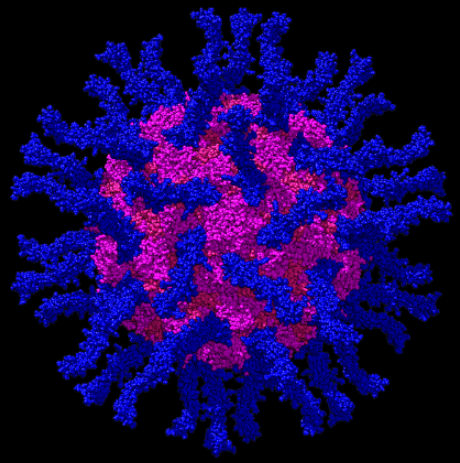


VIROLOGY LIVE

WITH VINCENT RACANIELLO

Attachment and Entry

Session 5

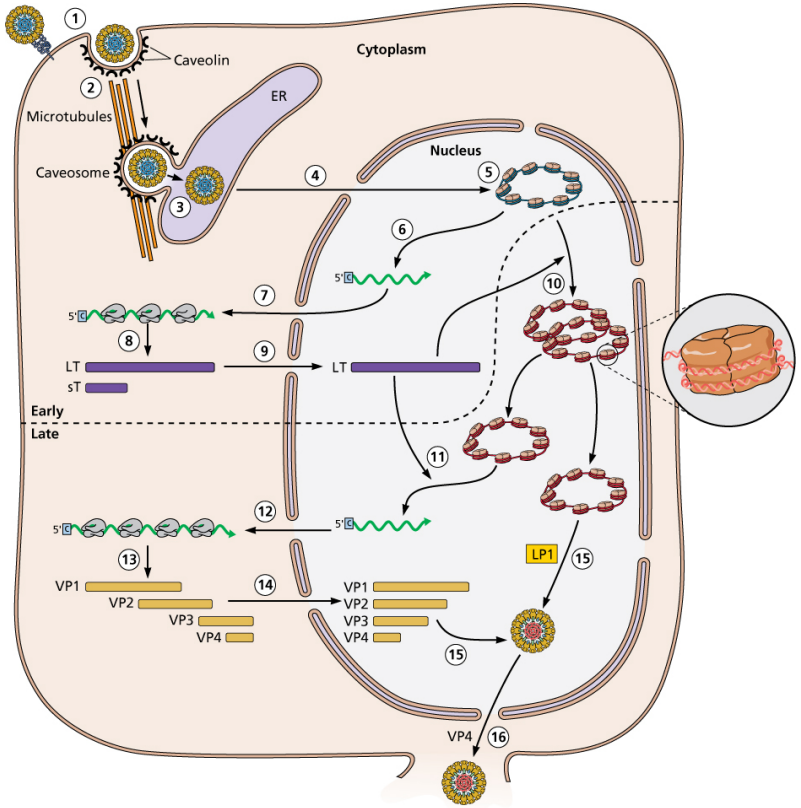
Virology Live

Fall 2021

Who hath deceived thee so often as thyself?

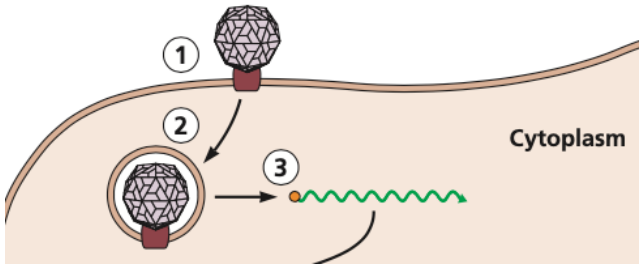
--Benjamin Franklin

Viruses are obligate intracellular parasites



Virus particles are too large to diffuse across the plasma membrane

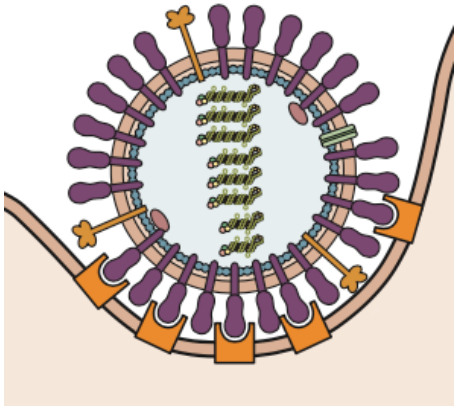
Finding the 'right' cell receptor



- Step 1: adhere to cell surface (random collisions & electrostatics)
  - No specificity
- Step 2: Attach to specific receptor molecules on cell surface
  - More than one receptor may be involved
- Step 3: Transfer genome inside the cell

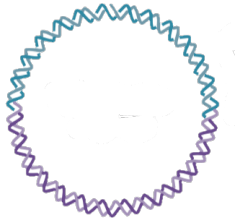
Cellular receptors for viruses

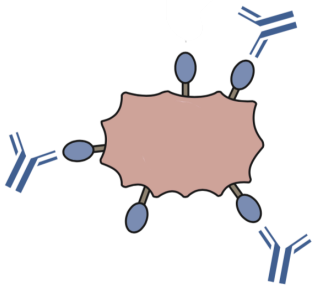
- Essential for all viruses except those of fungi (no extracellular phases) and plants (enter cells by mechanical damage)
- 1985: one receptor known, sialic acid for influenza virus

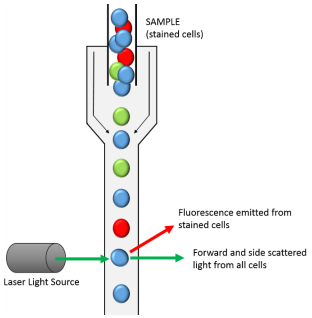


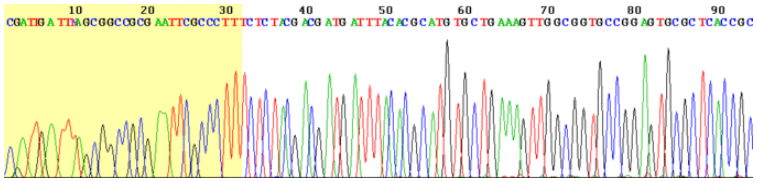
Enabling technologies

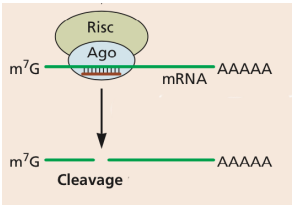
- Recombinant DNA
- Monoclonal antibodies
- Flow cytometry
- Nucleotide sequencing
- siRNA
- CRISPR/Cas9

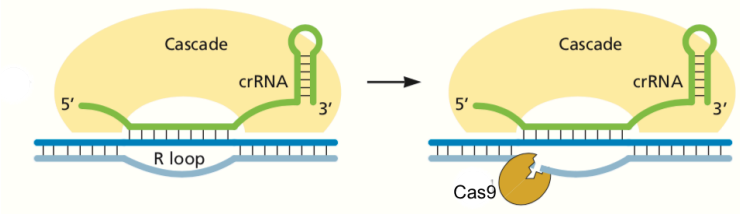






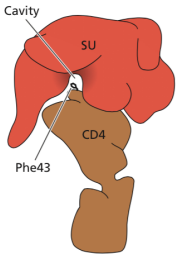


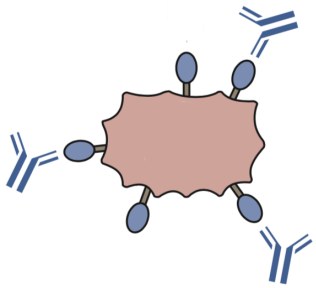


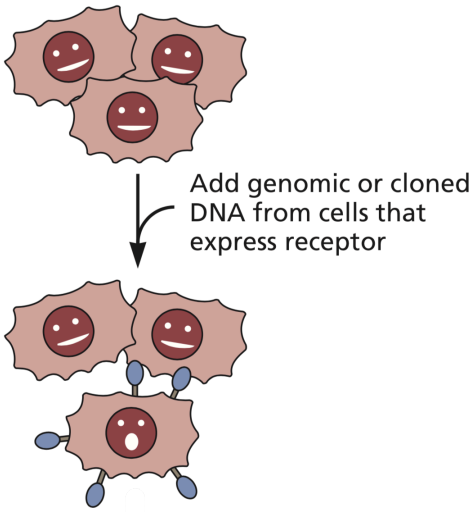


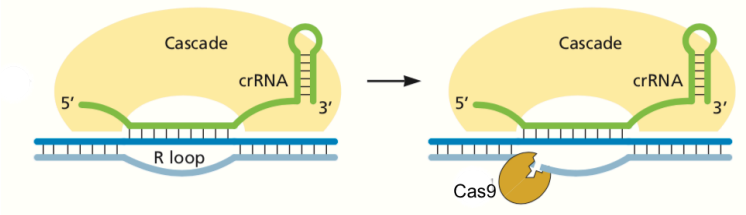
Criteria for identifying cell receptors for viruses

- Receptor binds virus particle
- Antibody to receptor blocks infection
- Receptor gene confers susceptibility
  - More than one receptor may be involved
- Disruption of receptor gene blocks infection

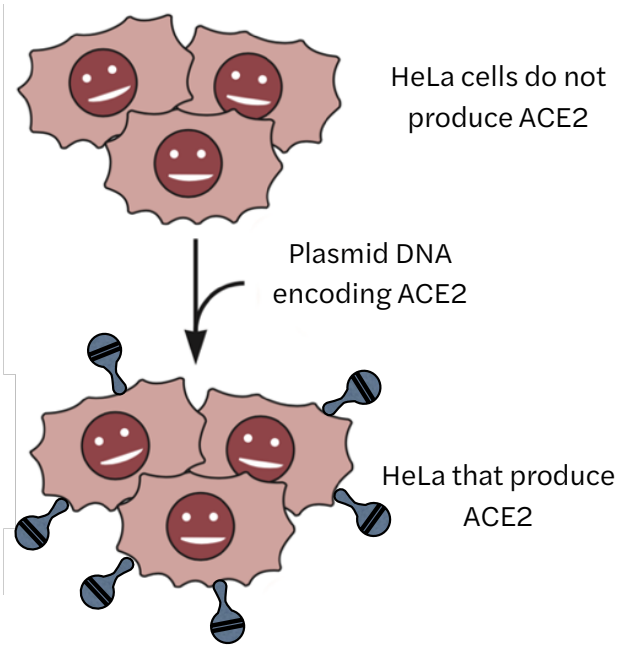


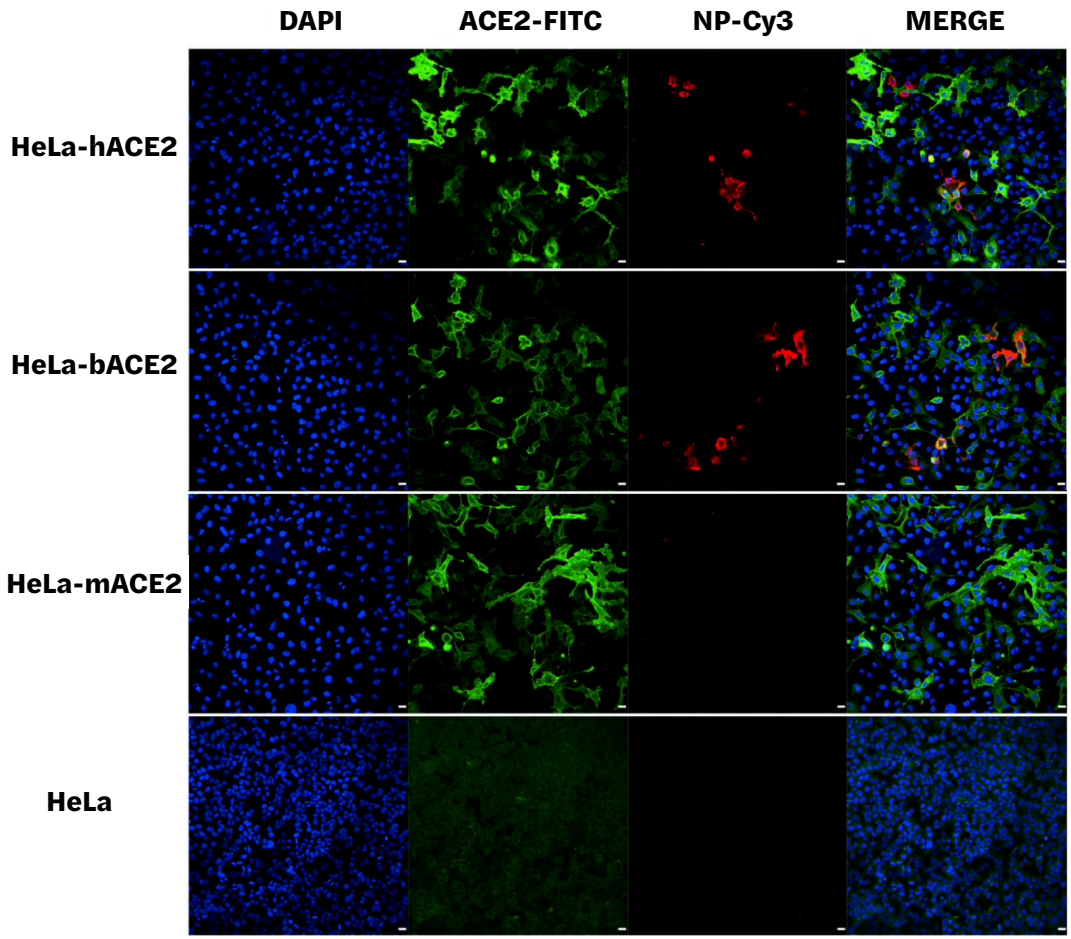




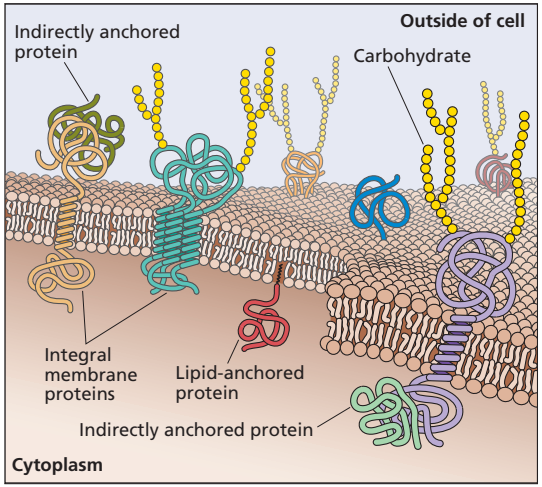


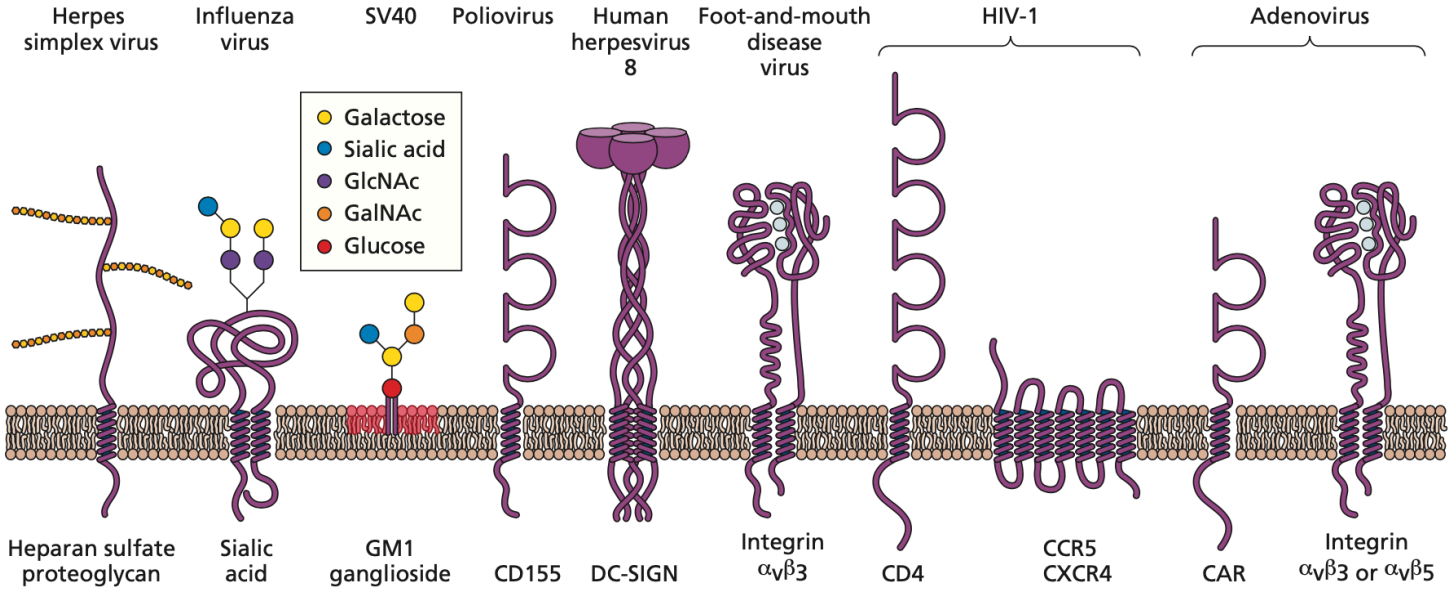
ACE2 is receptor for SARS-CoV-2





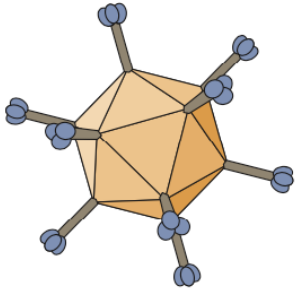
https://www.nature.com/articles/s41586-020-2012-7

