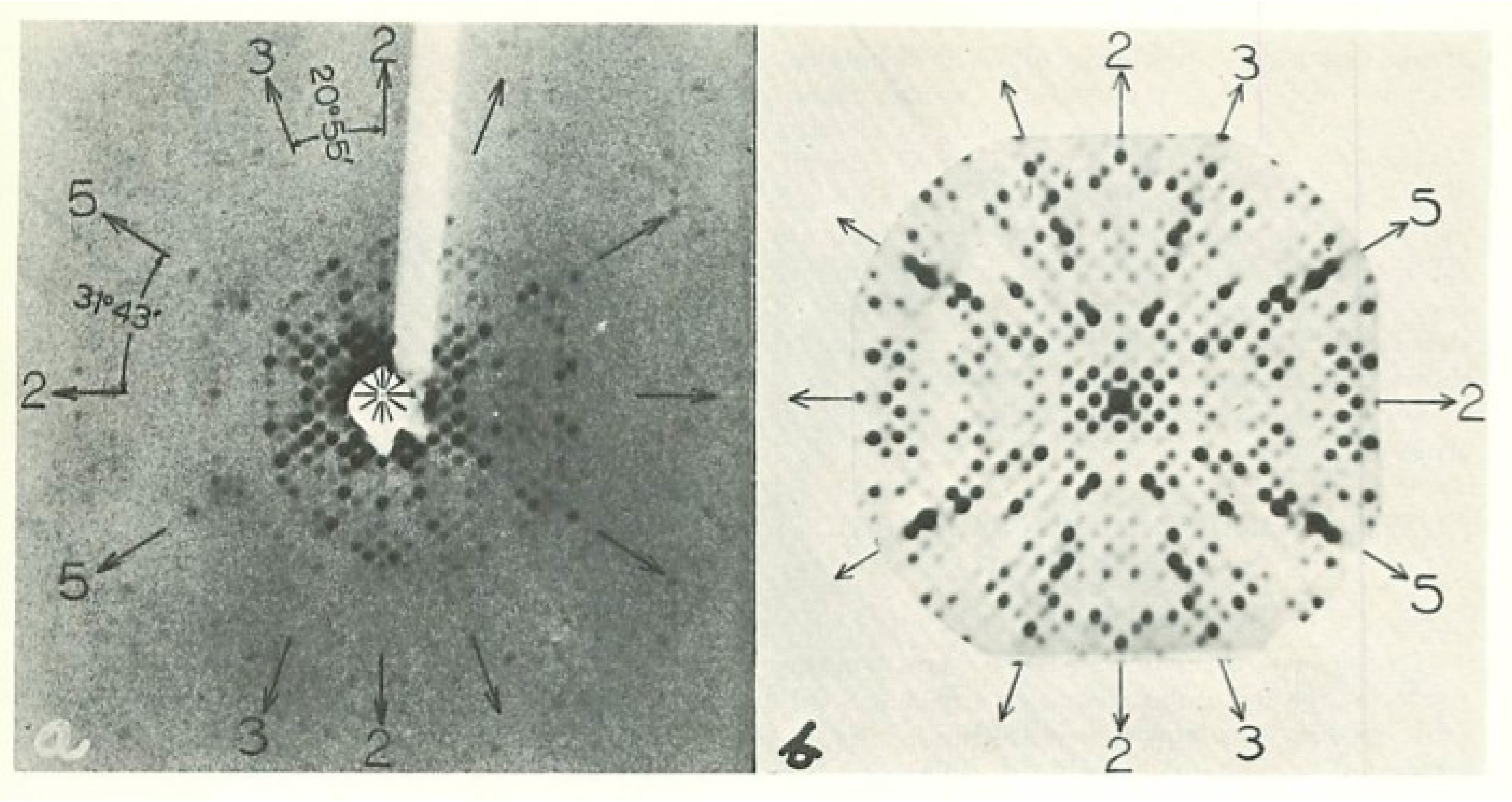


A
B
C
D
E
F
a
b



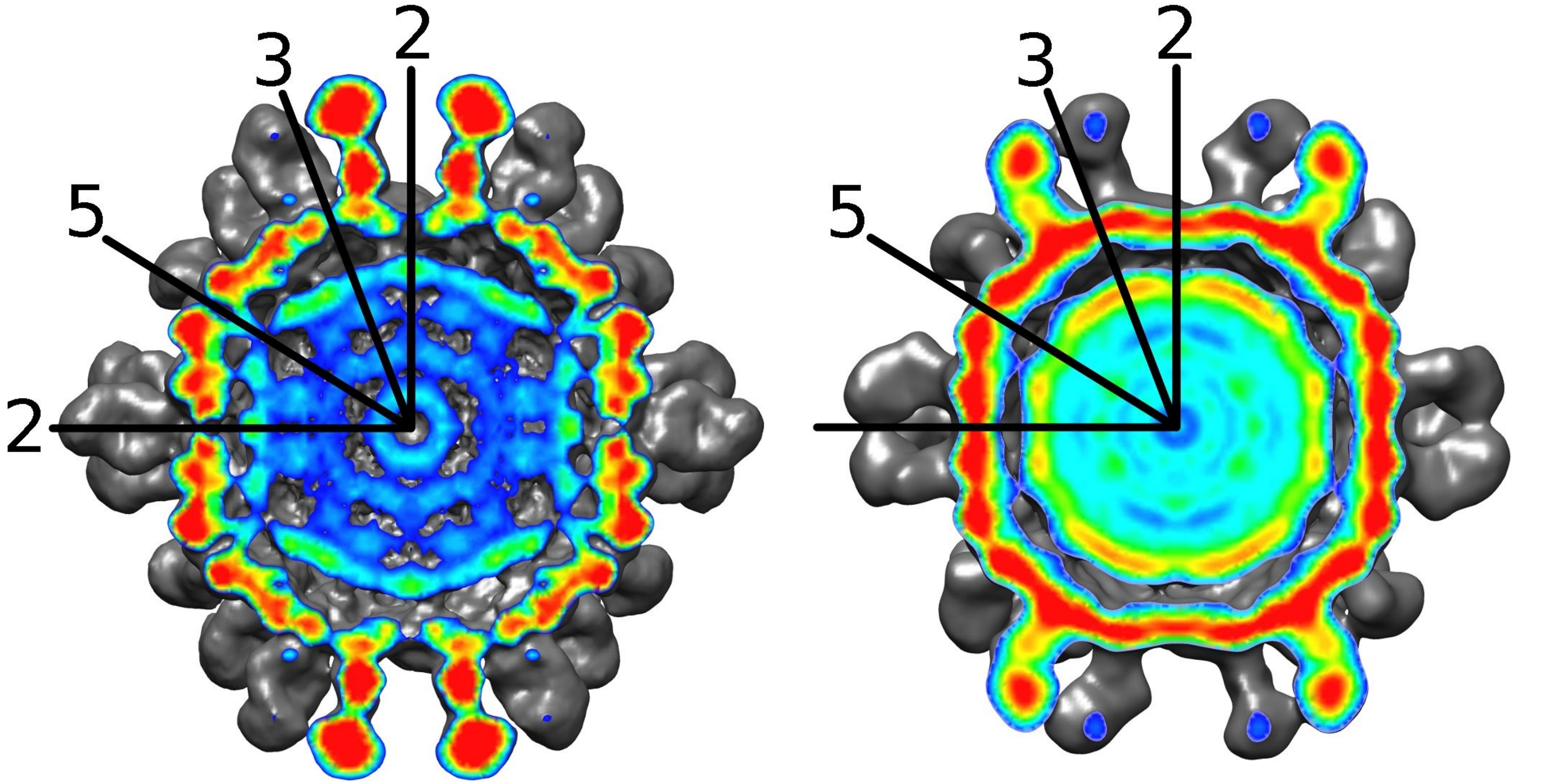
C B
D
A
E F
a
b

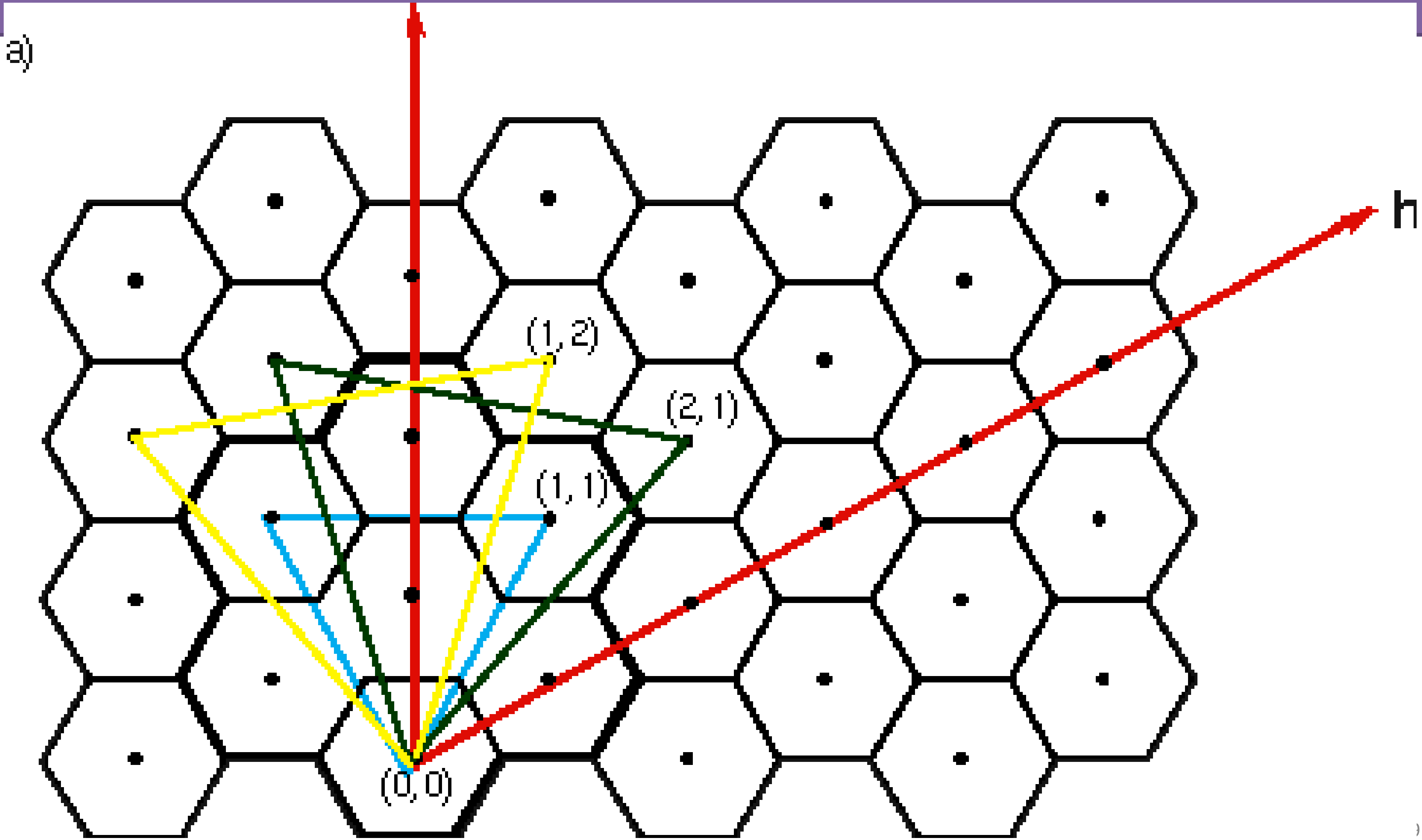



3
2
20°55'
5
