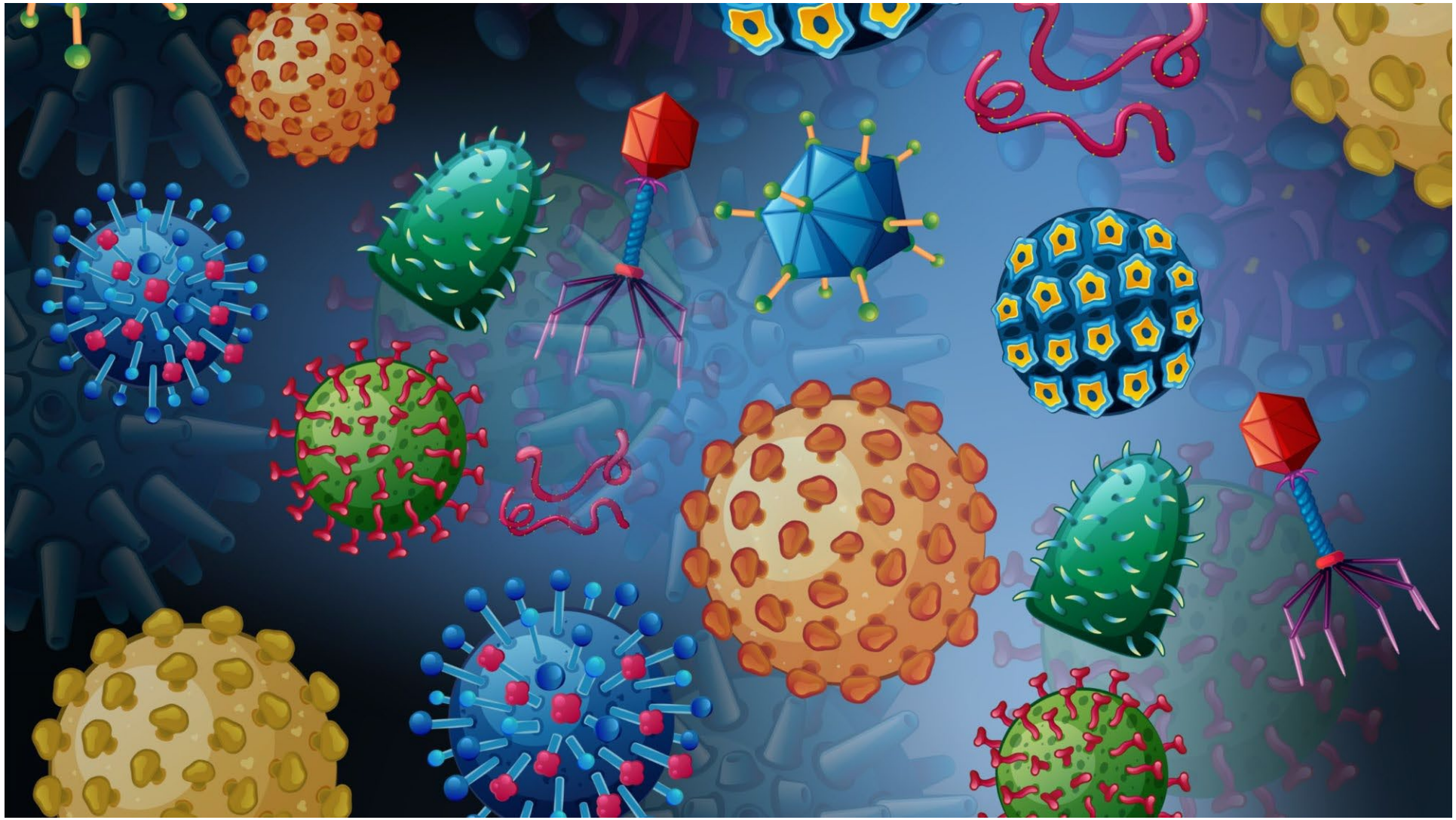
VIRUSES, CELL PARASITES



Viral diseases and anti-viral medicines have been known for thousands of years.

- 2500 BC: the Chinese recognized the nature and specific characteristics of smallpox

- 1400 BC: representation of polio patients in Egypt

- 460 BC: mumps described by Hippocrates

Viral pathologies: recurrent diseases

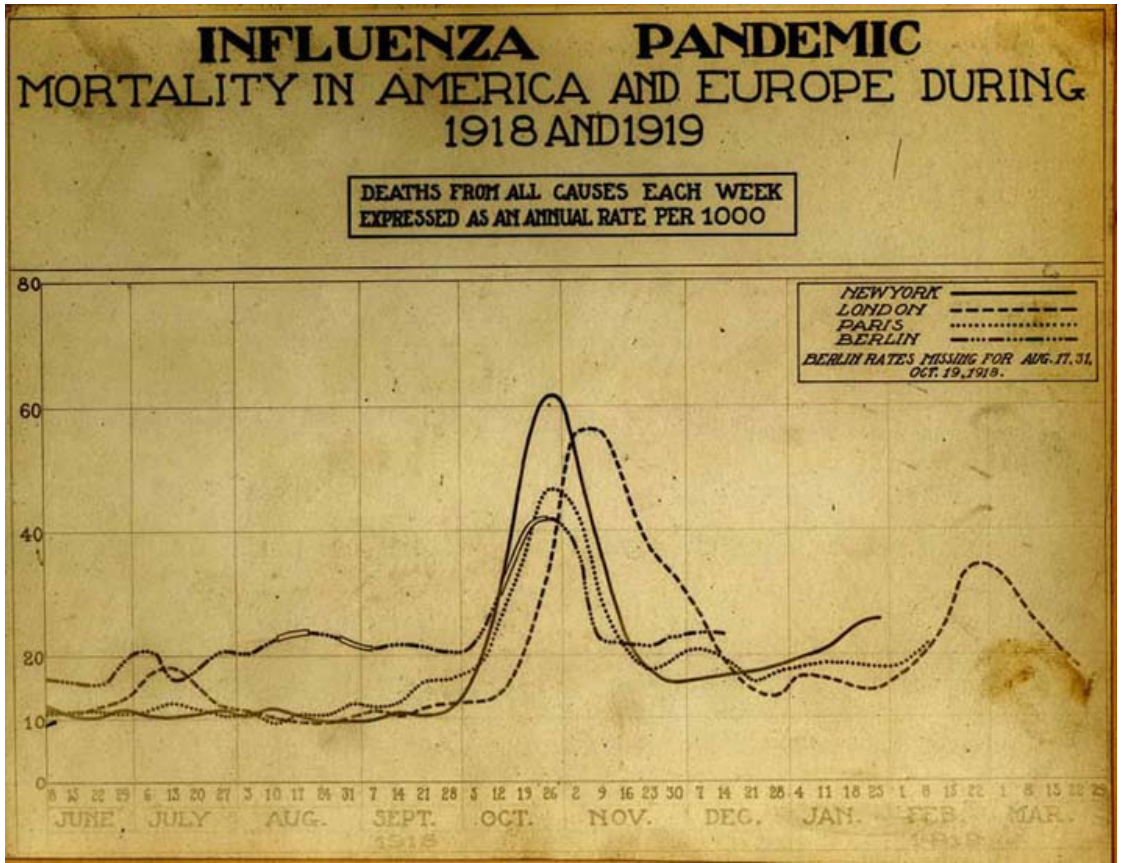
Measles

According to the World Health Organization (WHO), measles caused 8 million deaths worldwide every year until the 1970s.
Thanks to vaccination, deaths fell to 535,300 in 2000, 242,000 in 2006 and 139,000 in 2010. Measles remains a scourge, **representing one of the main causes of infant mortality for children who do not have access to the vaccine.**

Influenza

Spanish flu:

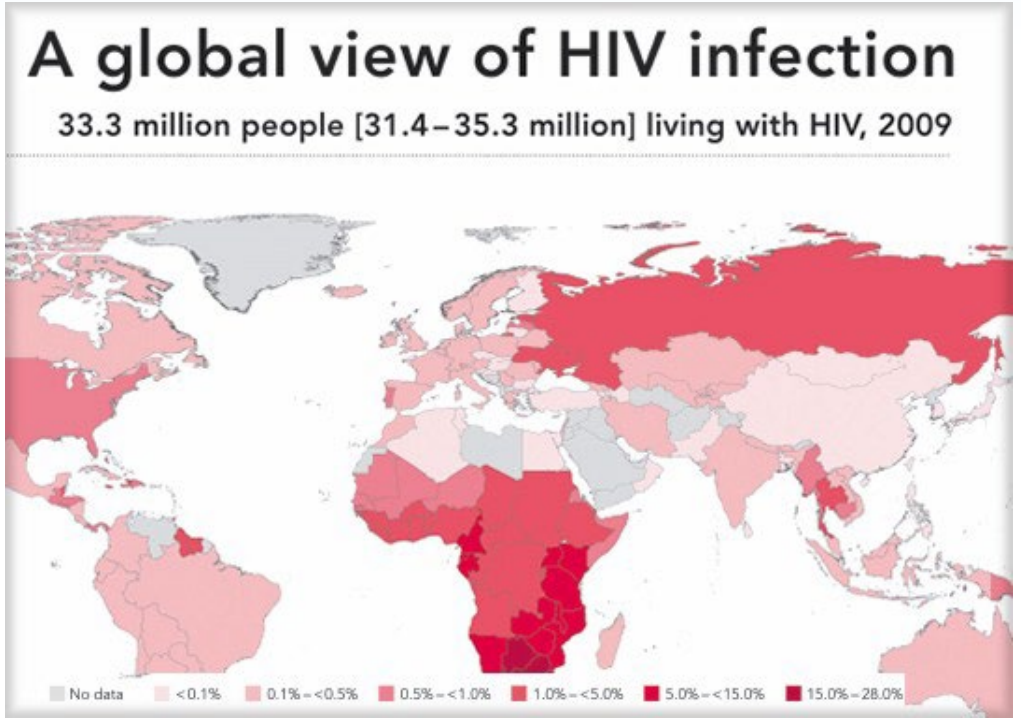
40-100 million deaths!



Viral pathologies: new diseases

AIDS

Virus identified in 2001
Late 2015:
- 78 million people have been infected since the start of the epidemic
- 35 million people have died since the start of the epidemic.



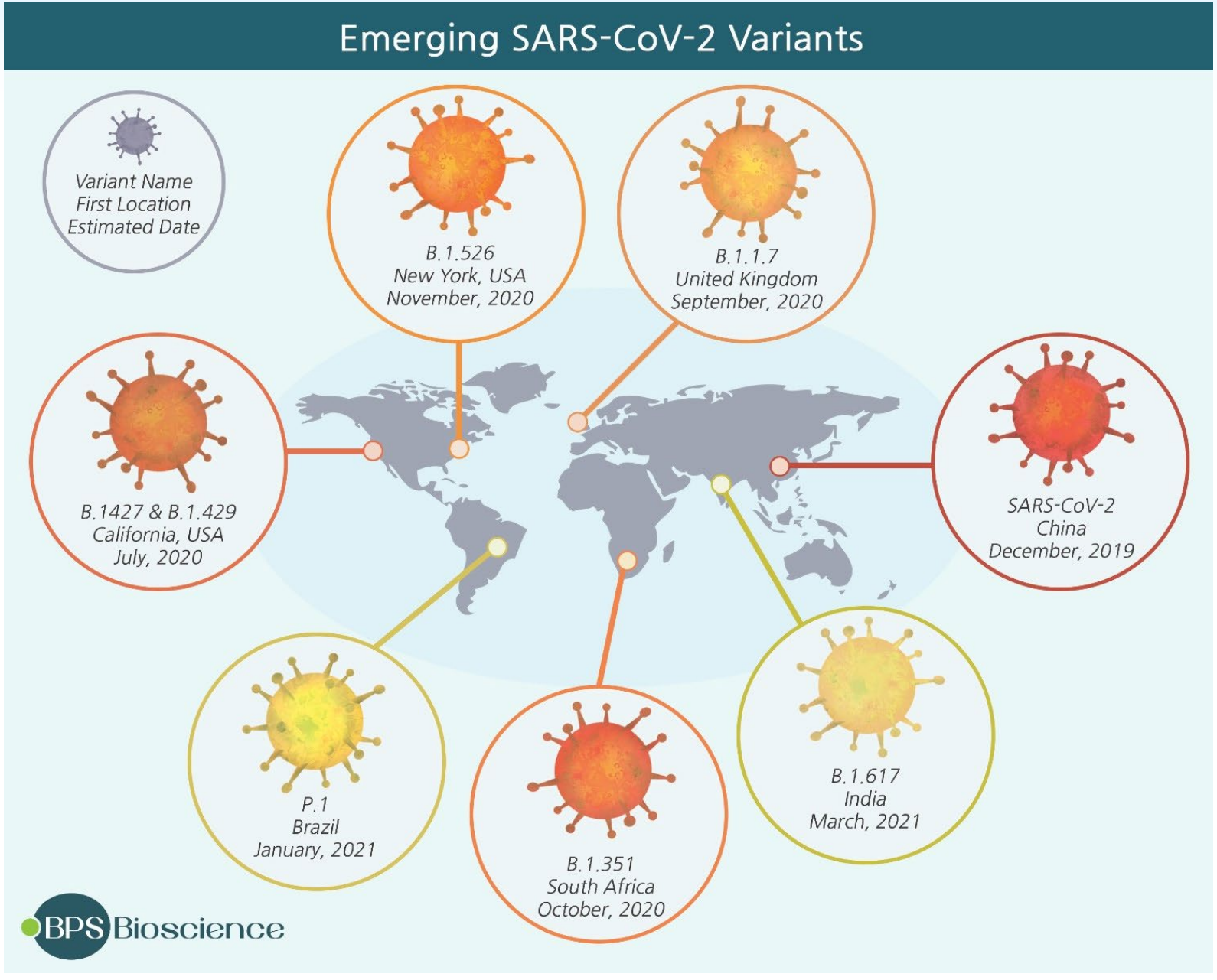
Ebola

According to the World Health Organization (WHO), the Ebola virus is one of the most deadly viruses affecting humans. The mortality rate varies from 25% to 90% depending on the type of virus and the conditions under which it is treated. 28,636 cases and 11,315 deaths during **the 2014-2015 epidemic**.

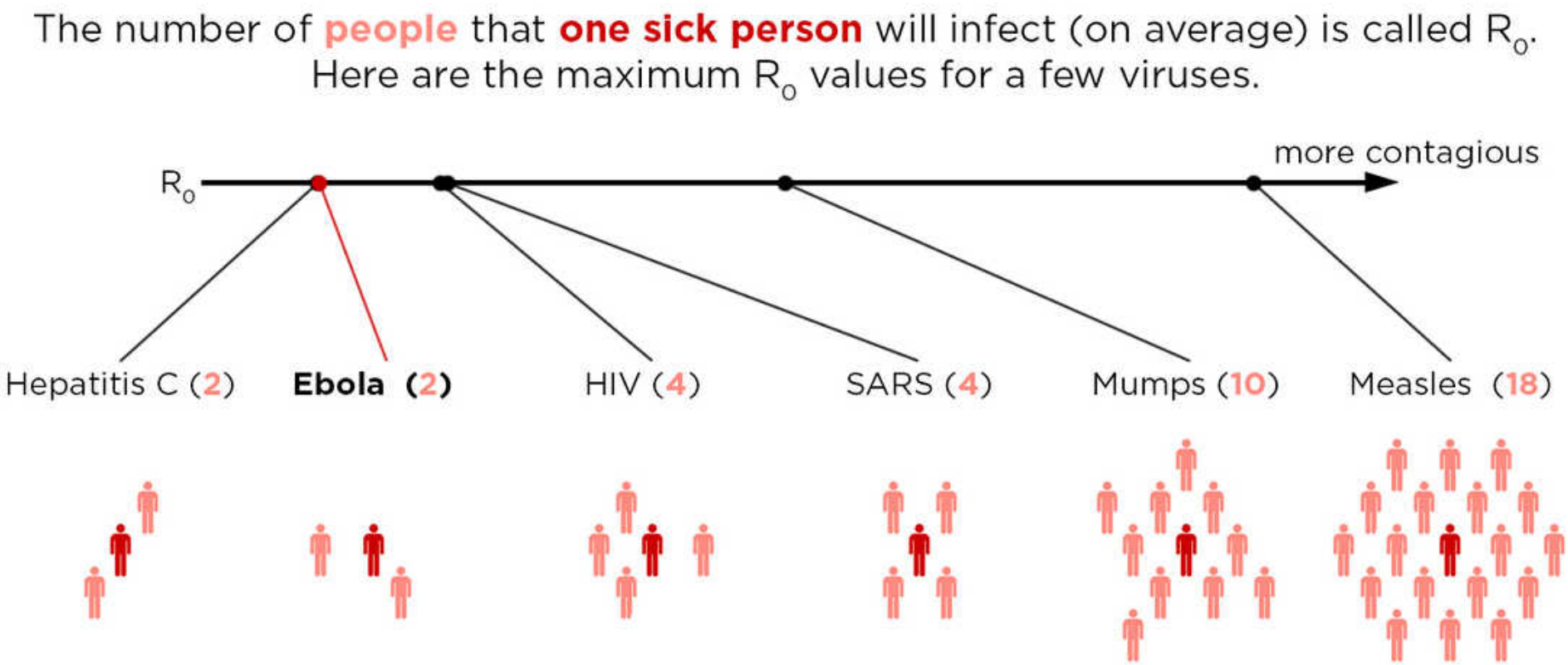
Causing "haemorrhagic fevers", it takes its name from a river in the north of the Democratic Republic of Congo (DRC), where it was first spotted in 1976.

Viral pathologies: emmergent diseases

COVID-19

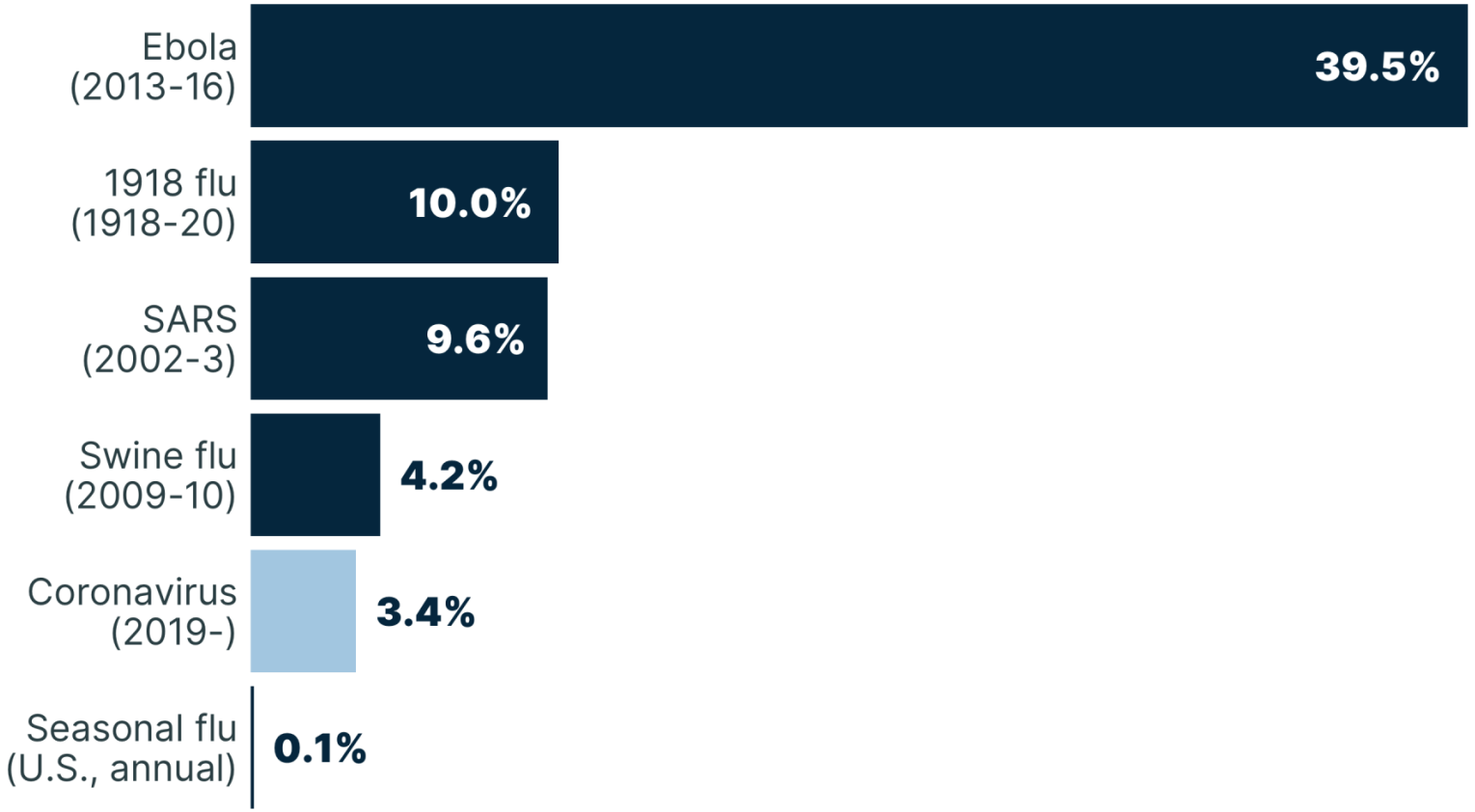


Viral transmission



Viral disease mortability rates

Share of reported infections leading to death. All figures are worldwide unless specified. Coronavirus mortality figures are being revised as more data comes in on the ongoing outbreak.



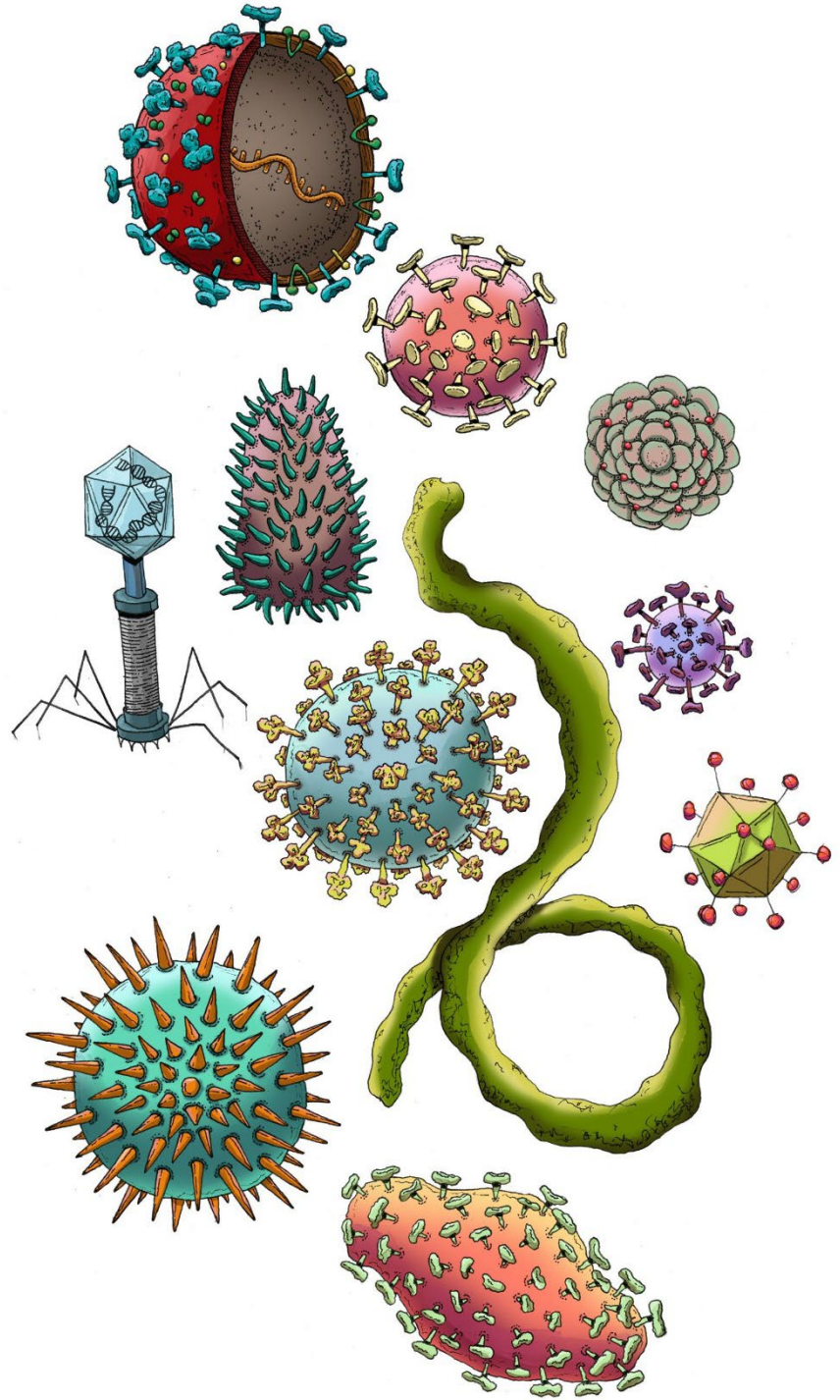
Outline

I- Nature and diversity of viruses

II- Virus entry into the cell

III- The viral cycle

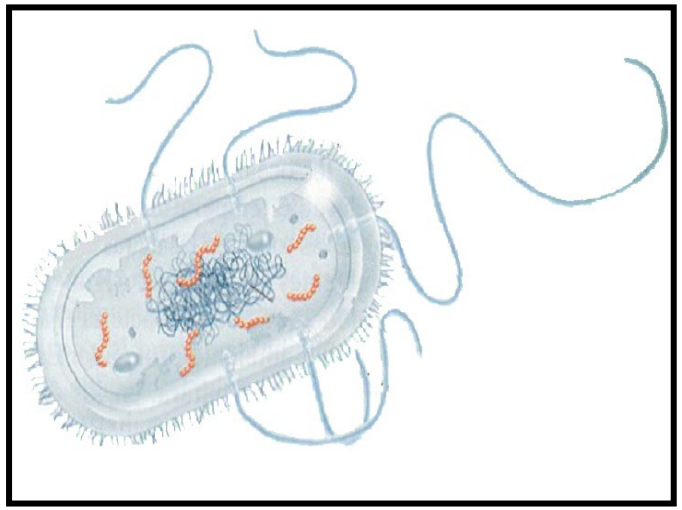
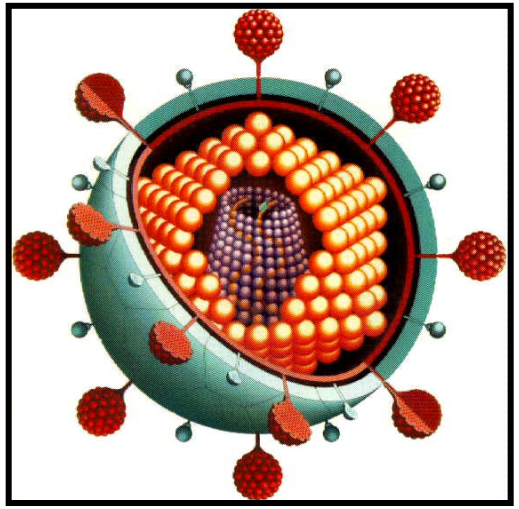
I- Nature and diversity of viruses



Virus characteristics

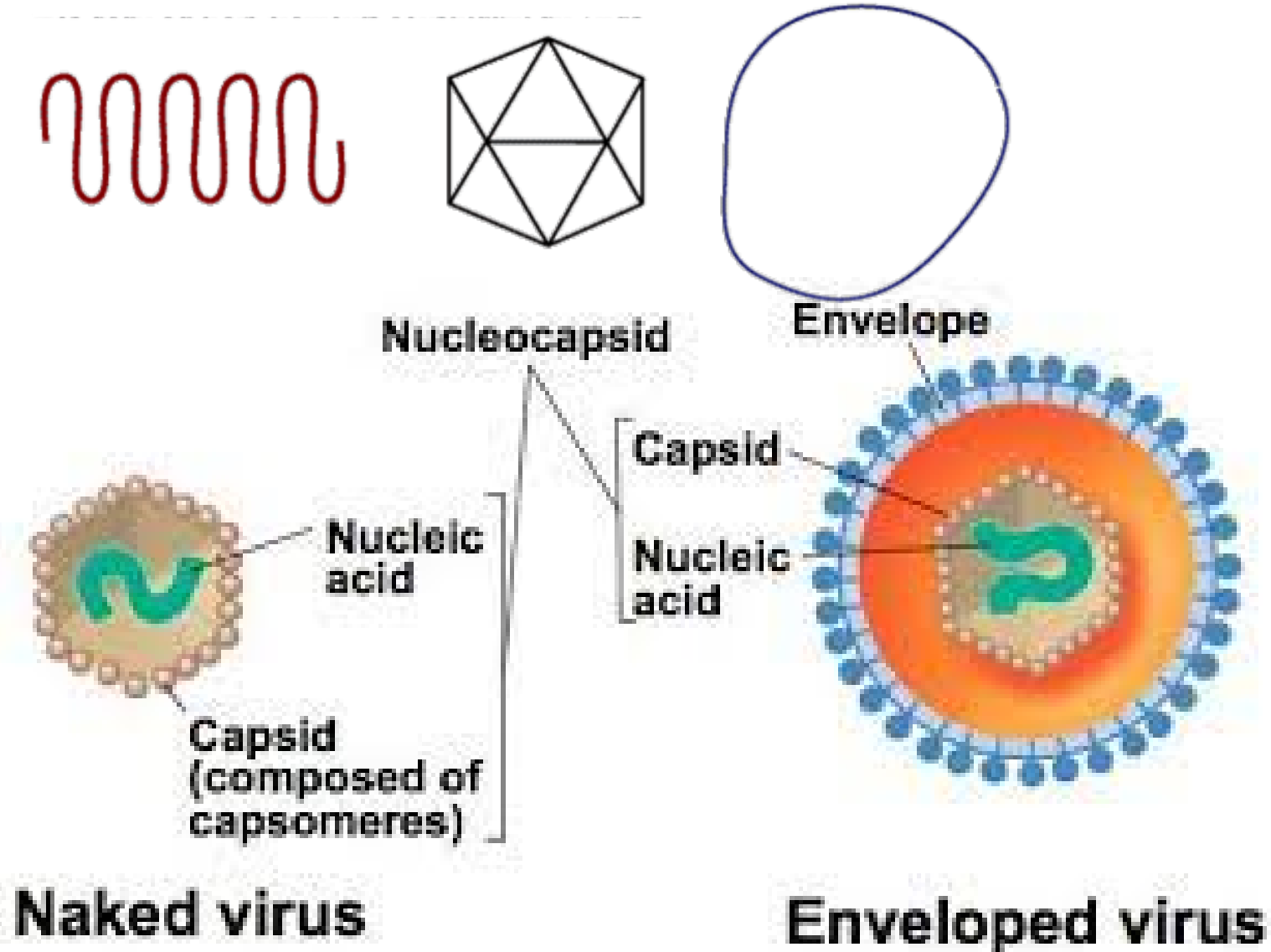
Viruses are infectious agents but Koch's postulates (1884-1890) are difficult to apply to viruses.