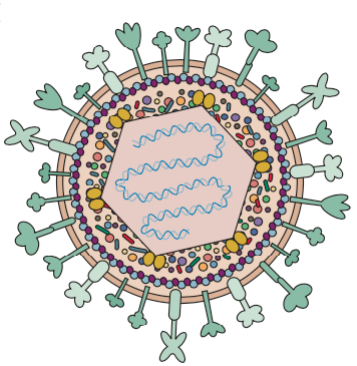




Cell functions!

Different viruses can bind the same receptor

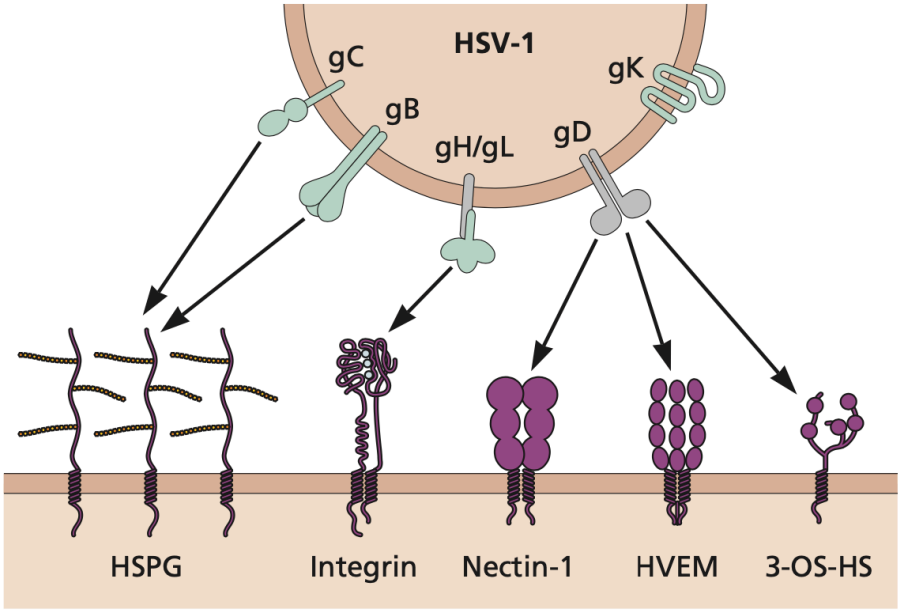




- Adenovirus and Coxsackievirus B3 have common primary receptor
- The swine herpesvirus, pseudorabies virus, binds same receptor as human poliovirus

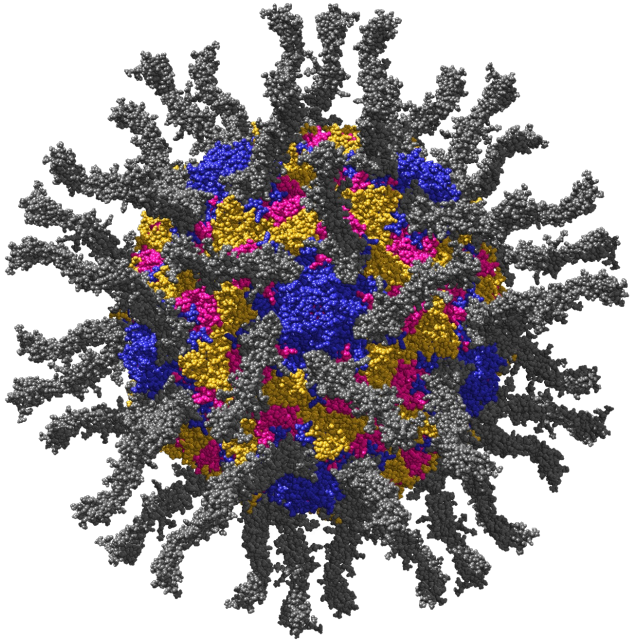
Viruses of the same family may bind different receptors

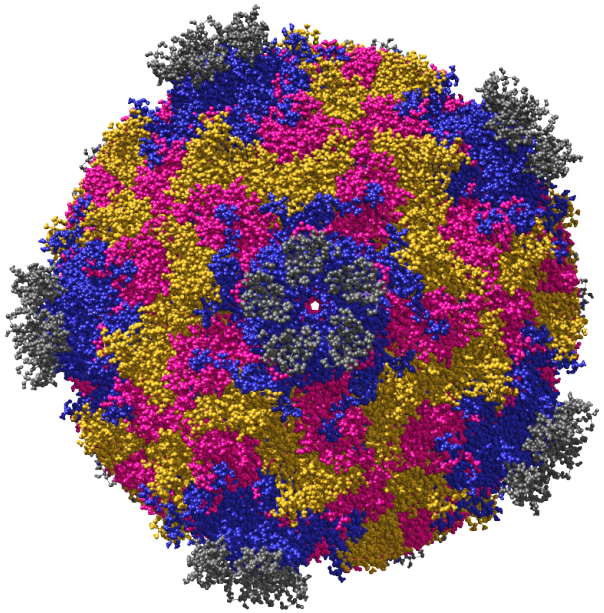
- Rhinoviruses (3), retroviruses (16)
- One virus may bind multiple receptors



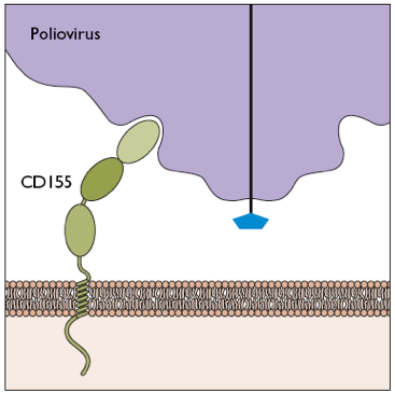
How do virus particles attach to receptors?