31°43'
2
5
3
2
a
2
3
5
2
5
b
2
3

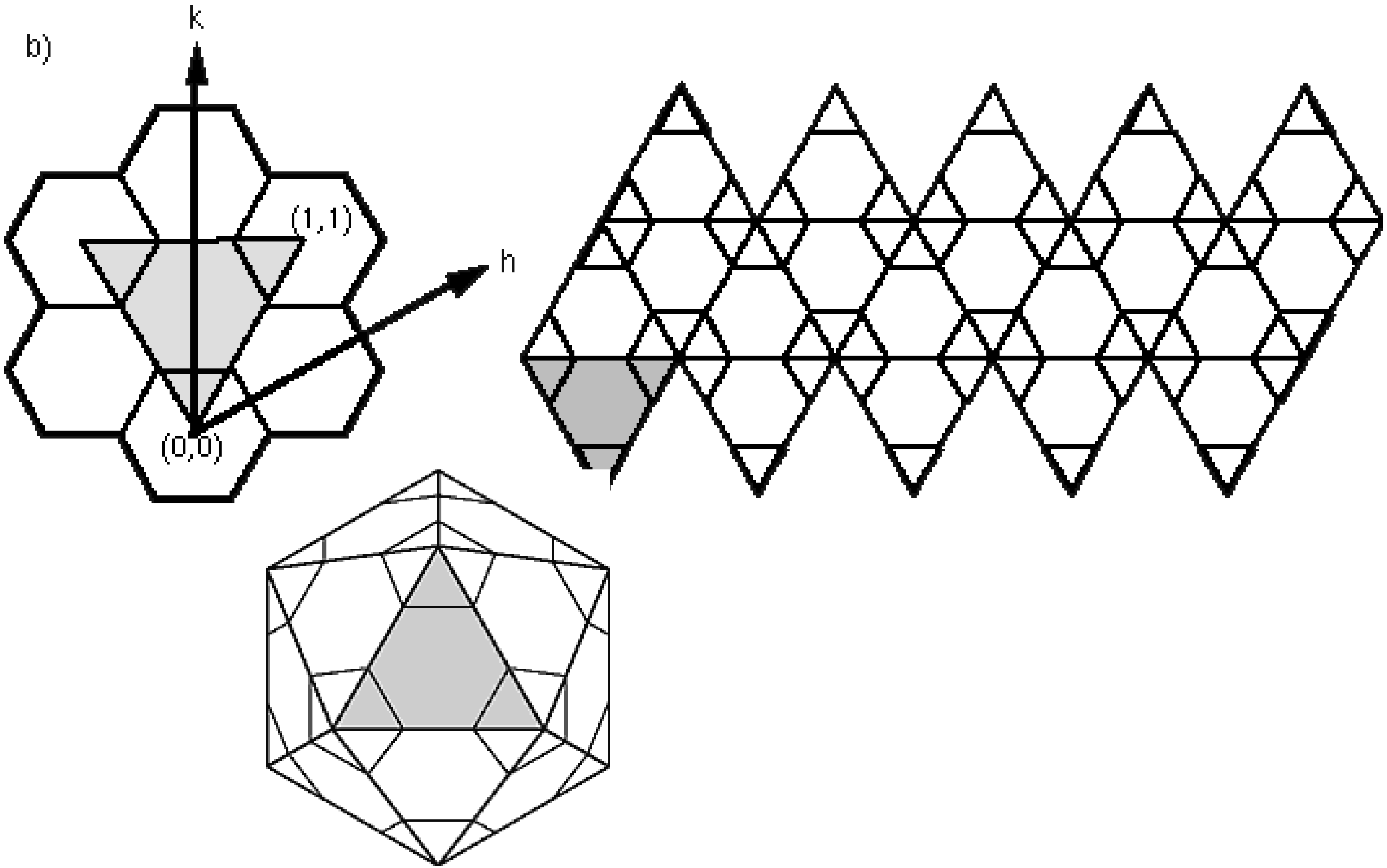
E18 Fab

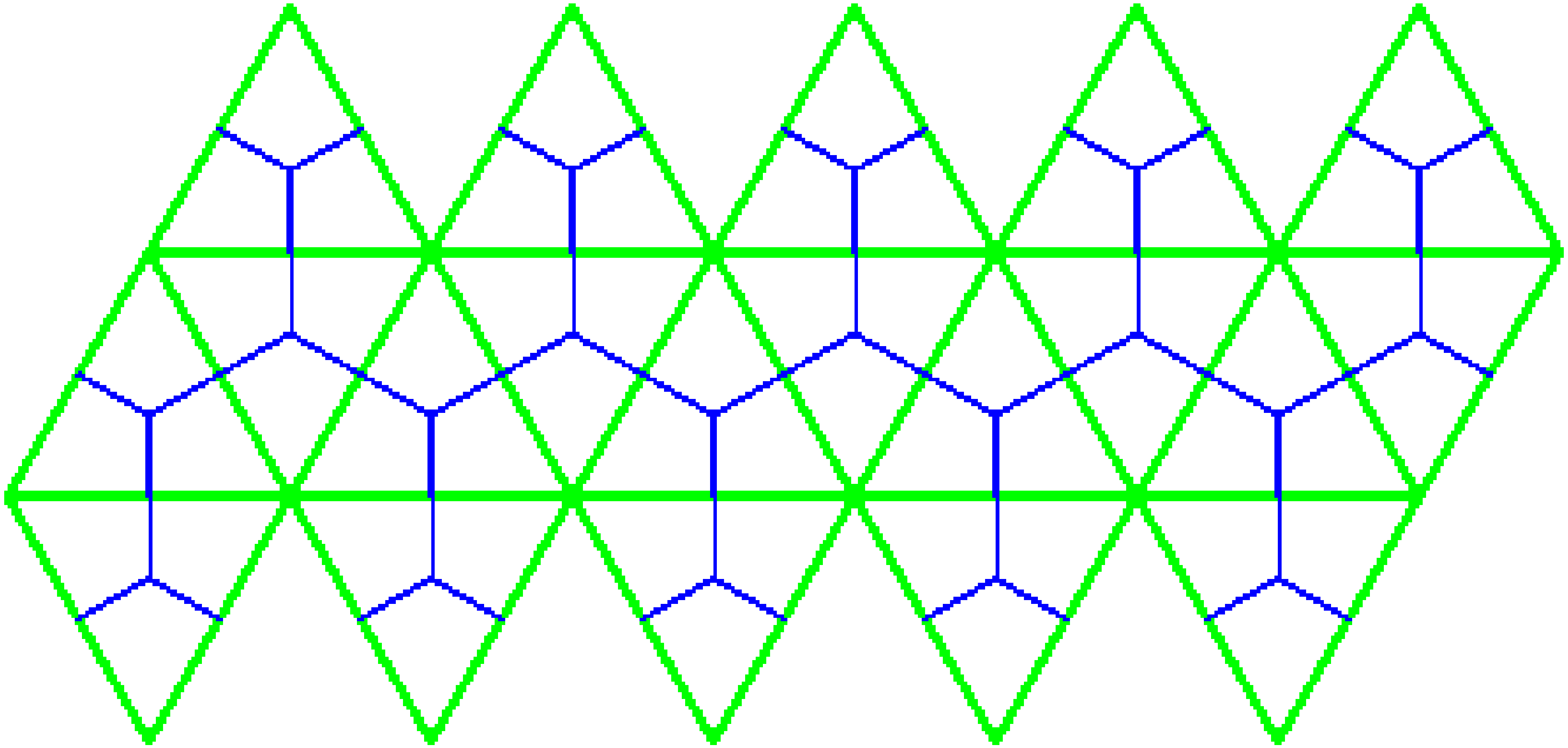
E19 Fab

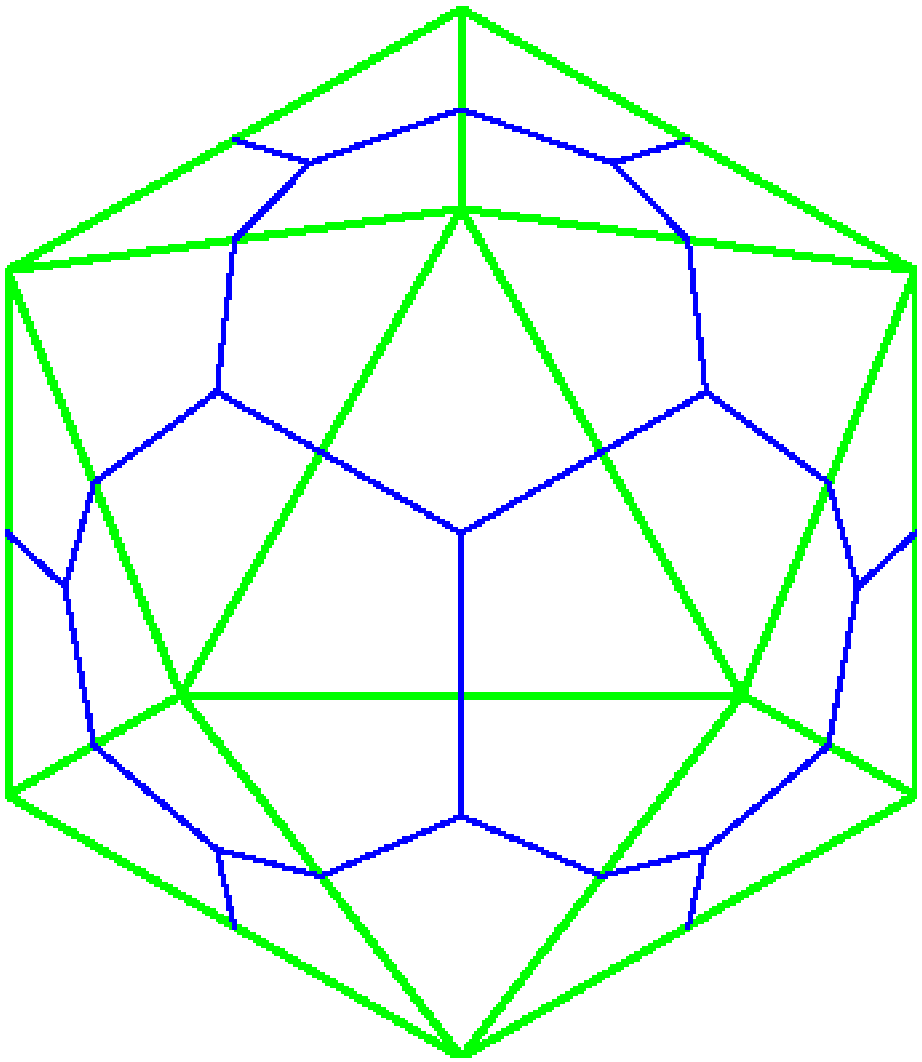