- Invisible under an optical microscope, only observable under an electron microscope.
- Cannot be grown in pure culture
- Ultrafiltrable: i.e. not retained by bacterial filters

	Bacteria	**Viruses**
		
Muliplication	Stand-alone	Host-dependent
Breathing	Aerobic/Anaerobic	Absence
Nutrition	Carbon source	No nutritional requirements

What is a virus?

A complete viral particle, called a virion, is defined by a structure consisting of two (or three) elements.



Viral genome diversity

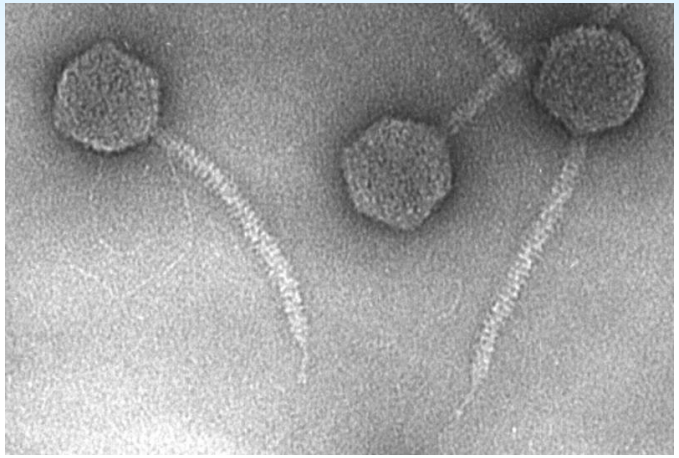
The nature of the genome differs considerably from one virus to another.

There are viruses at :

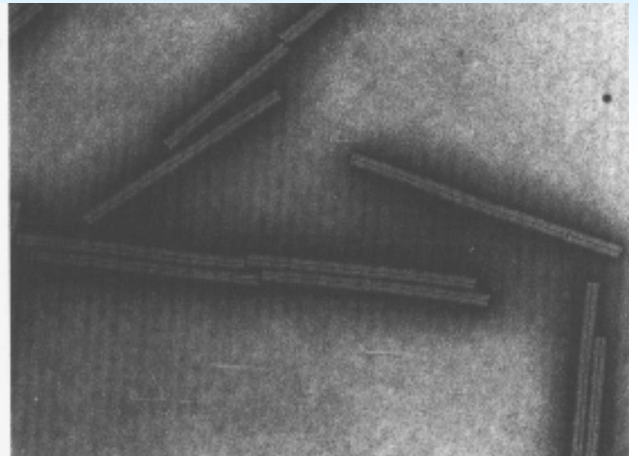
- Double-stranded DNA
- Single-stranded DNA
- Single-stranded RNA
  - with positive polarity
  - with negative polarity
- Double-stranded RNA

Genome size differs considerably between viruses.
From 1 (HDV) to 1200 genes (mimivirus)
It is generally larger for DNA viruses (3 to 300 kb) than for RNA viruses (10 to 20 kb).

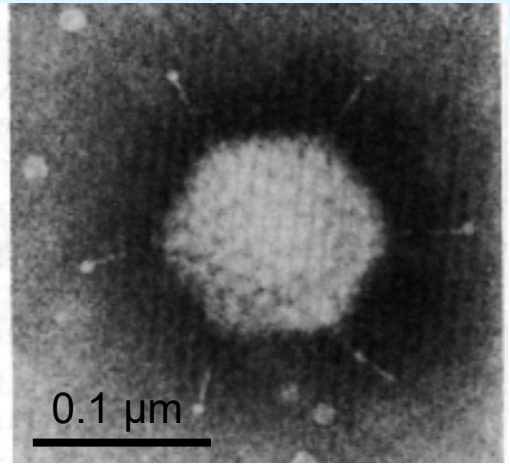
Capsid diversity



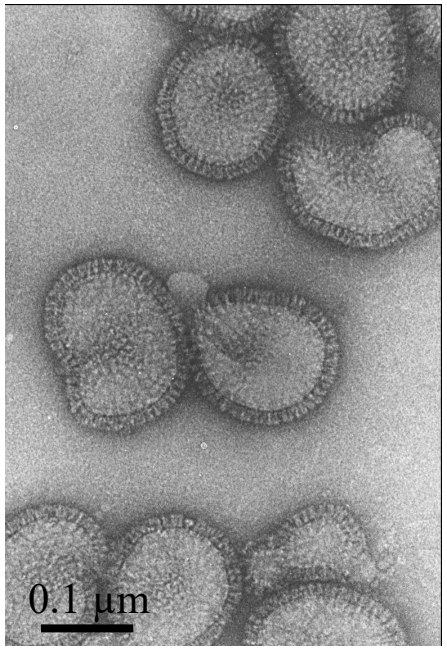
Bacteriophages (phage λ)



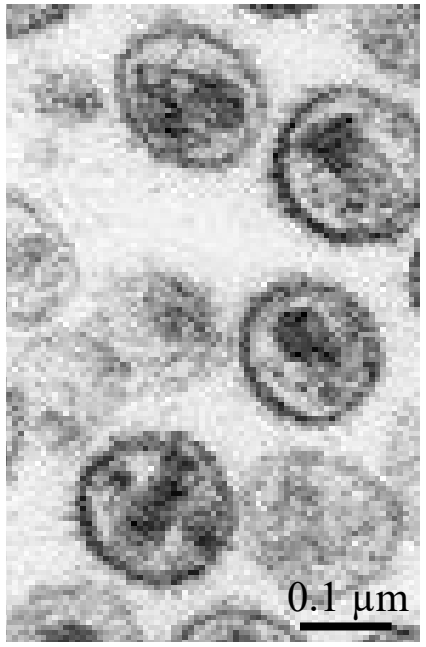
Tobacco mosaic virus



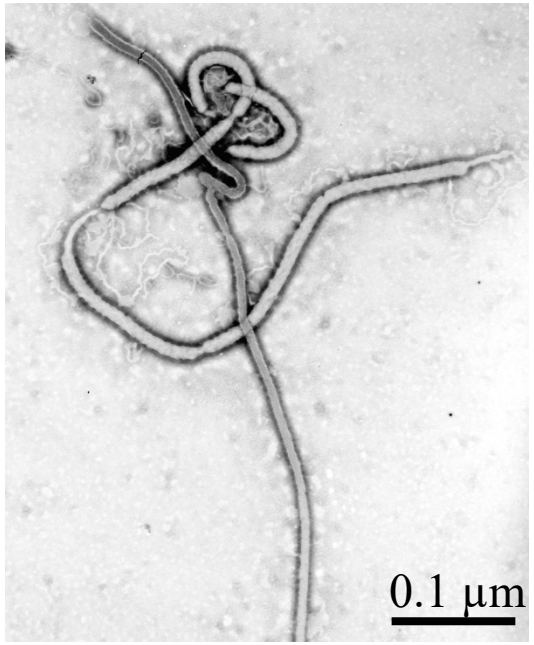
Adenovirus



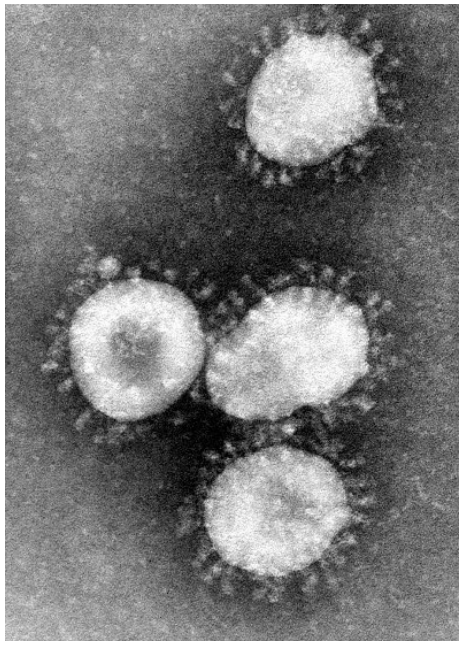
Influenza virus



HIV virus



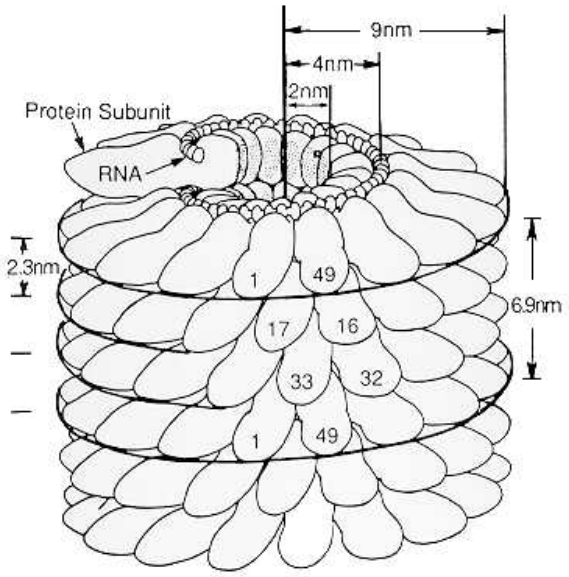
Ebola virus

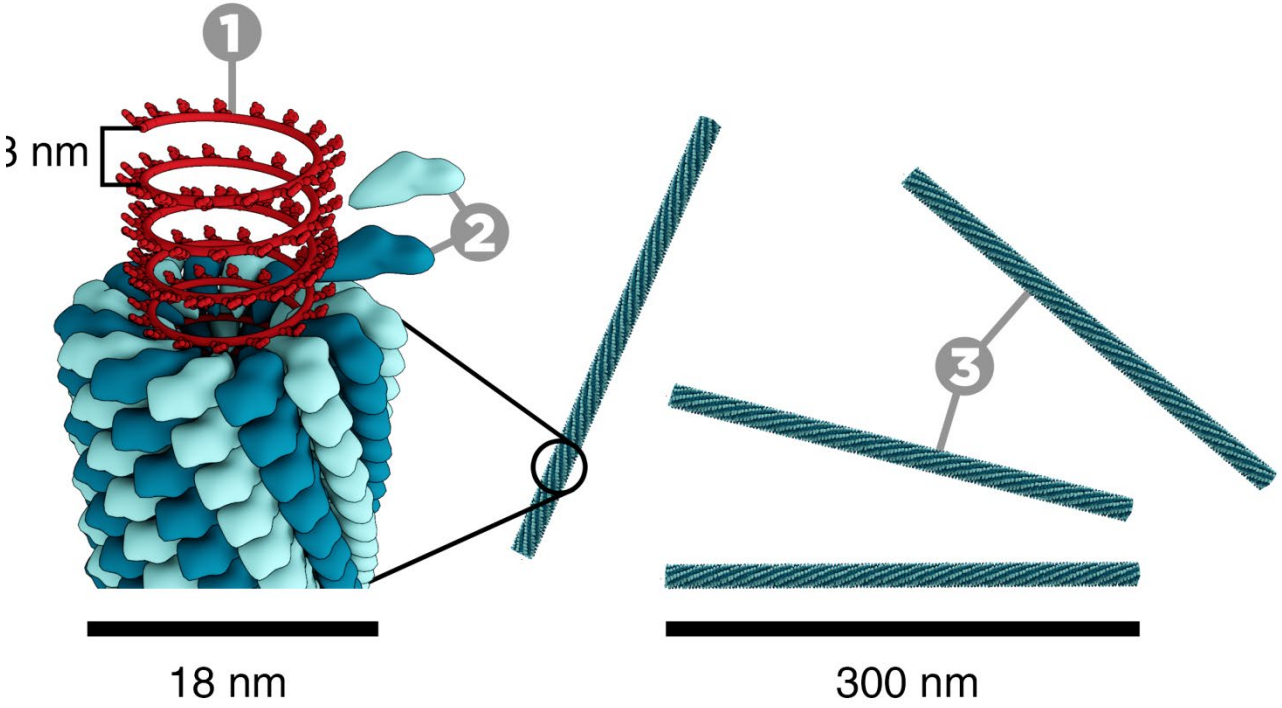


SARS-CoV virus

Helical symmetry

Helical symmetry is described by *u* (the number of subunits per helix turn) and *p* (the displacement along the axis per subunit).





Tobacco mosaic virus.

1- nucleic acid (RNA)
2- capsomer (protomer)
3- capsid

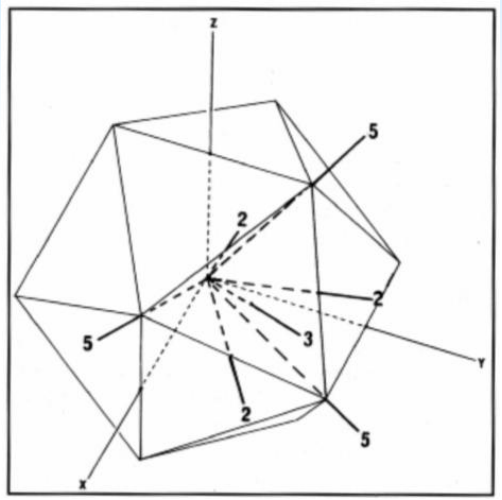
Icosahedral symmetry

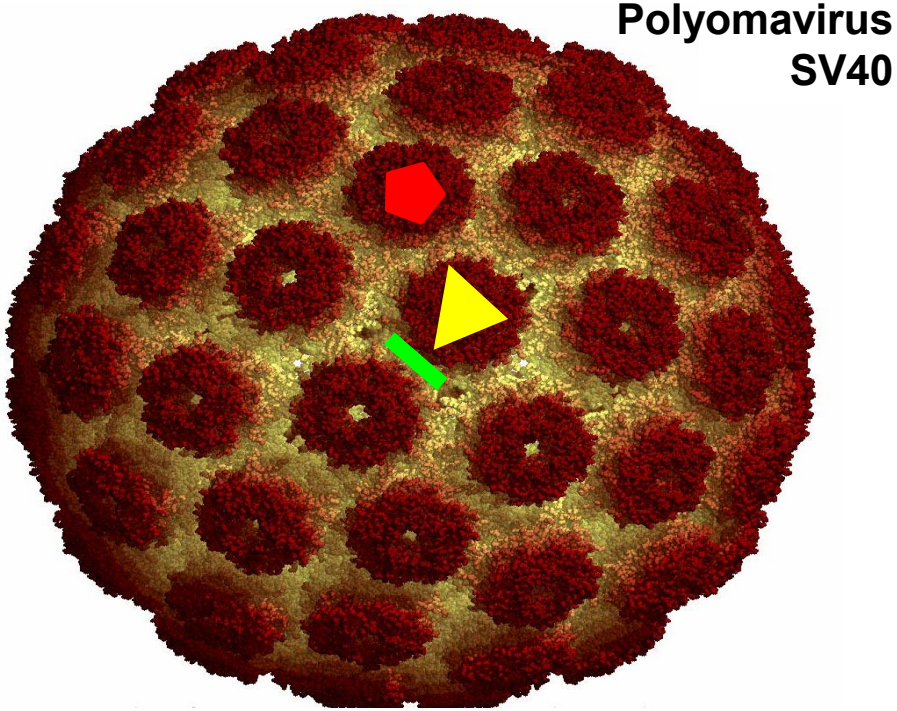
The icosahedron is made up of at least

20 faces (10 axes of rotation of order 3),

with 12 vertices (6 axes of rotation of order 5),

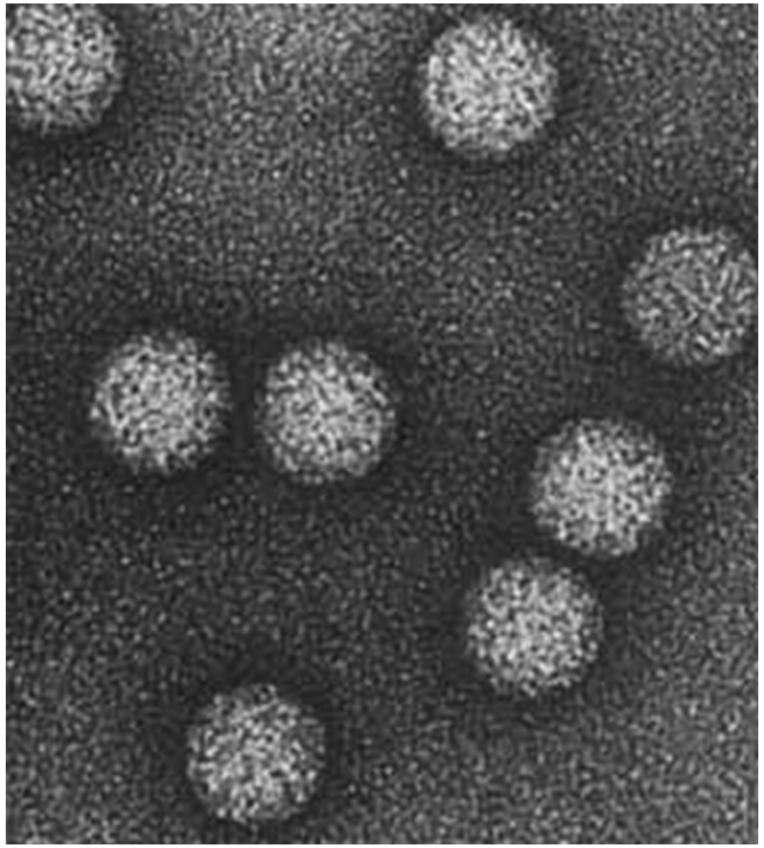
and 30 edges (15 axes of symmetry of order 2)



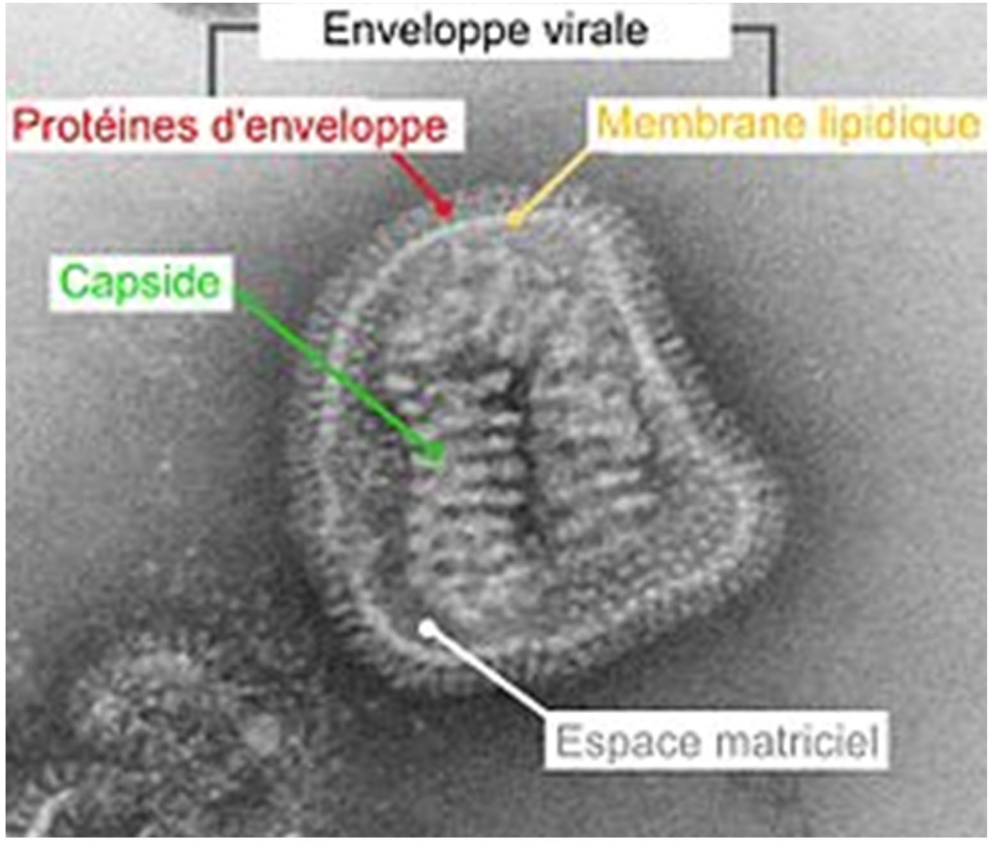


Viruses with or without envelopes

Polio virus



Influenza virus (Orthomyxo viridae)



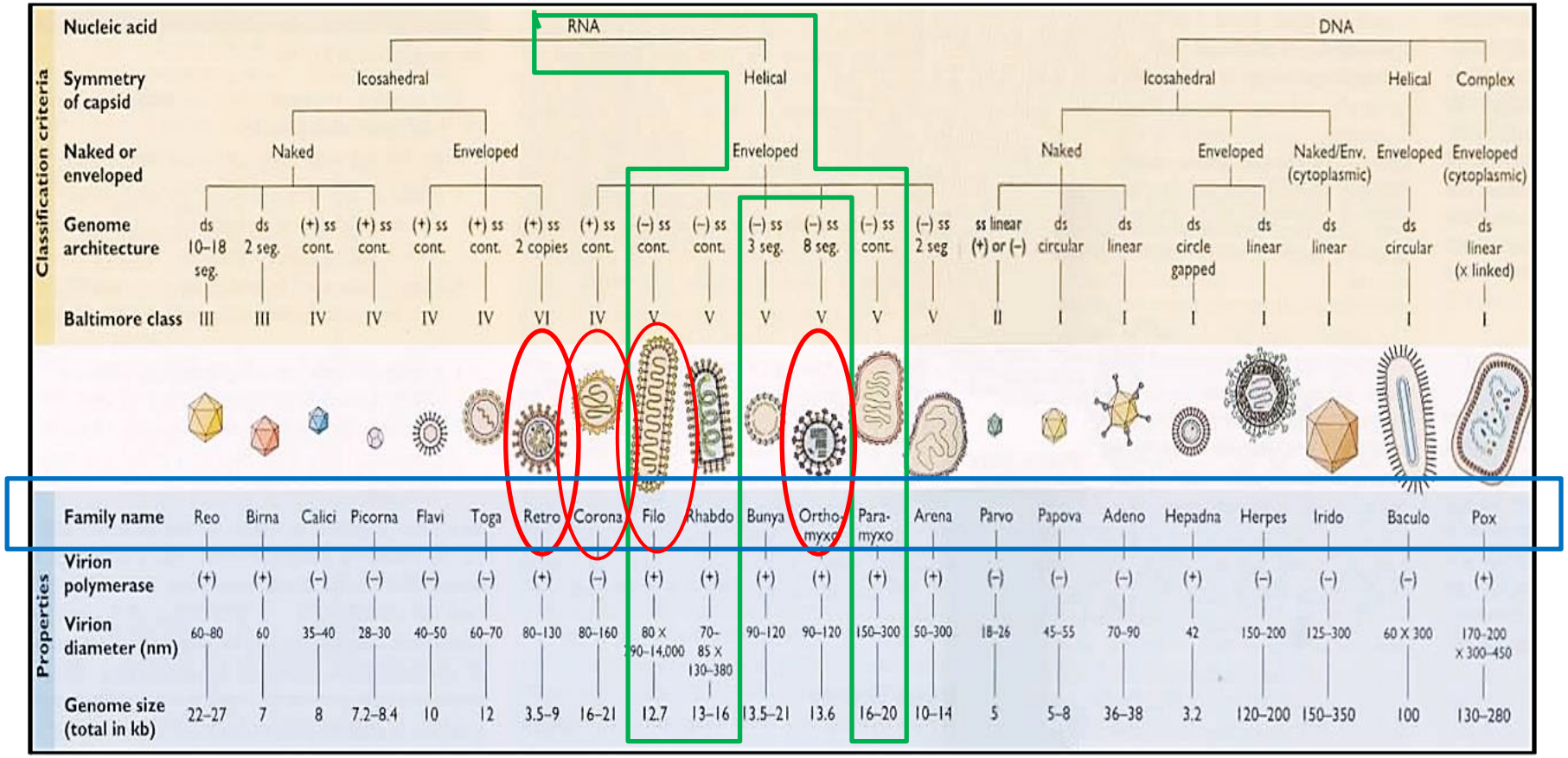
The presence or absence of an envelope largely determines how diseases are transmitted.