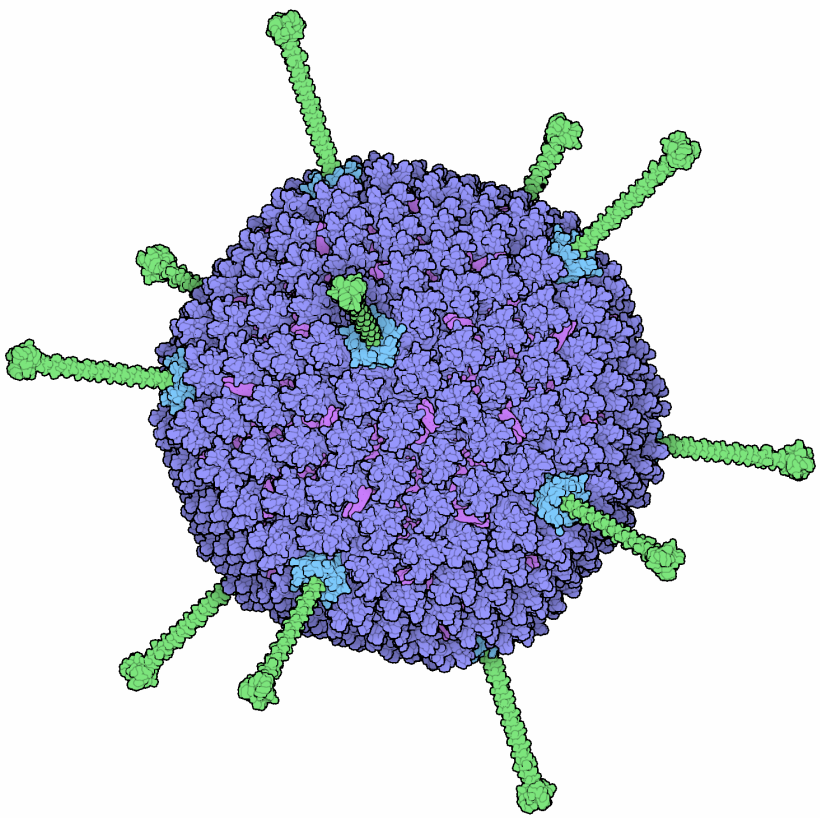
Poliovirus





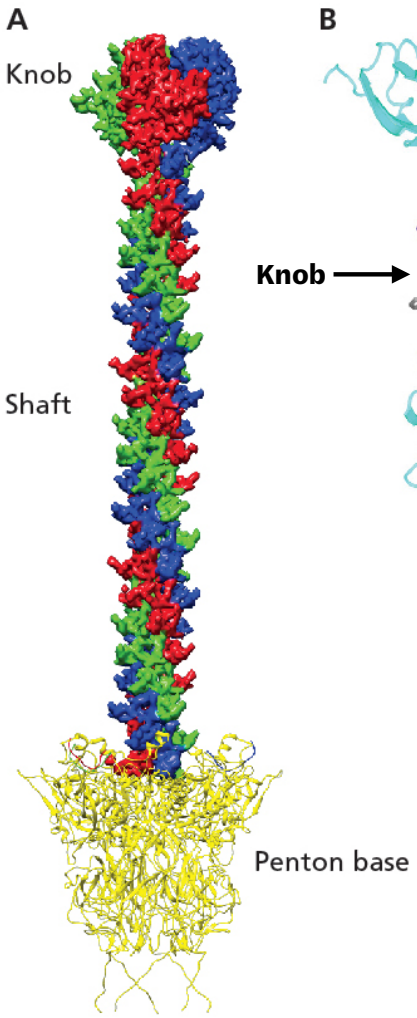
Rhinovirus 2

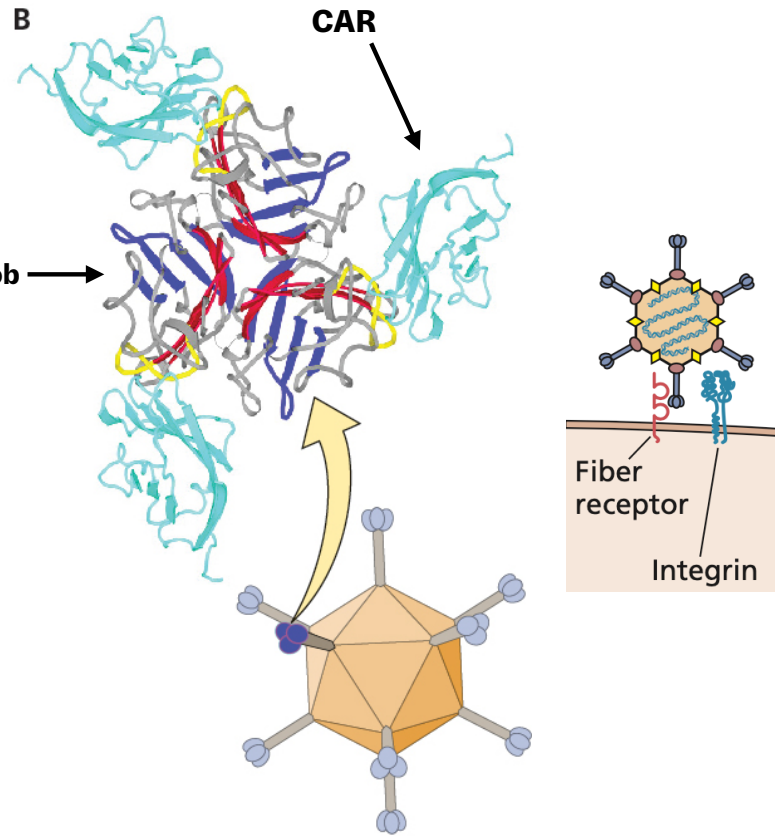




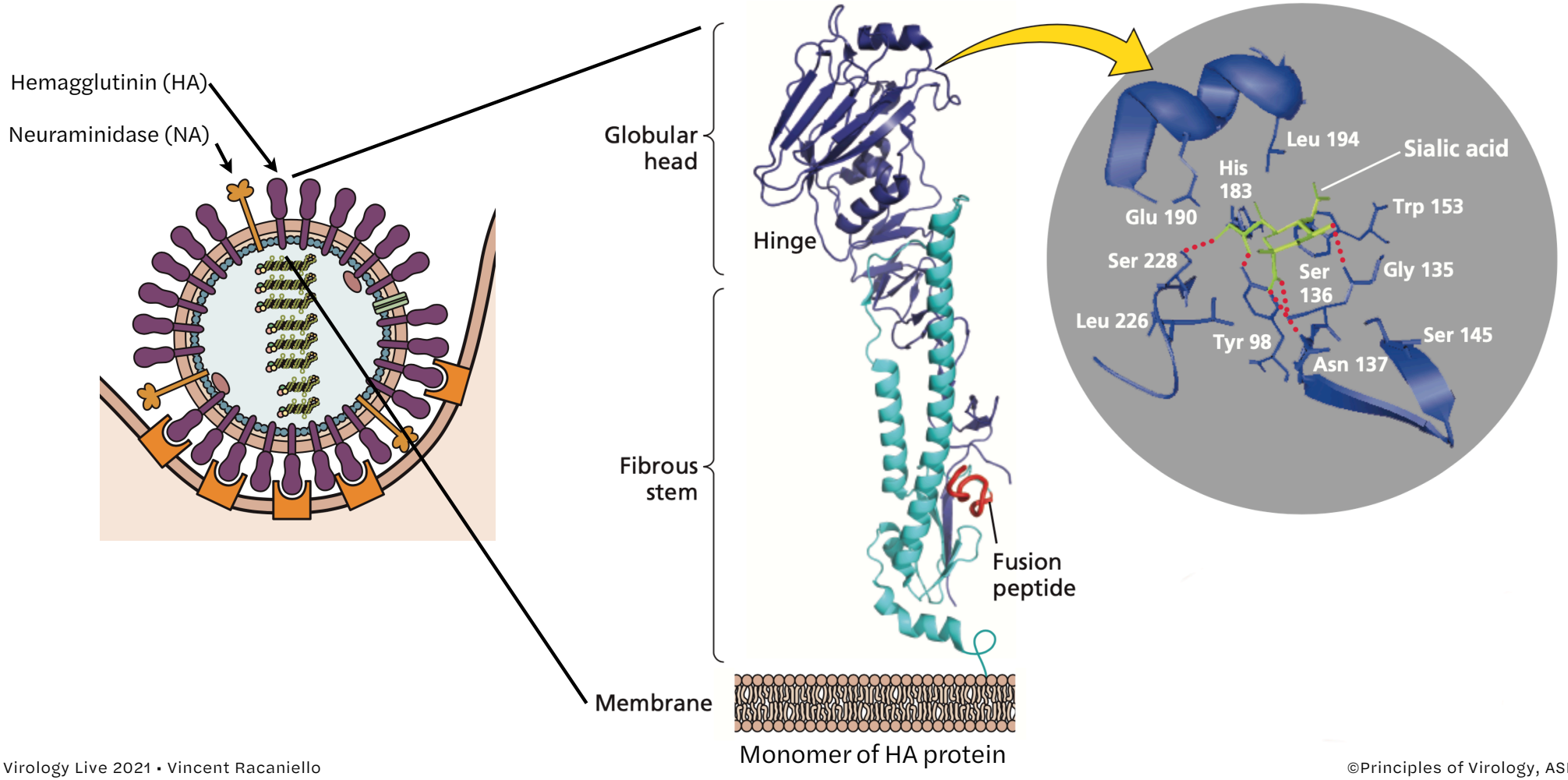
Adenovirus

http://pdb101.rcsb.org/motm/132

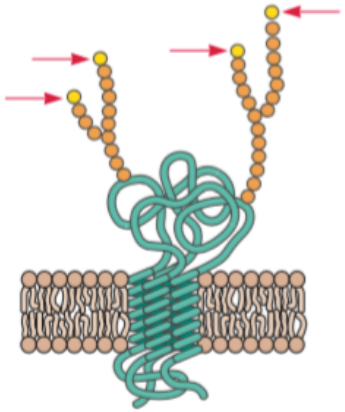




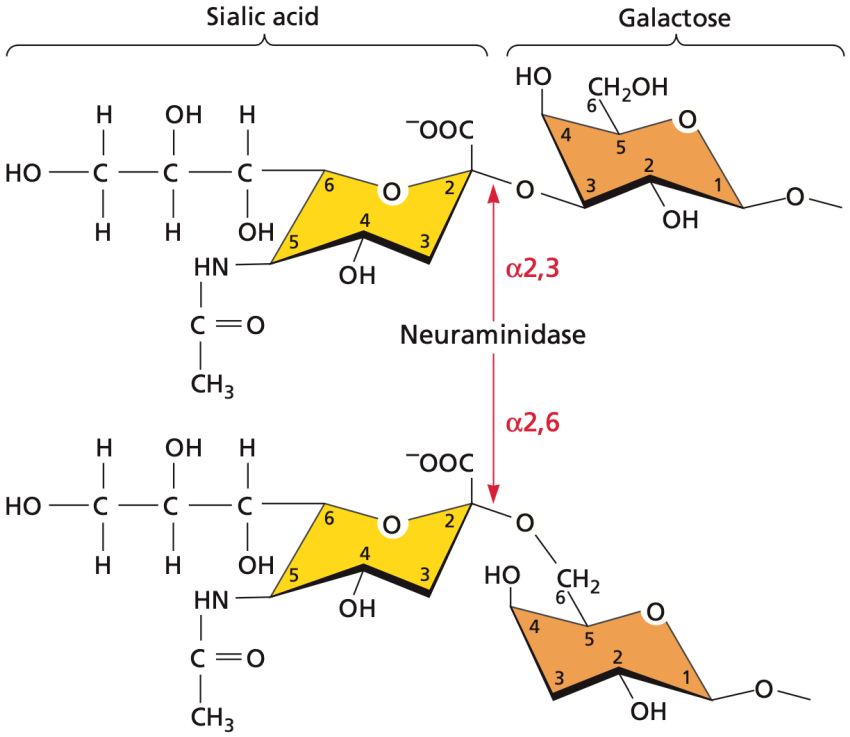
Influenza virus attachment to cells

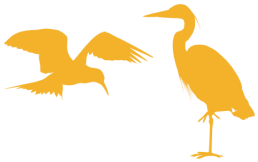


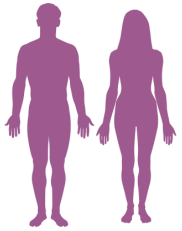
Sialic acid: receptor for influenza viruses



Transmembrane glycoprotein

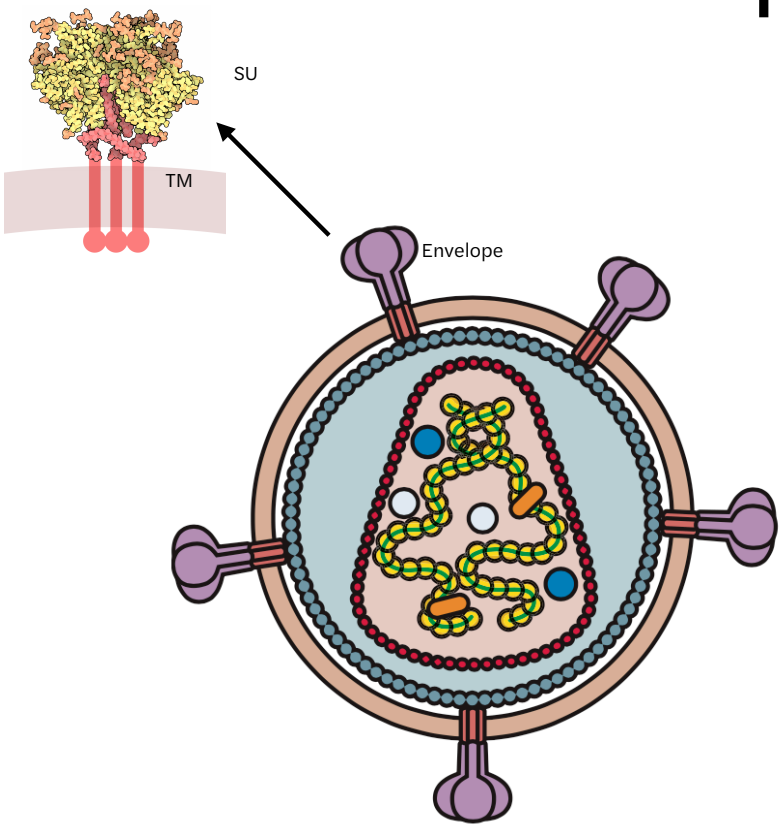


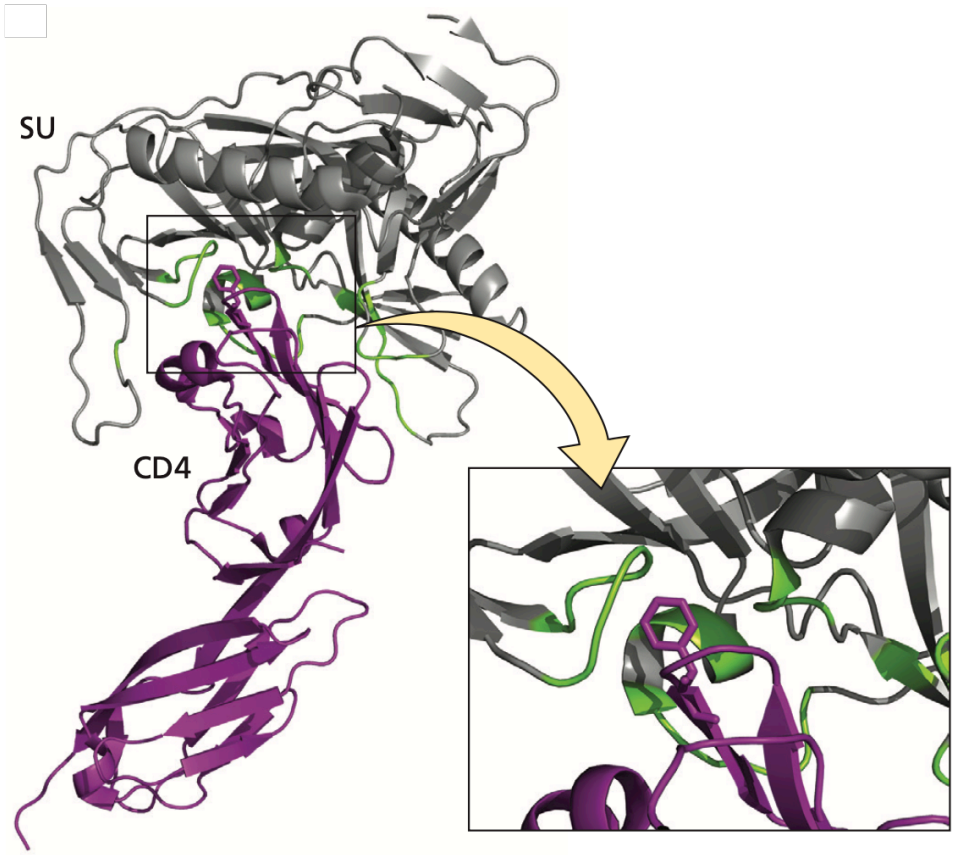




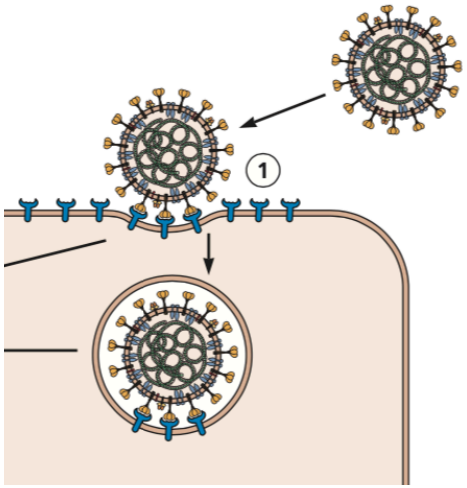
- Sialic acids: N-acetylneuraminic acid (A,B); 9-O-acetyl-N-neuraminic acid (C)
- α(2,6) preferentially bound by human strains, α(2,3) by avian

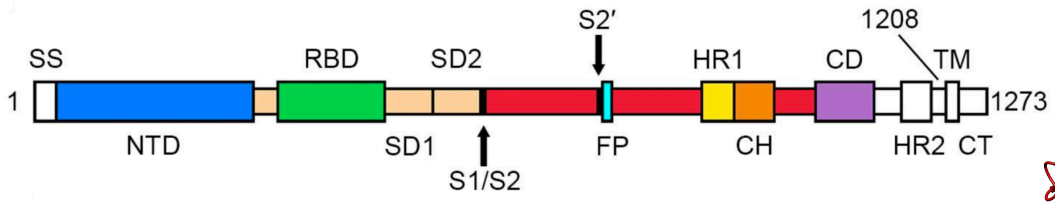
HIV-1 attachment



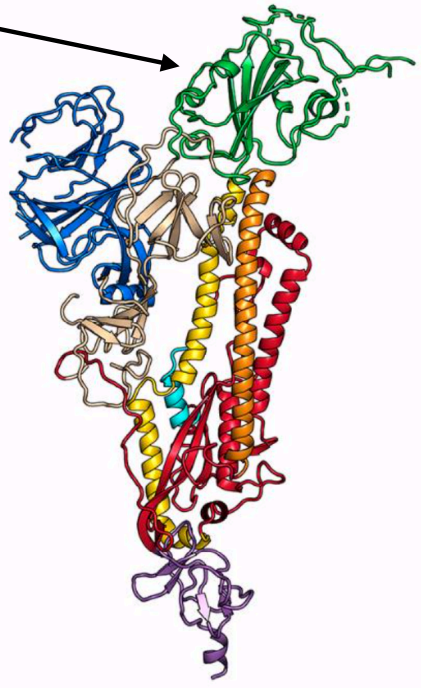


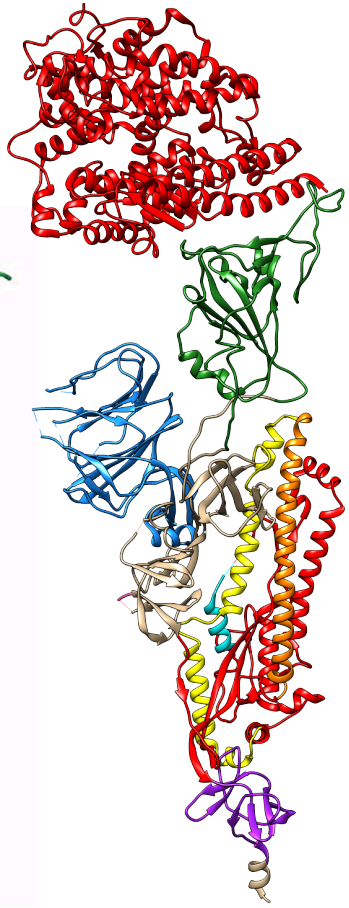
SARS-CoV-2 attachment





Receptor binding domain
(ACE2)





Coronavirus cell receptors

HCoV-229E	Human amino peptidase N
HCoV-NL63	Angiotensin converting enzyme 2
HCoV-OC43	N-acetyl-9-O-acetylneuraminic acid
SARS-CoV	Angiotensin converting enzyme 2
MERS-CoV	Dipeptidyl peptidase 4
SARS-CoV-2	Angiotensin converting enzyme 2

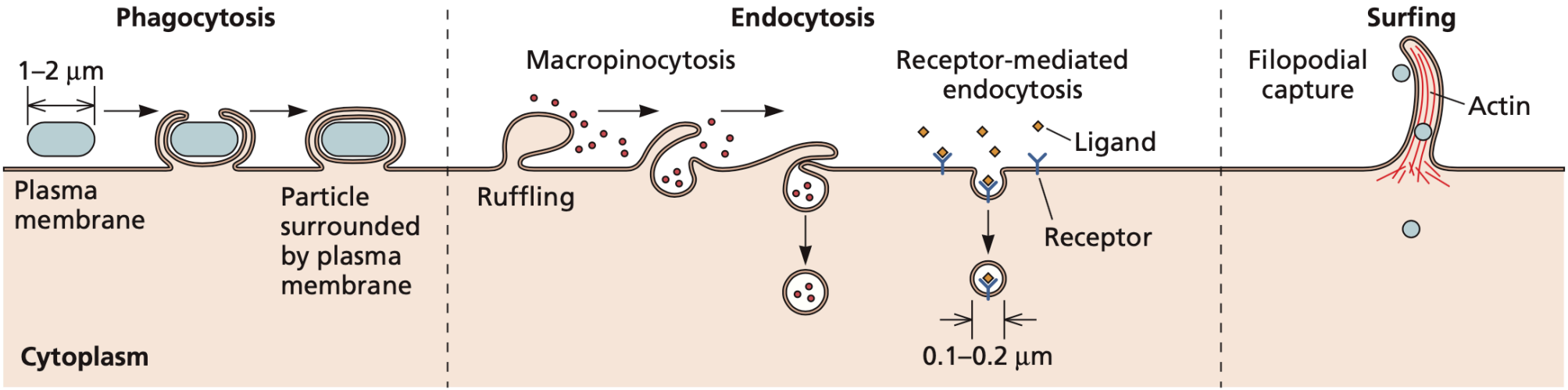
Go to:

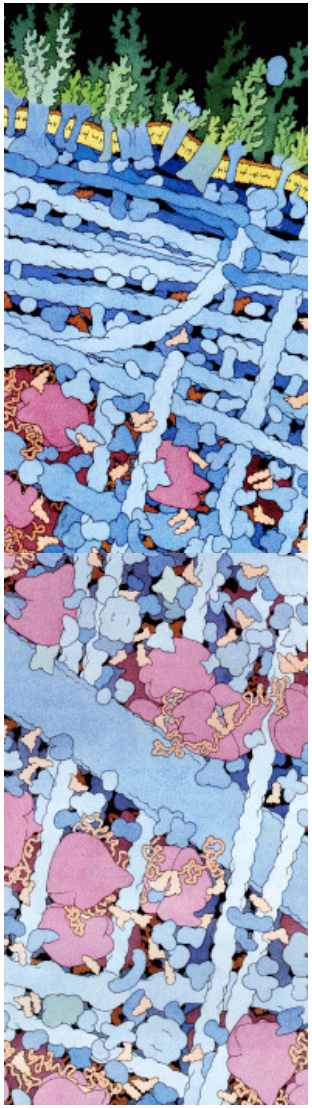
b.socrative.com/login/student
room number: virus

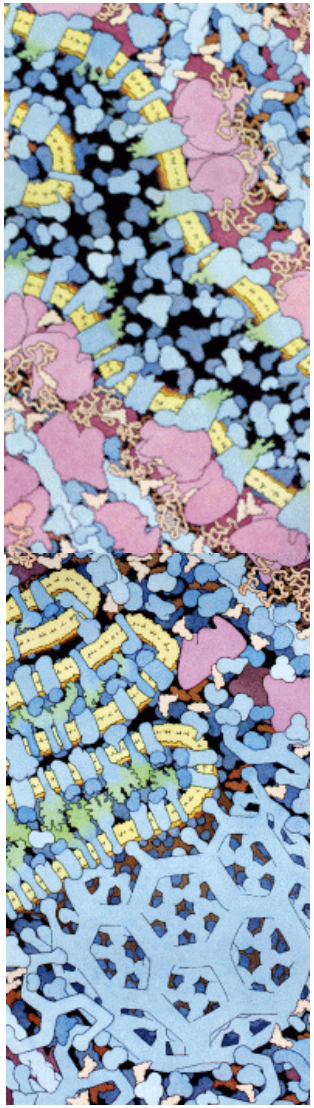
Viral receptors on the cell surface:

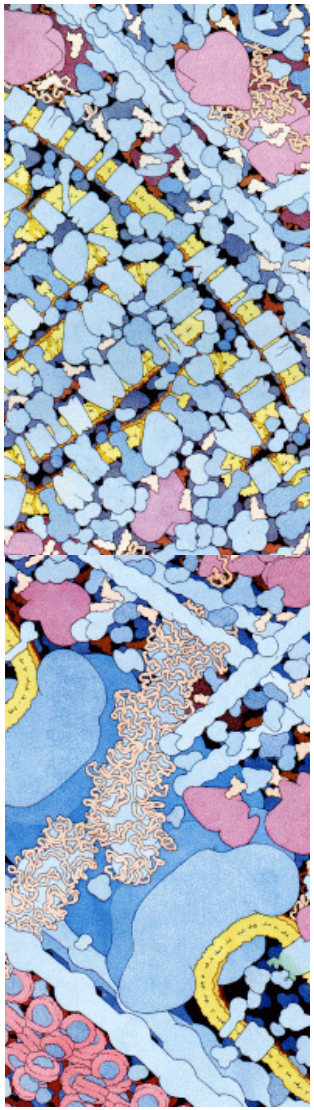
A. Can bind directly to icosahedral virus capsid proteins
B. Interact with glycoproteins of enveloped viruses
C. Can be carbohydrate or protein molecules
D. Have cellular functions
E. All of the above

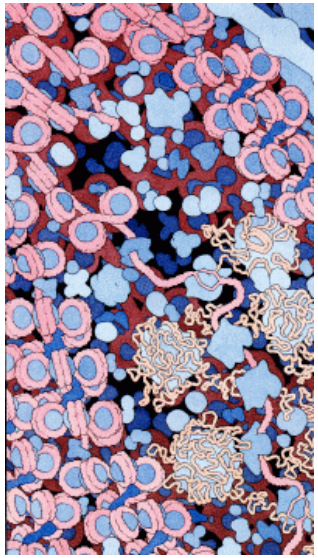
Entry into cells









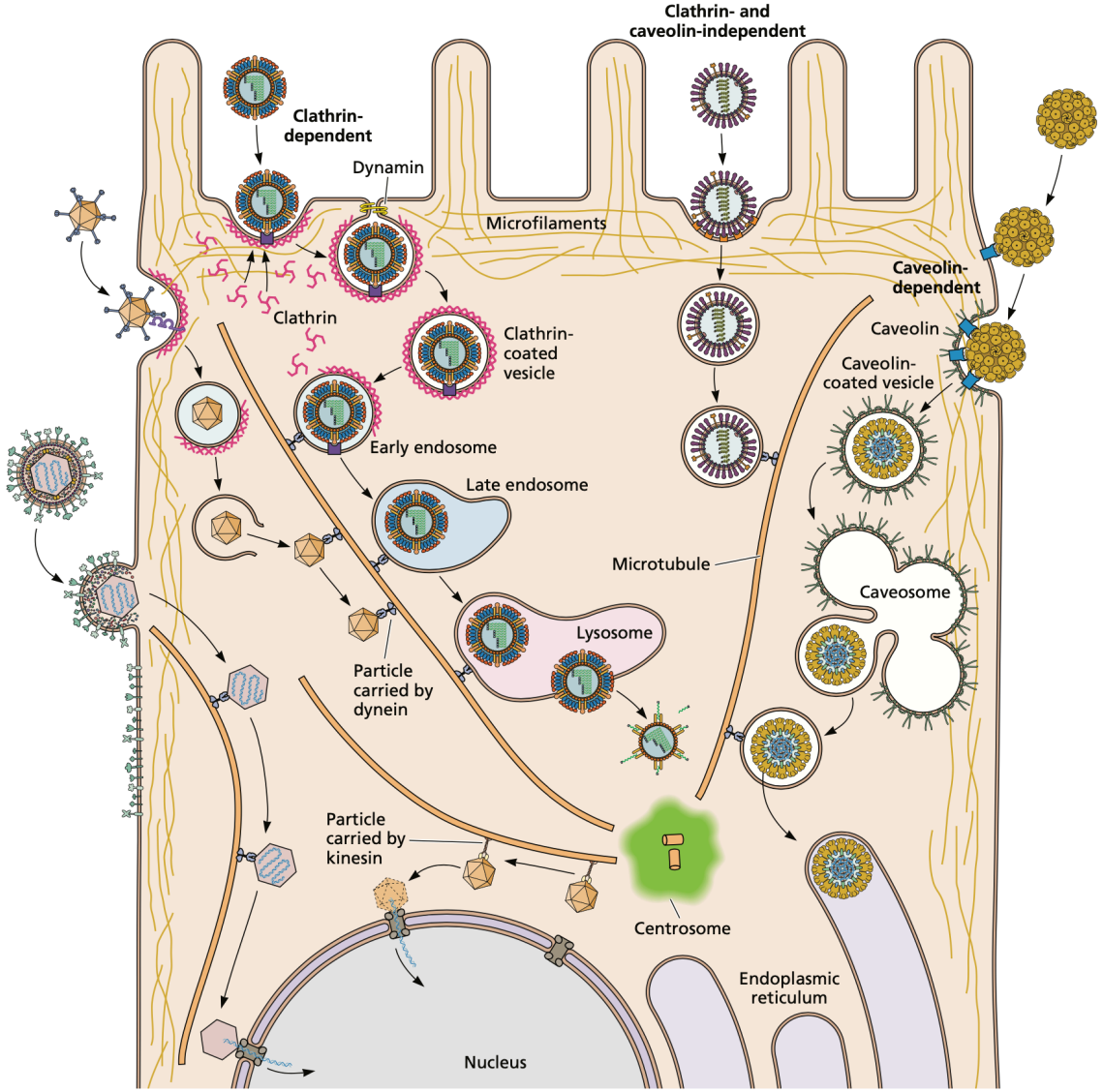


https://ccsb.scripps.edu/goodsell/

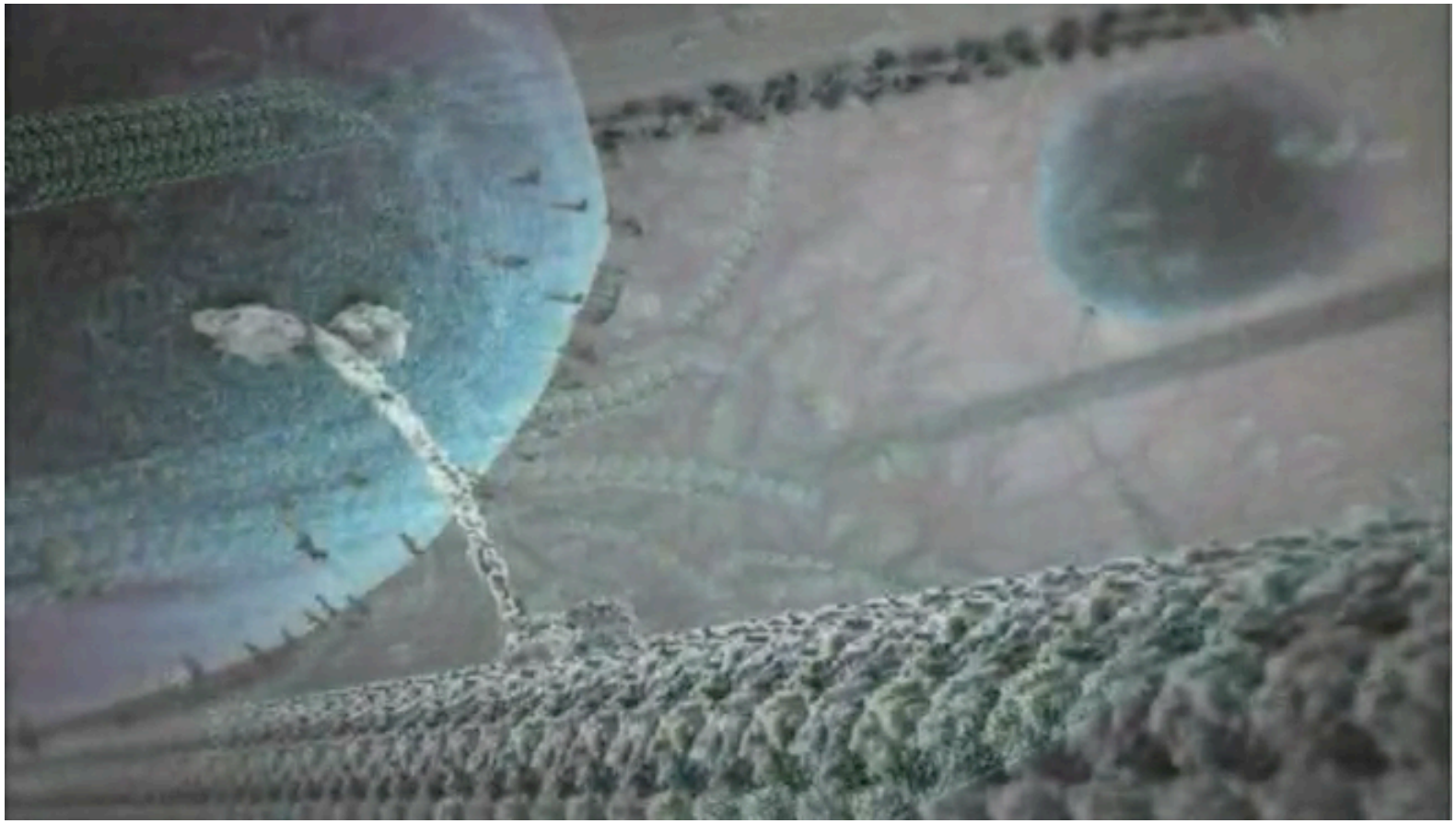
The cytoplasm is crowded!

Movement of large protein complexes will not occur by diffusion!

Fusion of viral and host cell membranes mediated by viral fusion proteins

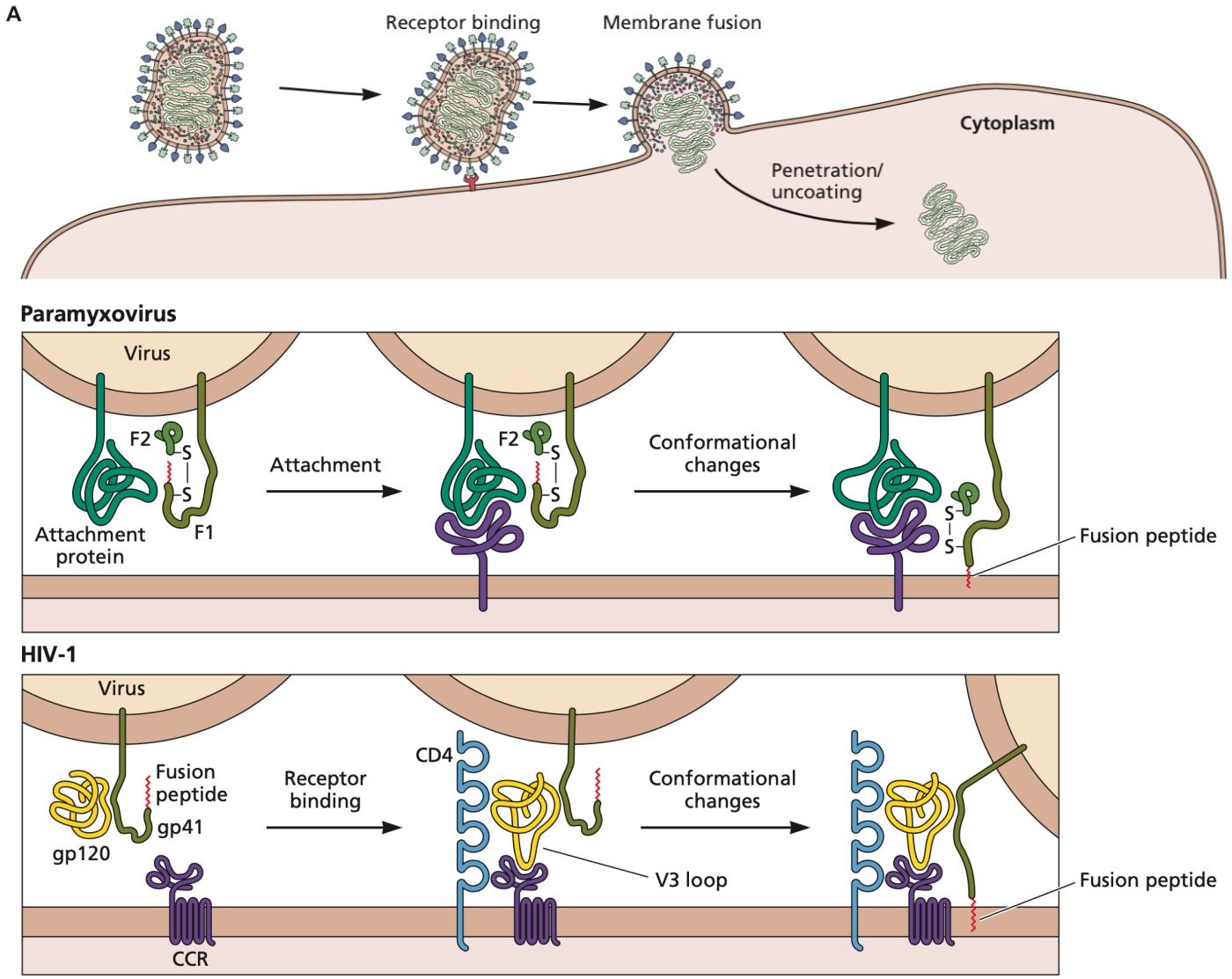


Movement of endosomes



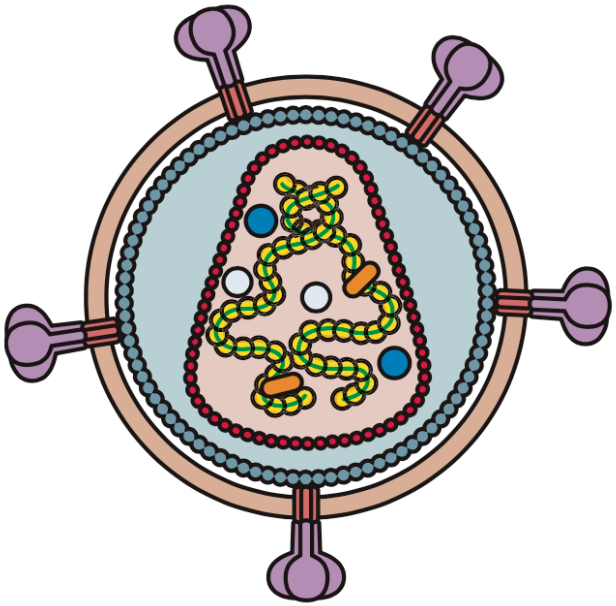
XVIVO Scientific Animation http://www.xvivo.net/