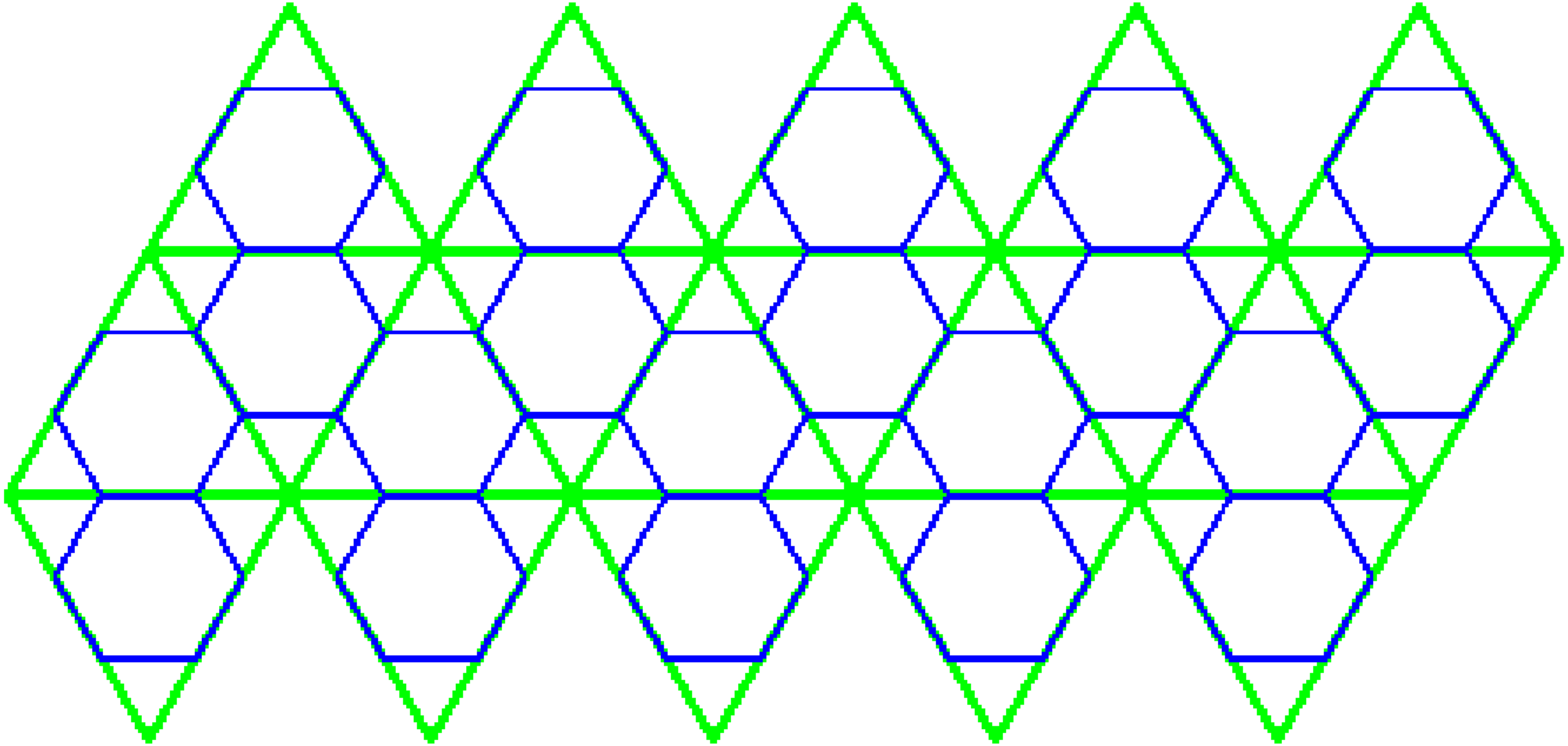


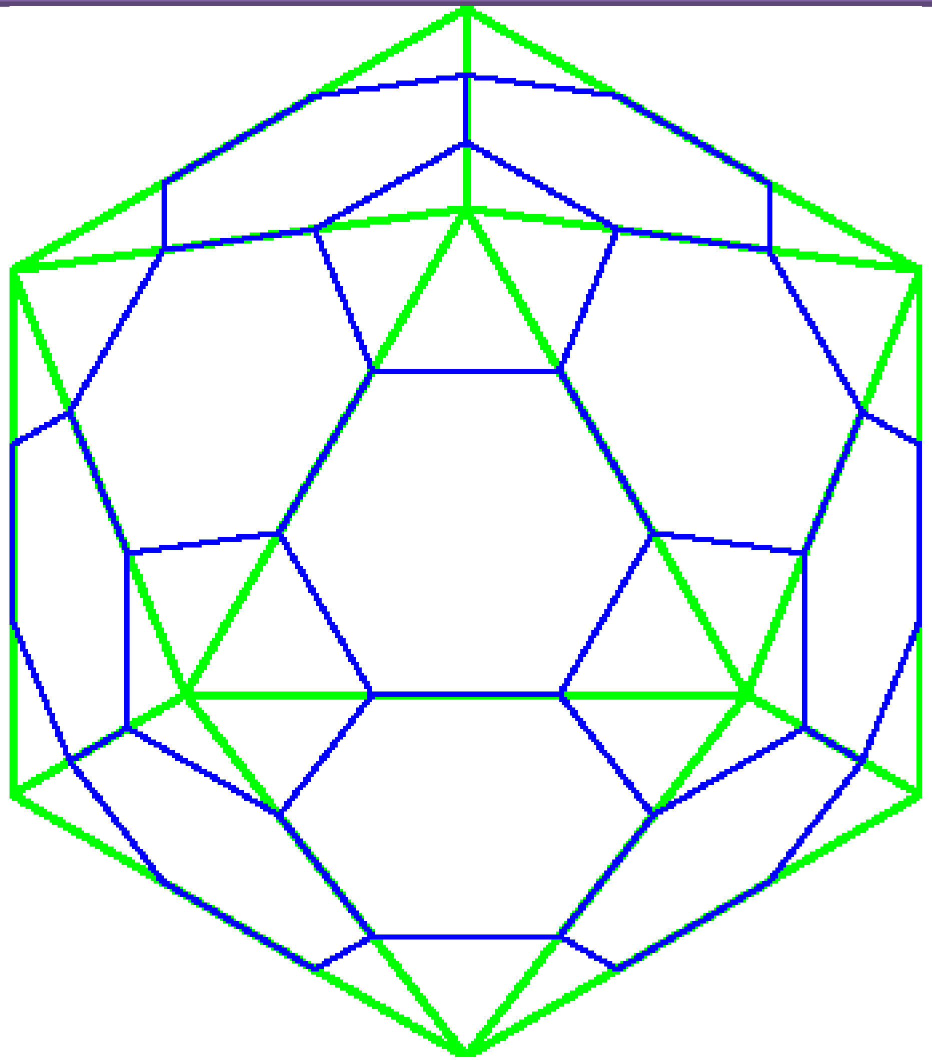
a)
h
(1, 2)
(2, 1)
(1, 1)
(0, 0)

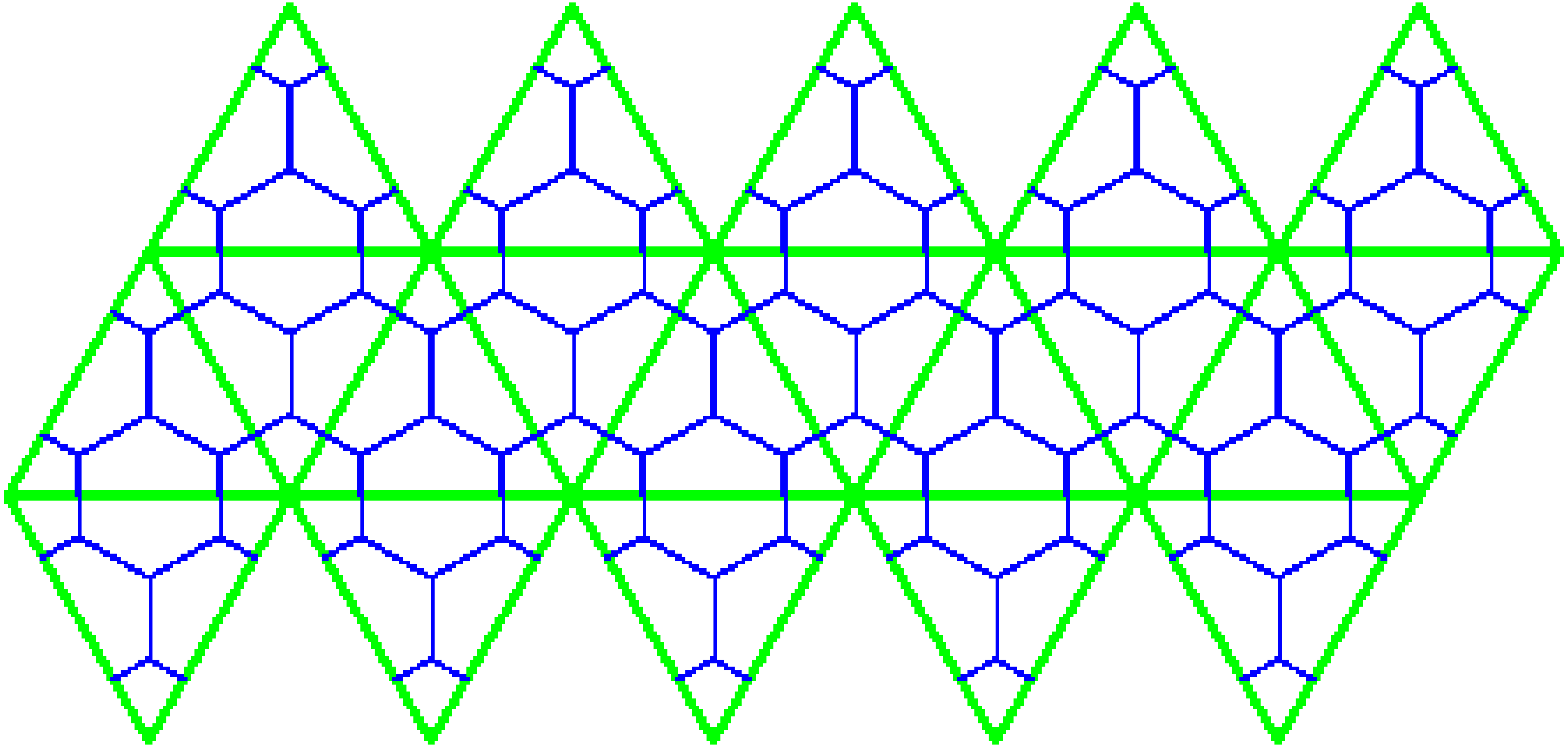

b)
k
h
(1,1)
(0,0)