Taxonomy

The classification of viruses, established in 1962 by Lwoff, Horne and Tournier, takes account of the following:

- the nature of the genome's nucleic acid (DNA or RNA),
- capsid conformation (tubular or icosahedral)
- the presence or absence of an envelope.
- genomic organization

These classification criteria enable viruses to be grouped into Families (...viridae).



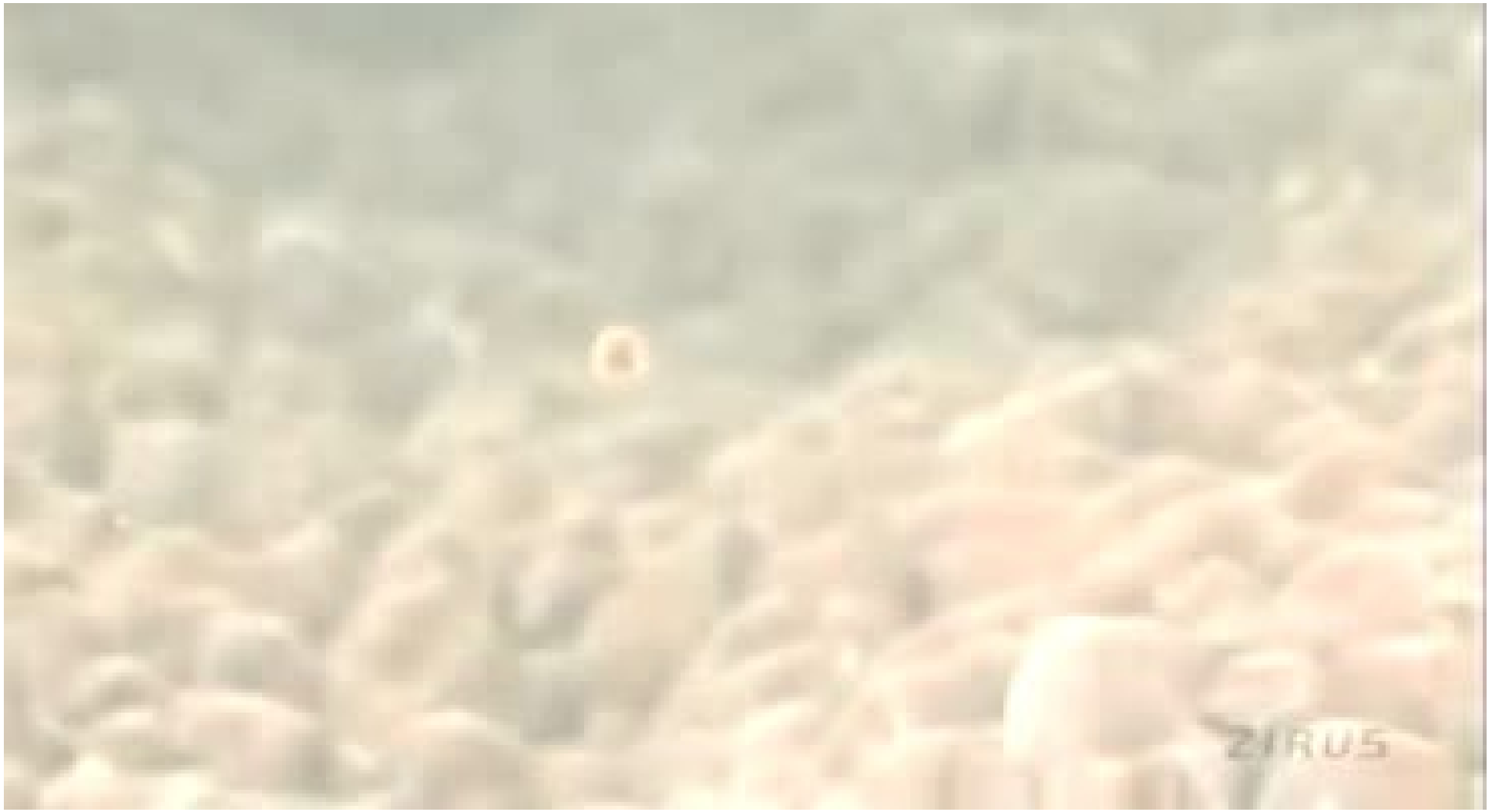
Conclusions I

I- Nature and diversity of viruses

- Viruses are not living organisms.
- Viruses are obligatory cell parasites.

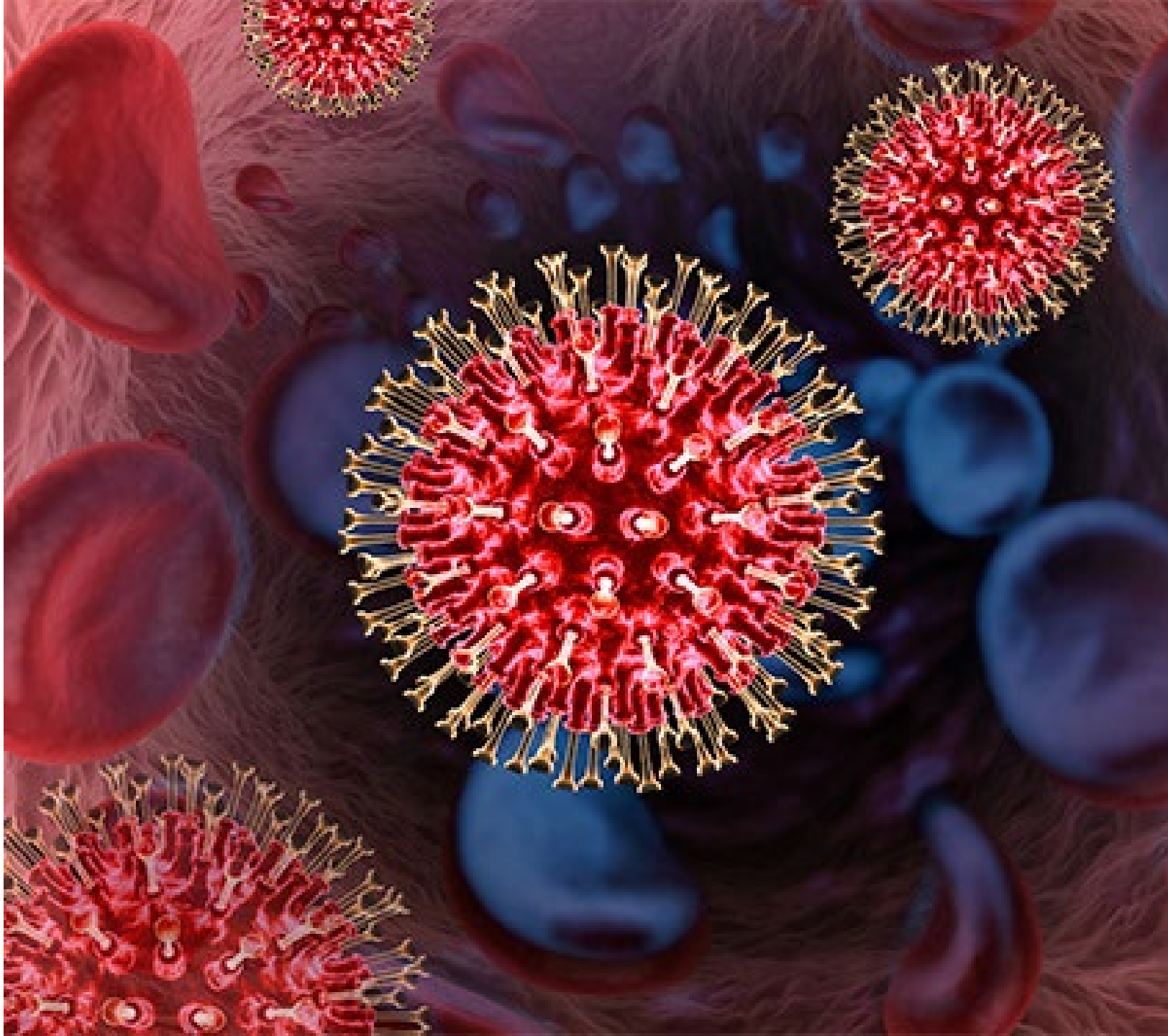
- Viruses are extremely diverse.
- All viruses contain genetic material, protected by a protein capsid. They may be surrounded by a membrane envelope.
- The genetic material may be DNA or RNA. The genome can be single-stranded or double-stranded.
- The nature of the genetic material determines viral replication and translation strategies, and the way viruses spread.
- Capsids frequently have helical or icosahedral symmetry.
- Viral taxonomy is based on the nature of the genetic material, capsid structure and envelope presence.

What does a virus do?



II- Virus entry into cells

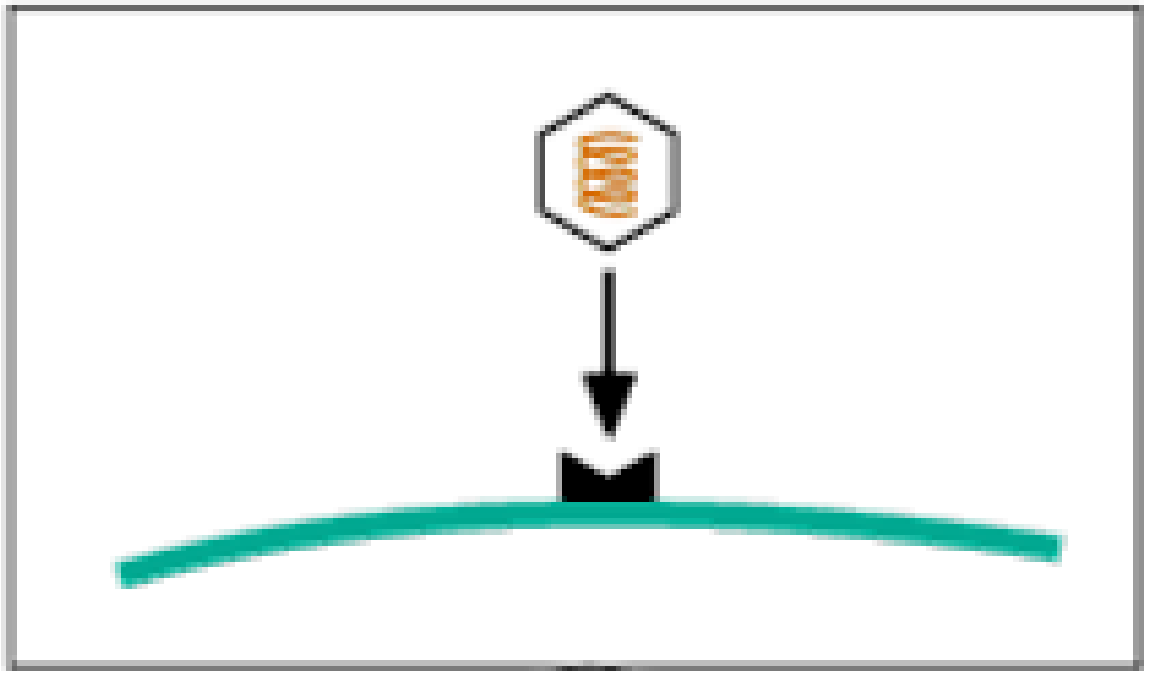
1. Receptors



Virion-target cell interaction

The virus interacts with a specific receptor expressed on the surface of target cells.

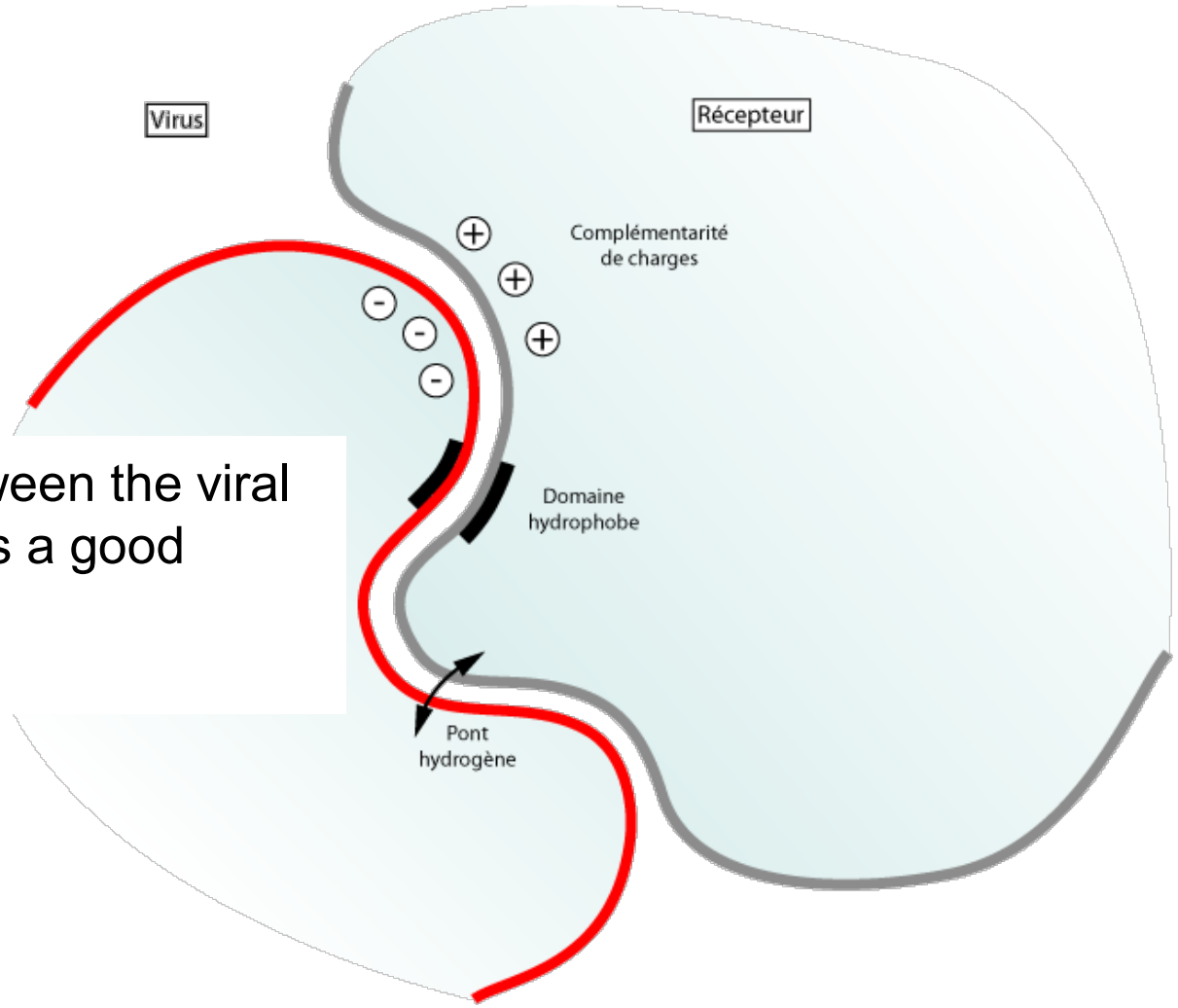
- The receptor is generally a cell surface protein.
- The cellular receptor is recognized by viral envelope glycoproteins (enveloped viruses) or capsid proteins (naked viruses).



Nature of the genome Virus	Viral protein	Receiver	Cellular function
Double-stranded DNA			
HSV1	gC	Heparan sulfates	Cell adhesion and recognition
	gD	HVEM	?
Adenovirus	Fiber	CAR	?
	Penton	integrins	Cell adhesion
Retrovirus			
HIV	Gp120	CD4	Interaction between T helper lymphocytes and antigen-presenting cells
	Gp120	CXCR4, CCR5	Chemokine receptors
Negative RNA			
Influenza virus	HA	Sialic acid	Present on cellular glycoproteins
Measles virus	H	CD46	Regulator of complement activation
Rabies virus	G	p75	Neurotrophin receptor
		NCAM	Neuronal and cellular adhesion
Positive RNA			
Transmissible swine gastroenteritis virus	S	Aminopeptidase N	Intestinal protease
Human rhinovirus		ICAM	Intercellular adhesion molecule
Polio virus		PVR	Member of the immunoglobulin superfamily

Virion-receptor interaction

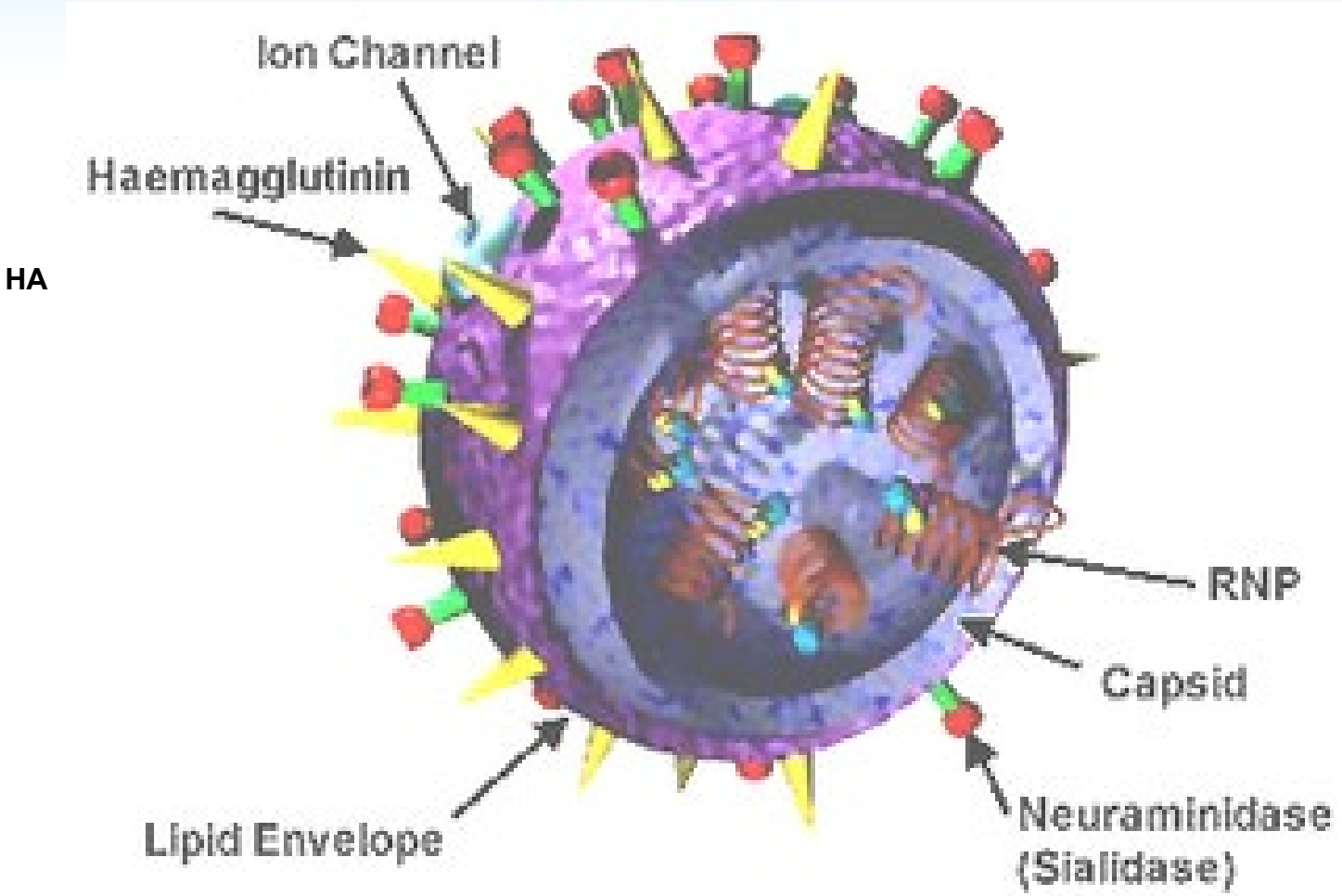
The interaction between virion and receptor is a physical one involving weak bonds



The contact surface between the viral protein and its receptor is a good indicator of affinity.

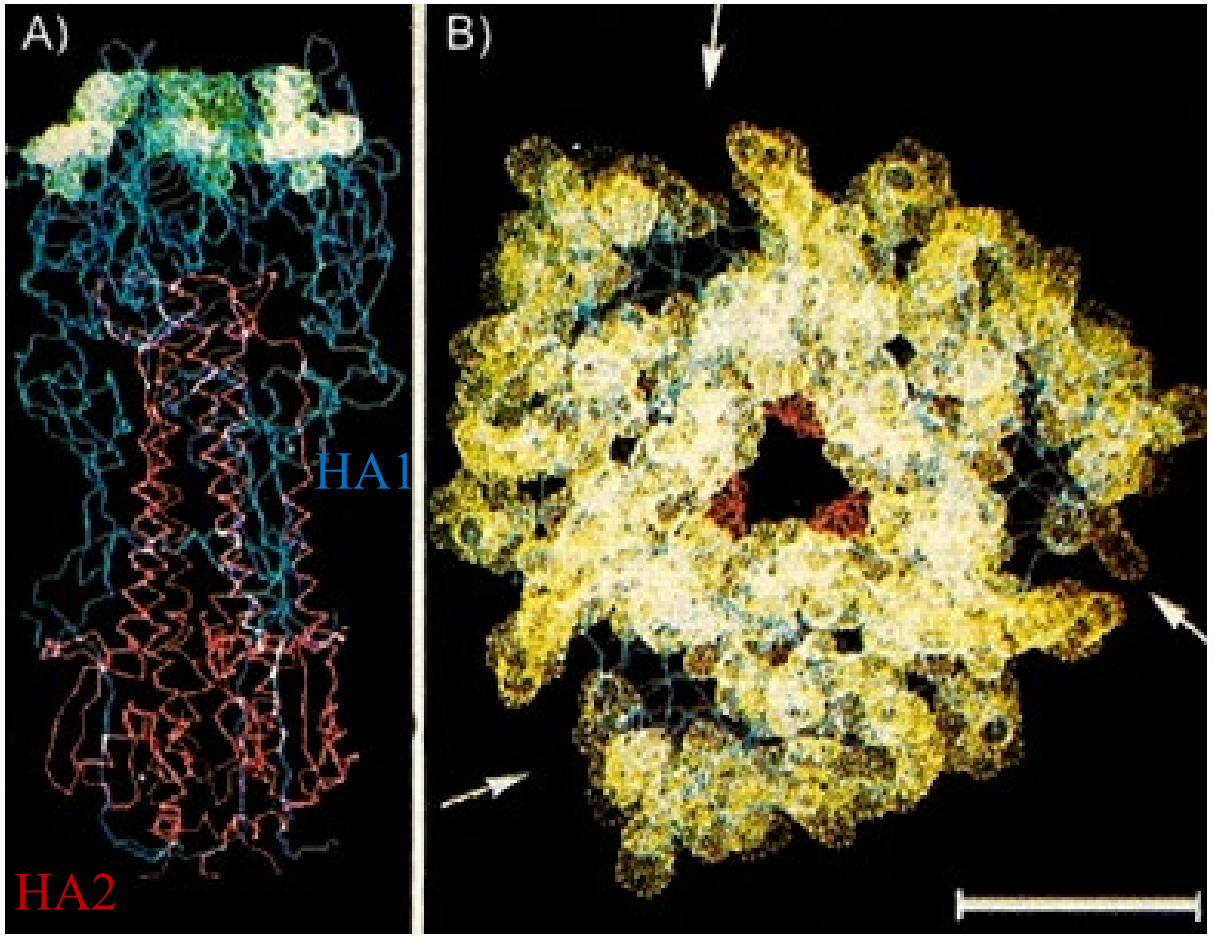
Influenza family portrait

The Influenza virus consists of
- of genetic material, in the form of eight single-stranded RNA of negative polarity.

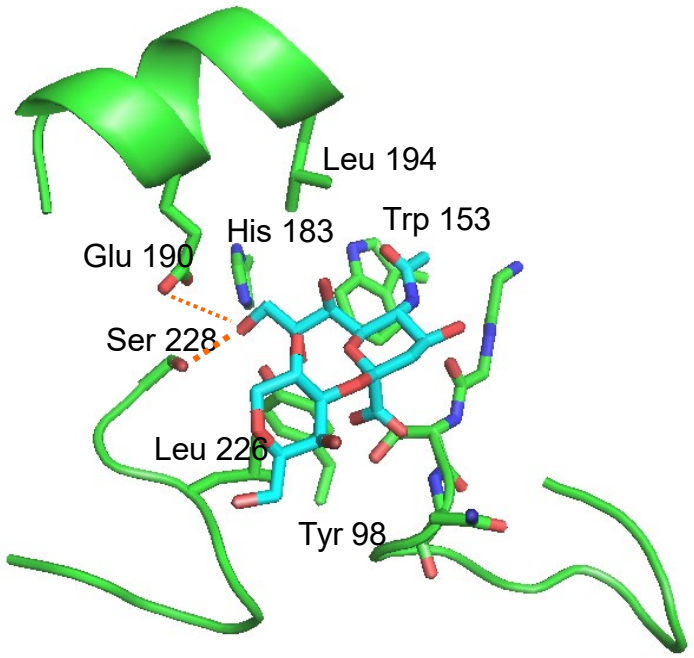


- an envelope derived from the host cell membrane.
It has three major viral proteins that project outwards and possess antigenic properties:
a small protein (M2), hemagglutinin (H 1-16), neuraminidase (N 1-9).

Interaction between influenza virus hemagglutinin and sialic acid



Three binding sites on the trimer that forms hemagglutinin

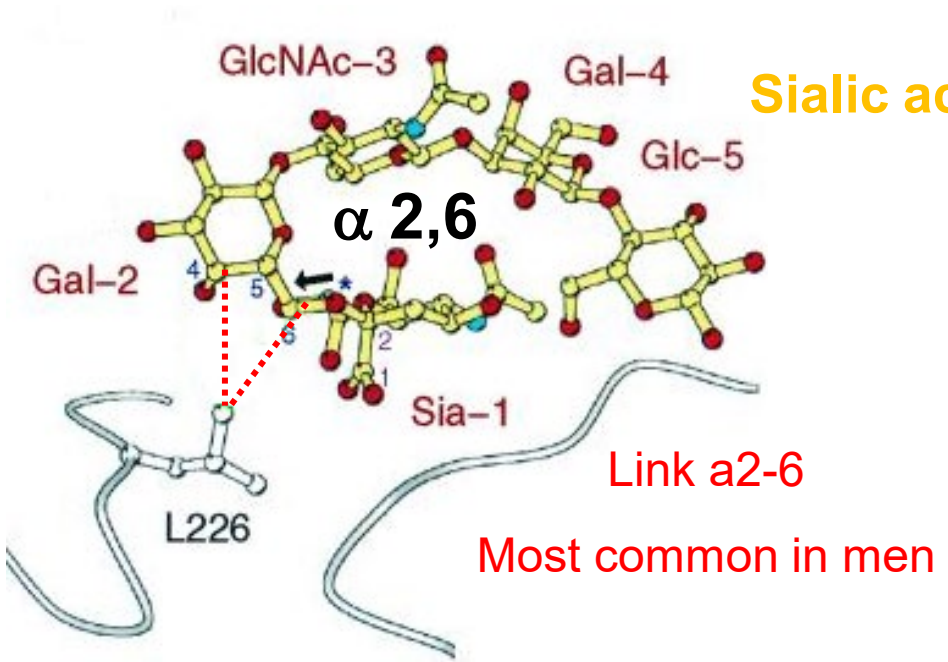


Sialic acid: sugar chain linked to a glycoprotein or lipid

The influenza receptor and species barrier

Expression of the virus receptor is often restricted to certain species, cell types or tissues.