Entry at plasma membrane

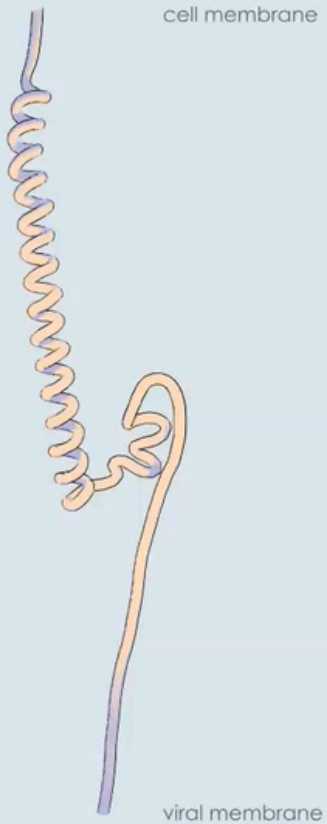


HIV-1

HIV-1 Env mediated fusion



During the fusion process, the C-terminal region that is anchored in the viral membrane undergoes an α-helical transition and runs alongside the outside of the core.



Go to:

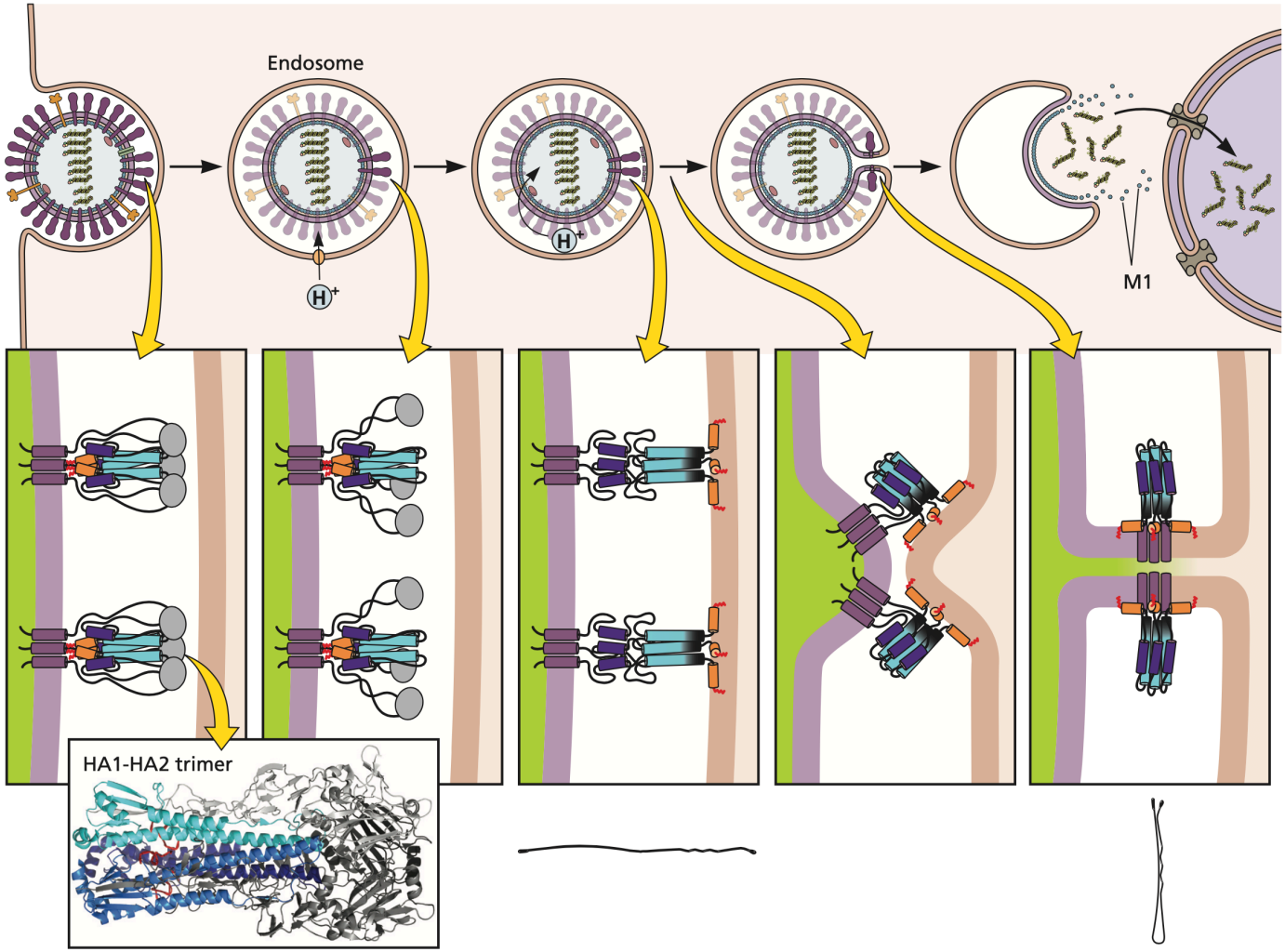
b.socrative.com/login/student

room number: virus

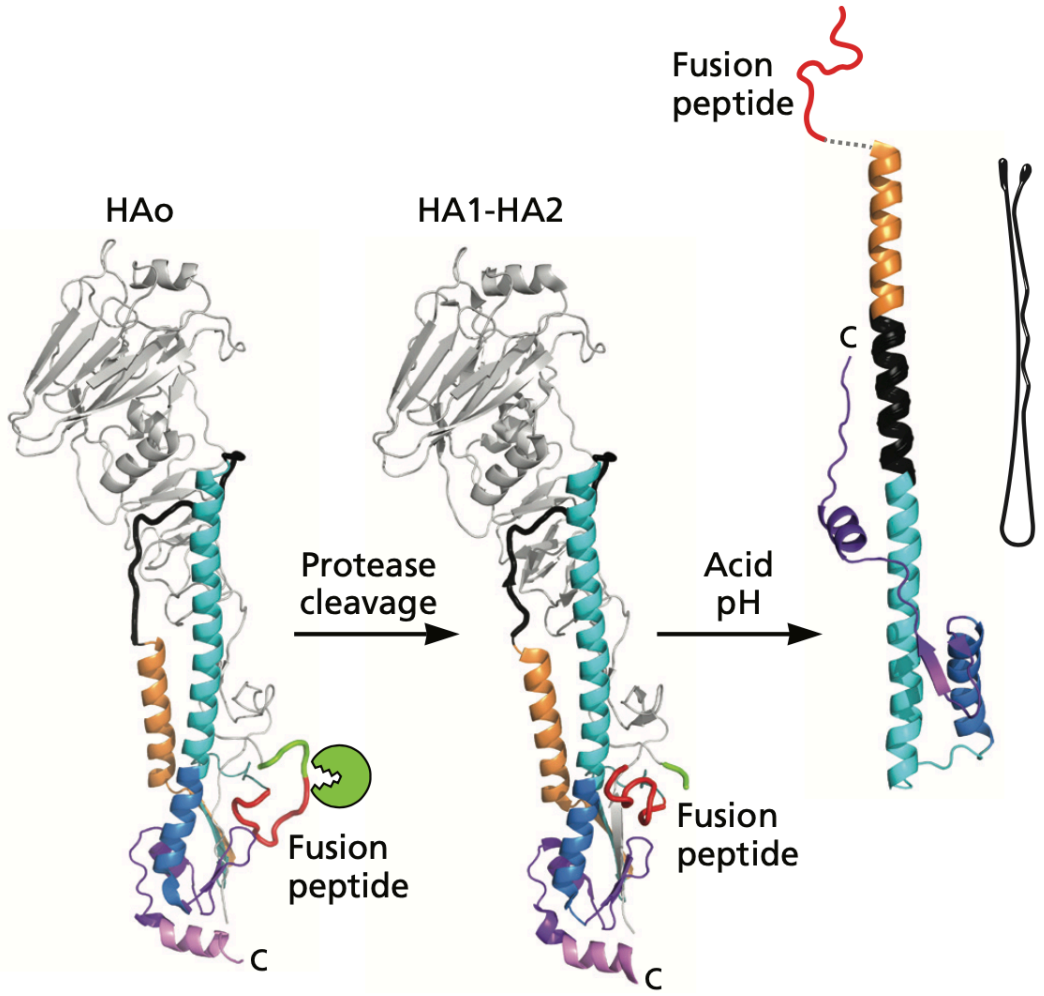
Which of the following does not play a role in virus entry:

A. Clathrin-mediated endocytosis
B. Fusion of viral and plasma membranes
C. Diffusion of virus particles in the cytoplasm
D. Microtubule-mediated transport
E. Sialic acids