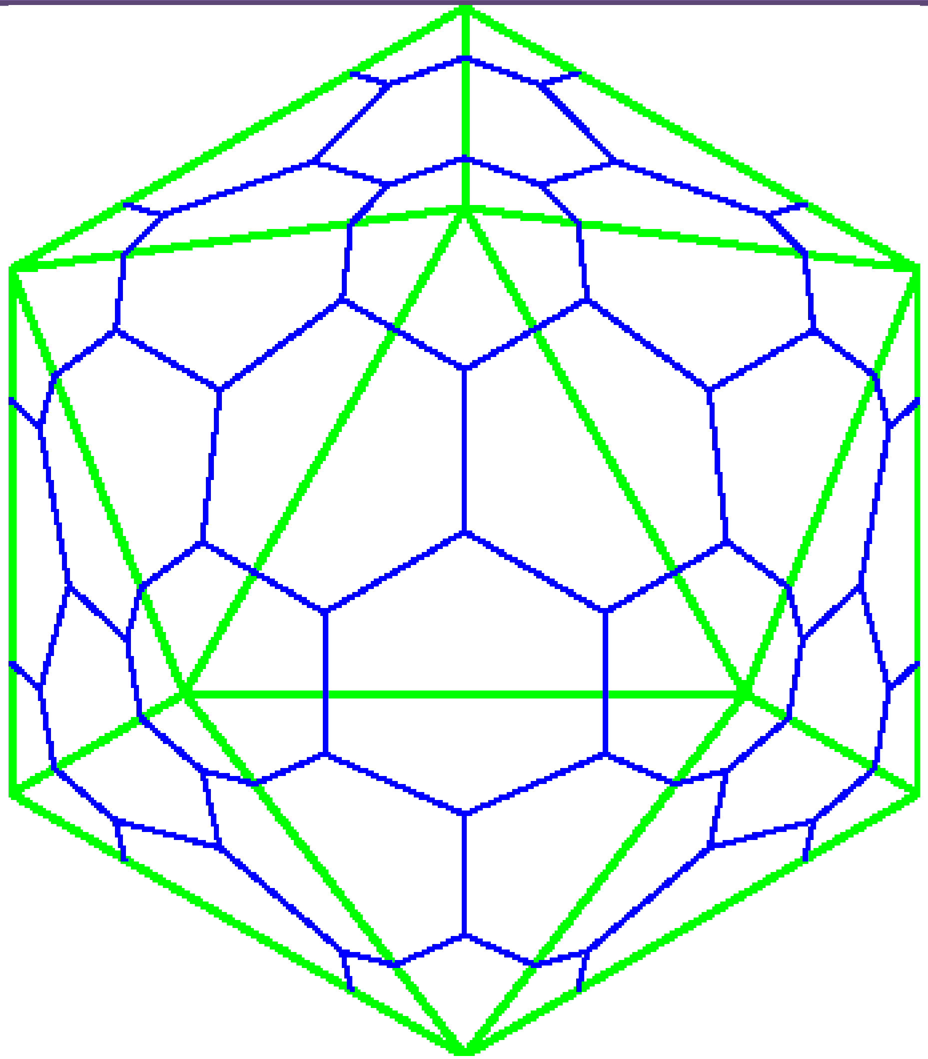


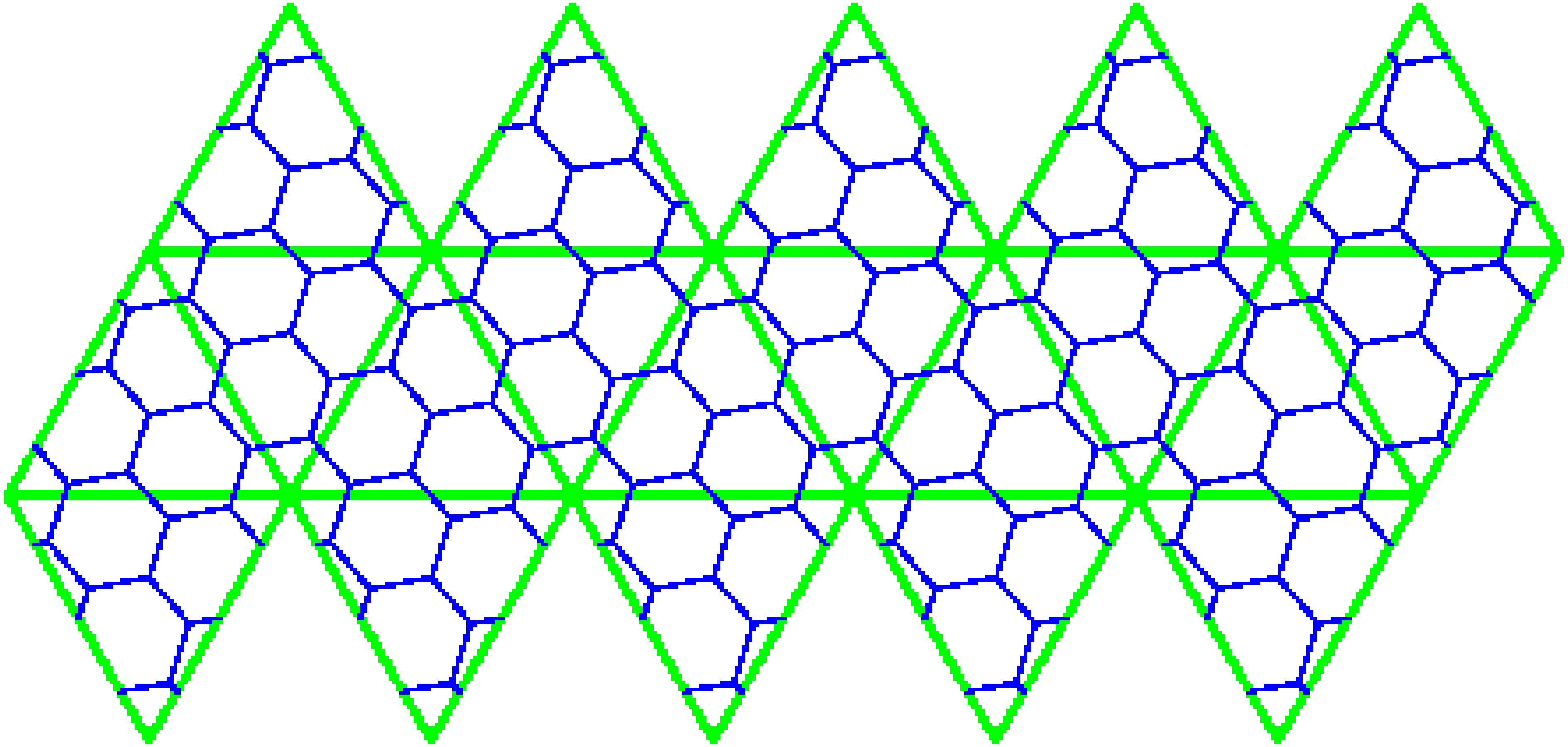


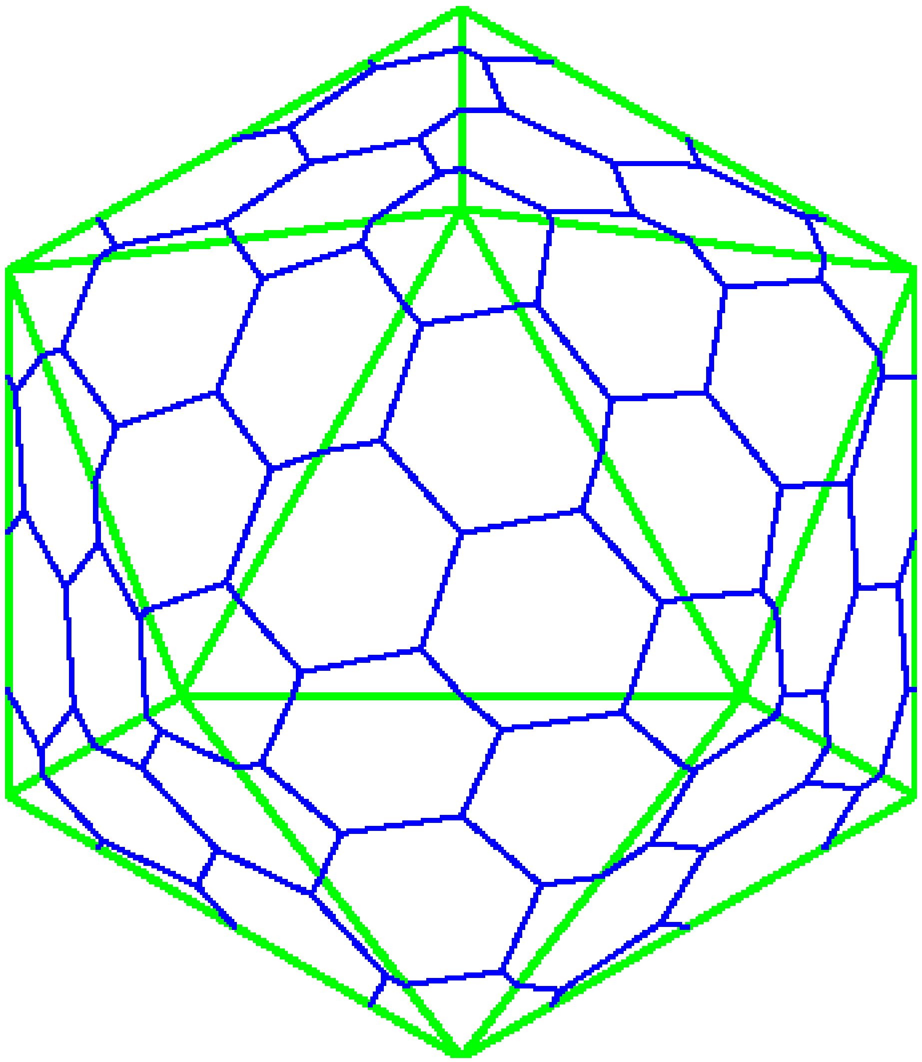












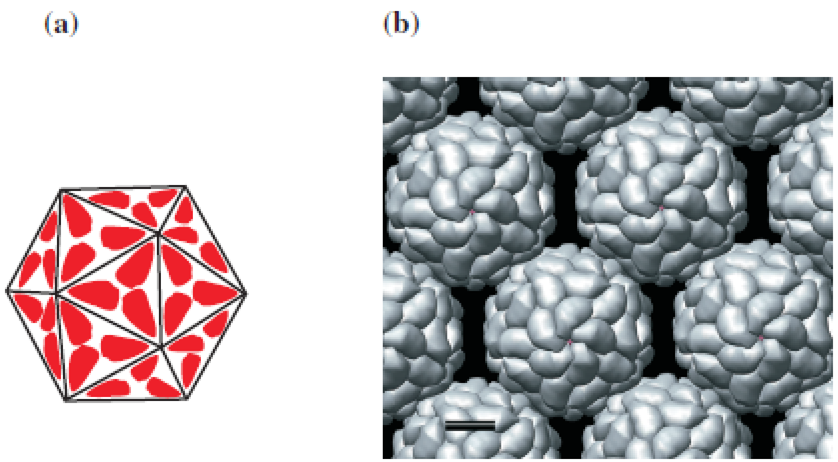


Figure 3.11 Capsid constructed from sixty protein molecules. (a) Arrangement of protein molecules, with three per triangular face. (b) Virions of satellite tobacco mosaic virus. The bar represents 5 nm. Image created with the molecular graphics program UCSF Chimera from the Resource for Biocomputing, Visualization, and Informatics, at the University of California, San Francisco.

Source: Courtesy of Tom Goddard.

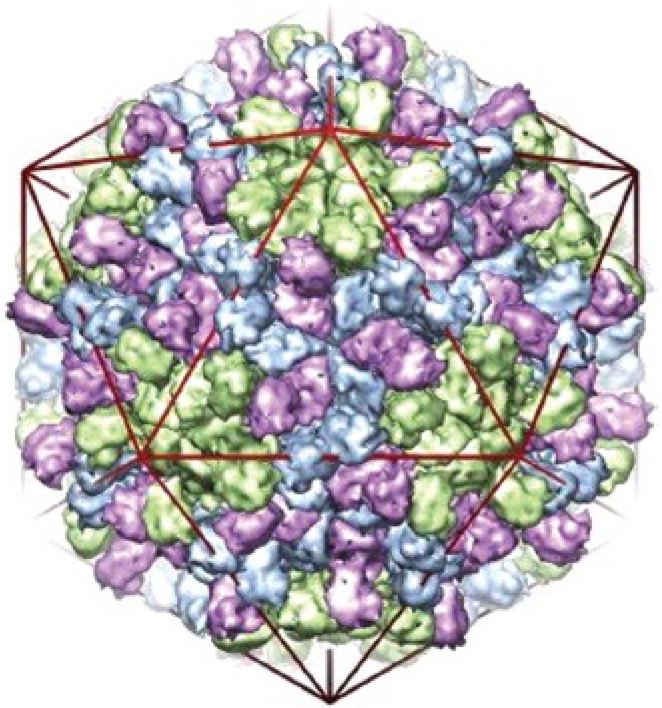


Figure 3.12 Turnip crinkle virus capsid. The capsid is built from 180 copies of the coat protein in three quasi-equivalent conformations; some protein molecules (green) are around the fivefold symmetry axes, while the remainder (pink and blue) are around the threefold symmetry axes. An icosahedron is superimposed.