The receptor is generally a crucial determinant of viral tropism (cells resistant or not to infection).

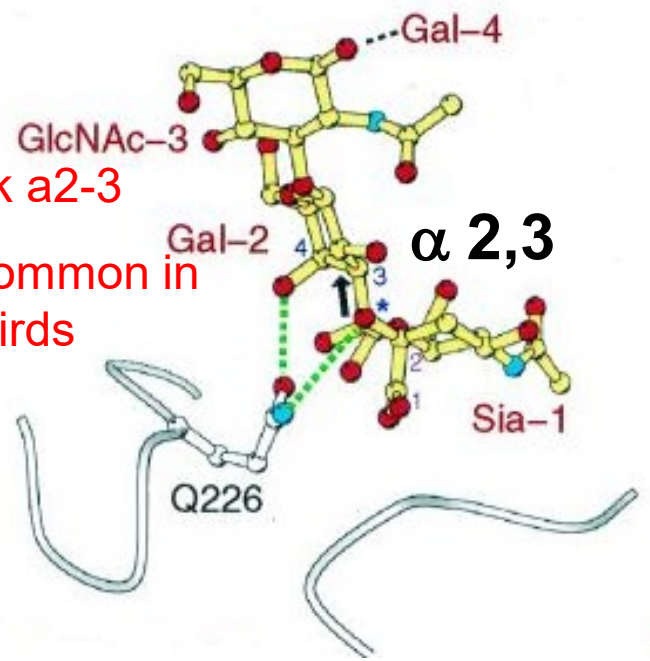


Sialic acid

Link a2-6

Most common in men

Hemagglutinin human viruses

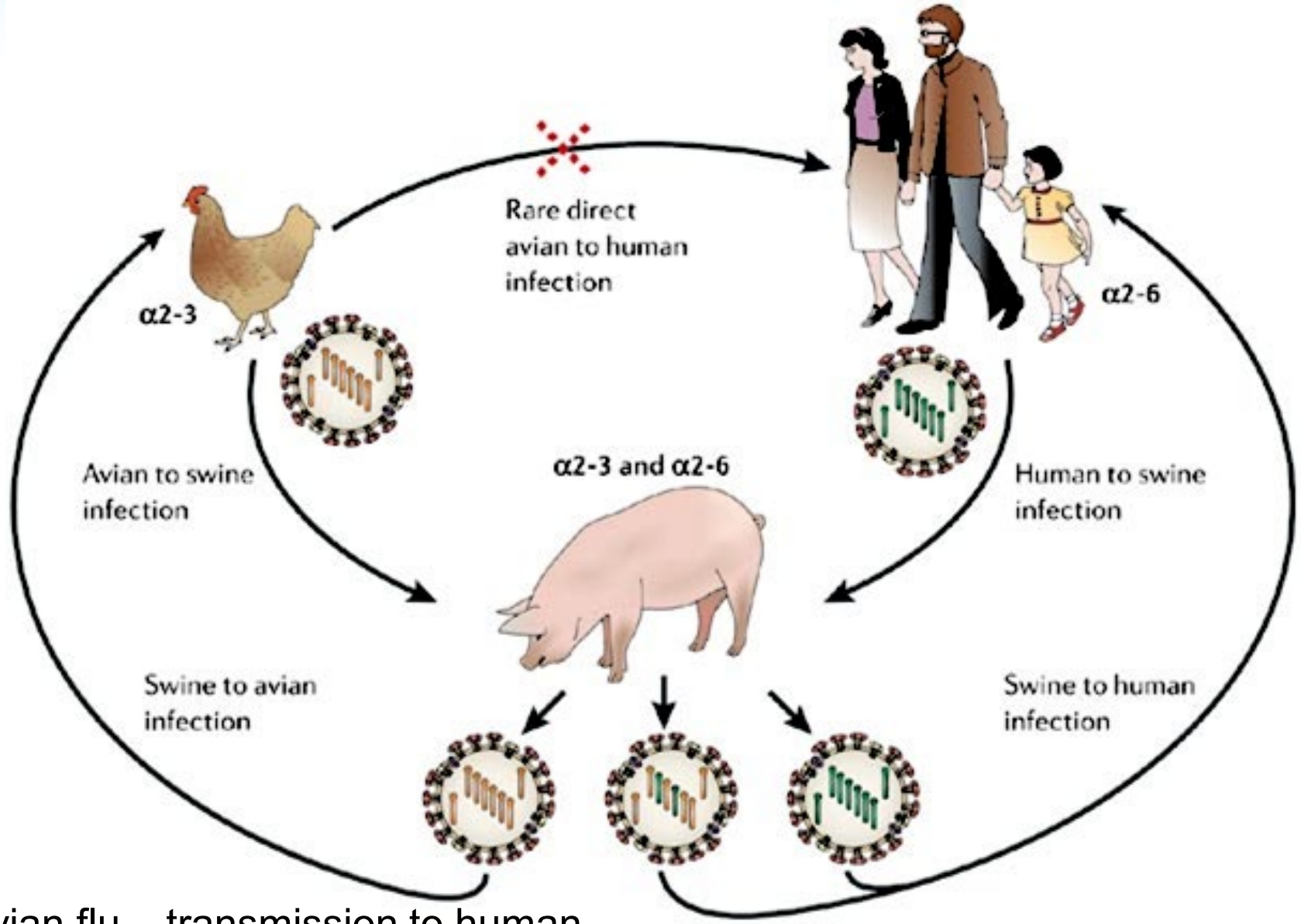


Link a2-3

Most common in birds

Hemagglutinin avian viruses

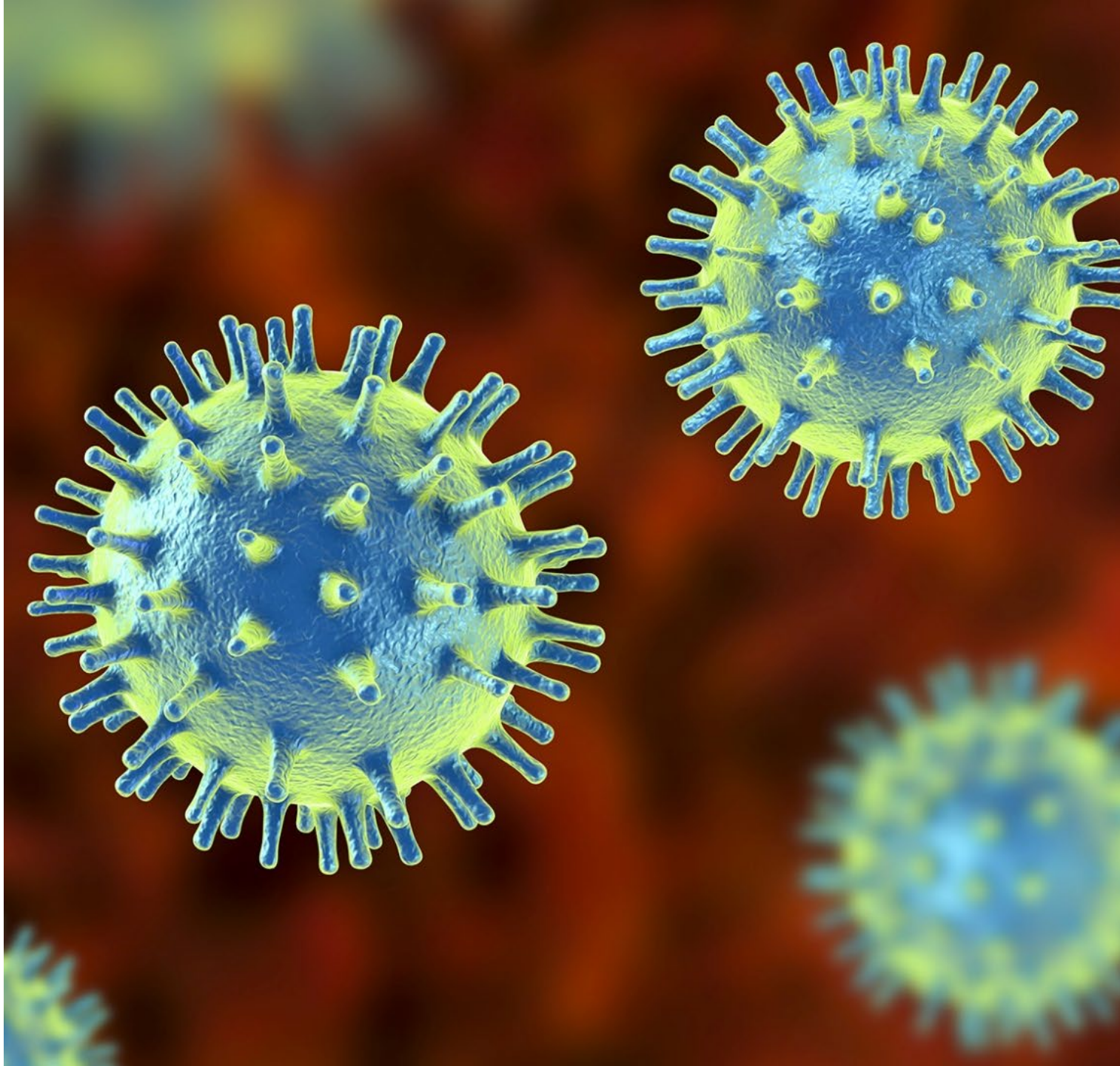
The influenza receptor and species barrier



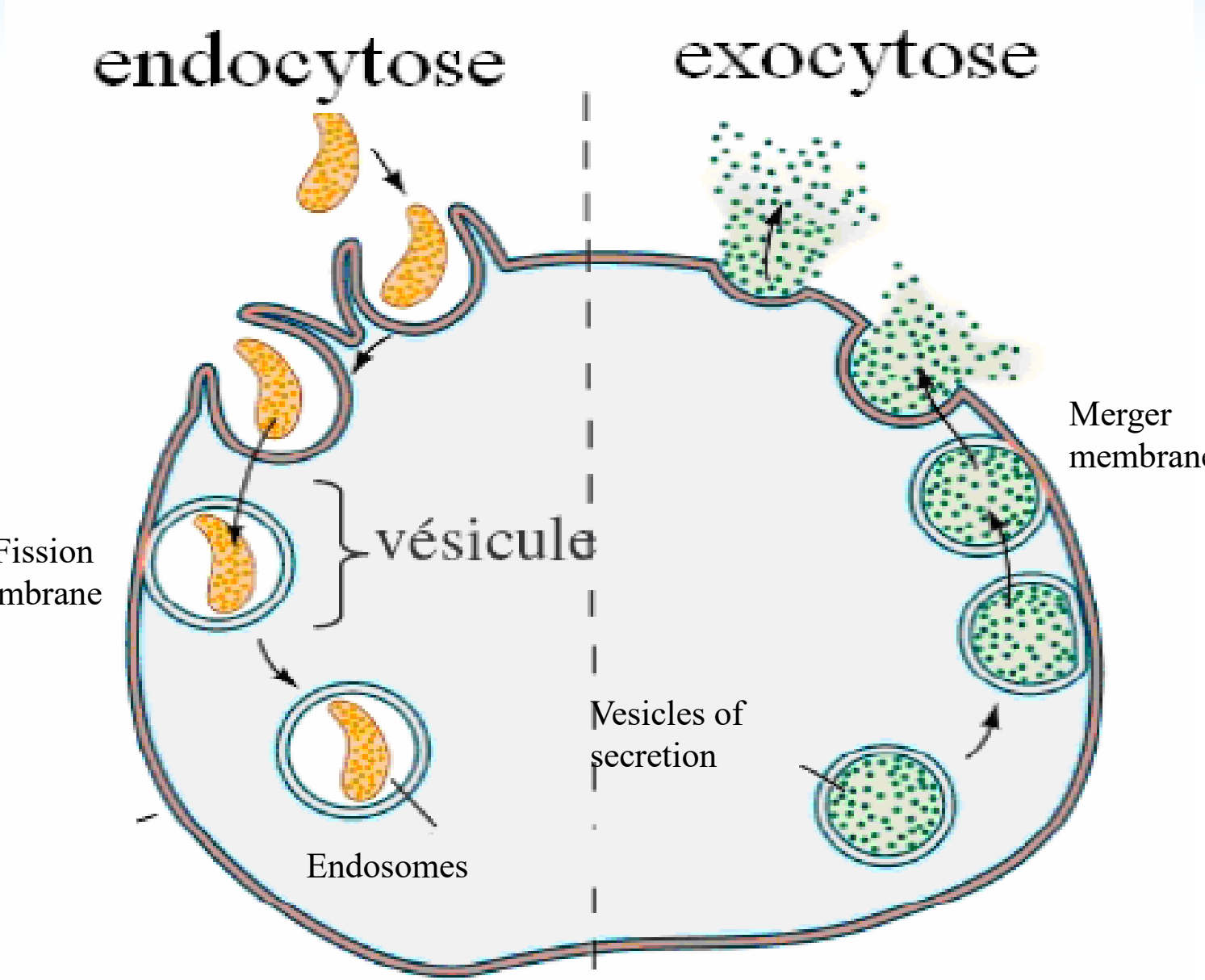
Avian flu – transmission to human

II- Virus entry into cells

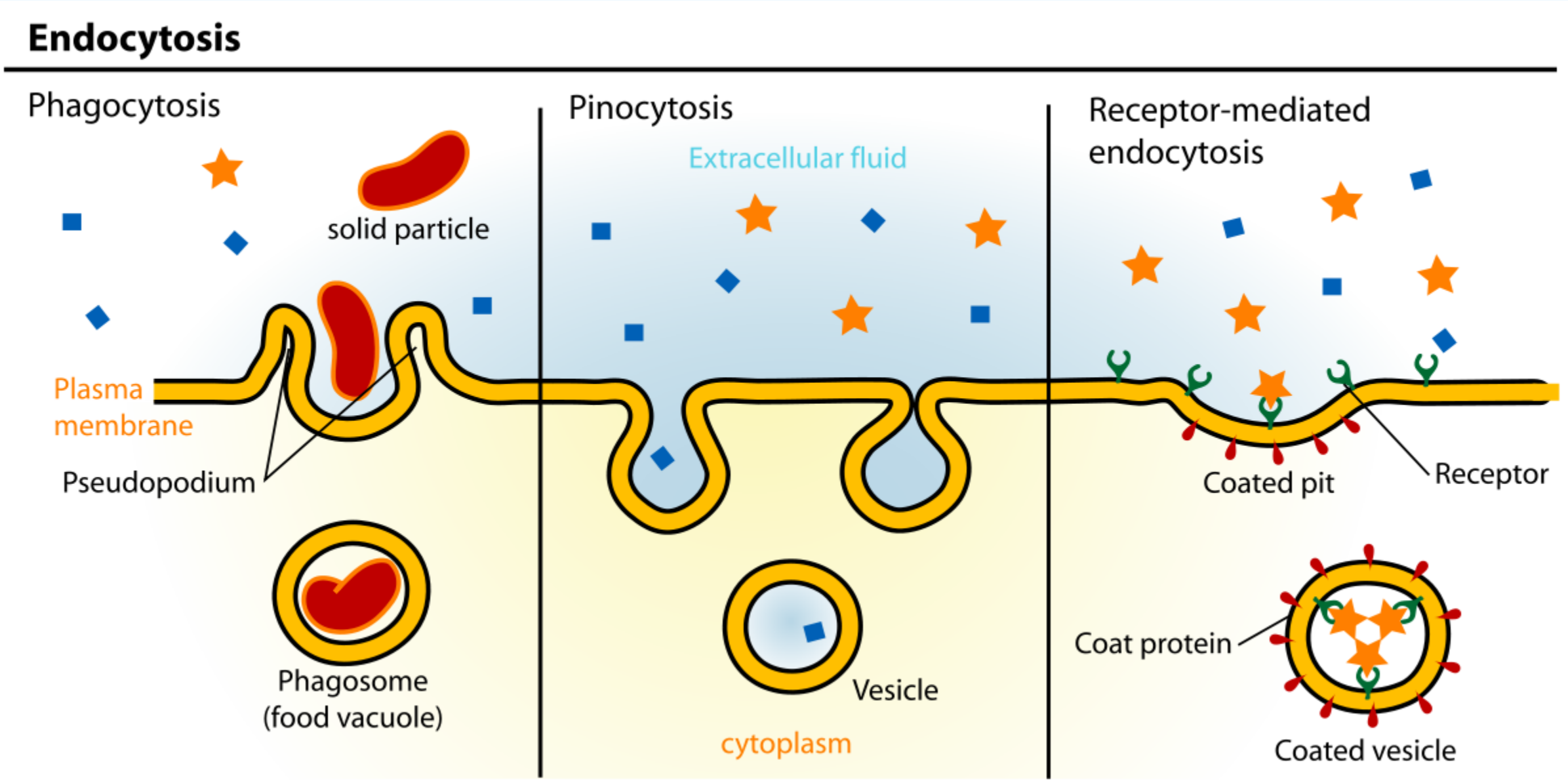
2. Entry in Eukaryotic cells



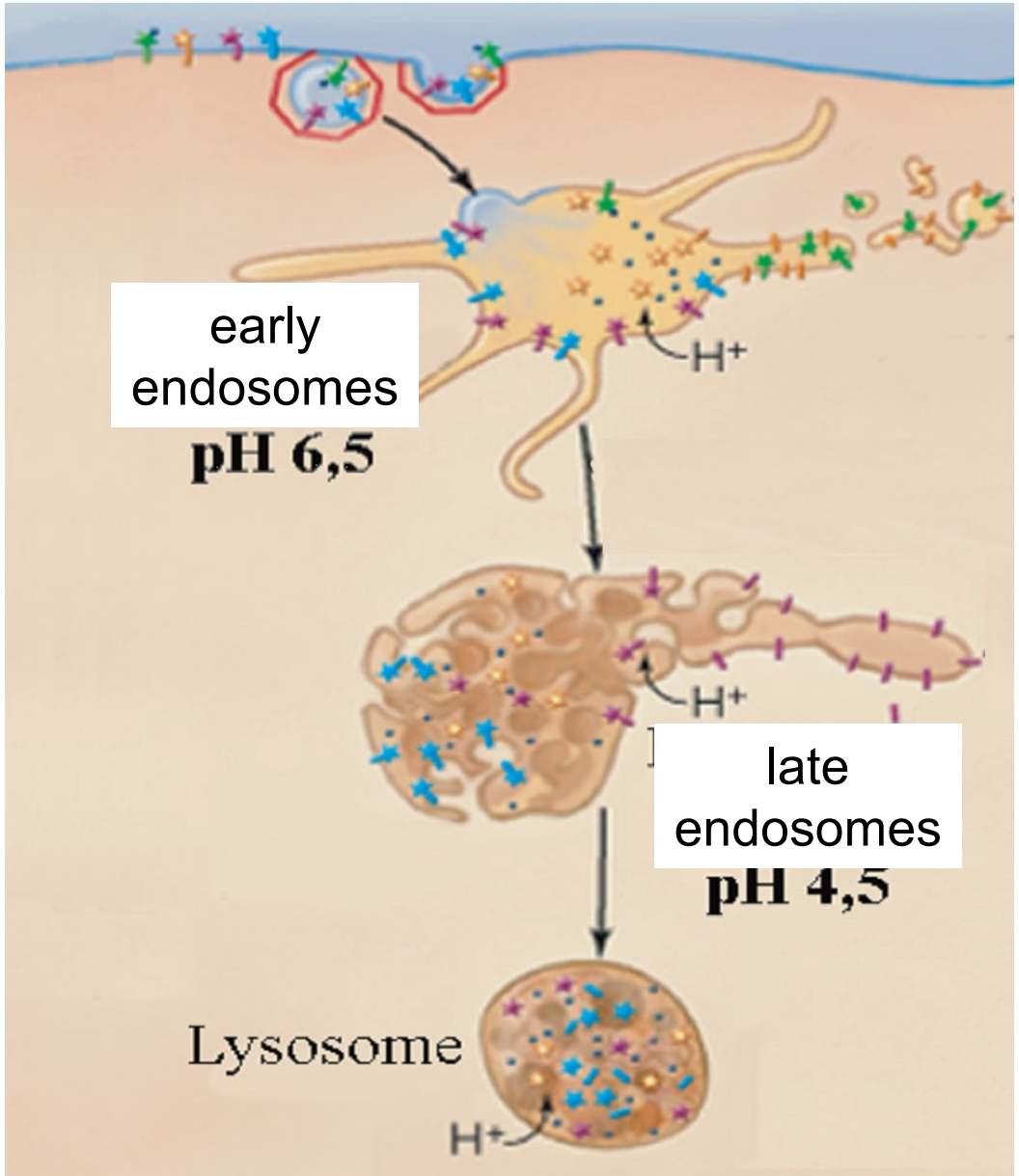
The endocytosis pathway enables internalization of molecules from the external environment in Eukaryotic cells



Internalization of molecules, fluids and particles: Endocytosis



Internalized molecules can be degraded in lysosomes ...



Decrease in Intravesicular pH

Endocytosis vesicles fuse to form late endosomes

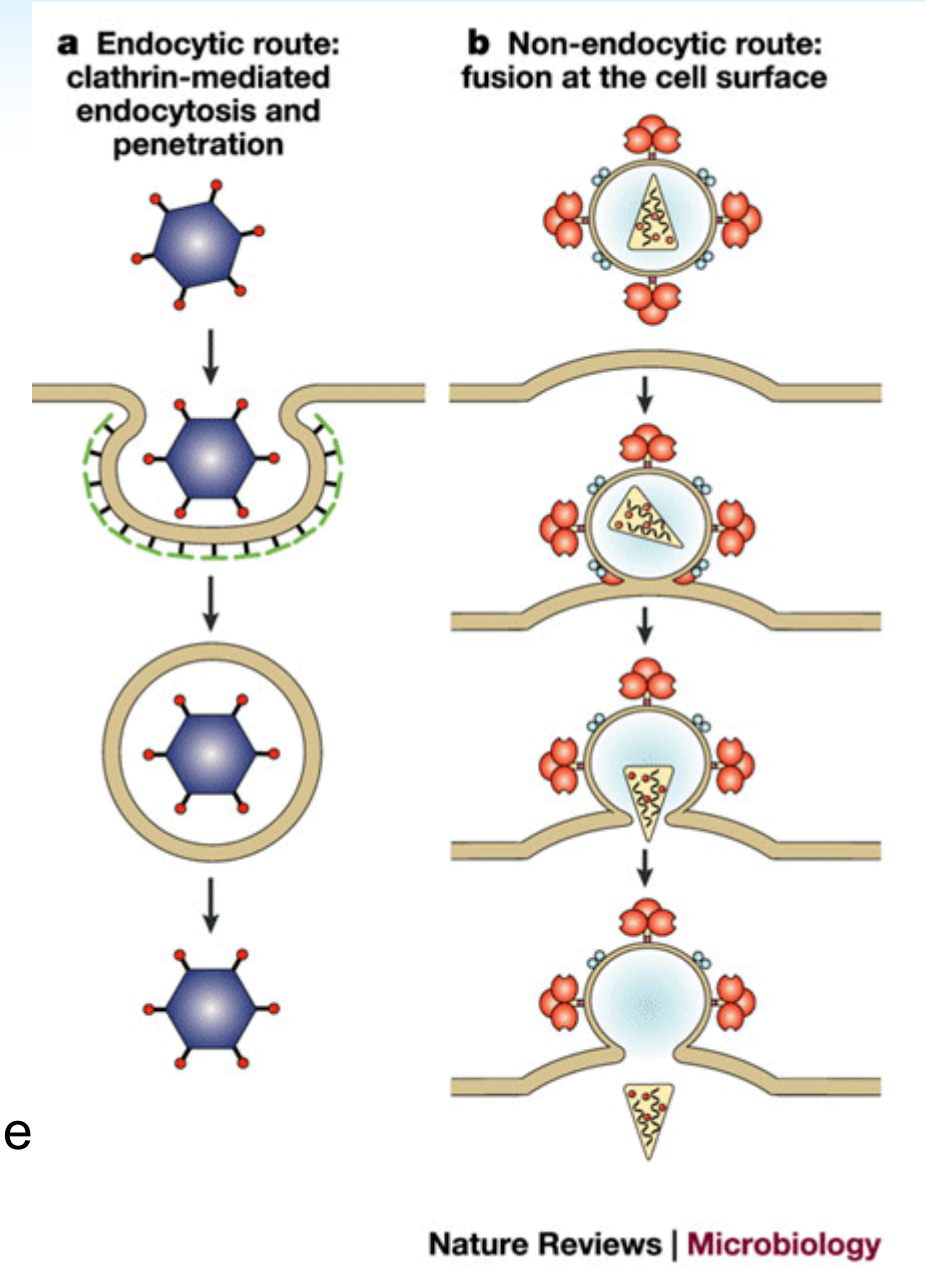
Lysosomes are responsible for (protein, lipid, sugar) degradation at acid pH.