Influenza virus membrane fusion

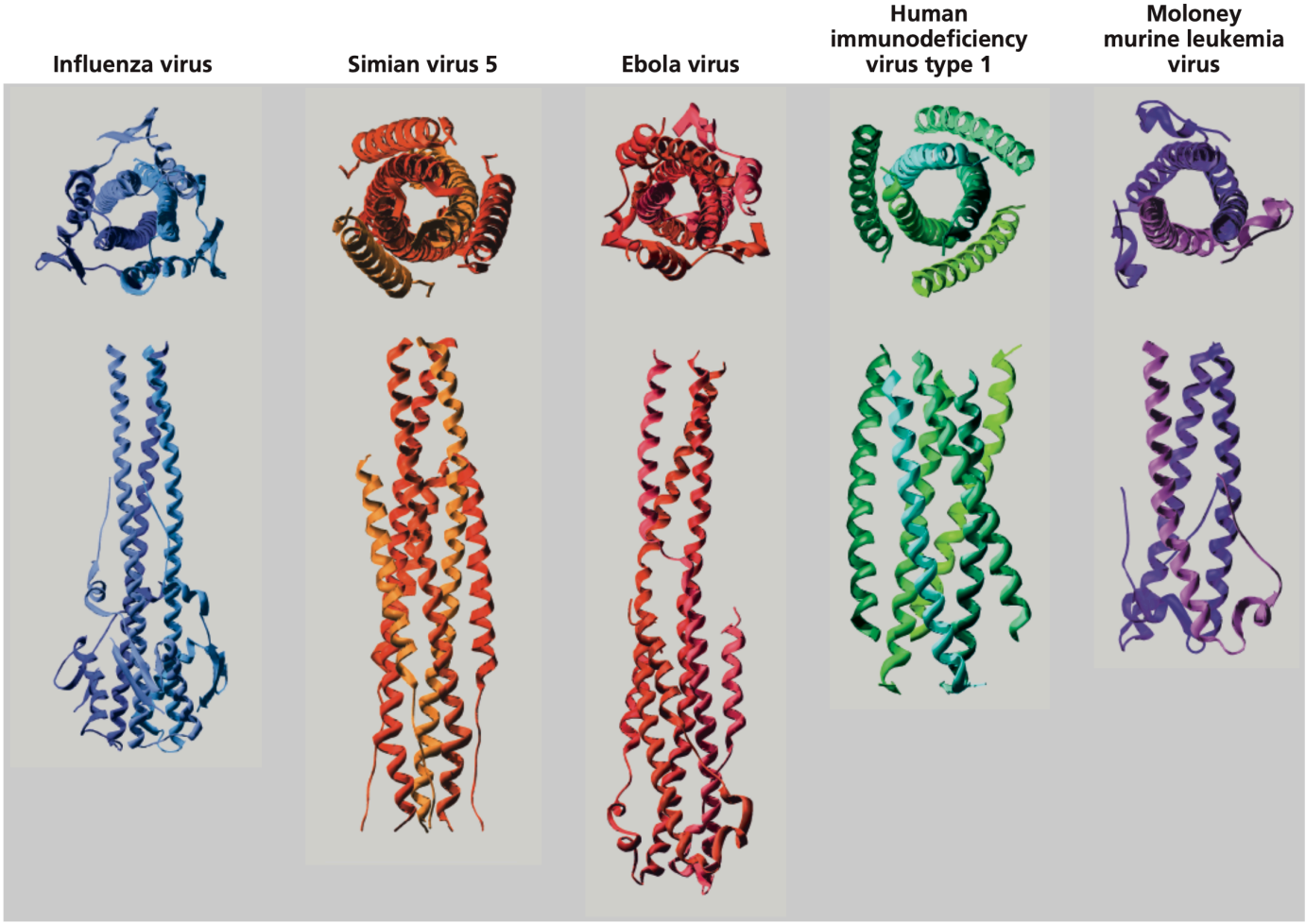


Three states of influenza virus HA



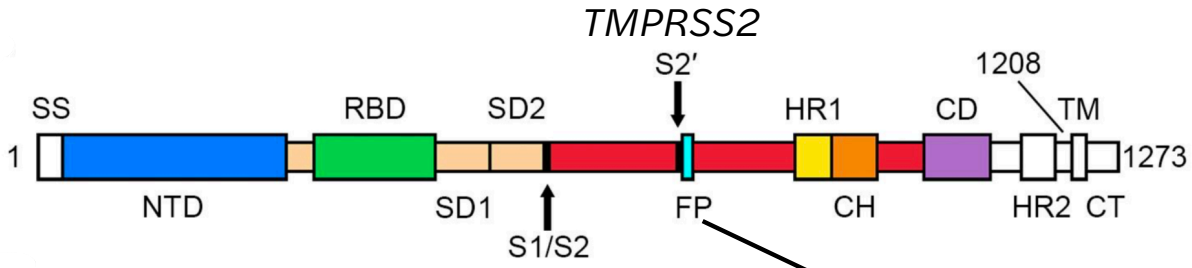
Class I fusion proteins

- Perpendicular to membrane - spikes
- Mostly α-helical
- Form trimers

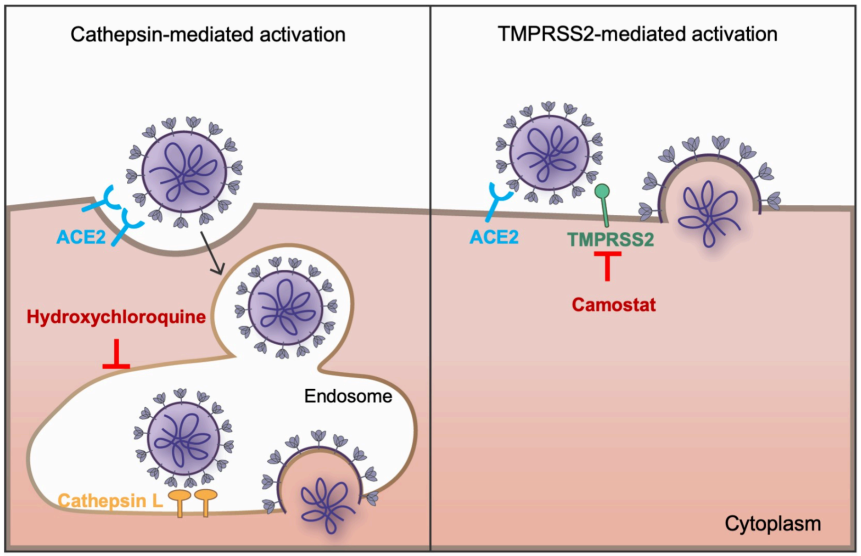


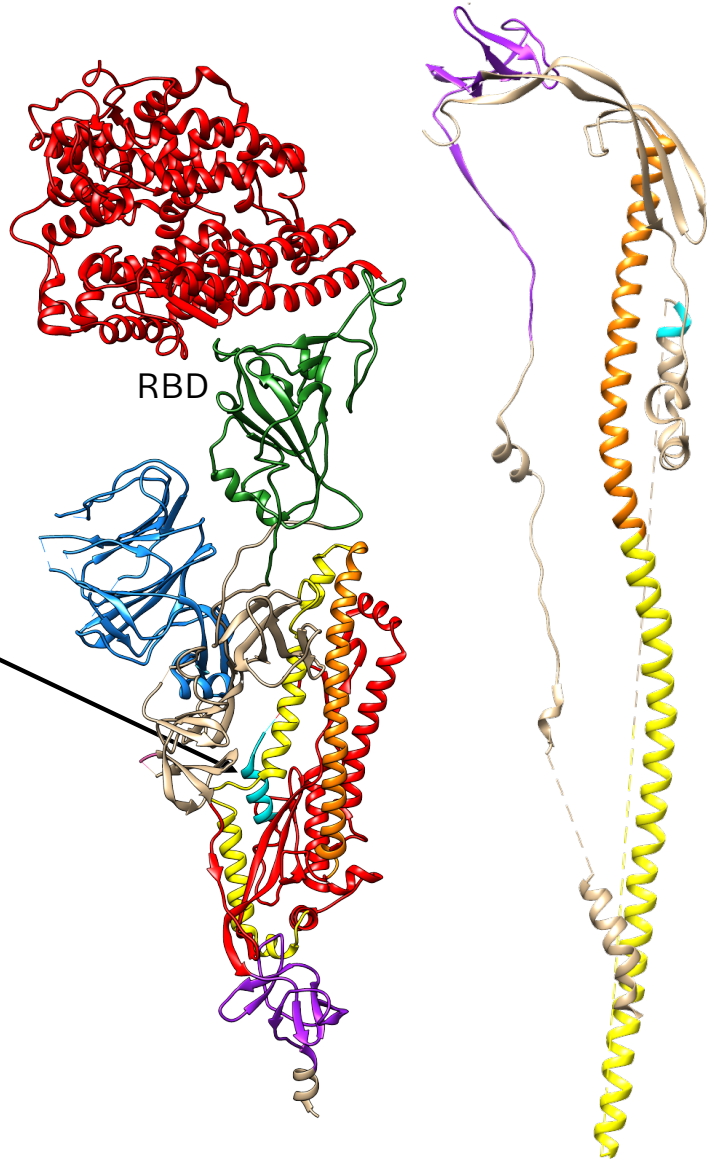
Influenza virus entry

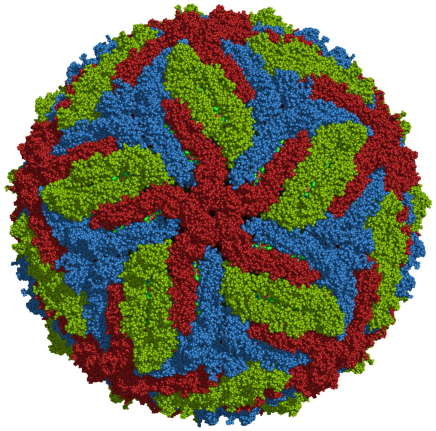
SARS-CoV-2 entry



Protease cleavage of spike needed for fusion: this process must be regulated

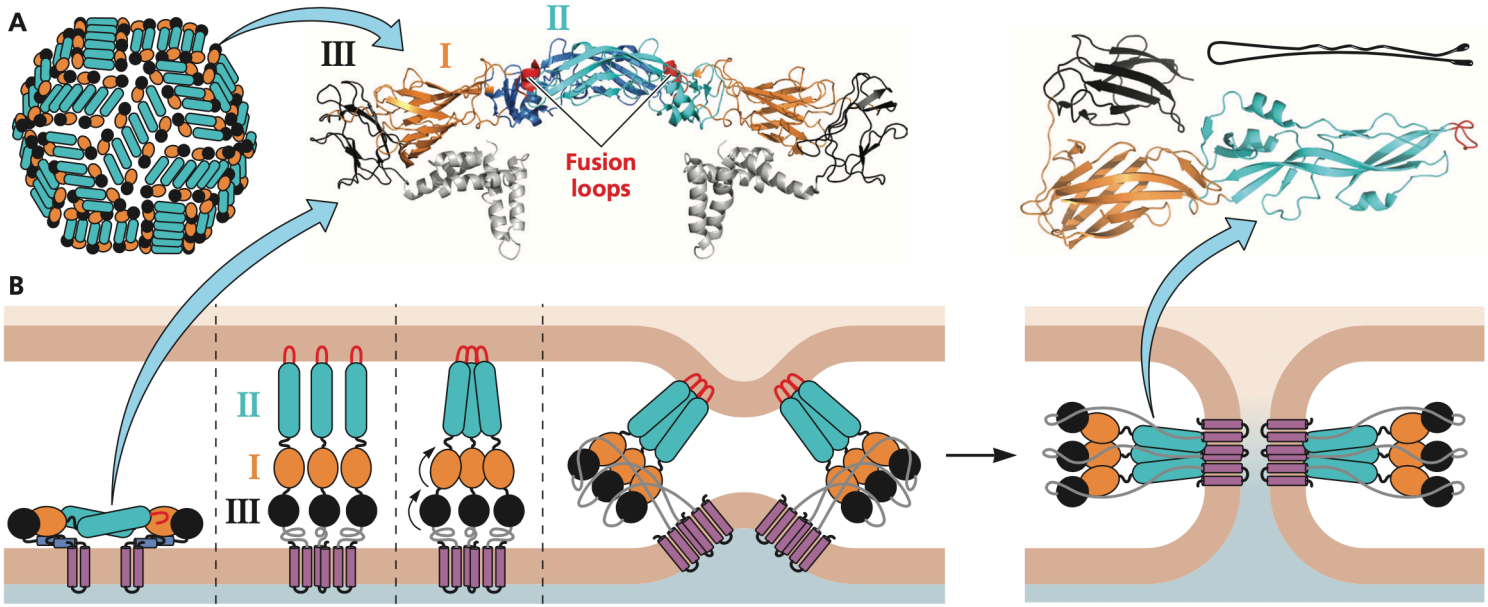




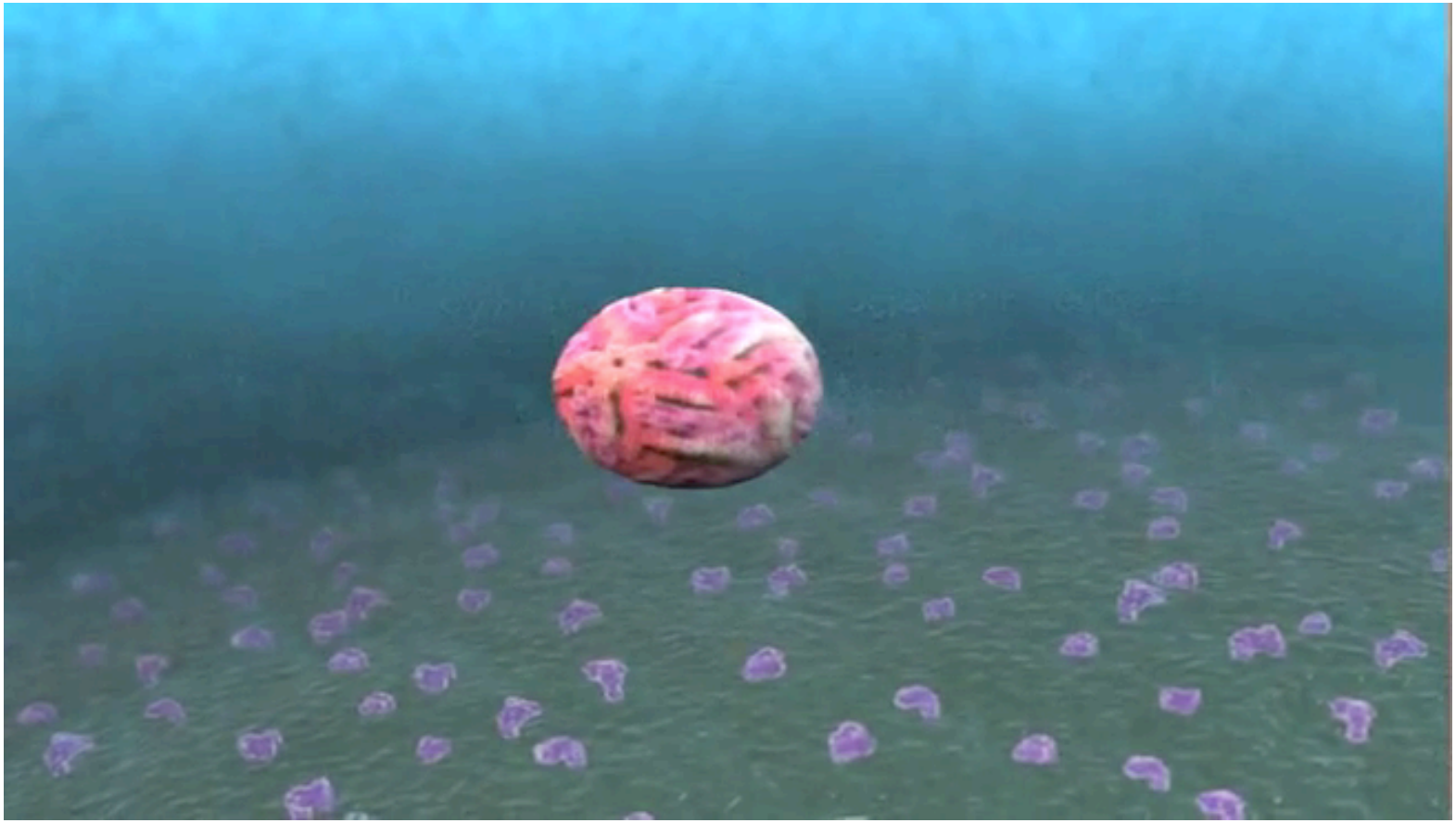


Class II fusion proteins

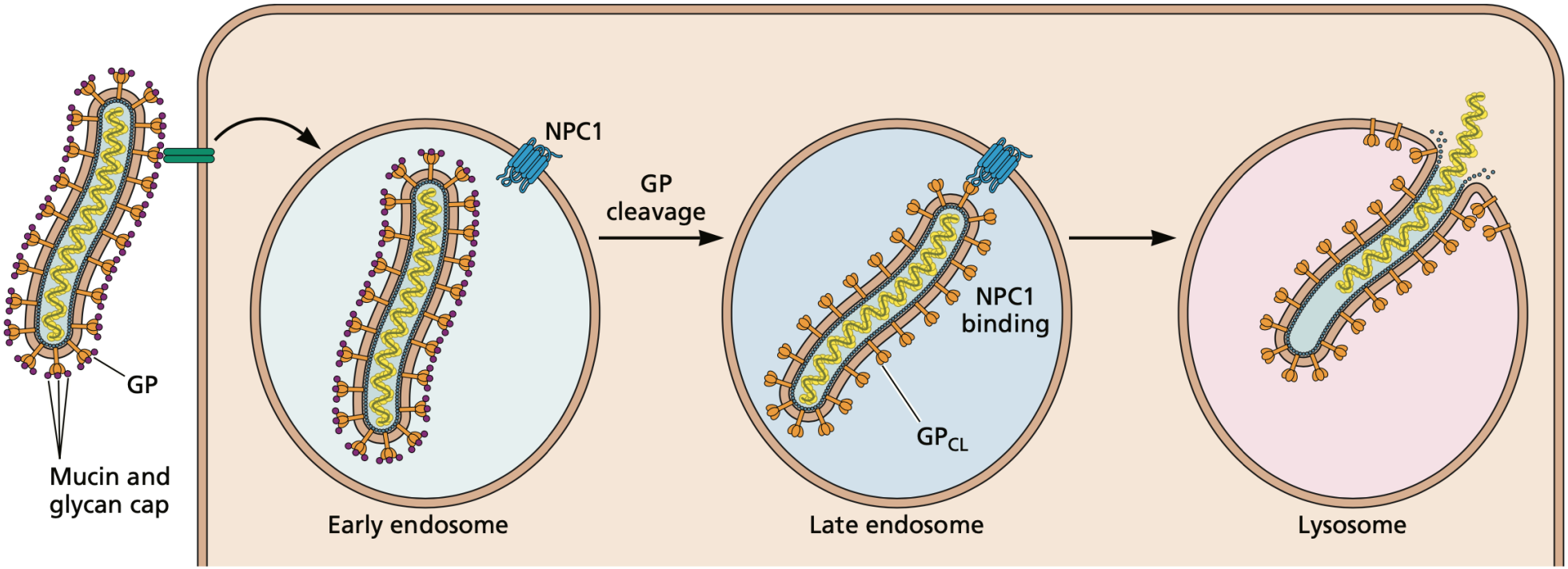
- Mostly β-sheet
- Form dimers
- Parallel to the membrane



Dengue virus entry

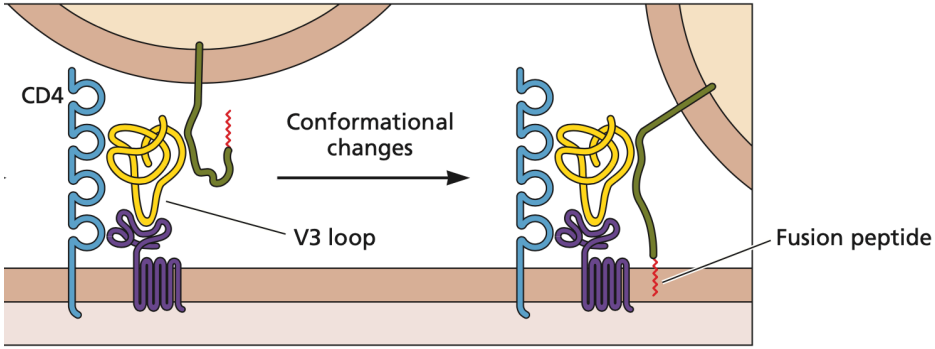


Ebolavirus entry



Fusion is regulated

- Must not occur in the wrong location
- Neutral pH (plasma membrane)
  - Second protein receptor interaction
- Low pH fusion
  - Proteolytic cleavage activates the fusion protein for cleavage (class I)
  - Endosome fusion receptor



Go to:

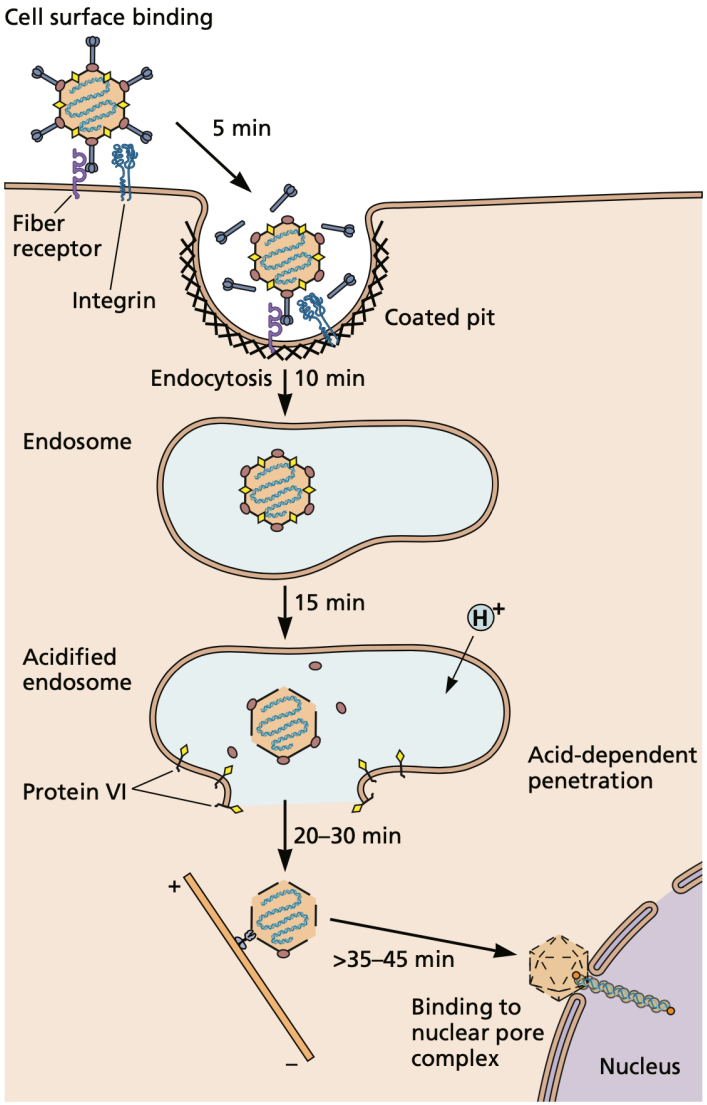
b.socrative.com/login/student
room number: virus

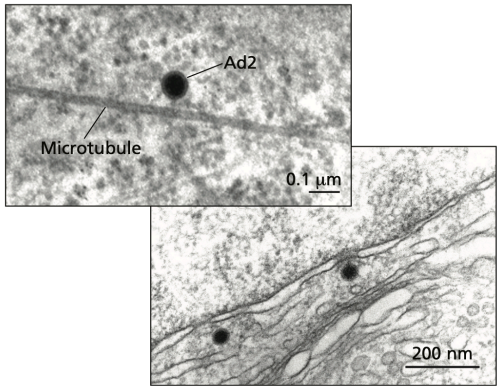
Viral fusion peptides are exposed for insertion into the host cell membrane when:

A. The virus particle is near a cell
B. The virus particle is in the cytoplasm
C. Trimers of the fusion peptides form
D. The endosome becomes acidified
E. The virus is docked on the nuclear pore

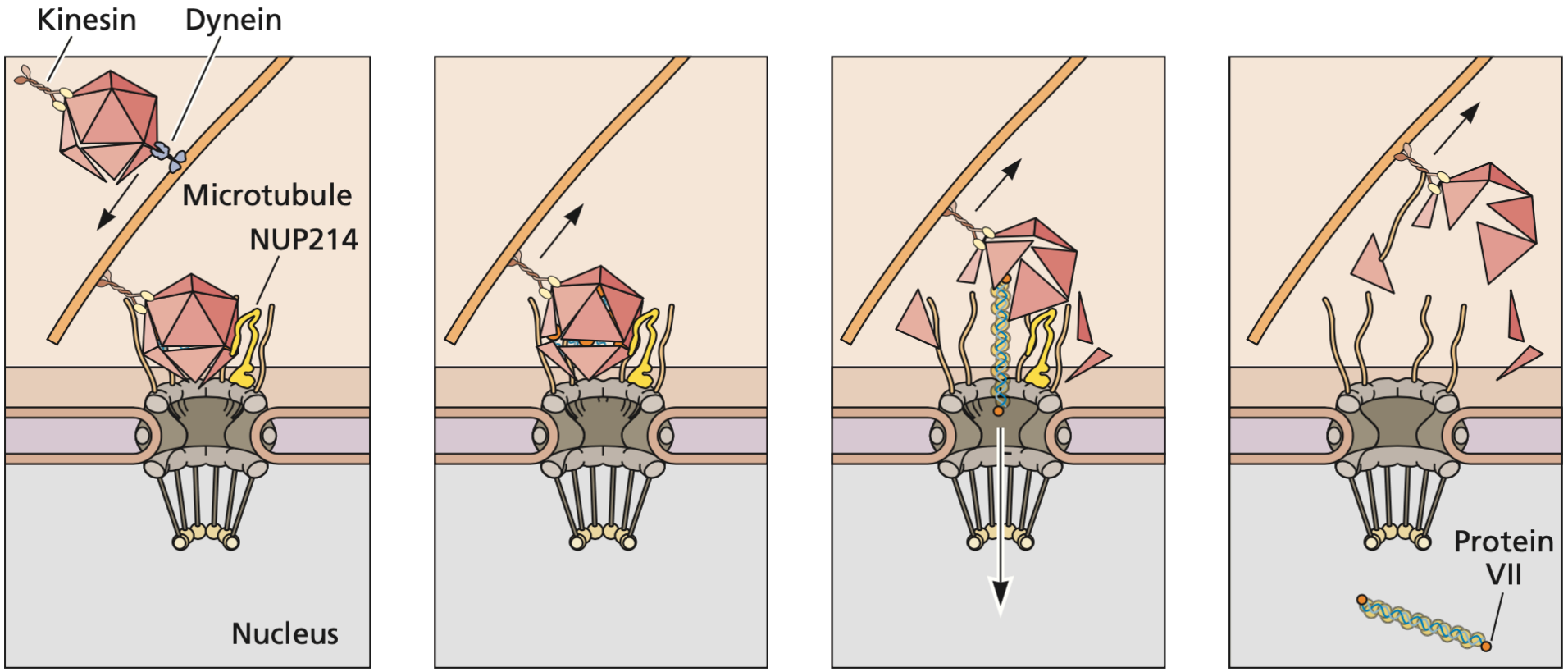
How are genomes released from non-enveloped viruses?

Adenovirus

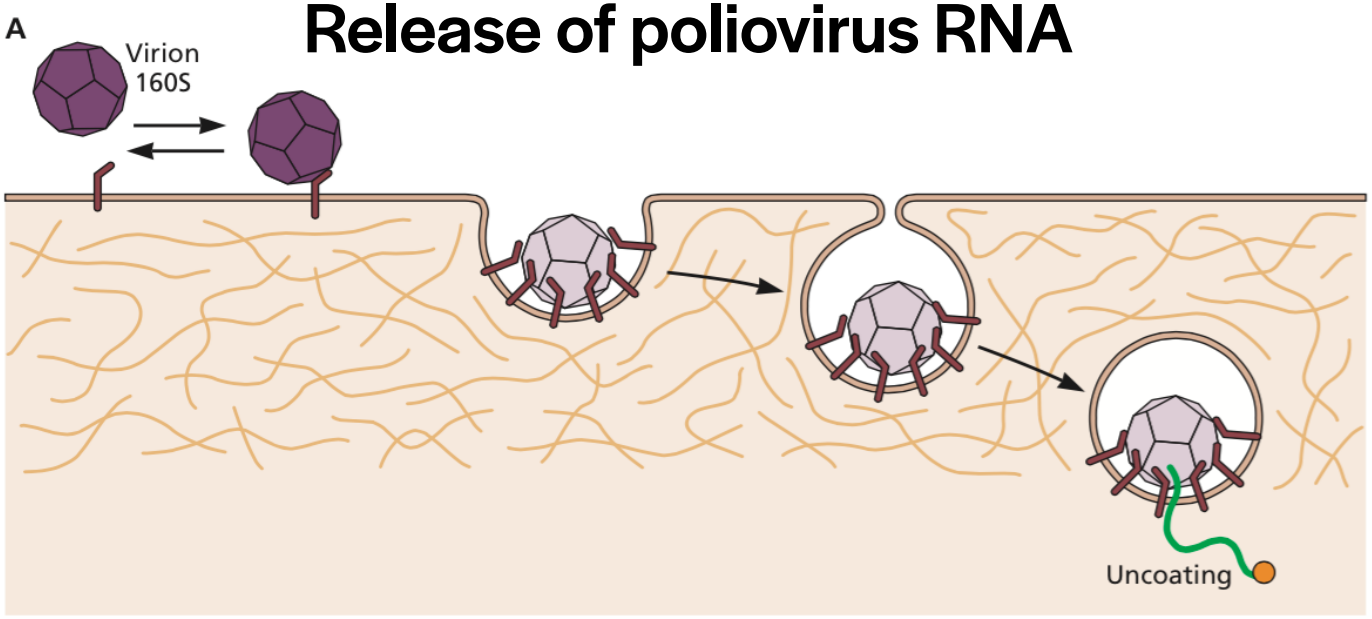


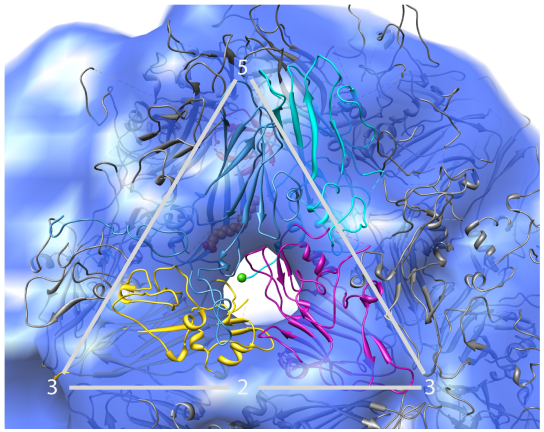


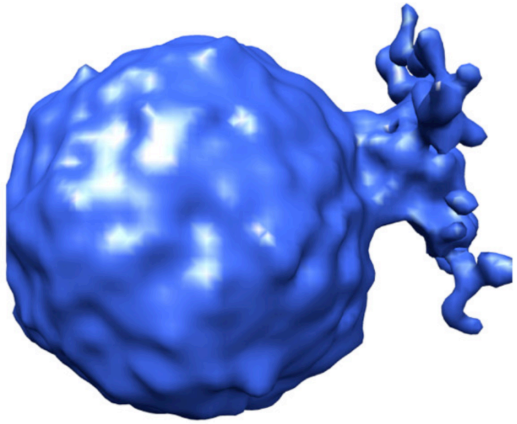
Uncoating of adenovirus at nuclear pore complex