Source: Bakker *et al.* (2012) *Journal of Molecular Biology*, 417, 65–78. Reproduced by permission of the authors and Elsevier Limited.

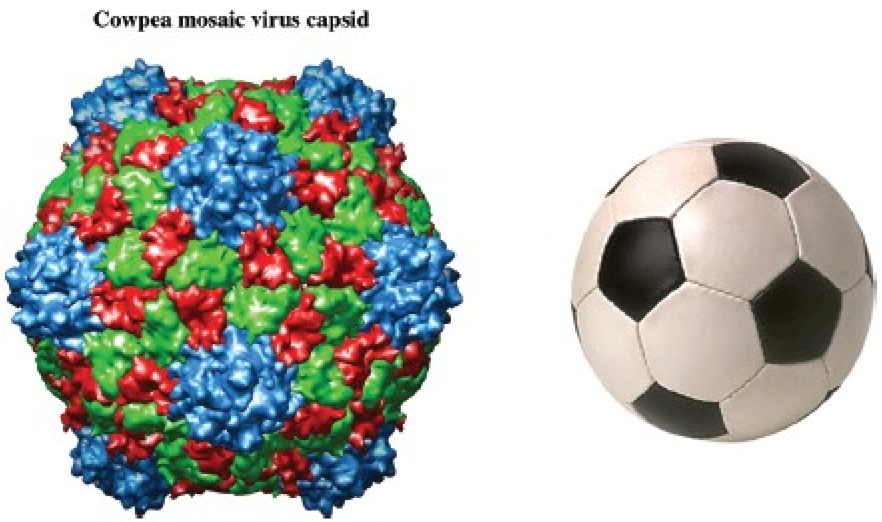
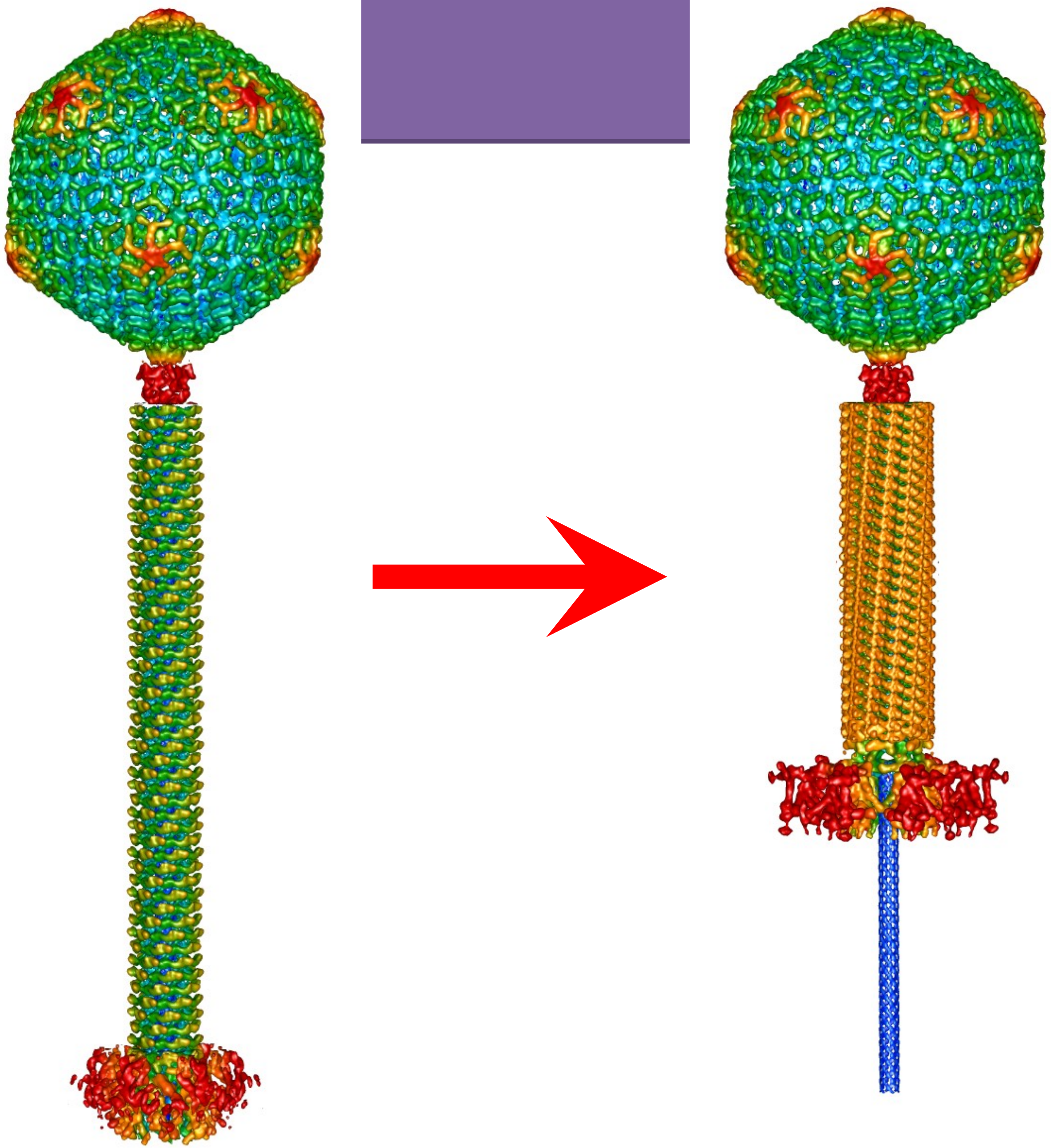
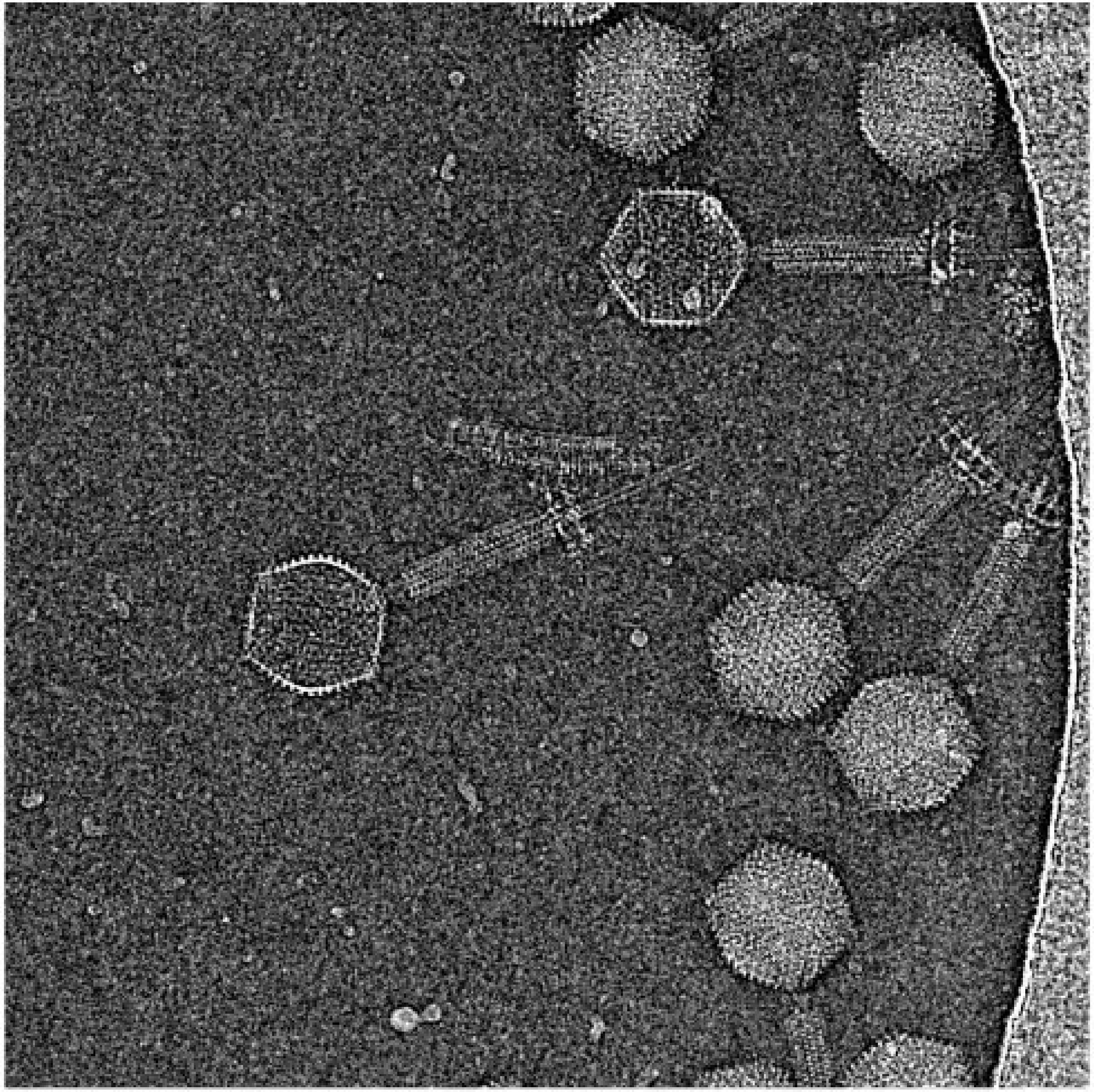


Figure 3.13 Capsid constructed from two protein species. The cowpea mosaic virus capsid is constructed from one protein species (blue) that forms 12 “pentamers,” and from a second protein species with two domains (green and red) that forms 20 “hexamers.” The football is similarly constructed from 12 “pentamers” and 20 “hexamers.” The cowpea mosaic virus image is from the VIPER database (Shepherd *et al.*, 2006).

Source: The image was reconstructed using the data of Lin *et al.* (1999) *Virology*, 265, 20. Reproduced by permission of Elsevier Limited.