Naked virus entry

Naked (uncoated) viruses bring their genome into the cell's cytoplasm

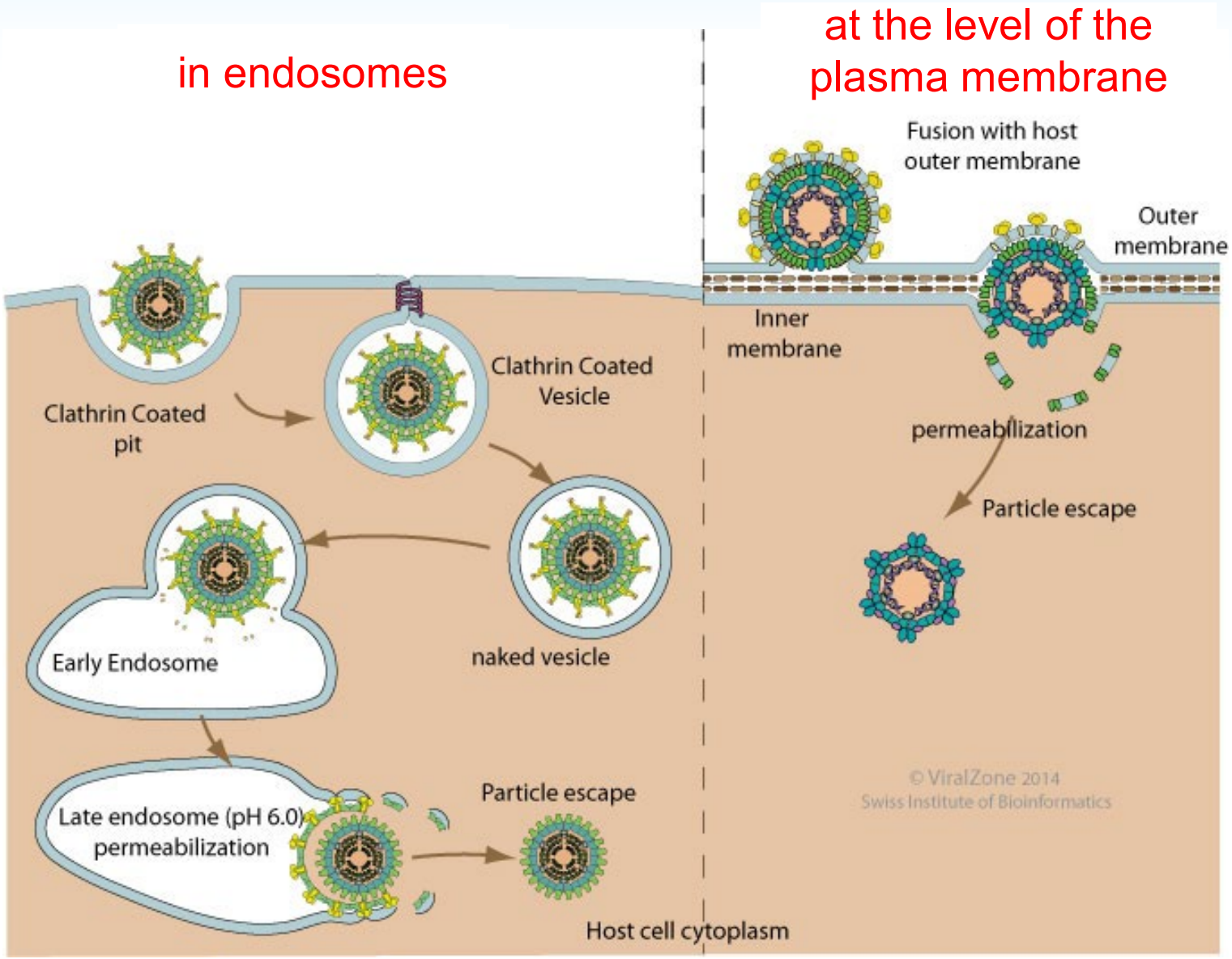
- by permeabilization of the endosomal membrane.

- by "injection" into the plasma membrane

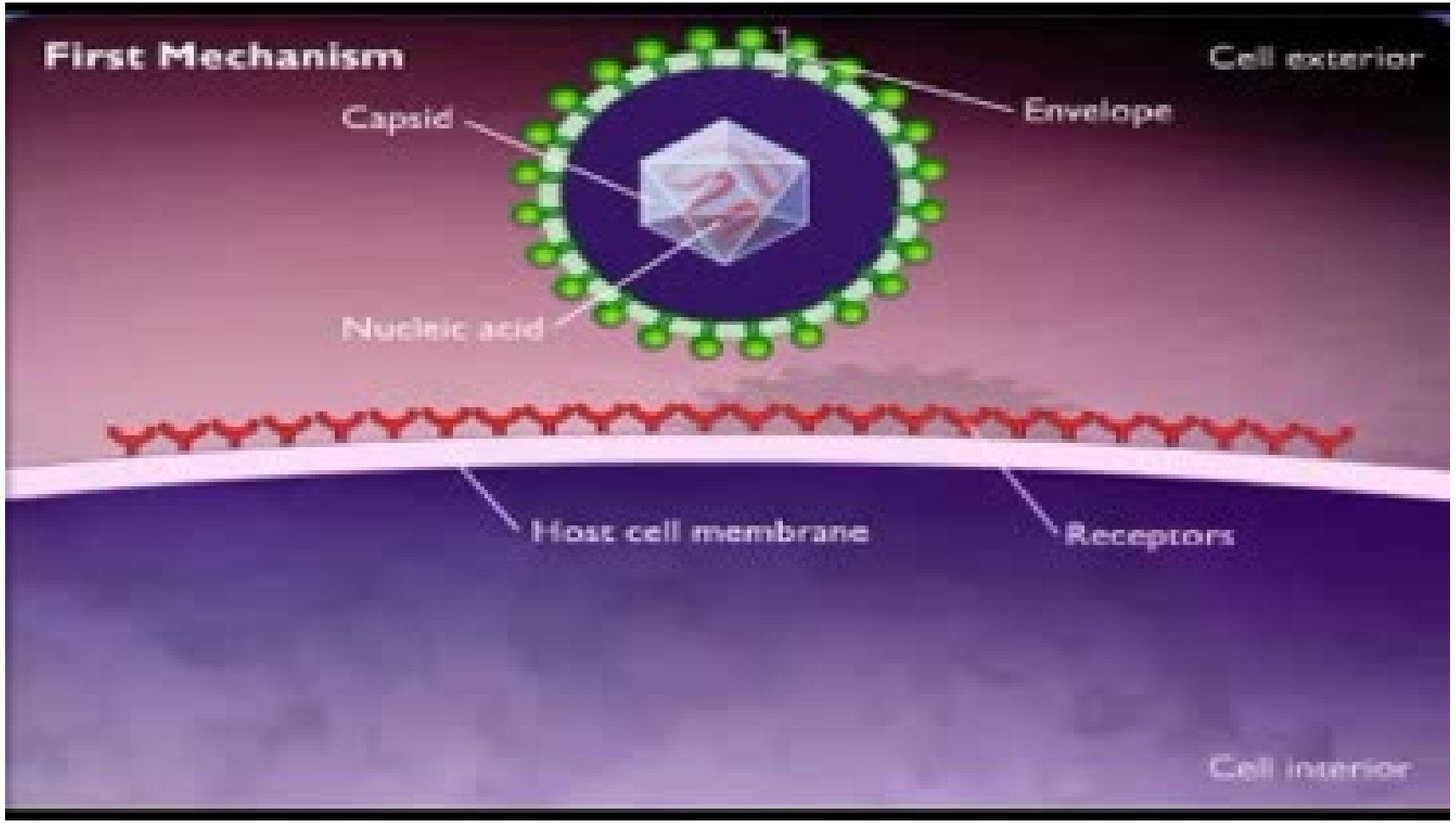


Two routes of entry for enveloped viruses

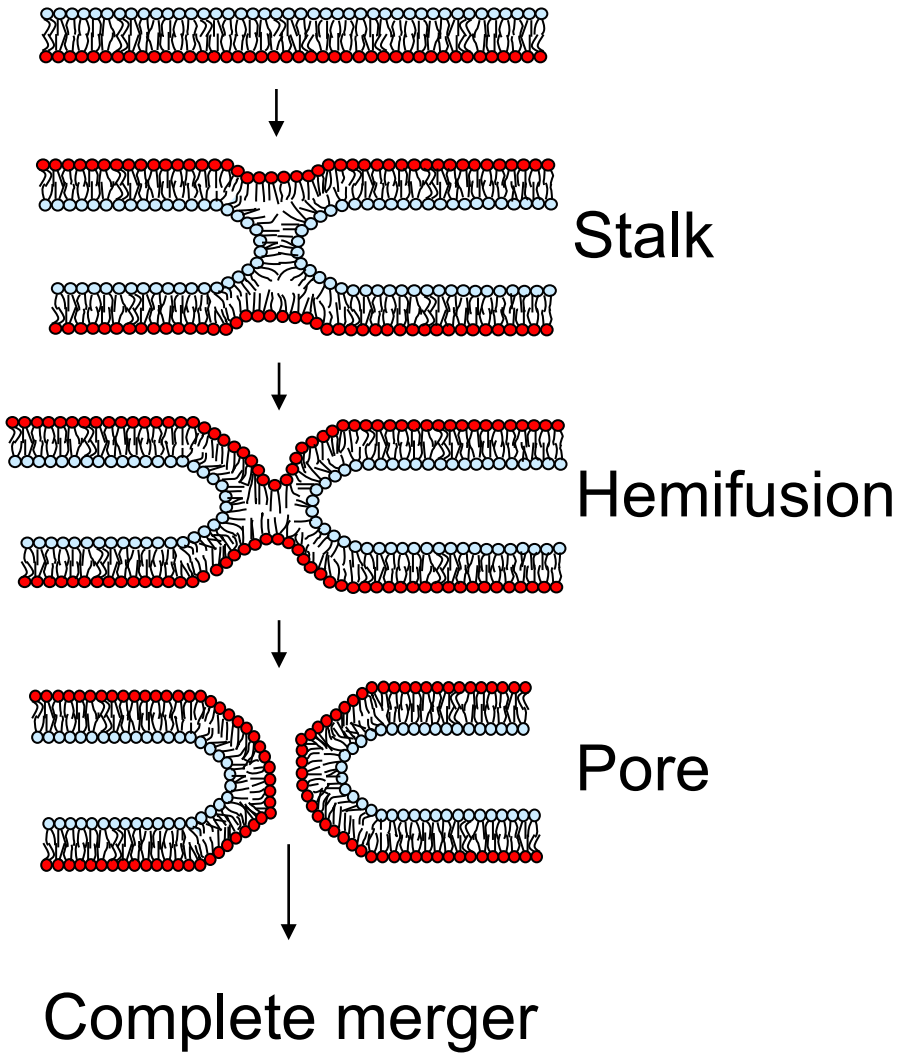
Enveloped viruses fuse with the cell membrane



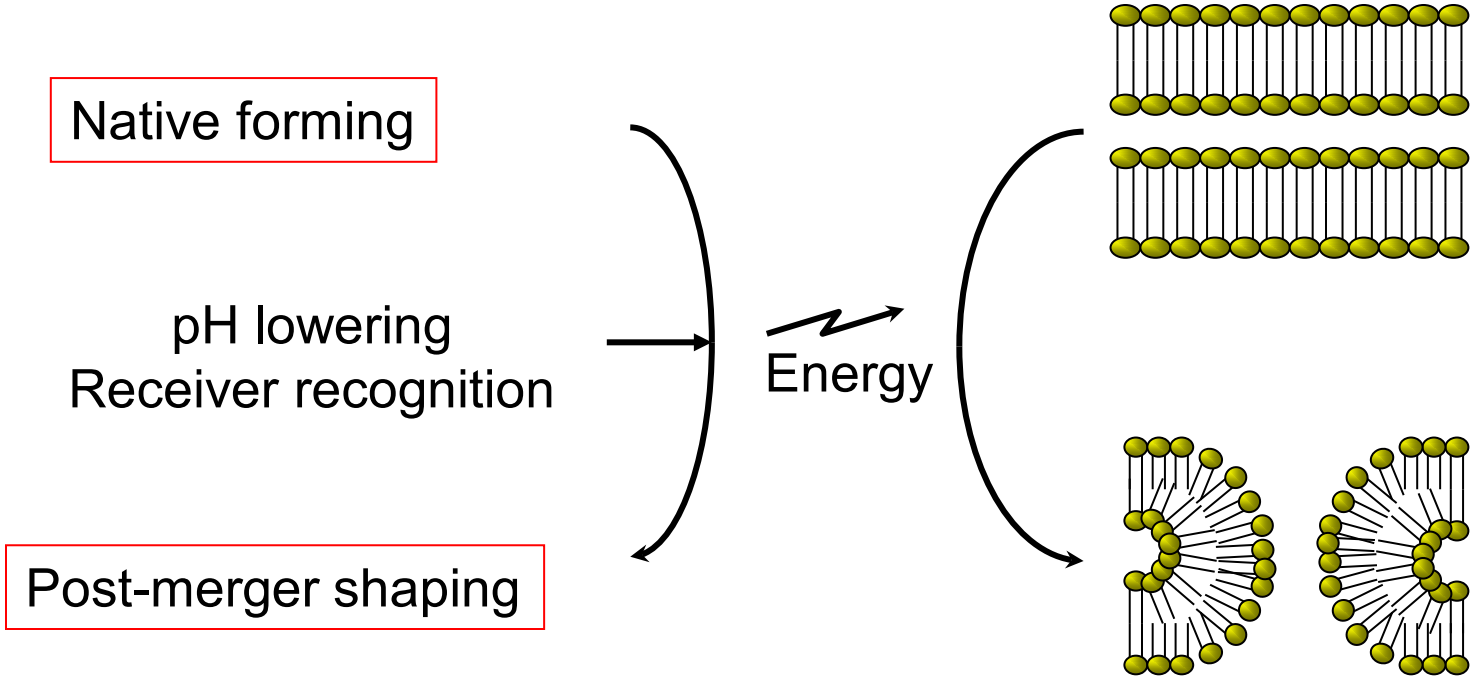
Two routes of entry for enveloped viruses



Fusogenic proteins provide energy for membrane fusion

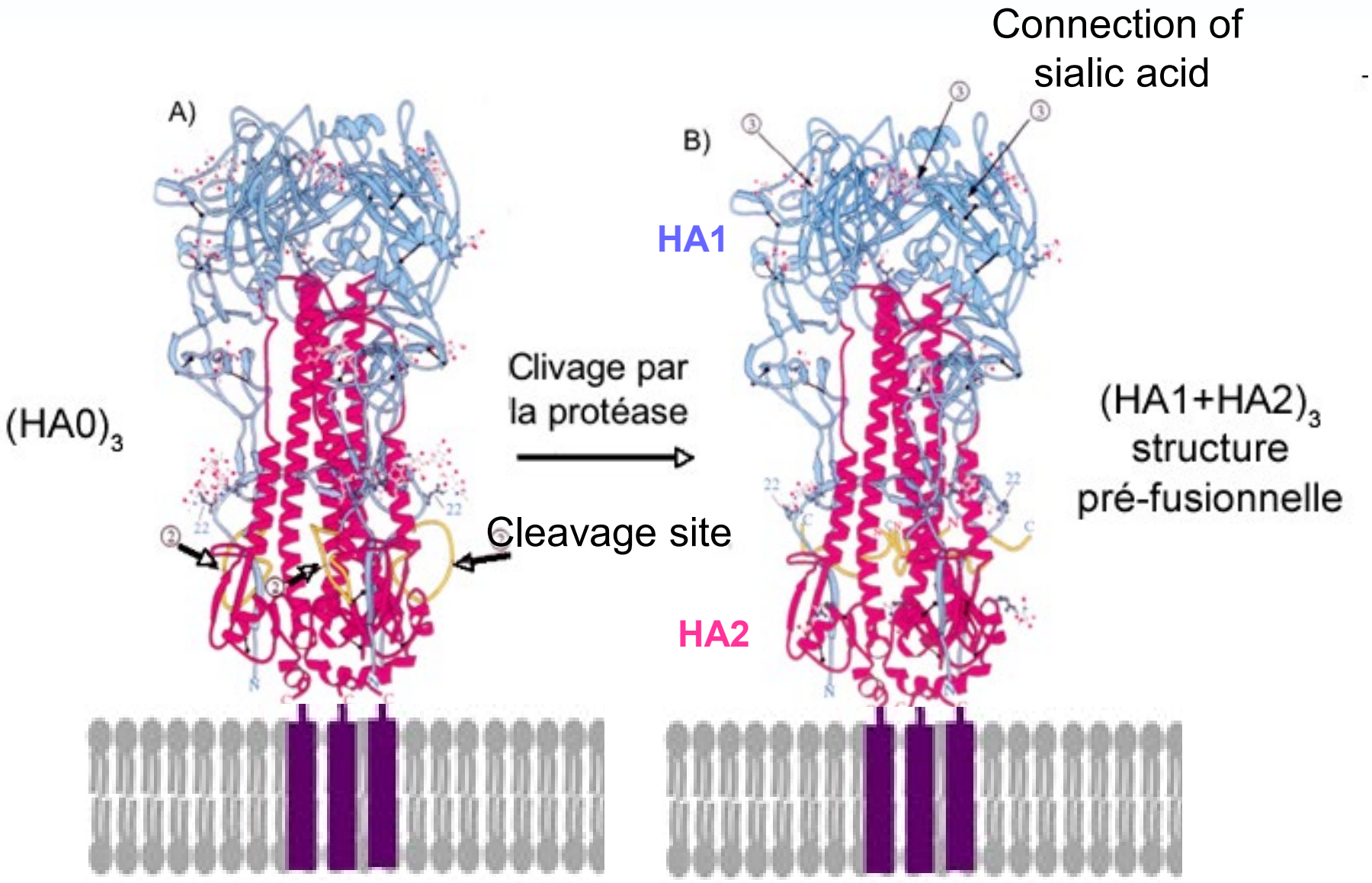


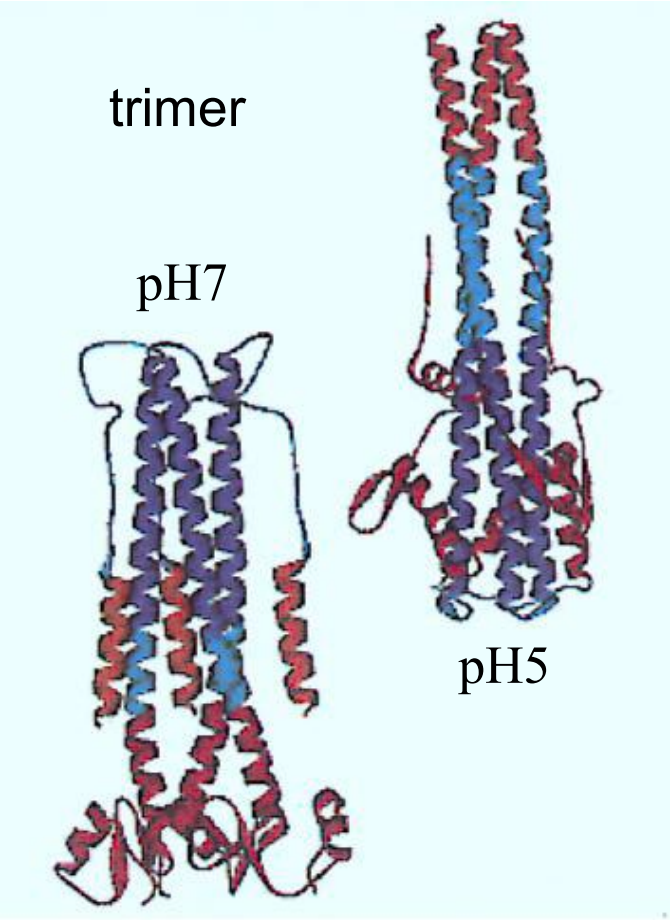
Fusion properties are carried by one or more viral transmembrane glycoproteins: fusogenic proteins.



Fusogenic proteins undergo major structural transitions triggered by interaction with a cellular protein (receptor) or acidification of the endosome.

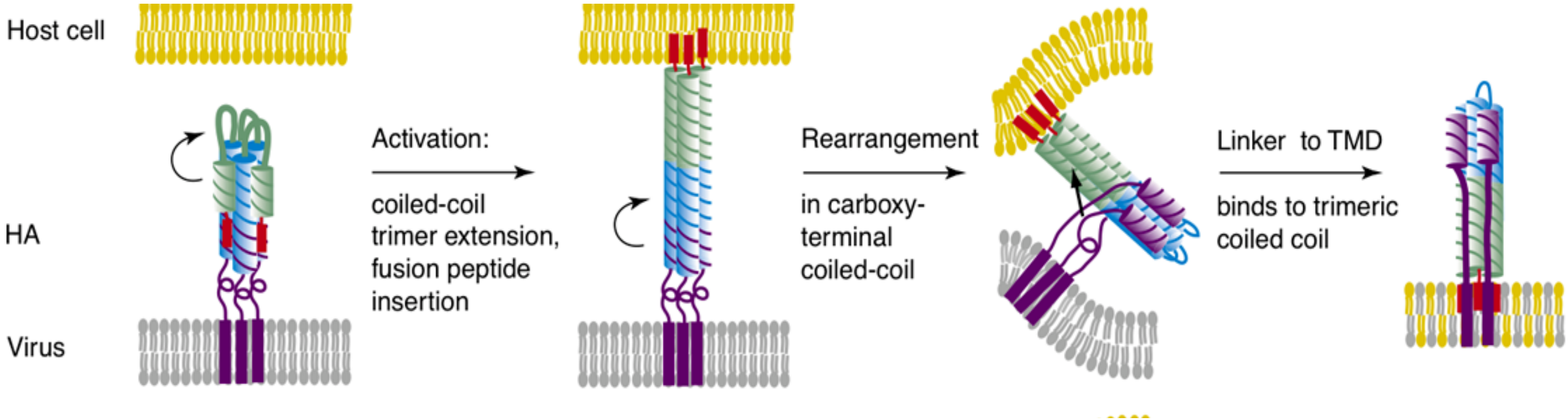
The influenza virus hemagglutinin: a fusion protein



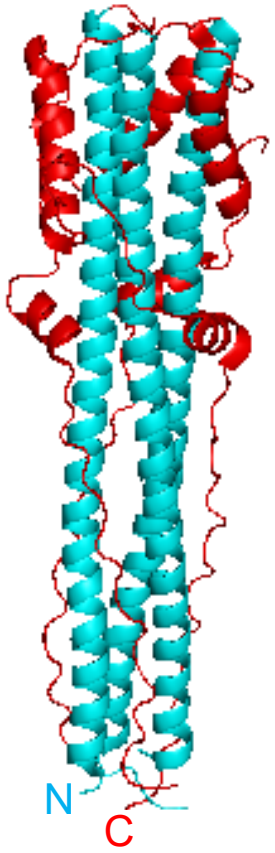


Lowering the pH causes a first change in the conformation of HA2

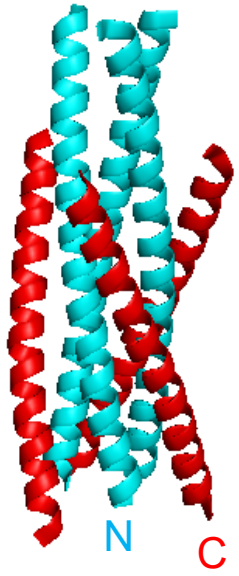
The 2ème conformational change in fusogenic viral glycoproteins promotes membrane fusion