Release of poliovirus RNA

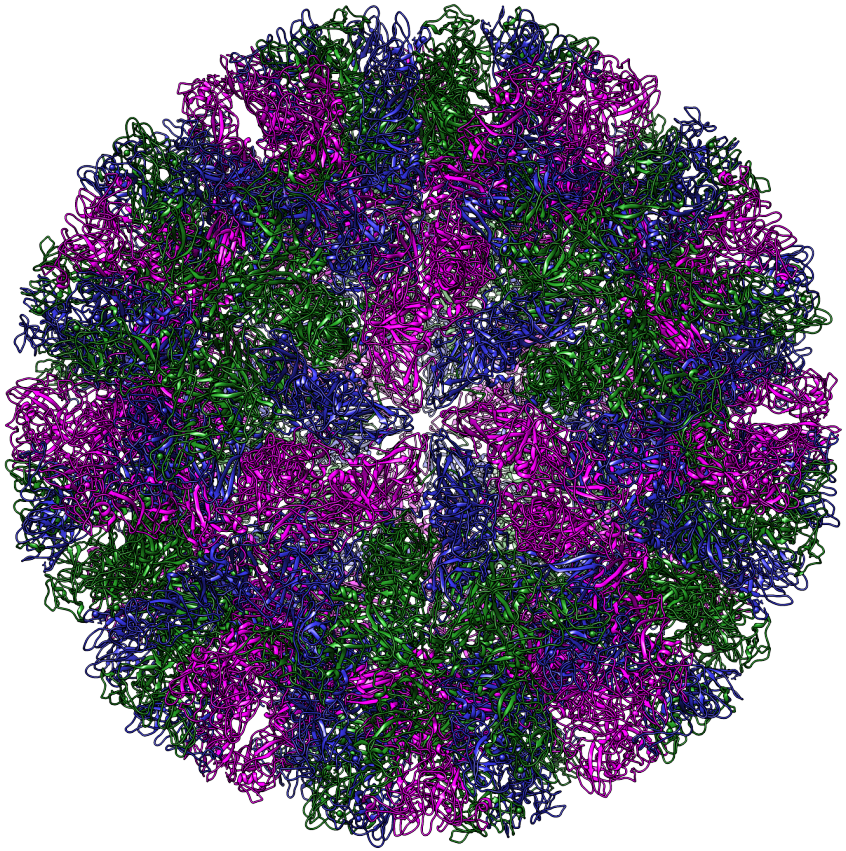


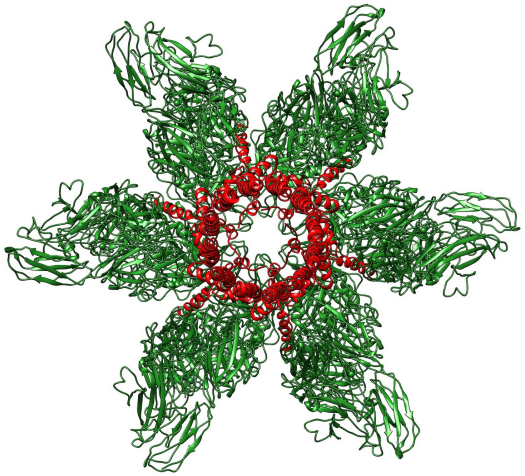


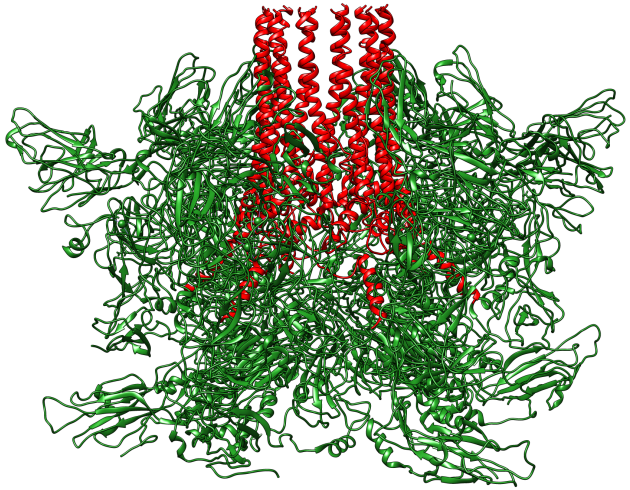


"Umbilicus" connector

Calicivirus portal on receptor engagement



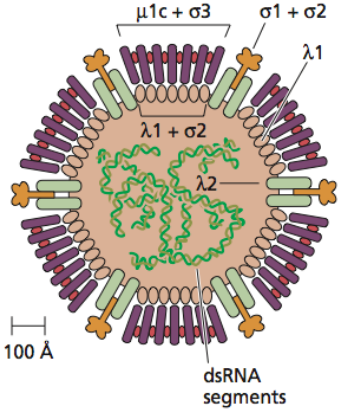


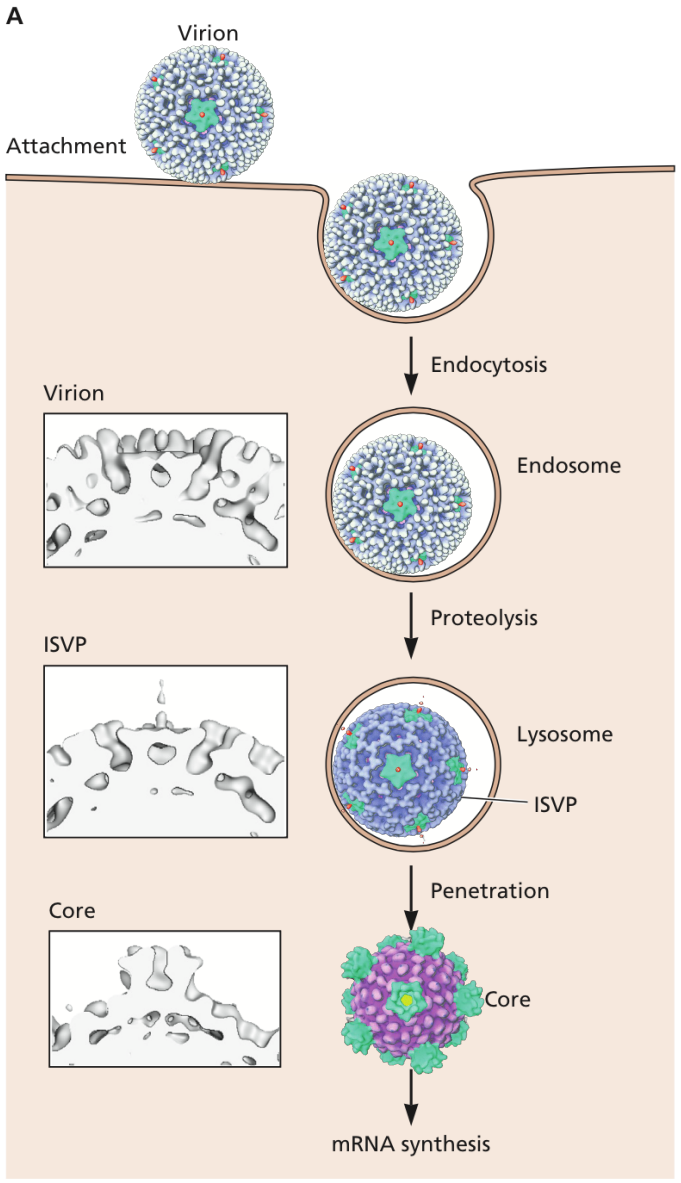


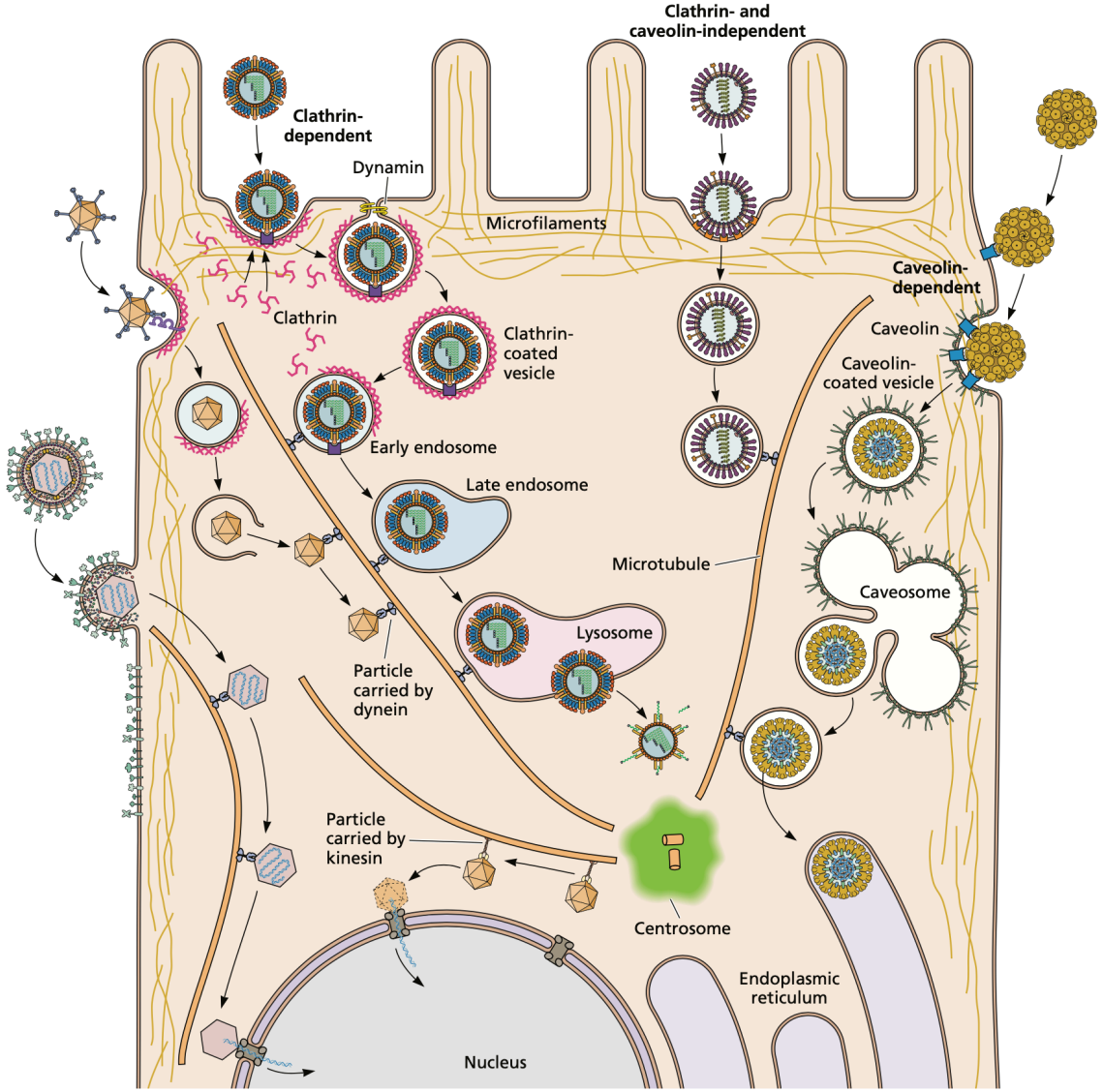
t=3, 180 copies of VP1

For poliovirus and calicivirus, only one portal is formed

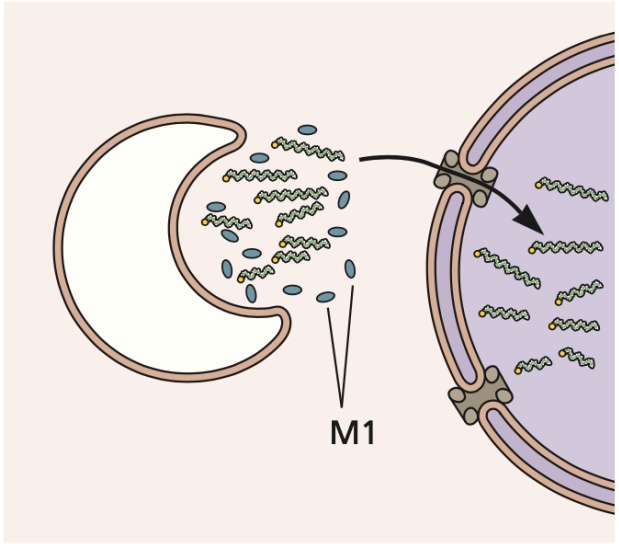
Reovirus entry

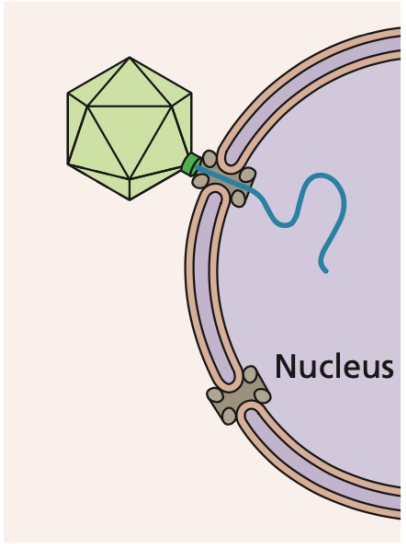


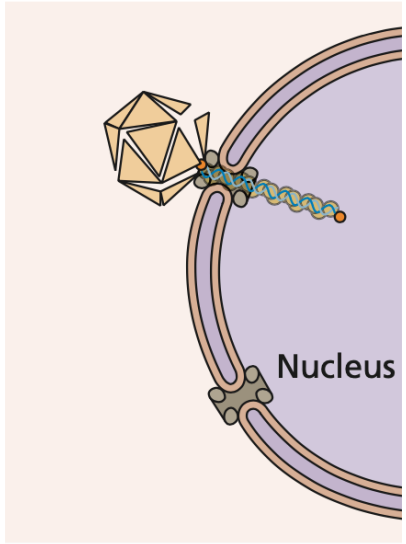


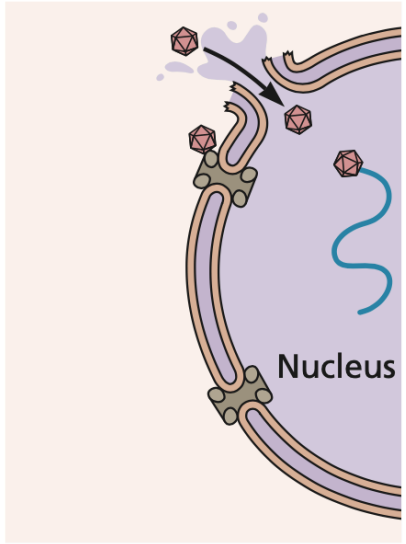


Four modes of nuclear entry





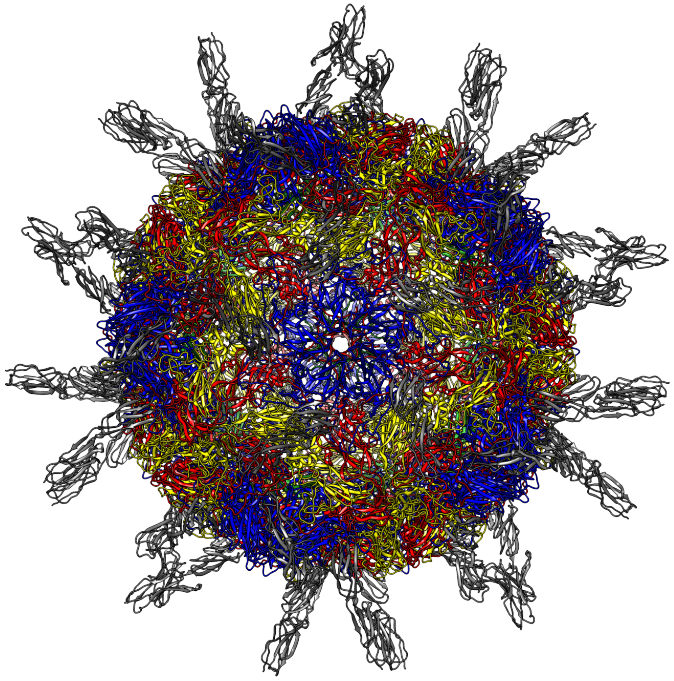


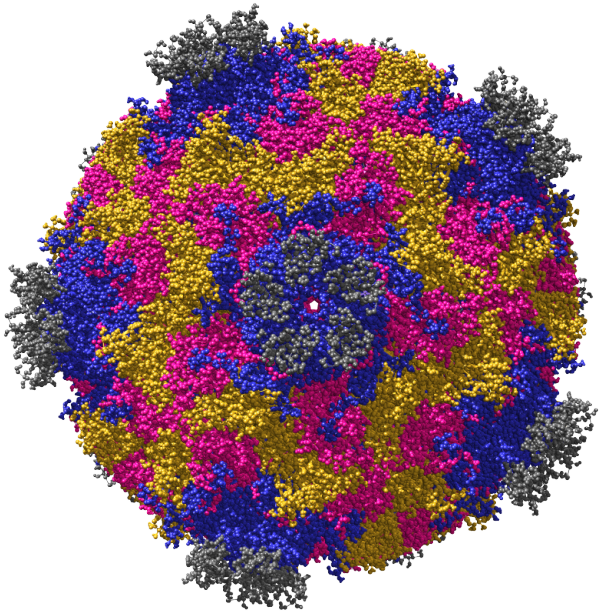


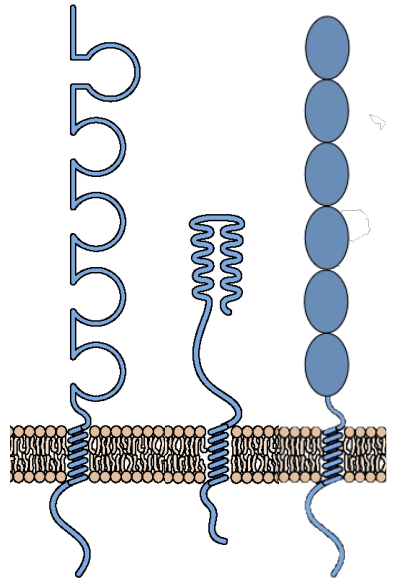
Receptor polymorphism and susceptibility

Rhinovirus receptors

- HRV A, B: 90 serotypes, ICAM-1; 10 serotypes LDLR
- HRV C: 49 genotypes, CDHR3

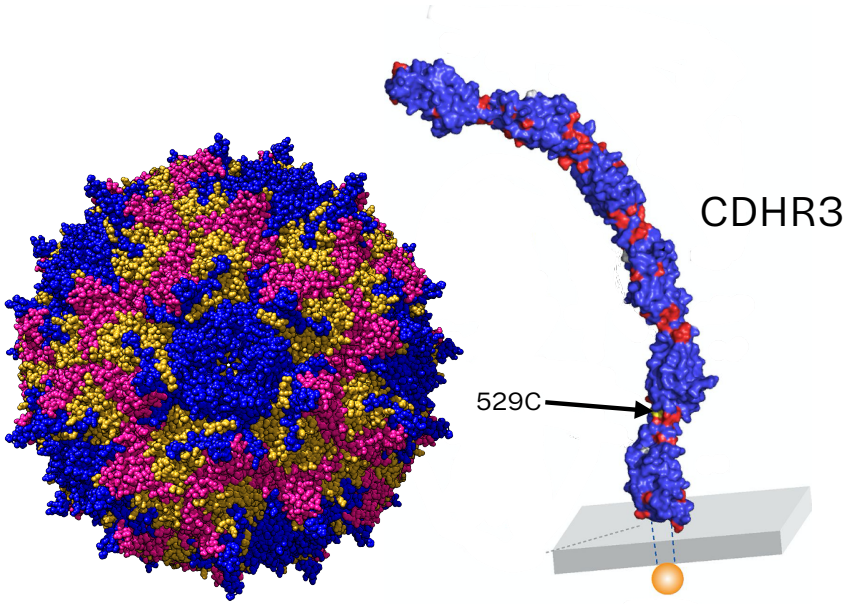






HRV-C

- 529Y in CDHR3 linked to increased surface production, increased risk of wheezing illnesses and hospitalization for childhood asthma
- Cells with CCHR3 529Y have 10x increased HRV-C binding and yields
- Risk factor for HRV-C wheezing illnesses
- 2013 outbreak of HRV-C in chimpanzees, Uganda: all homozygous 529Y
- Only humans have 529C, recently selected as Neanderthal and Denisovans have 529Y



Betty

Next time: RNA directed RNA synthesis