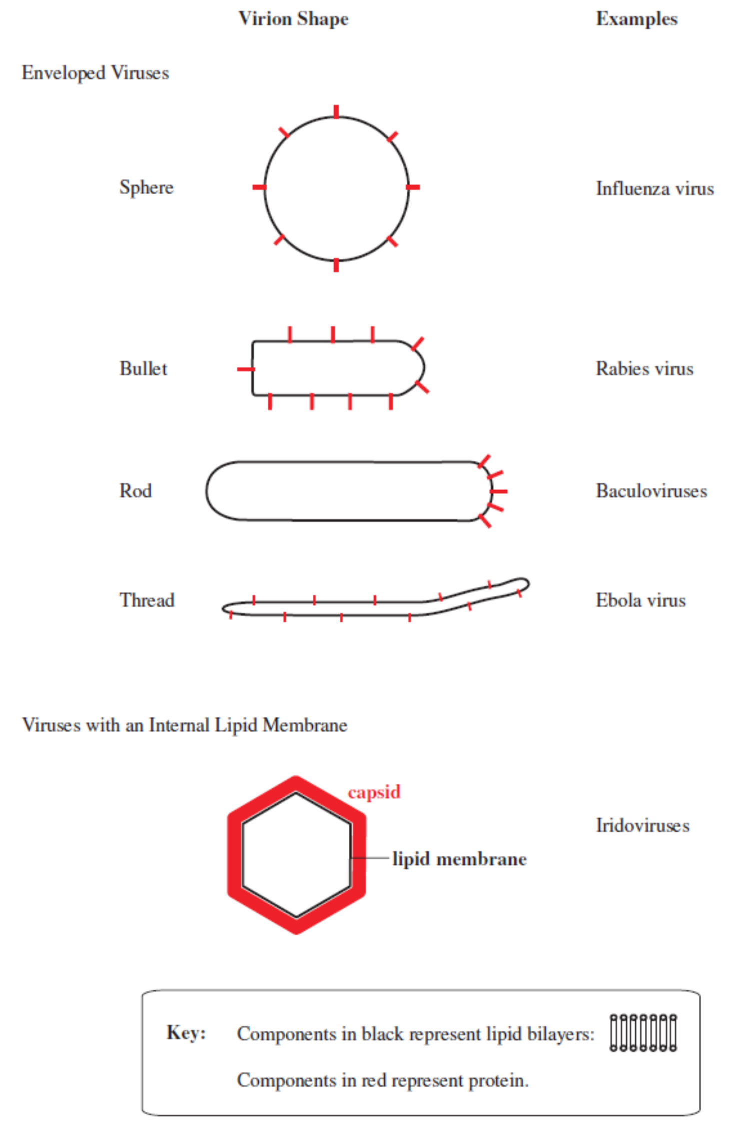


Figure 3.20 Lipid-containing viruses.

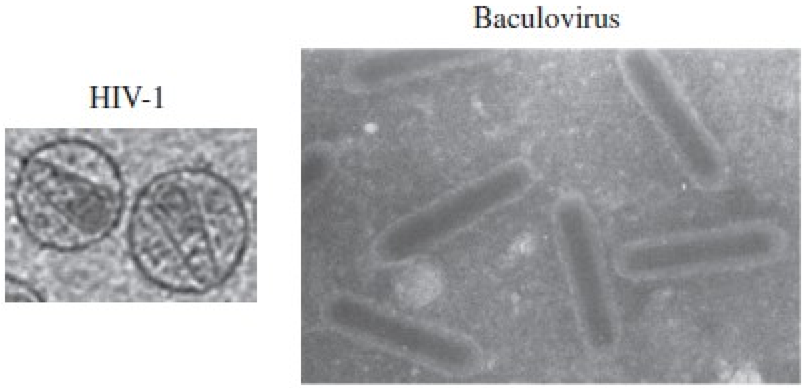


Figure 3.19 **Virions containing conical and rod-shaped capsids.**

Sources: HIV-1 virions from Wei and Yin (2010) *Journal of Structural Biology*, 172, 211. Reproduced by permission of Elsevier and the authors. Baculovirus virions are those of *Aglais urticae* nucleopolyhedrovirus. From Harrap (1972) *Virology*, 50, 124. Reproduced by permission of Elsevier Limited.

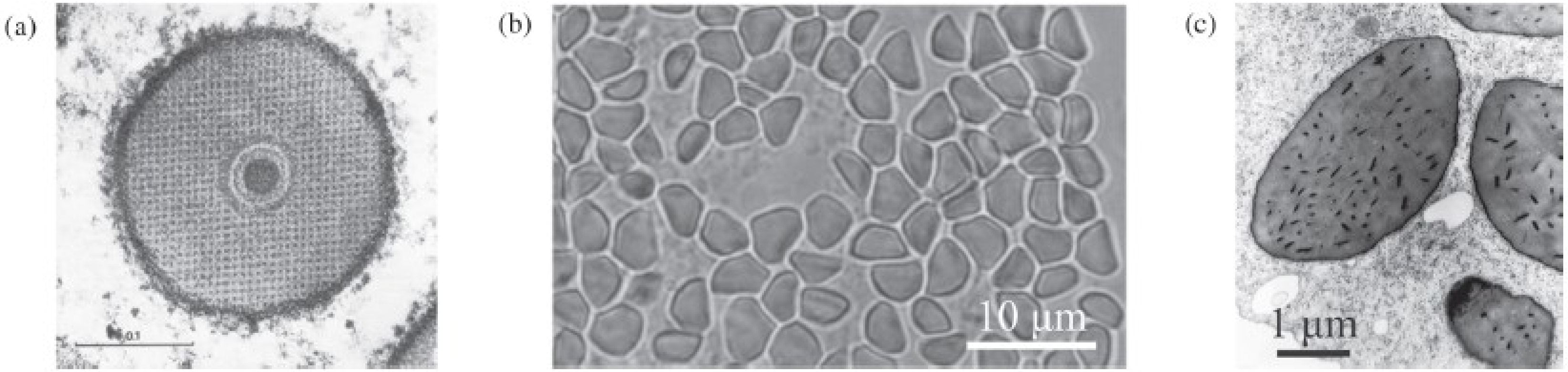
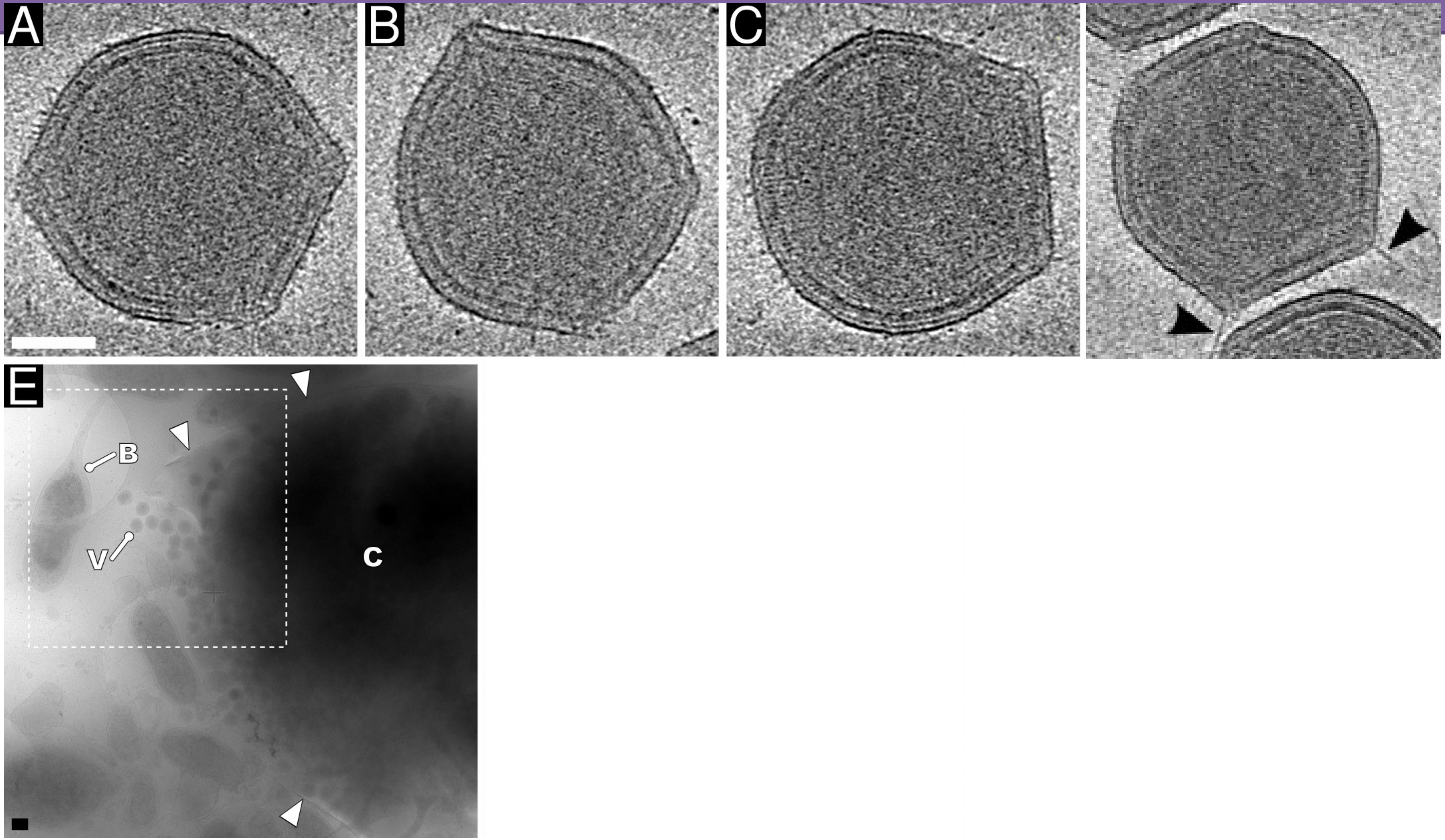


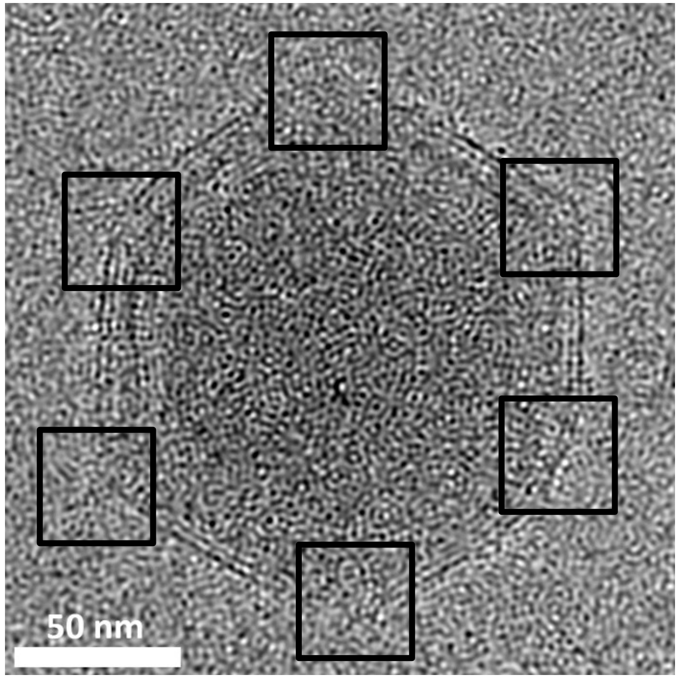
Figure 3.21 Baculovirus occlusion bodies. (a) Transverse section through an occlusion body of a granulovirus of the Indian meal moth (*Plodia interpunctella*). The nucleocapsid surrounded by its membrane can be seen at the center, surrounded by the crystalline protein that forms the occlusion body. The bar represents 0.1 µm. (b), (c) Nucleopolyhedrovirus of the leatherjacket (*Tipula paludosa*). (b) Light micrograph of occlusion bodies. (c) Electron micrograph of a thin section of an occlusion body. The virions are randomly embedded in the crystalline protein.