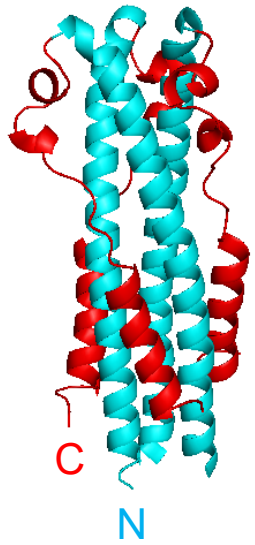
Structure of class 1 fusion proteins



HA2
flu



gp41
HIV

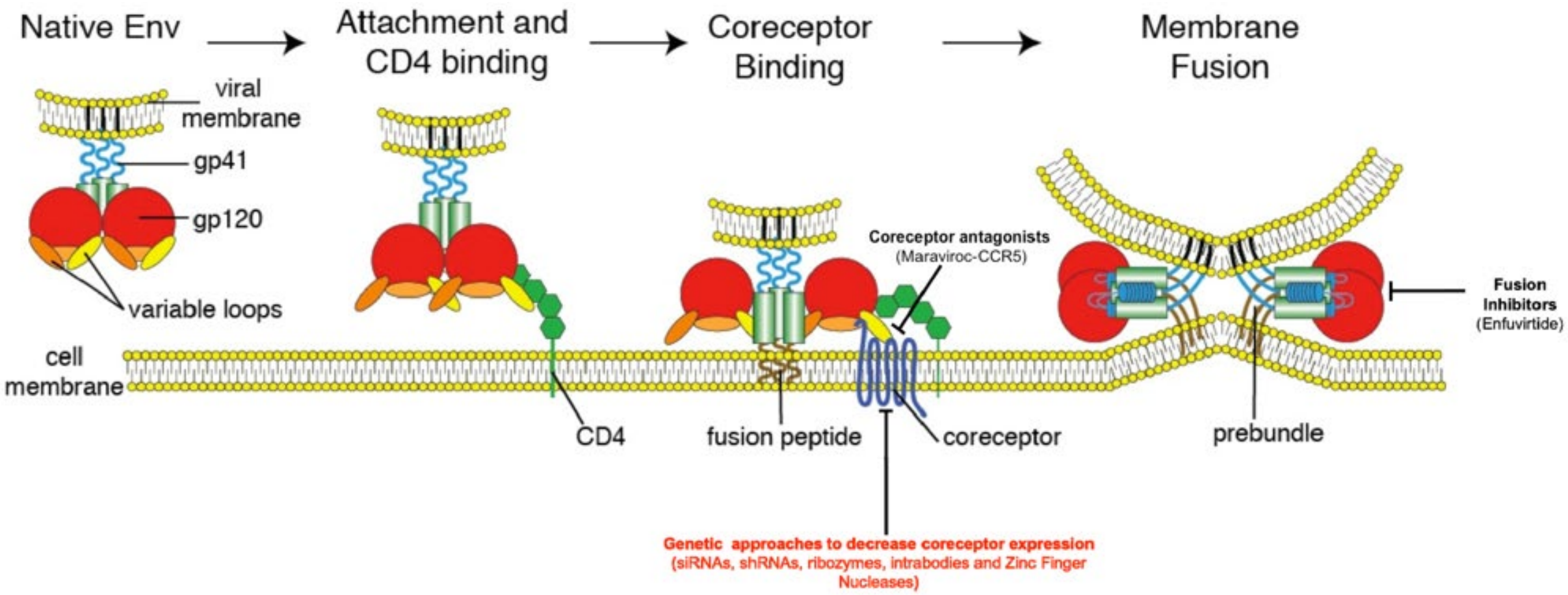


gp2
Ebola

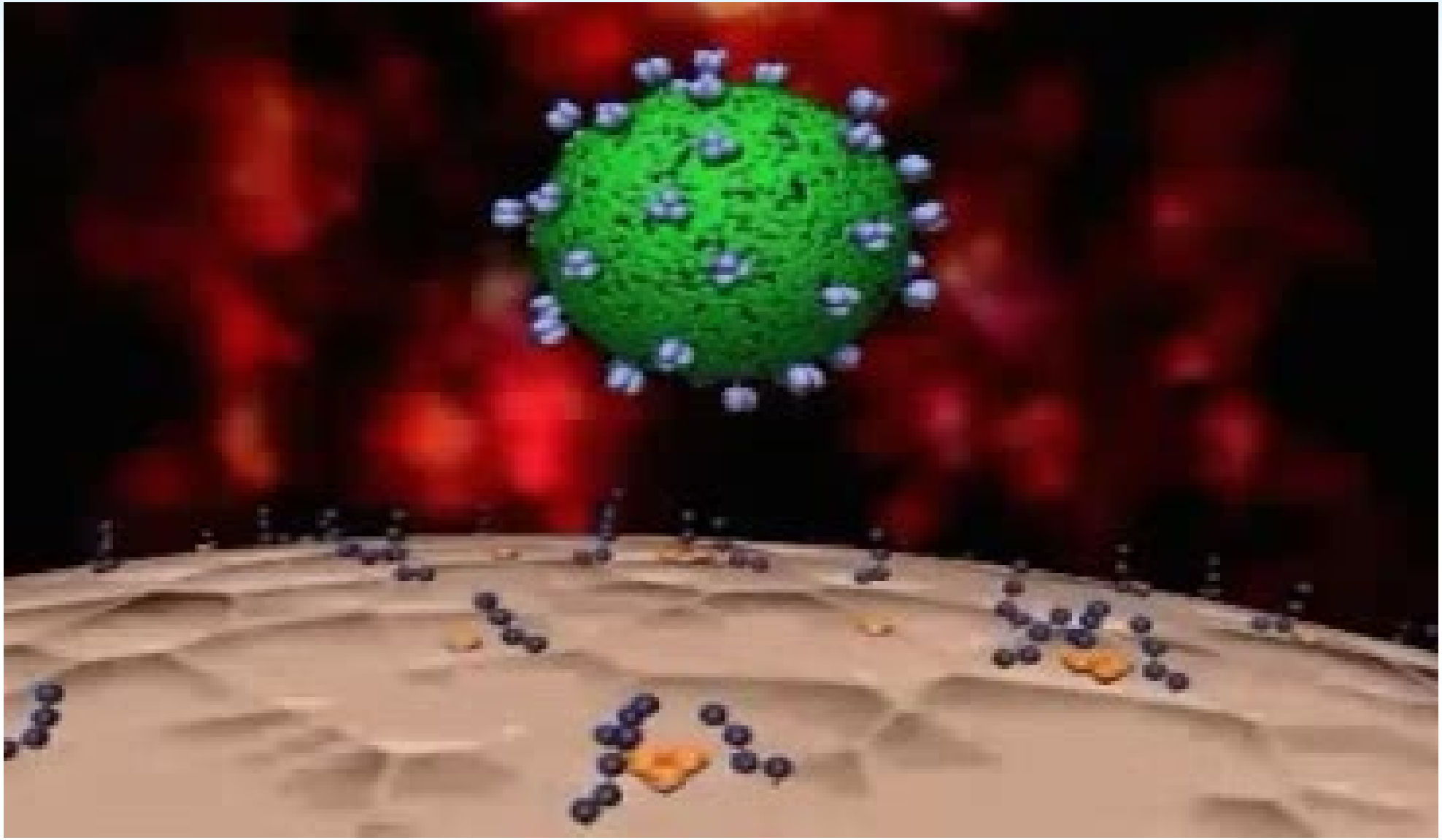
F
HPIV3

S
SARS

HIV enters the plasma membrane

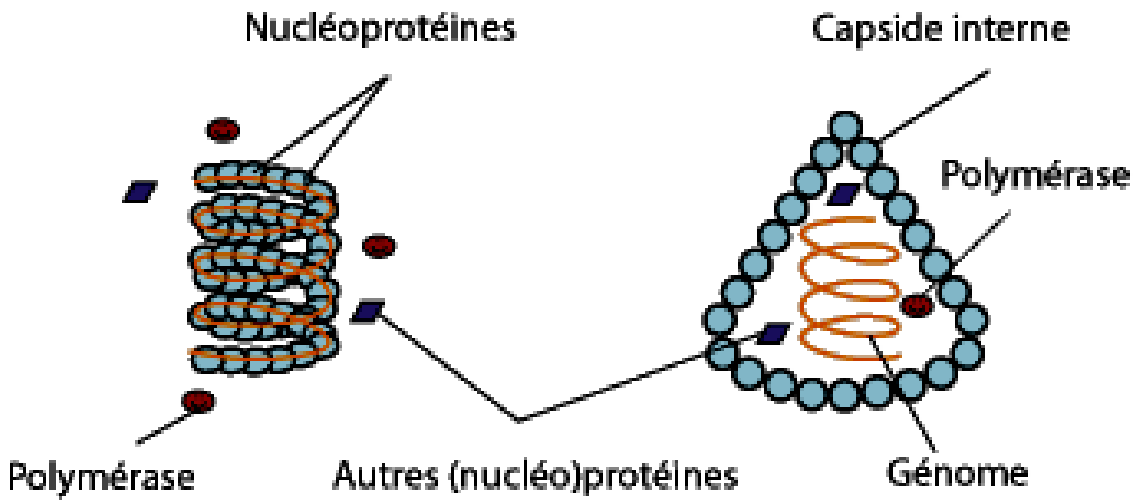


HIV enters the plasma membrane



The entry stage is followed by decapsidation

The viral genome is partially or totally stripped of the proteins protecting it in the virion: this process is called "decapsidation".



Nucléocapside "souple" d'un virus à ARN". Le génome est recouvert de "nucléoprotéines".

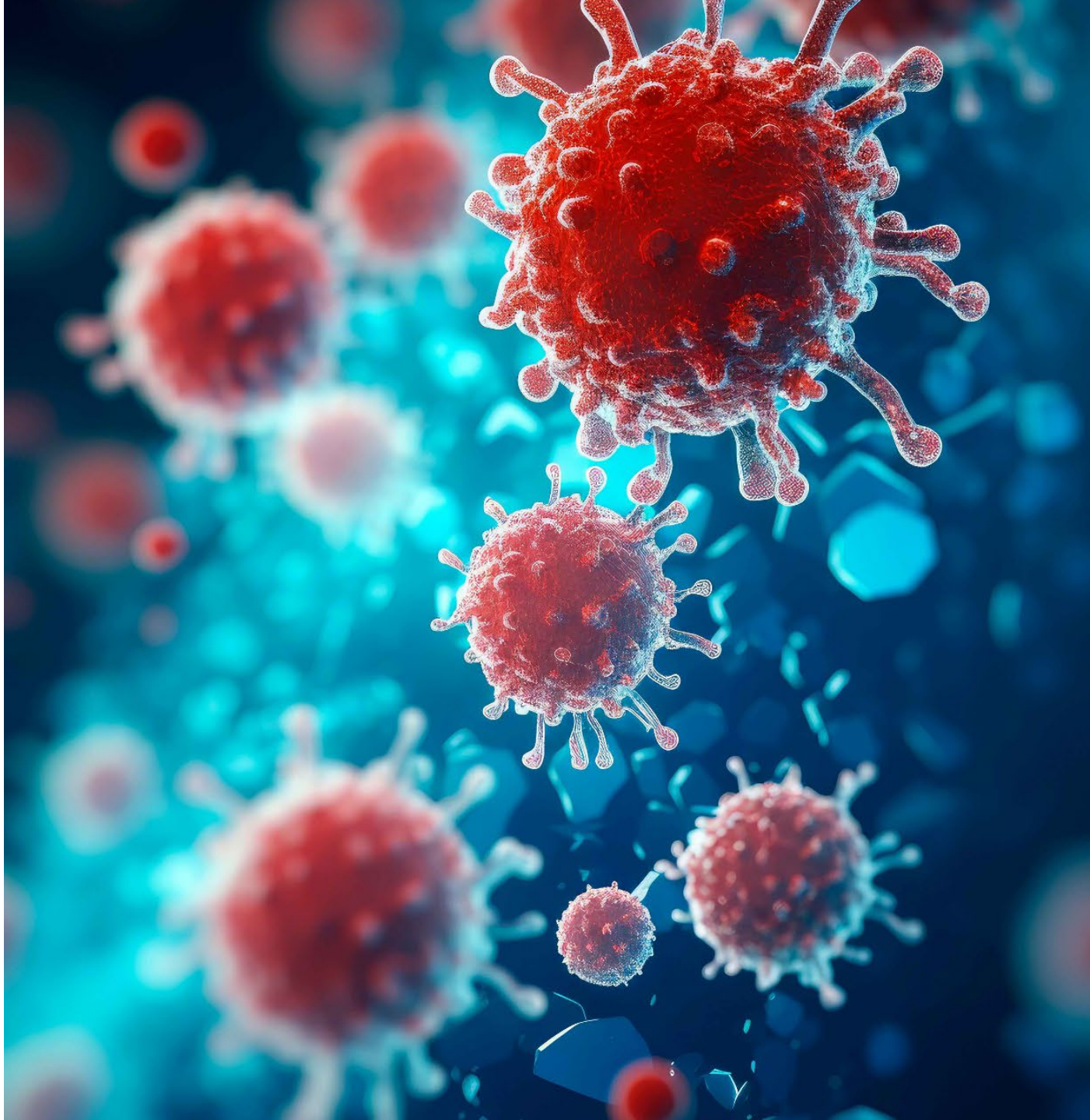
Nucléocapside d'un rétrovirus. Le génome, associé à quelques protéines est contenu dans une capside "semi-rigide".

Conclusions II

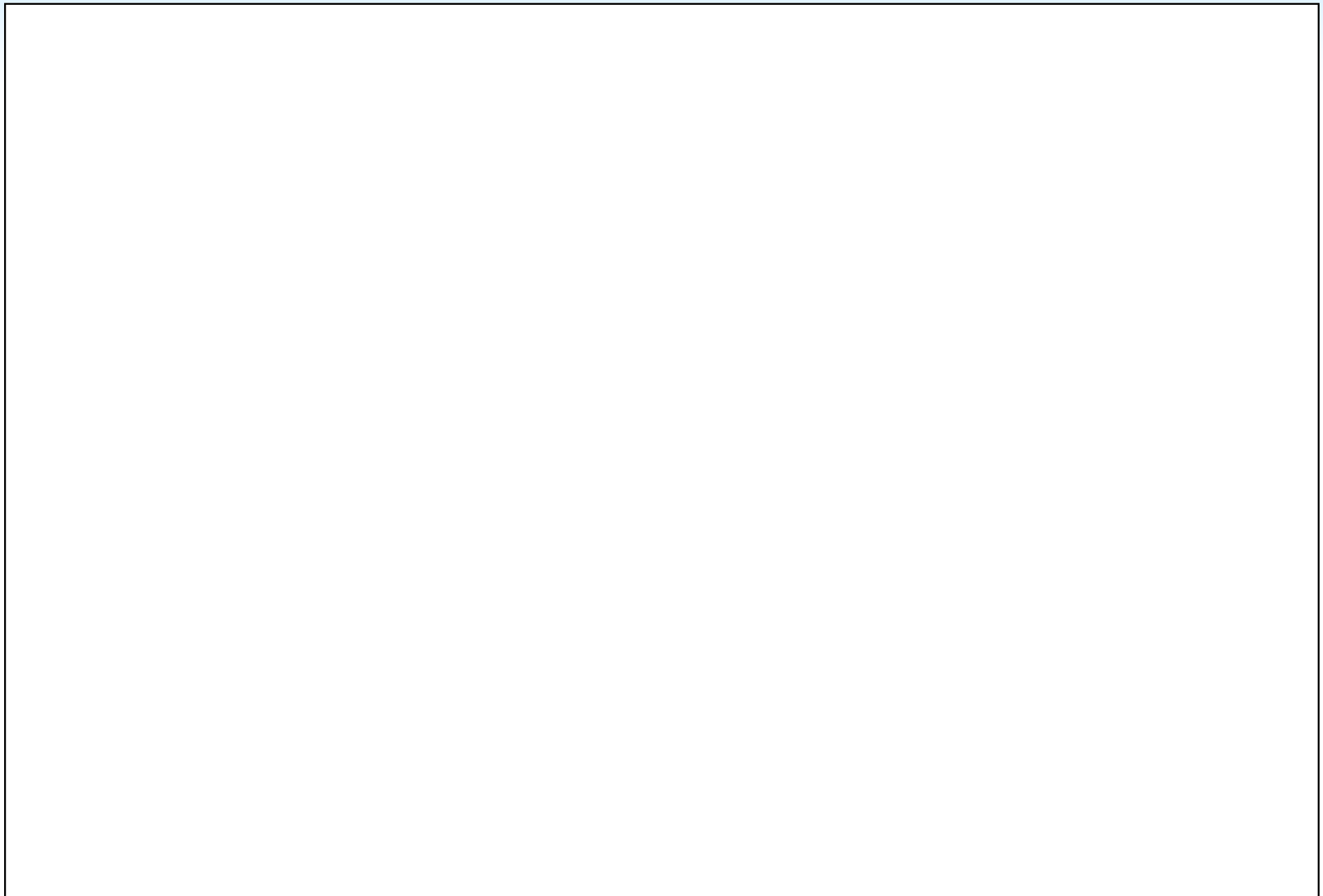
II- Entering the cell

- Virus entry into cells requires a recognition stage (adsorption), followed by entry of the genetic material into the cytoplasm.
- Viruses are adsorbed to the surface of host cells through the interaction of one of their surface proteins with a specific membrane receptor.
- Naked viruses inject their genome through the cell membrane or destabilize the cell membrane to penetrate the cytoplasm.
- Entry of enveloped viruses involves fusion of the viral envelope with the plasma or endosomal membrane.
- Fusogenic proteins, such as hemagglutinin, undergo major conformational changes that promote membrane fusion.
- Virus entry into the cell is followed by decapsidation, releasing the genetic material.

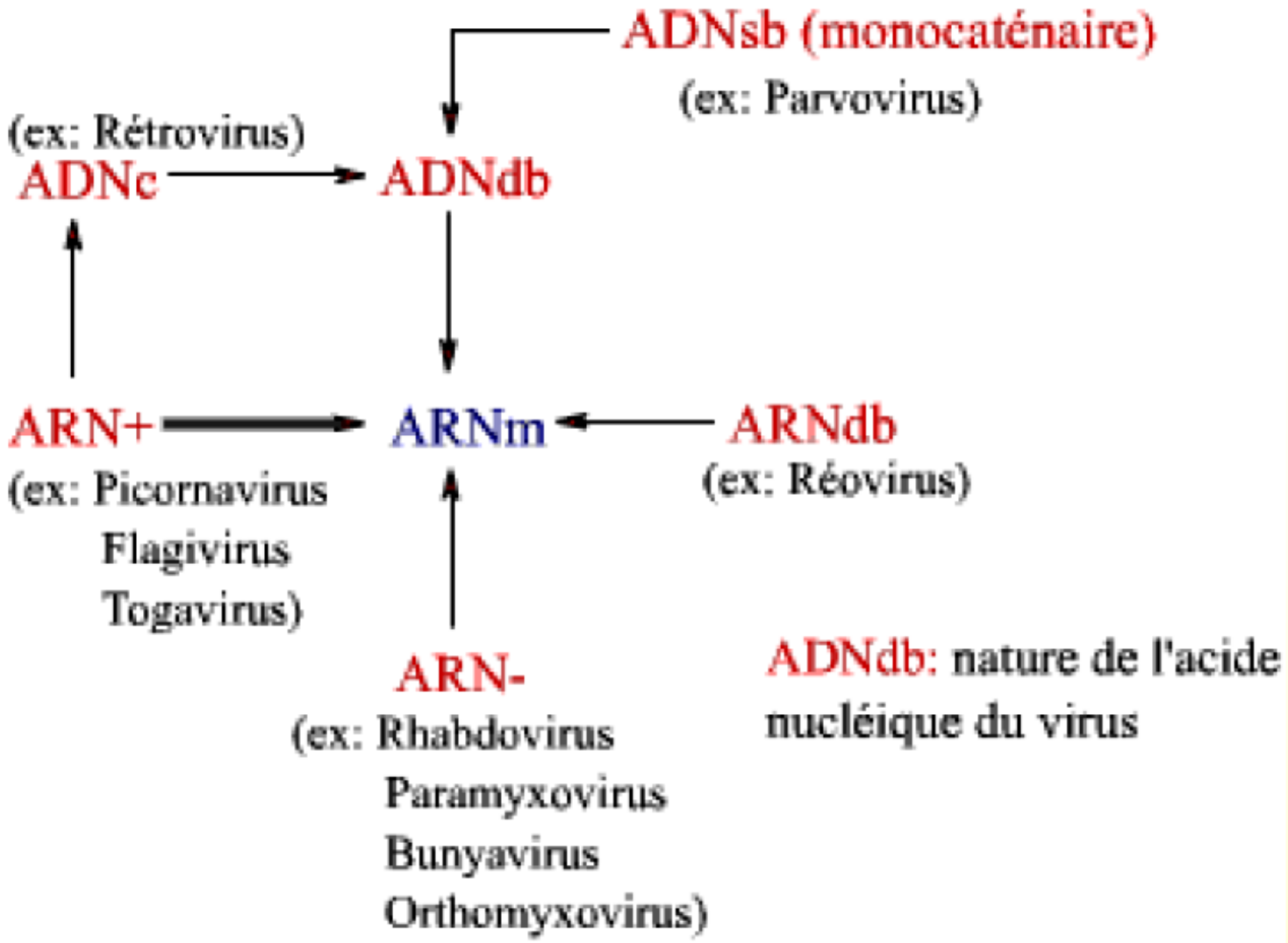
III- The viral cycle



Viral cycle



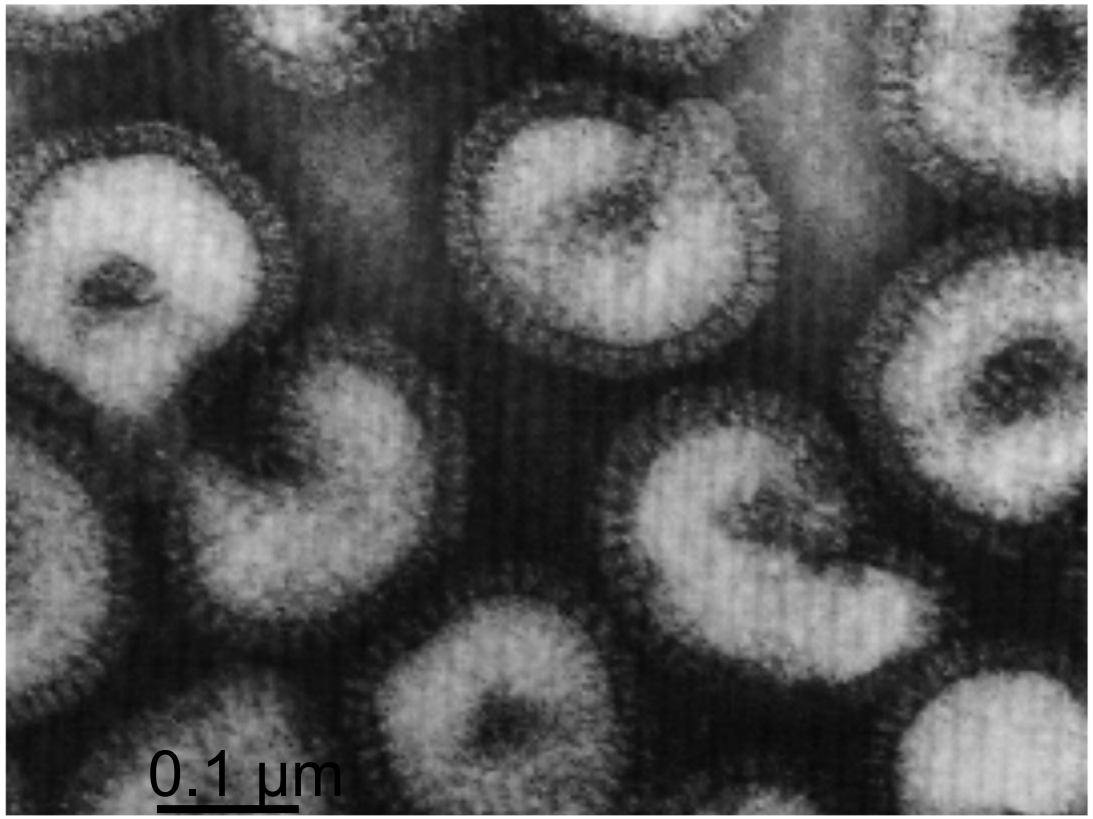
The nature of its genome dictates the virus' replication and transcription strategies.