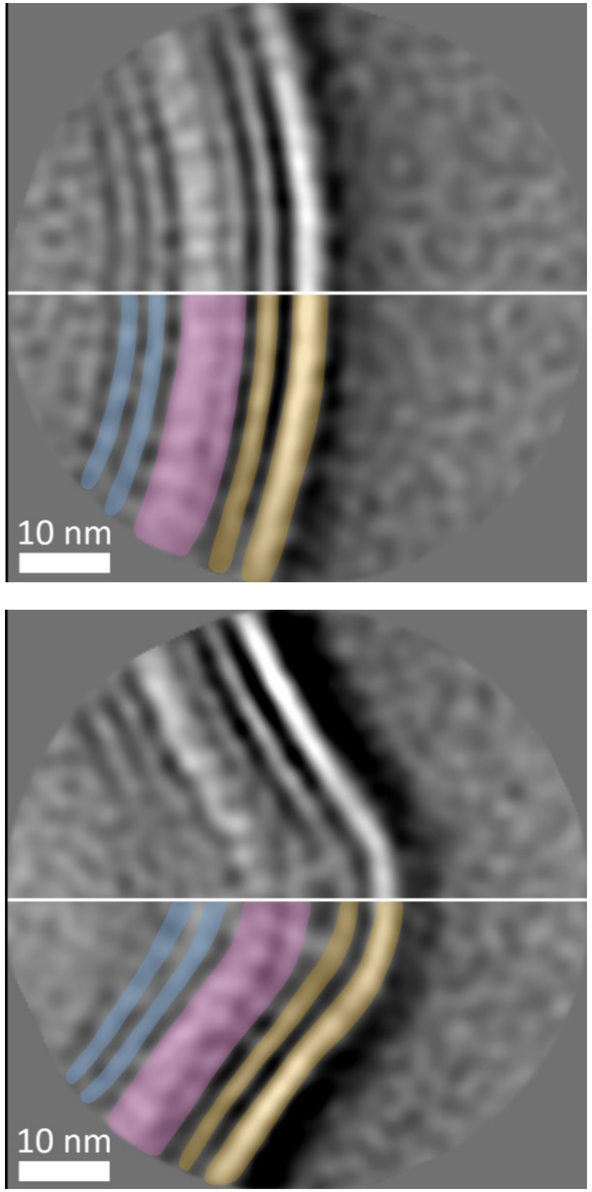
Sources: (a) Arnott and Smith (1967) *Journal of Ultrastructure Research*, 21, 251. Reproduced by permission of Elsevier Limited. (b), (c) Courtesy of Dr Liz Boslem.

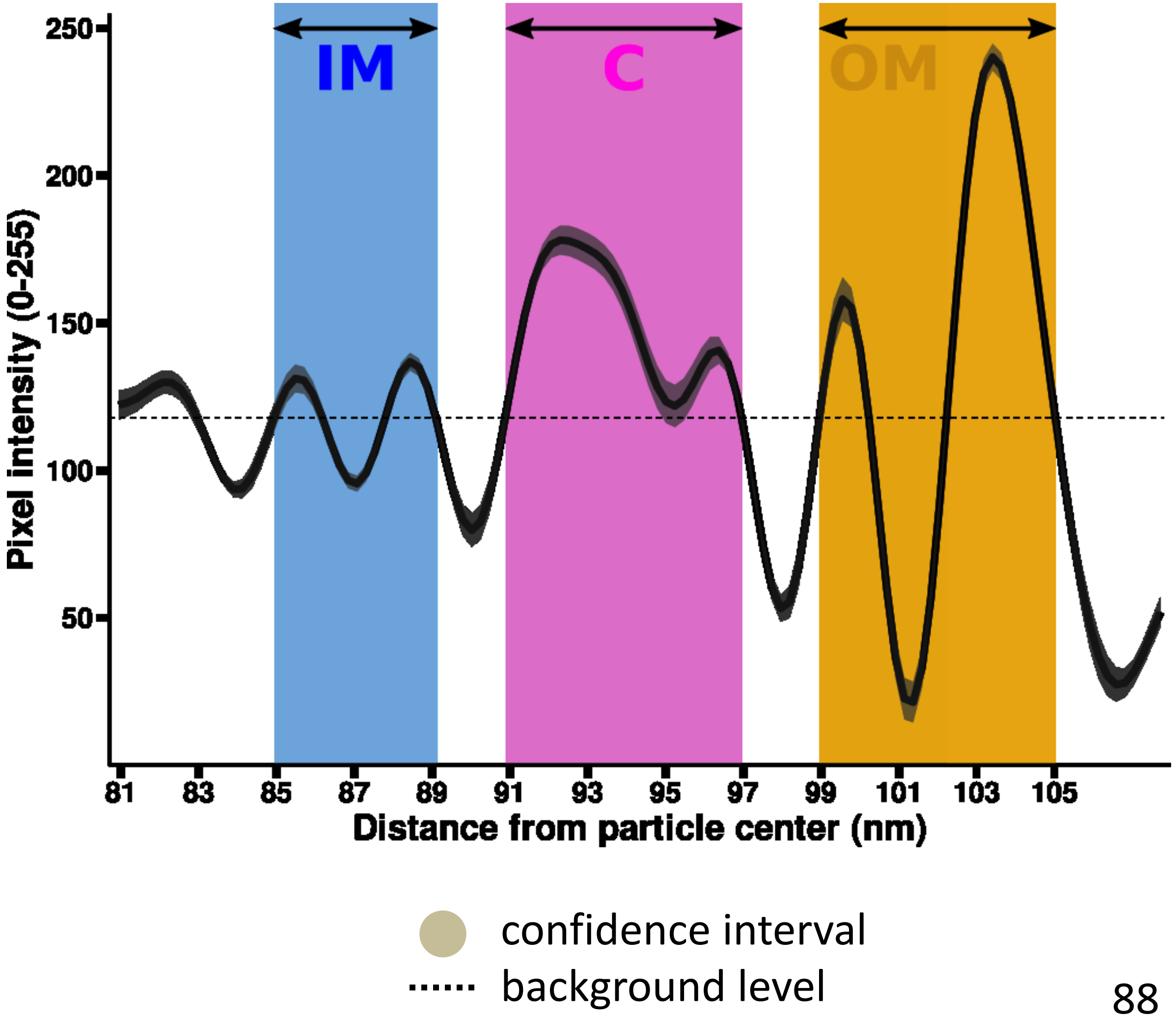
EhV-201 virions are pleiomorphic



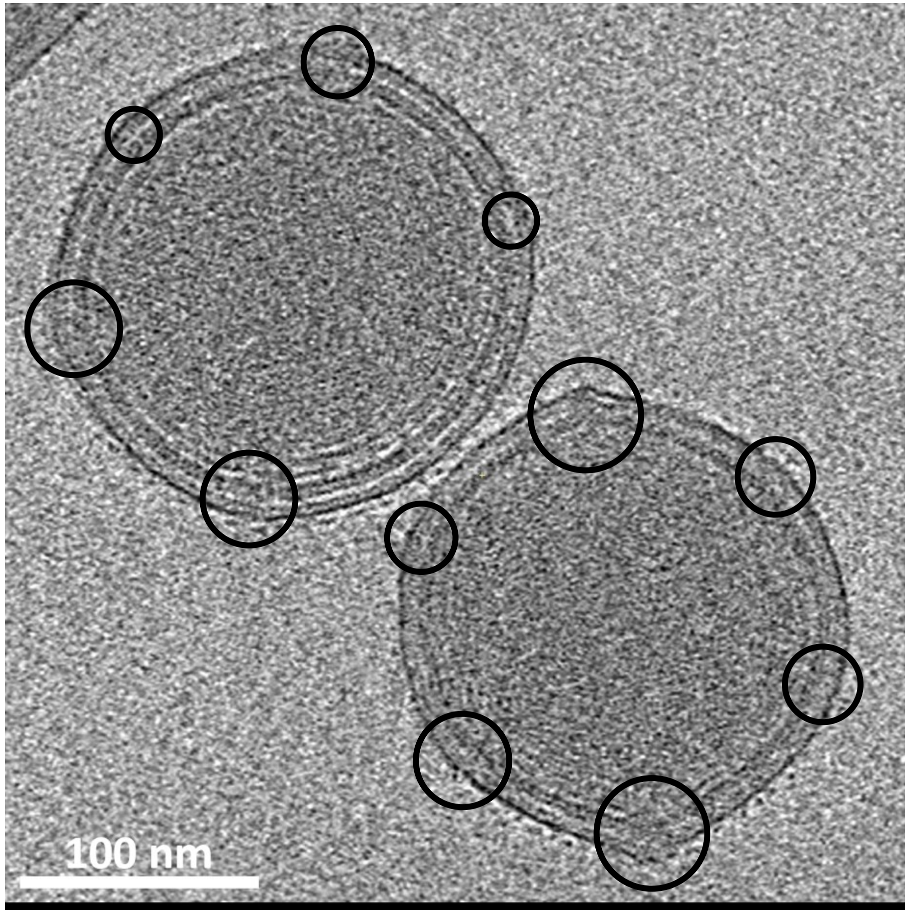
EhV-201 virions contain inner and outer membranes

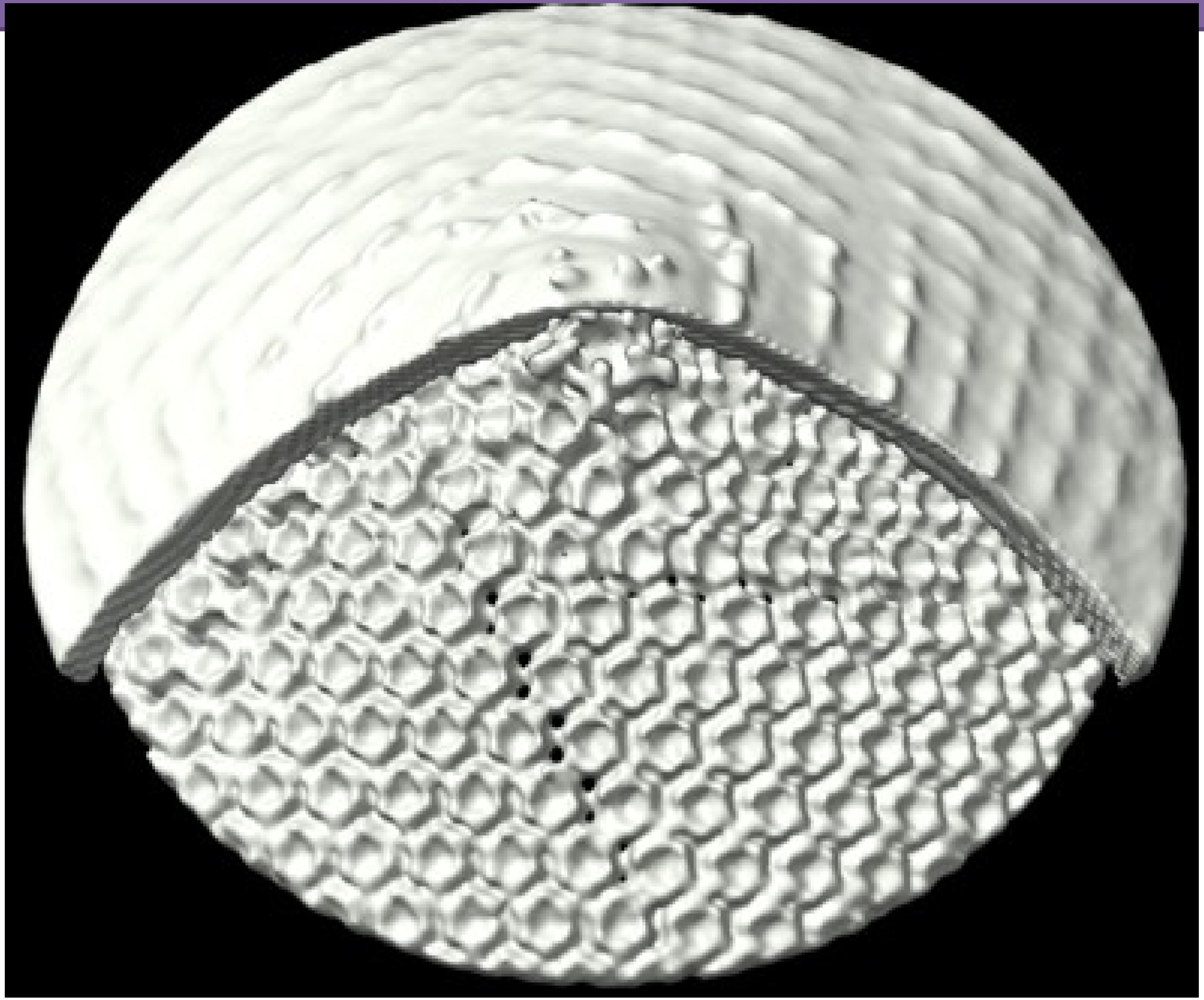




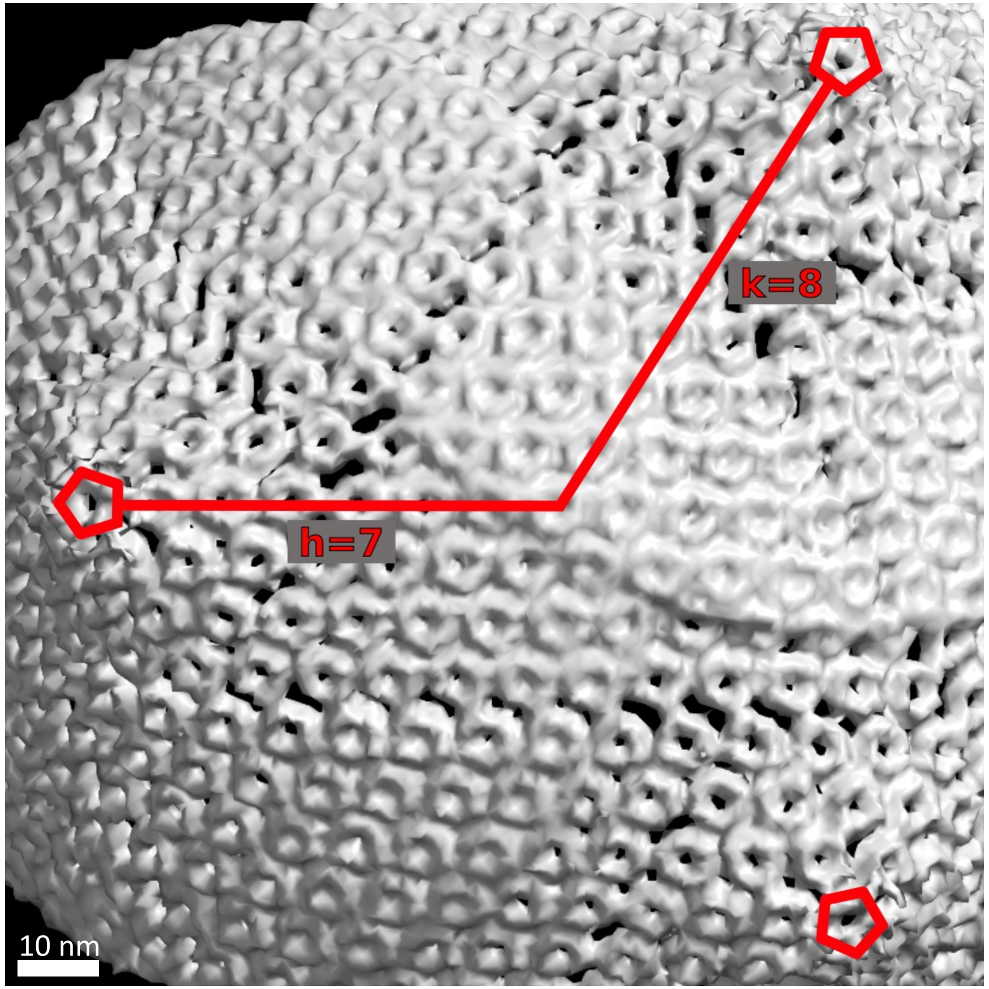


Sub-tomogram reconstruction of EhV-201 vertex

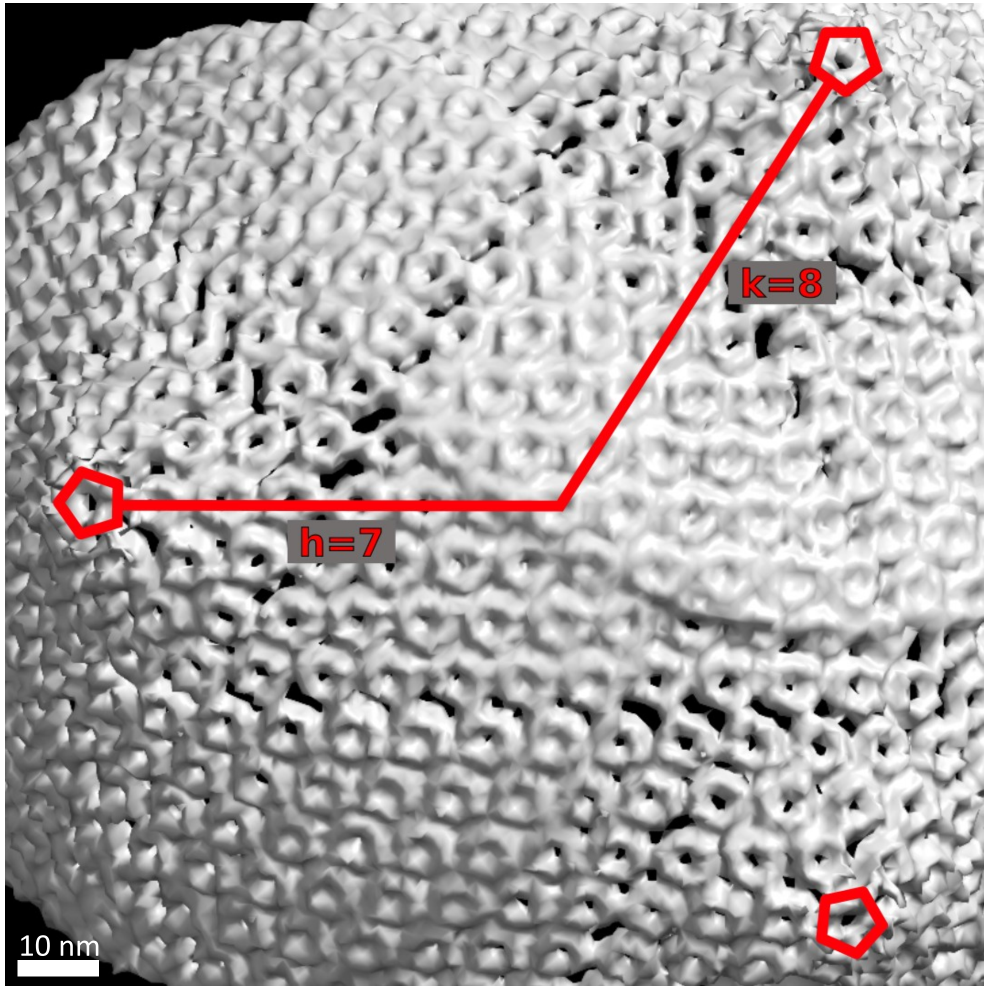


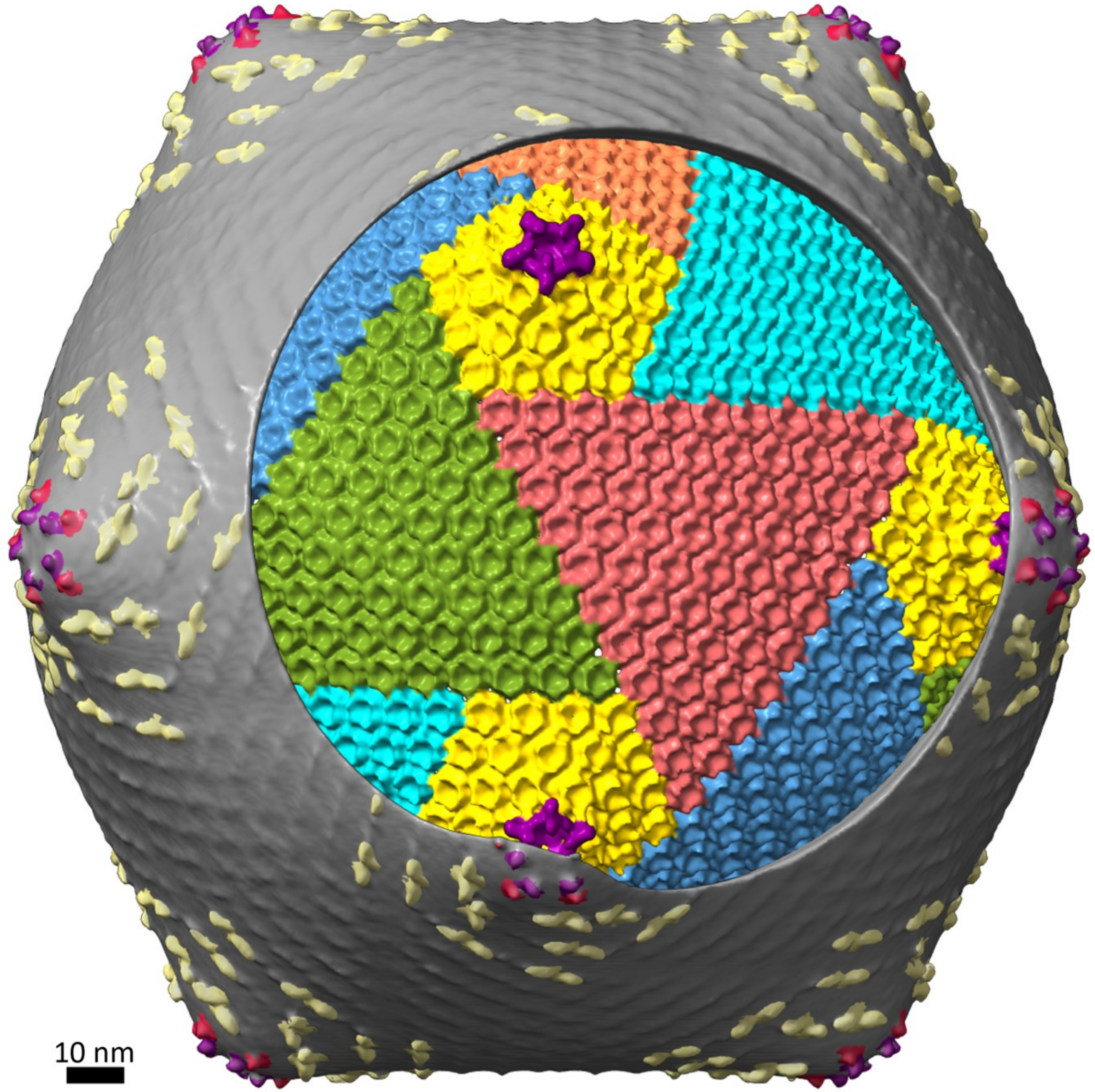


T-number and ideal EhV-201 virion



T-number and ideal EhV-201 virion





T-number and ideal EhV-201 virion