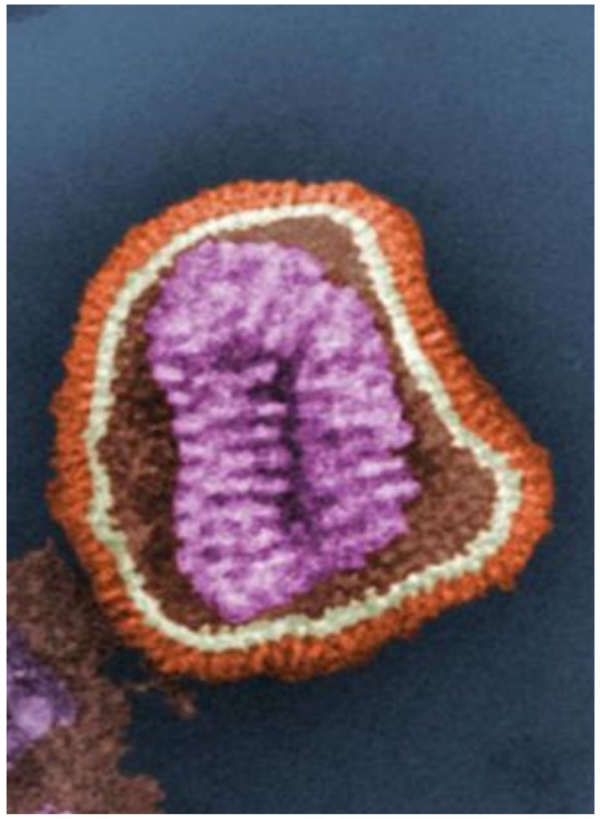


Influenza, the flu virus

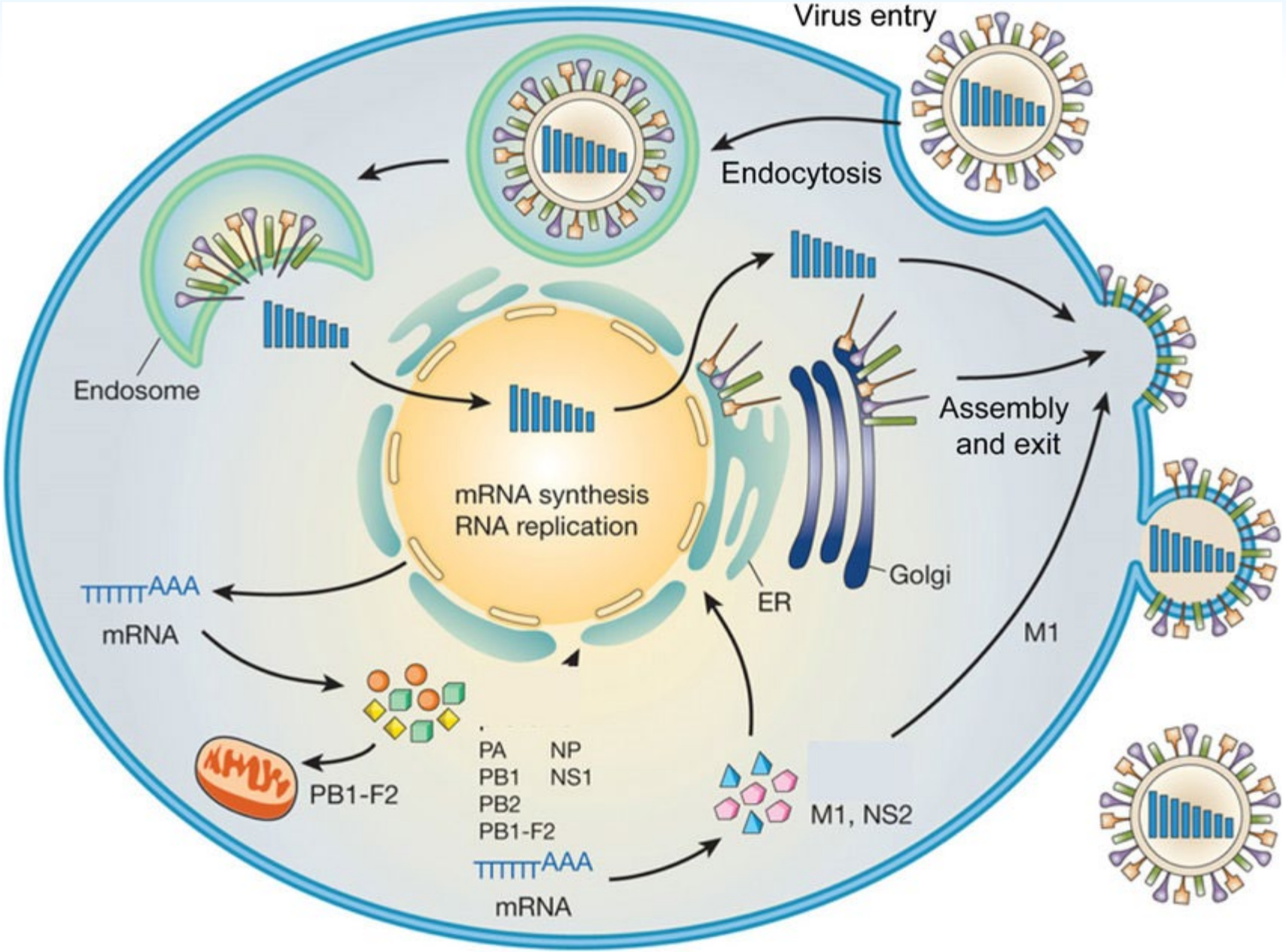
Negative polarity segmented RNA viruses

Family Orthomyxoviridae
Genus Influenzavirus
3 types A, B, and C

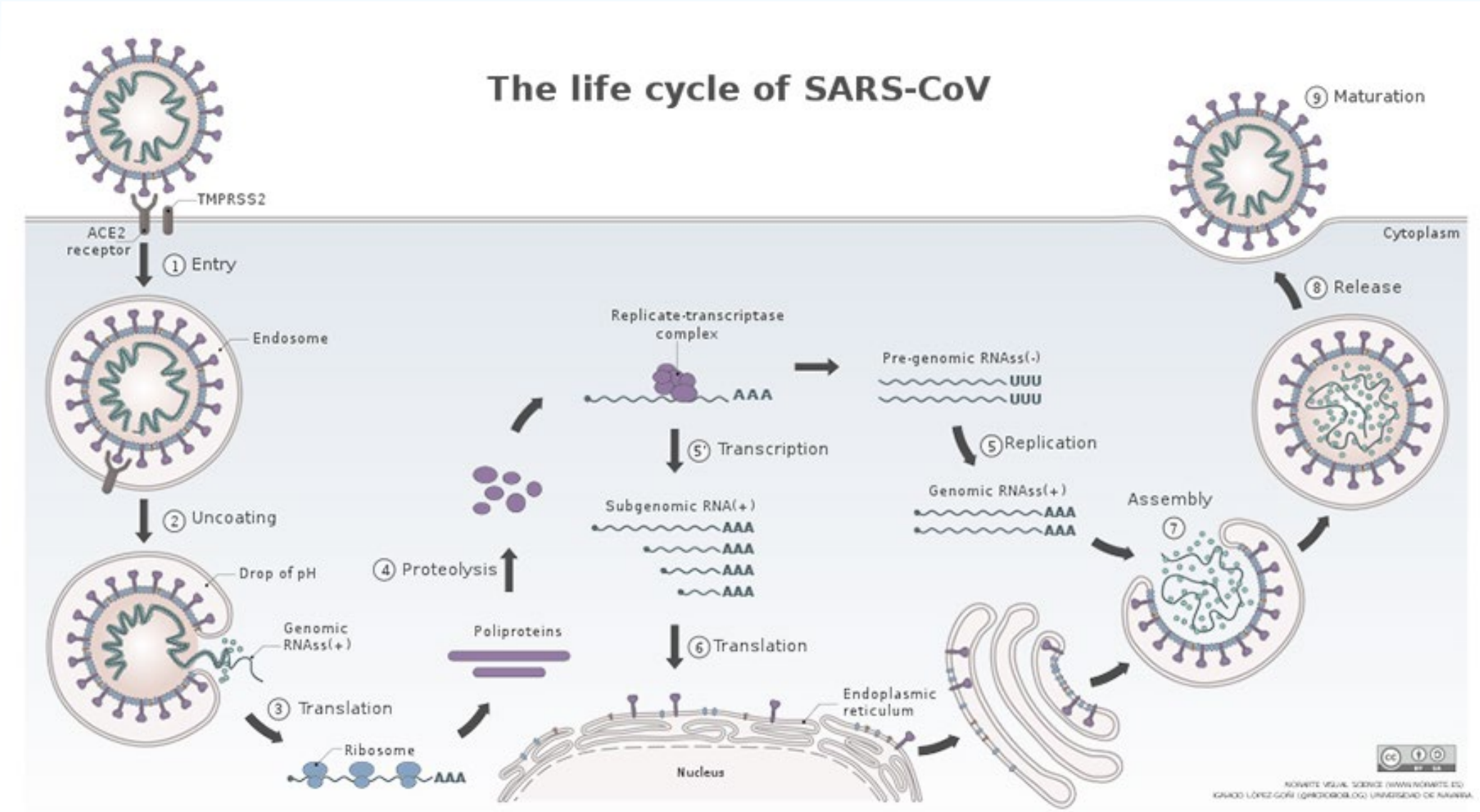




The Influenza viral cycle

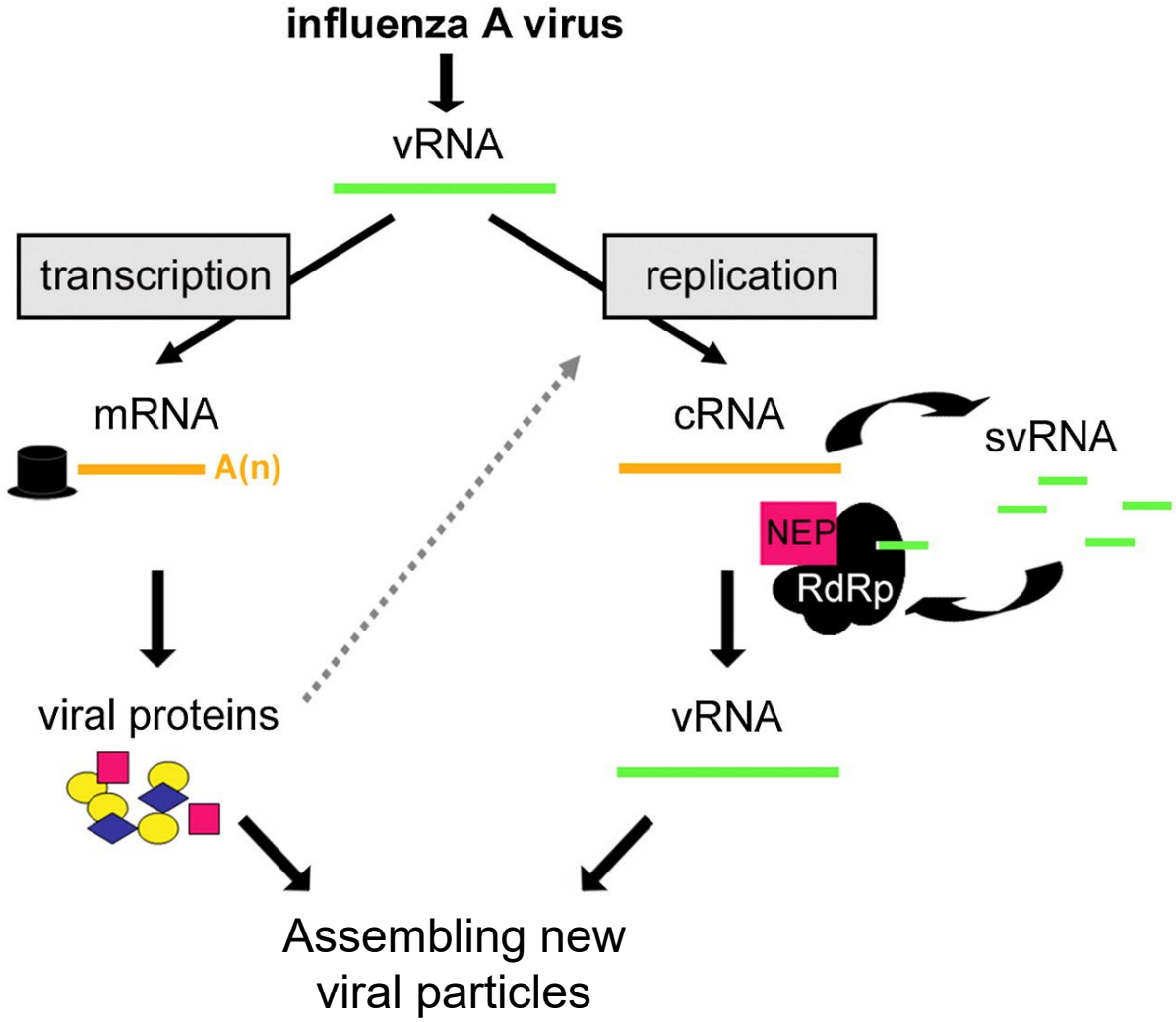


The SARS-CoV viral cycle

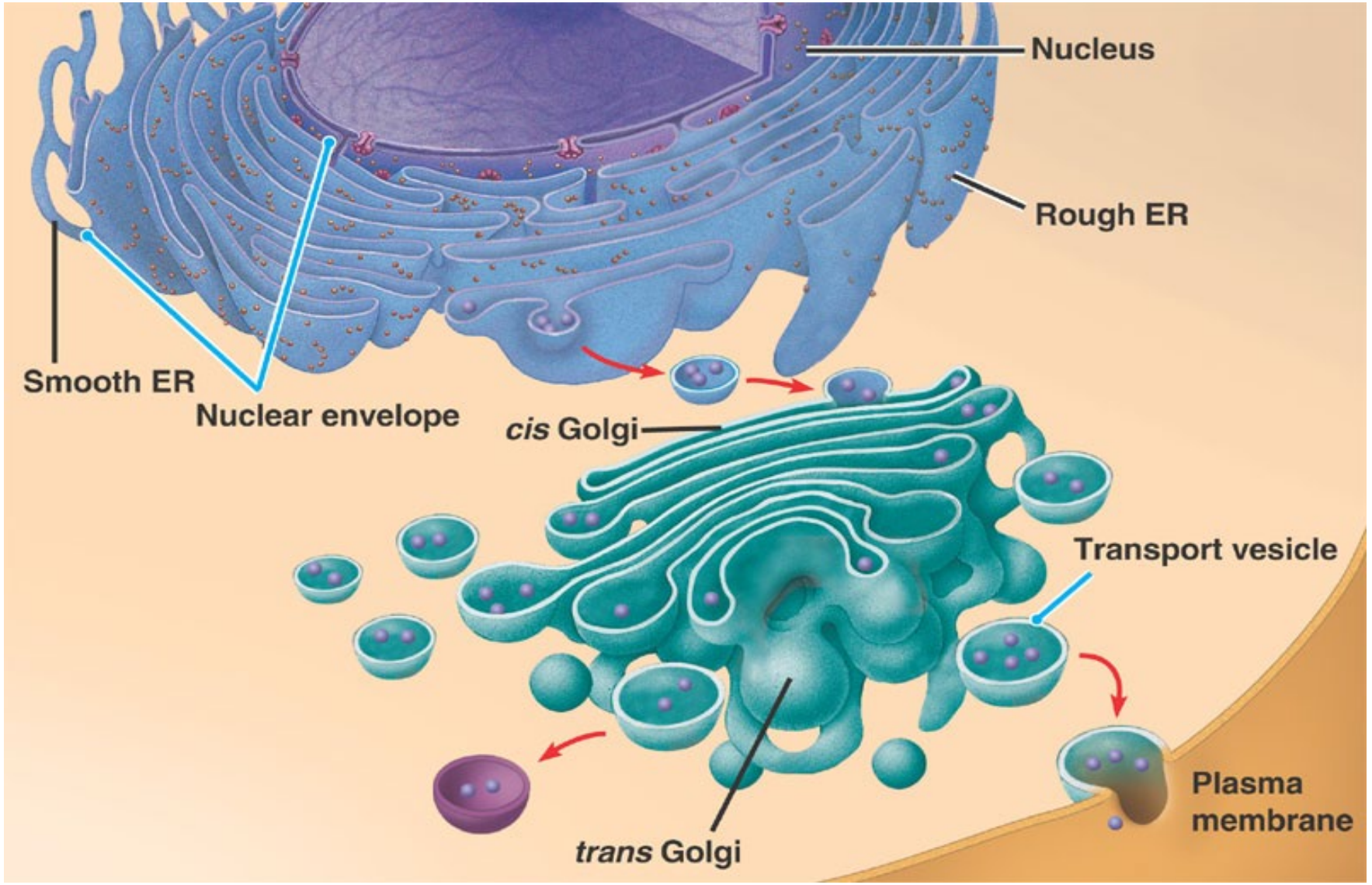


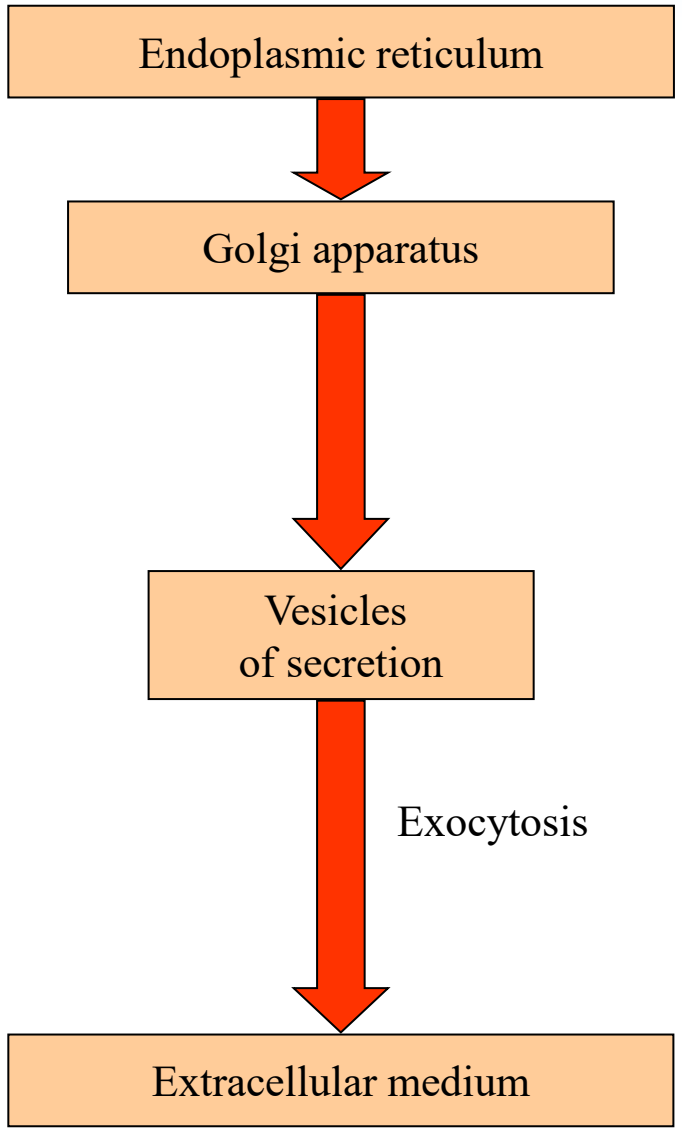
Replication and translation of Influenza

Genomic RNA (-) is replicated in the cell nucleus to form RNA (+), which is used to :
- or mRNA,
- as a template for the synthesis of new genomic RNAs



Enveloped virus use the secretory pathway to include its envelop proteins in the cell membrane



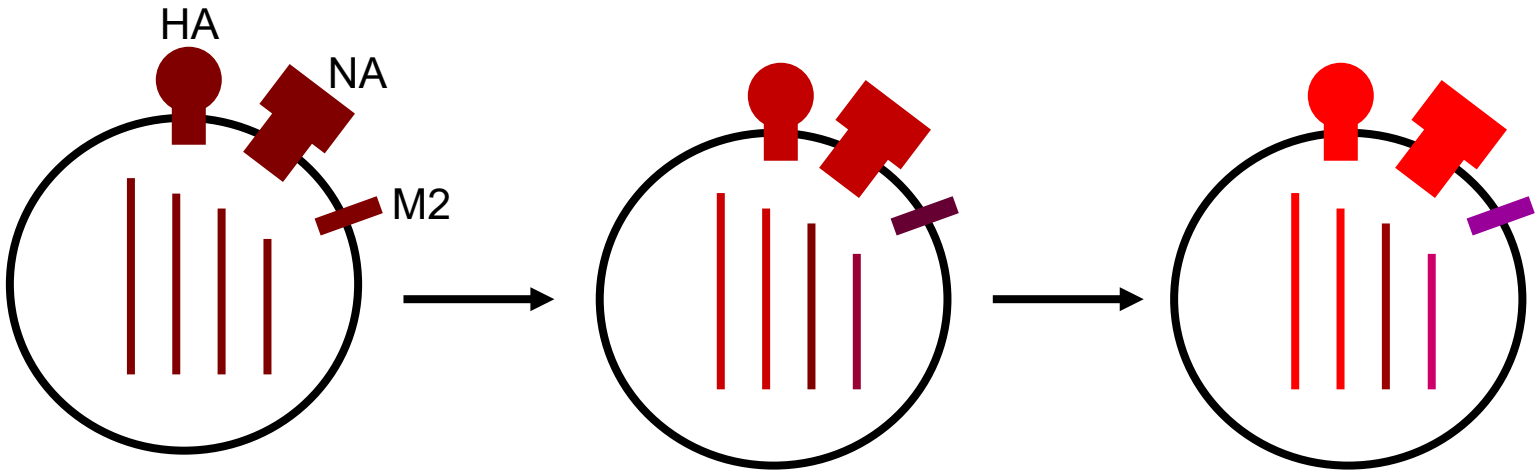


The Influenza viral cycle

Evolution of the influenza virus

Antigenic shift

The genome evolves slowly through the accumulation of mutations.
These are due in particular to the enzyme that ensures RNA replication, which is not faithful.

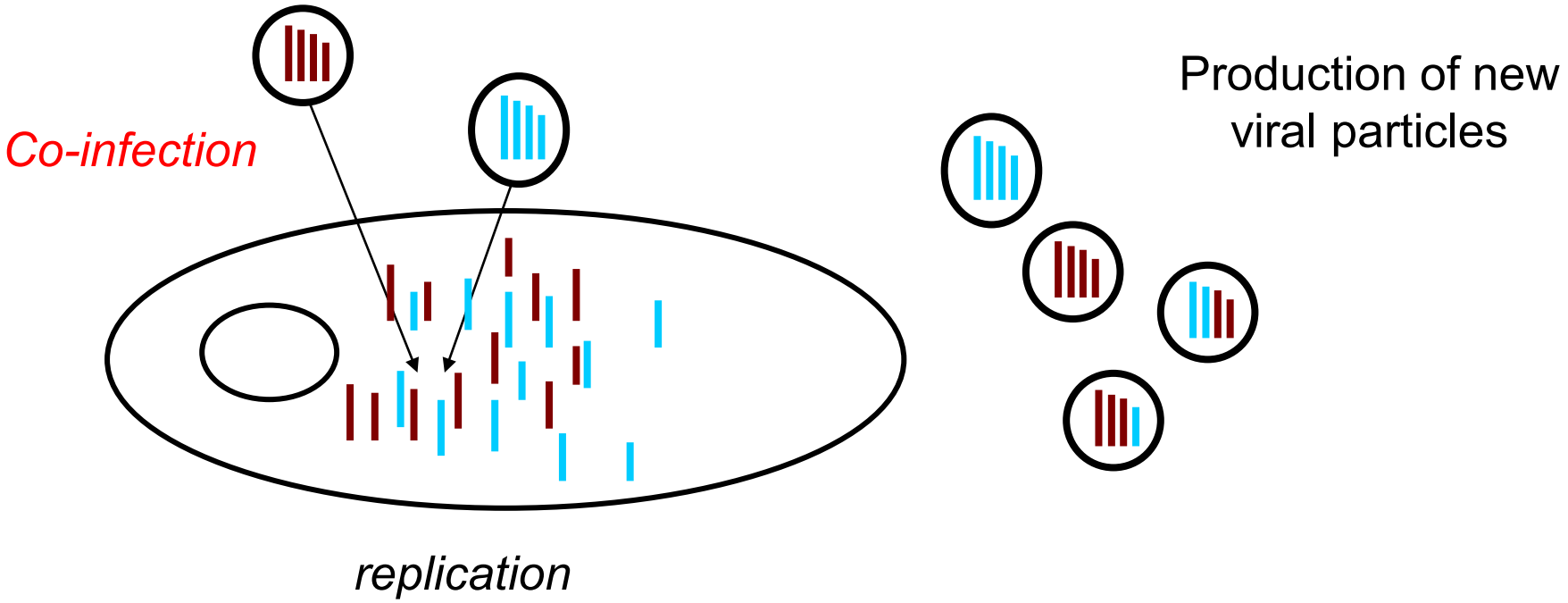


This mechanism enables the virus to evade the immune system, and is at the root of seasonal flu epidemics.

Evolution of the influenza virus

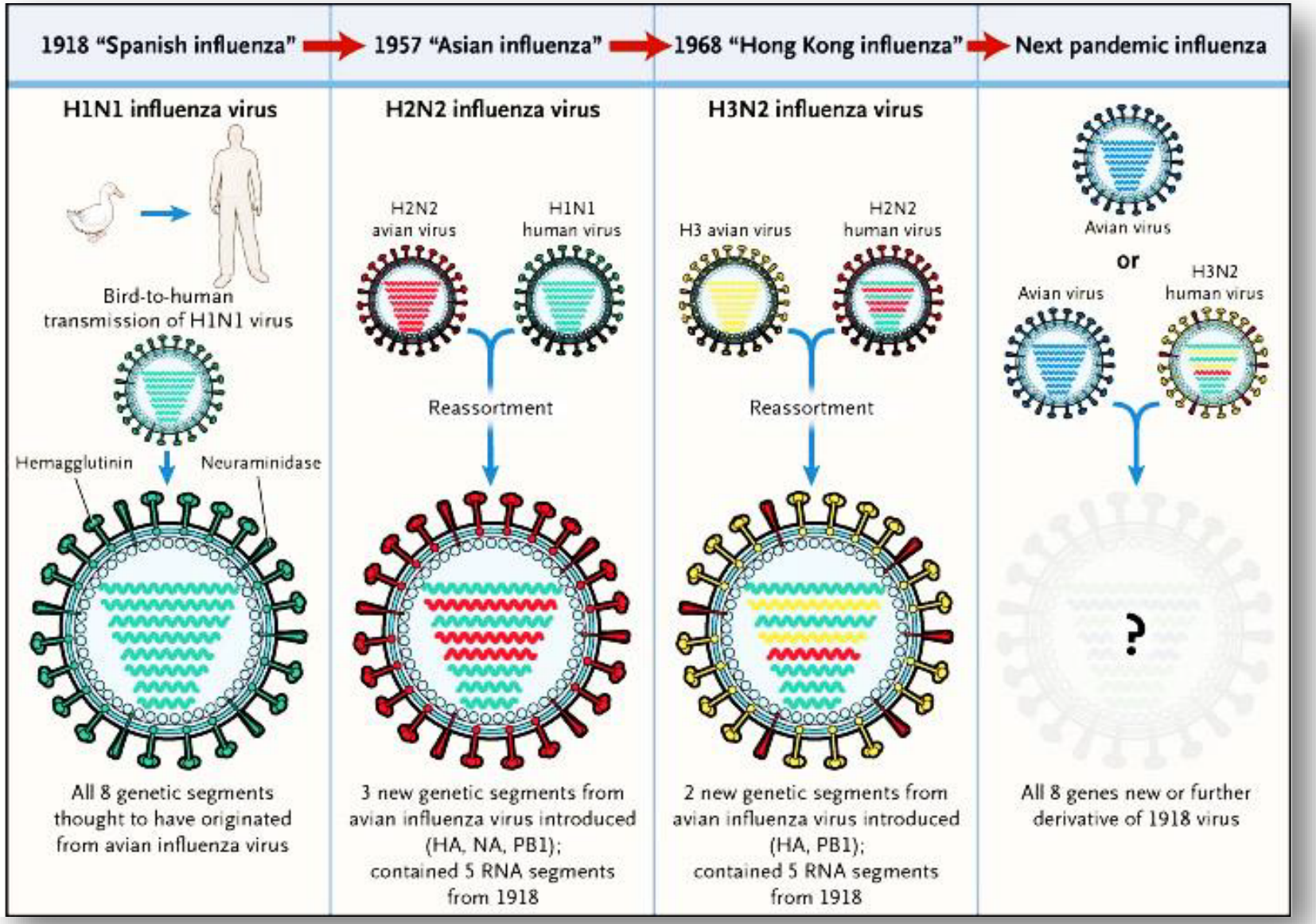
Antigenic breakage

The viral genome's multi-stranded RNA structure explains its high potential for variability. The exchange of fragments between viruses leads to abrupt evolution by segment exchange.
These changes are at the root of pandemics



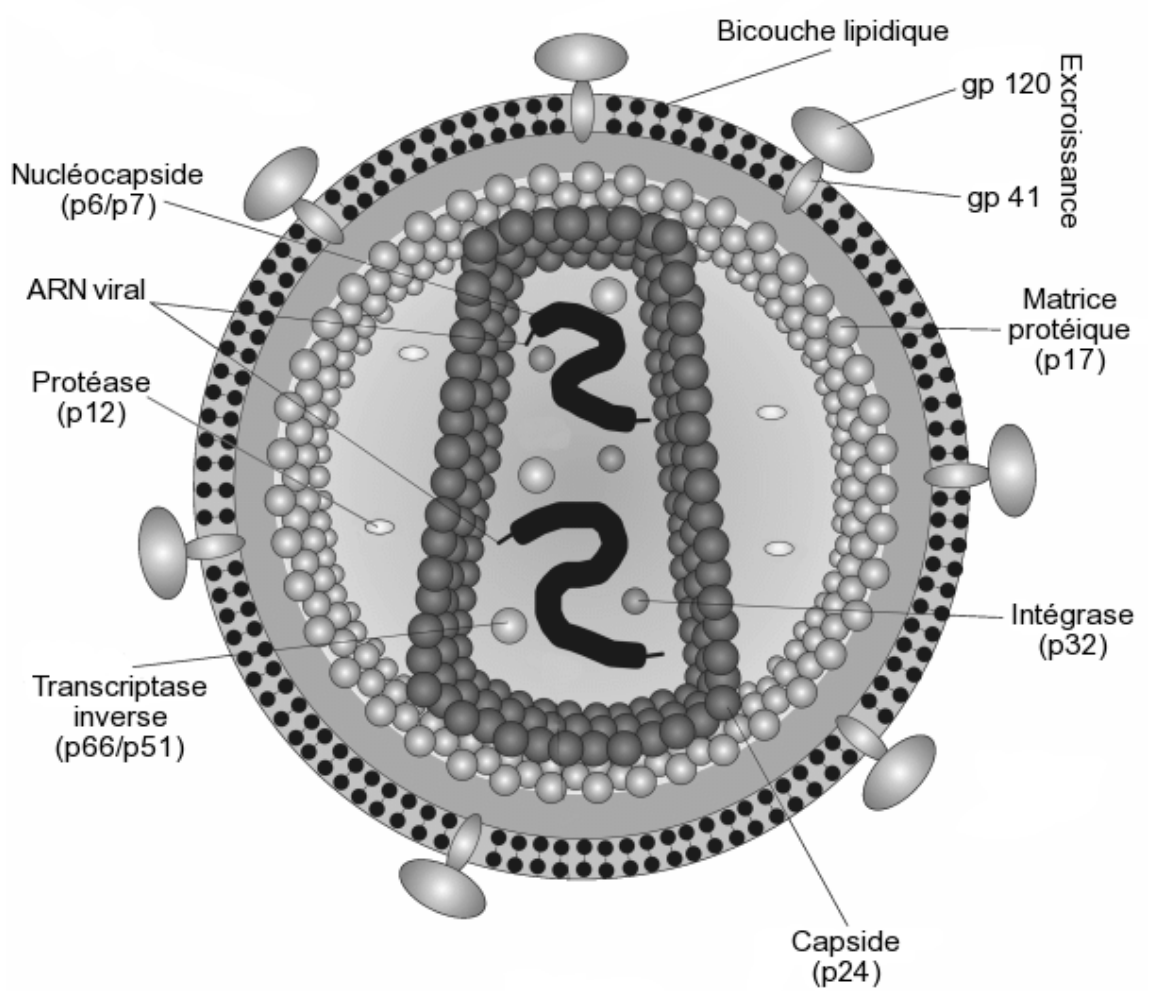
Evolution of the influenza virus

Antigenic breaks are at the root of emergencies and major pandemics



HIV profile

A diploid genome made up of two strands of RNA, accompanied by enzymes: a reverse transcriptase, an integrase and a protease.



Inside the envelope, a protein matrix and, still inside, the capsid.

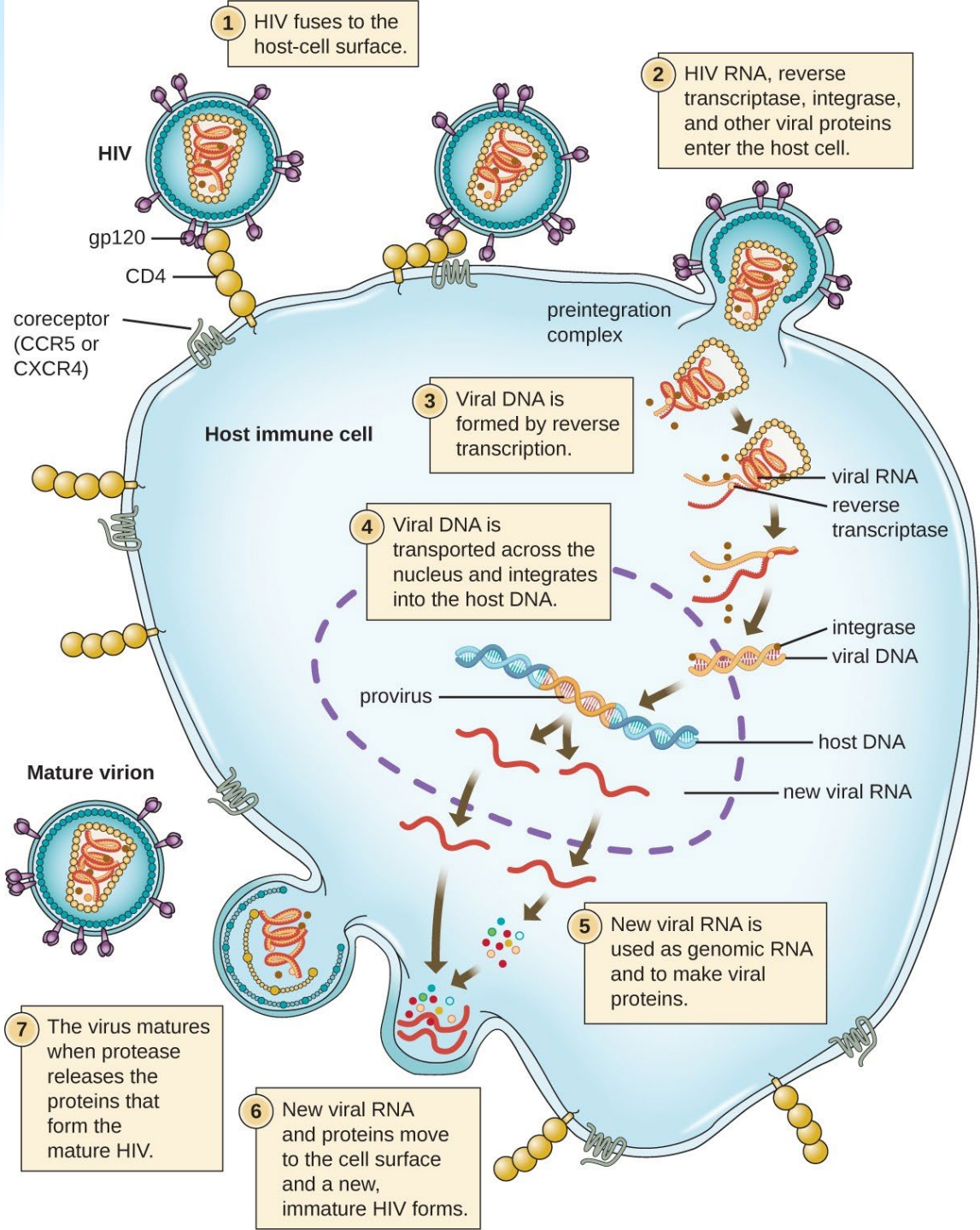
An envelope composed of a fragment of the infected cell's membrane and viral proteins.

HIV cycle

Viral RNA is retrotranscribed into single-stranded and then double-stranded DNA by reverse transcriptase.

Double-stranded DNA enters the nucleus. Viral DNA is integrated into cellular DNA by integrase.

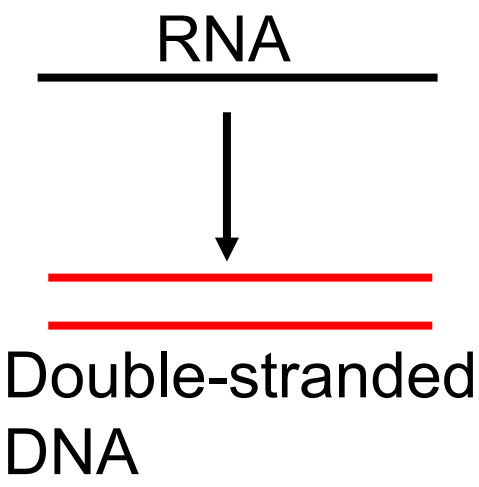
Viral DNA is transcribed into RNA, which serves as messenger RNA and genomic RNA.

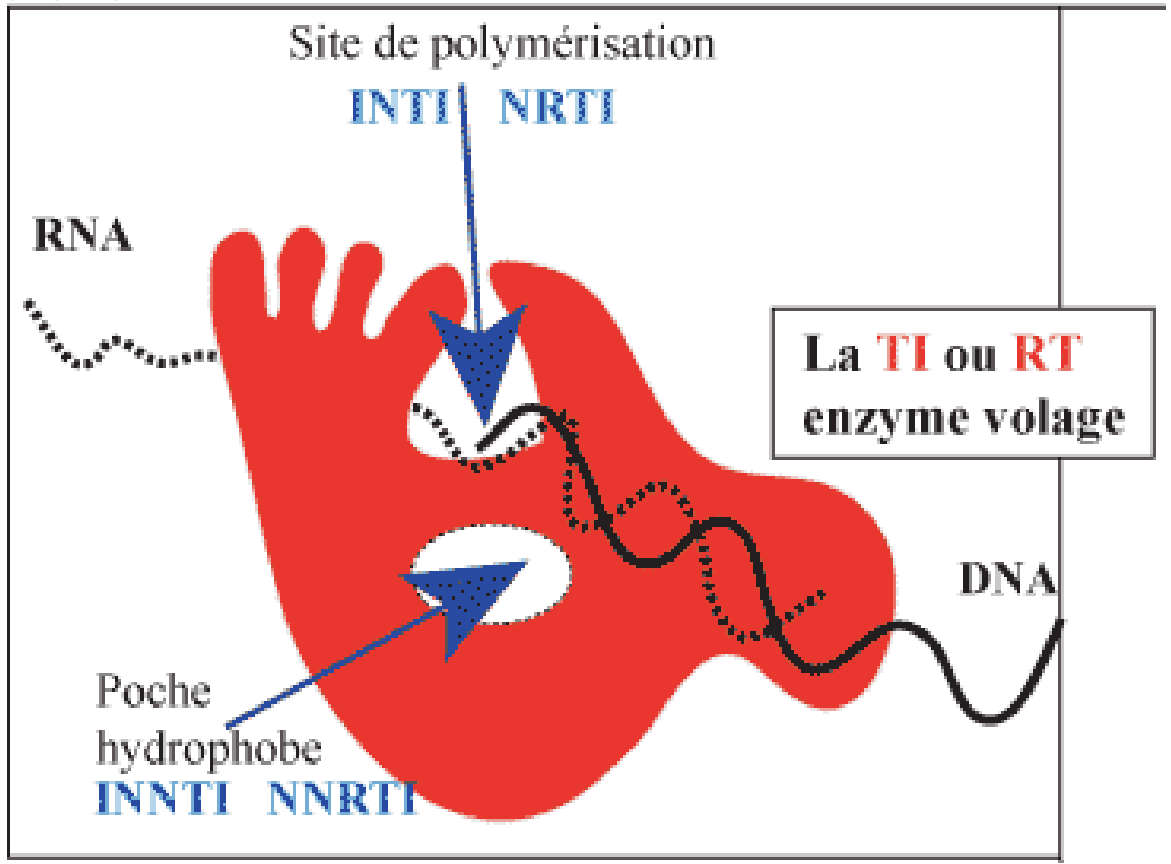


Reverse transcriptase

Reverse transcriptase :

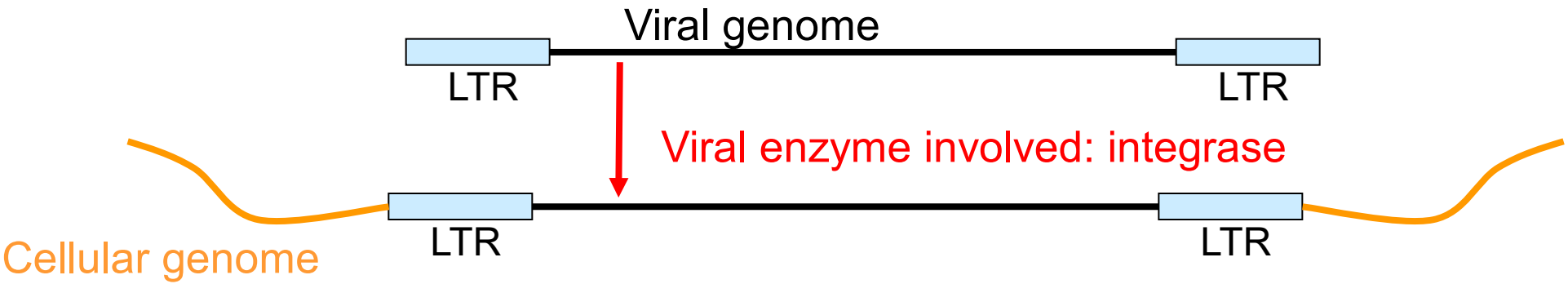
- copies RNA into a complementary strand of DNA (cDNA)
- hydrolyzes the RNA of the RNA/cDNA heteroduplex (RNAse H activity)
- synthesizes the complementary strand of cDNA





Integration of the viral genome into the cellular genome

Integrase is attached to the LTR (long terminal repear).
Integrase cuts both strands of cellular DNA to introduce viral DNA.

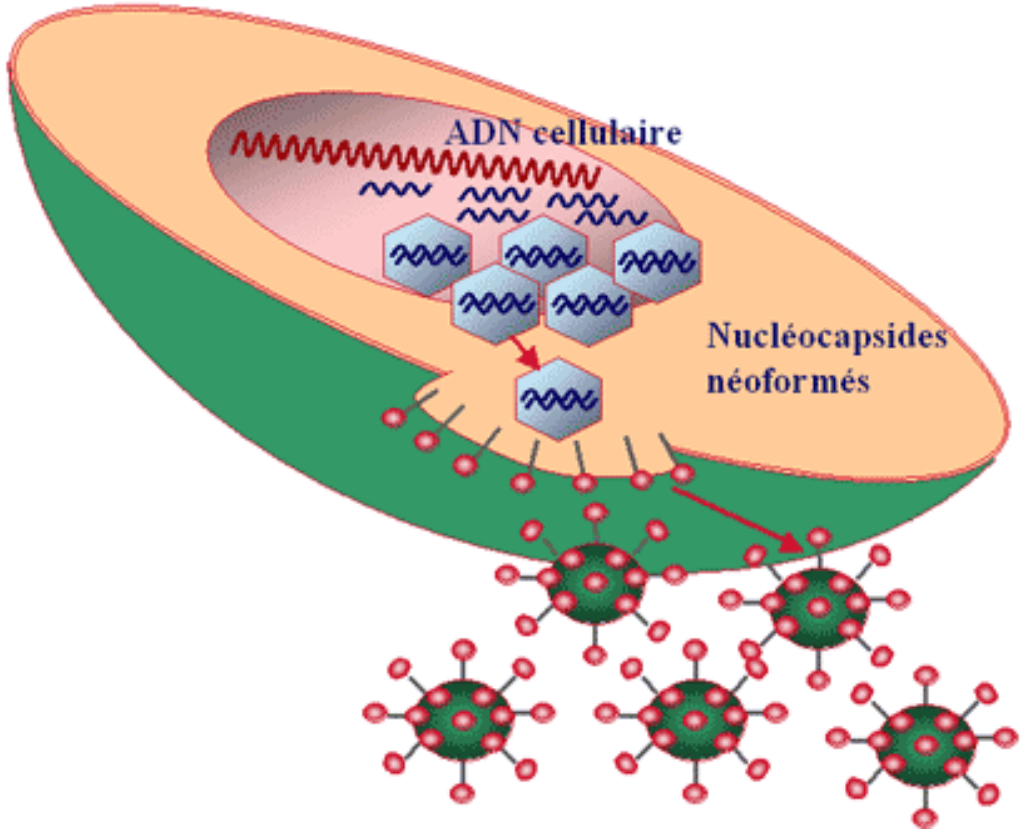


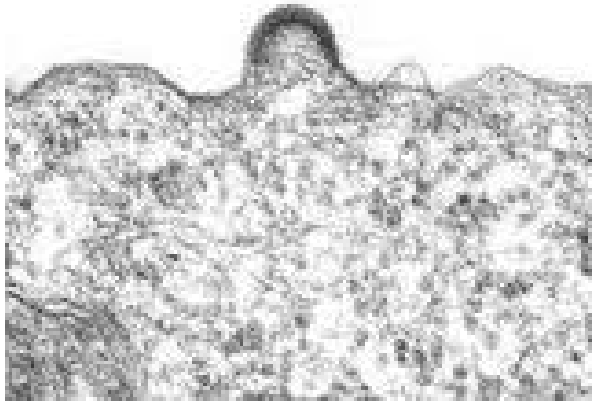
LTRs

- appear following the replication of RNA into dbDNA.
- are essential for integration. They bind to viral integrase
- in 5' it is a powerful promoter of transcription;
- in 3' it provides the cut-off signal preceding polyadenylation.

Virion formation at the plasma membrane

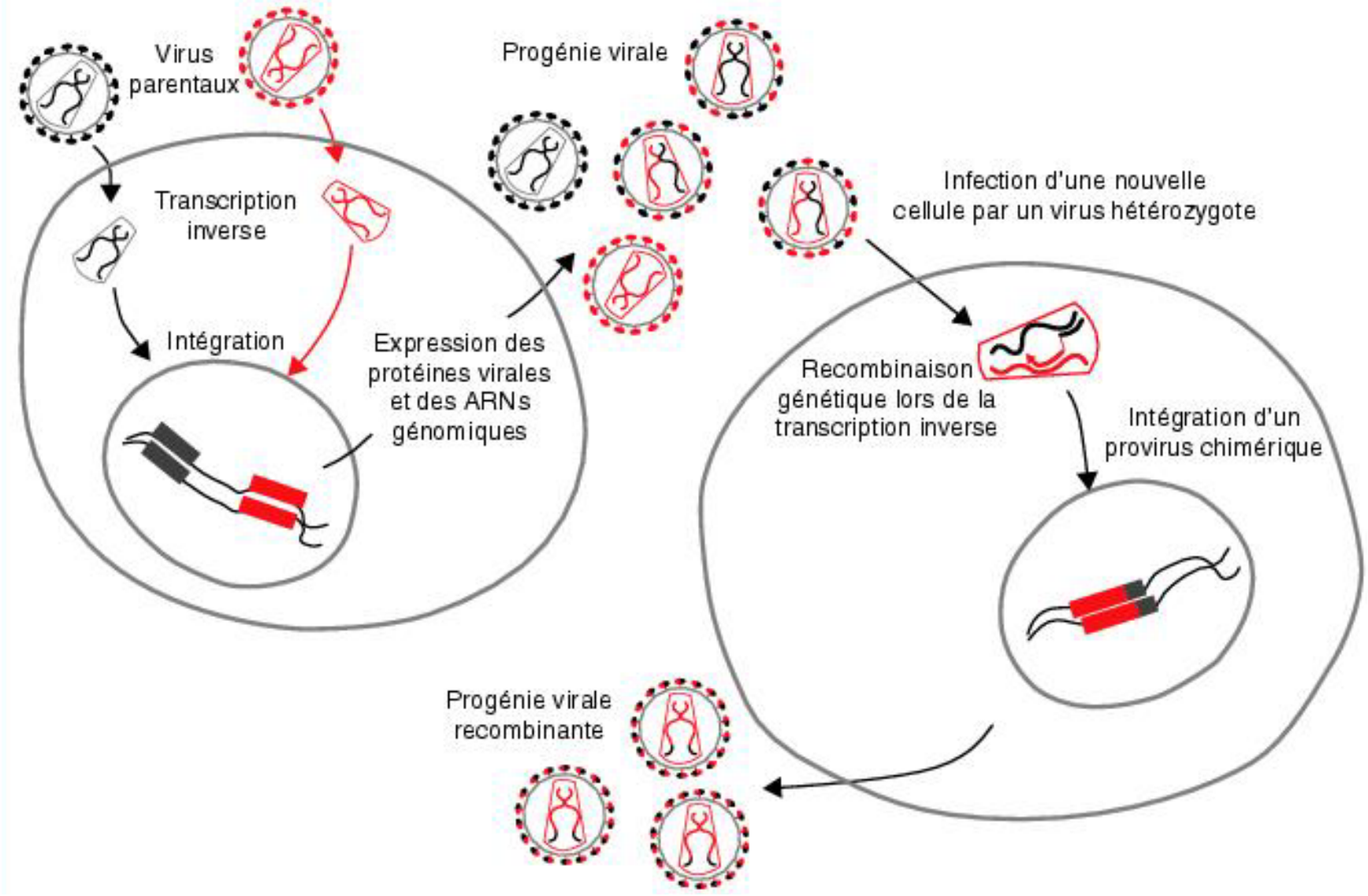
- Envelope proteins are integrated into cell membranes.
- Virus particles (virions) form by budding at the plasma membrane.





- Virus particles (virions) mature after budding

Recombination increases the variability of retroviral genomes



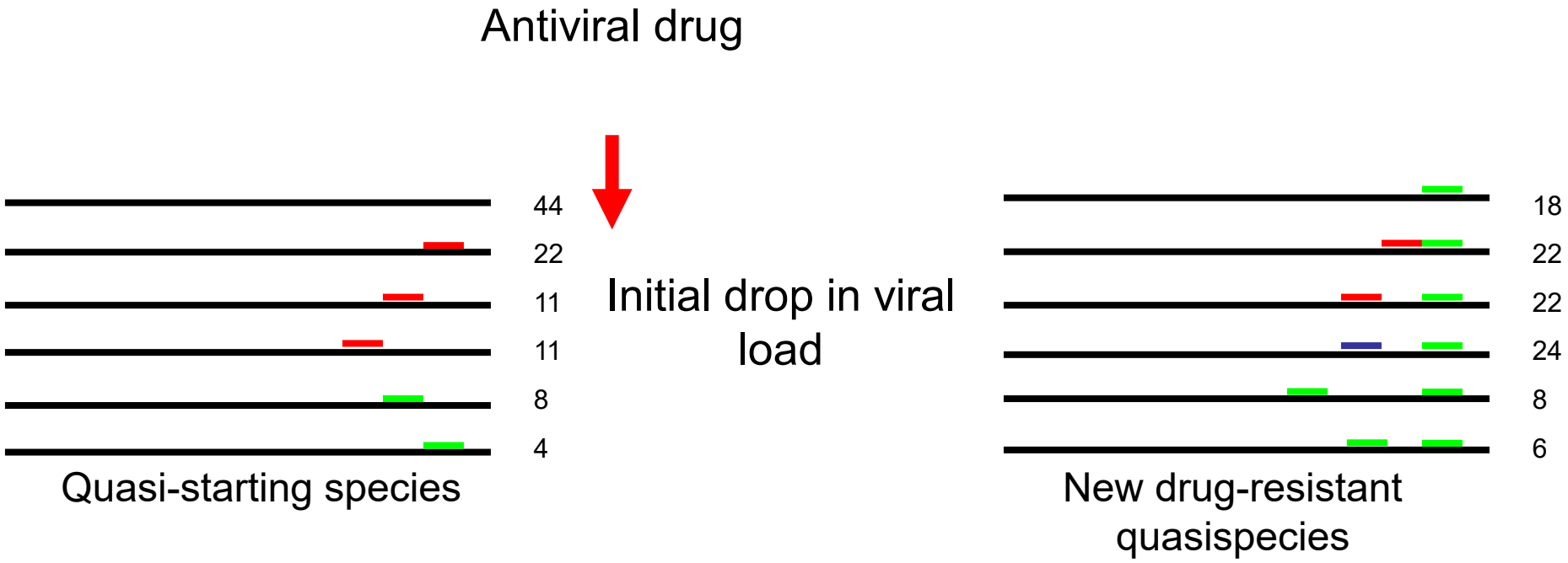
Virus species and quasispecies concepts

Viral polymerases (RNA viruses, retroviruses) are unreliable: they have no proofreading capability (error rate: 1/10000).

This error rate leads to the formation of quasispecies: a population of variants in dynamic equilibrium.

Quasi-species are highly adaptable, enabling them to evade antiviral therapies.

Variant selection during therapy



HIV viral cycle

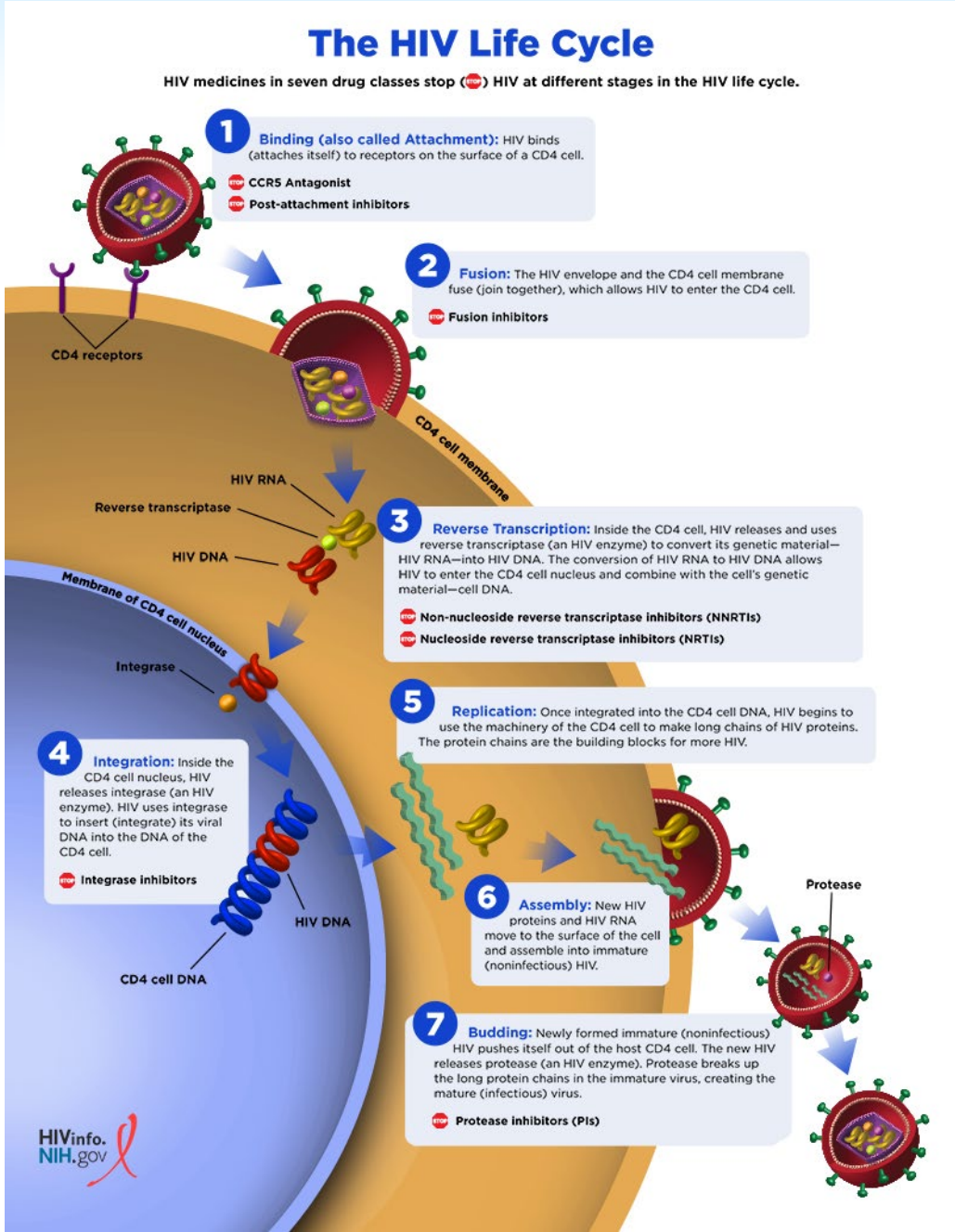
Antivirals target different parts of the viral cycle (HIV)

Problems encountered :

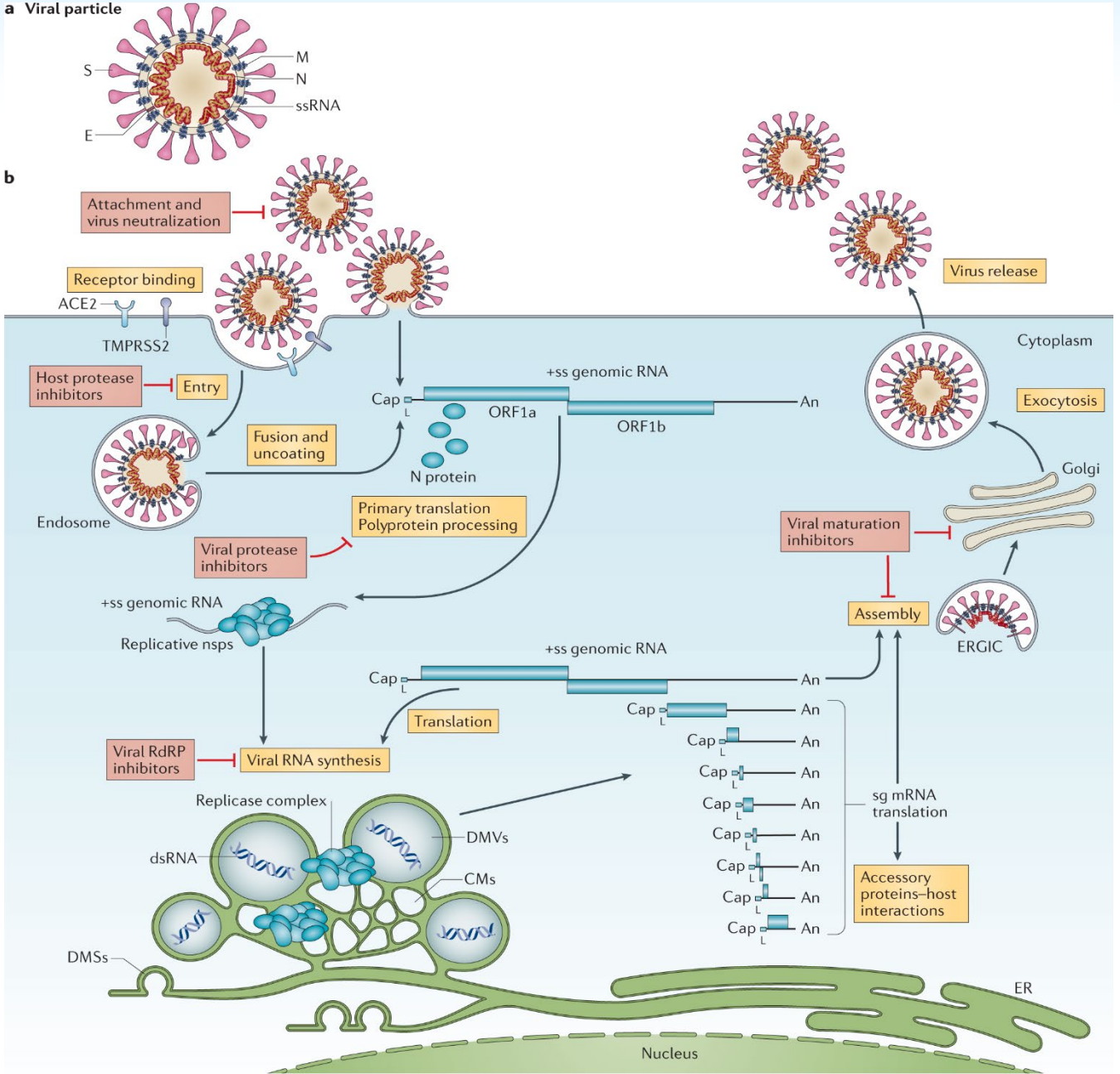
- Determining targets: viruses use cellular machinery, and viral enzymes often resemble those found in cells →Toxicité.

- Rapid emergence of resistance due to the existence of quasi-species → polytherapies.

- Cost of researching and bringing an antiviral to market.



Antivirals target different parts of the viral cycle (SARS-CoV



Conclusions III

III- The viral cycle

The viral cycle includes :

- the entry of viruses into the cell.

- the expression of genetic material (translation - see Part IV), which enables the synthesis of viral proteins needed for virus replication and survival in the cell and host organism.

- replication of genetic material.

- the assembly of new nucleocapsids.

- the exit of newly-formed viruses by budding or lysis of the host cell.

- possibly, the maturation of viral particles.