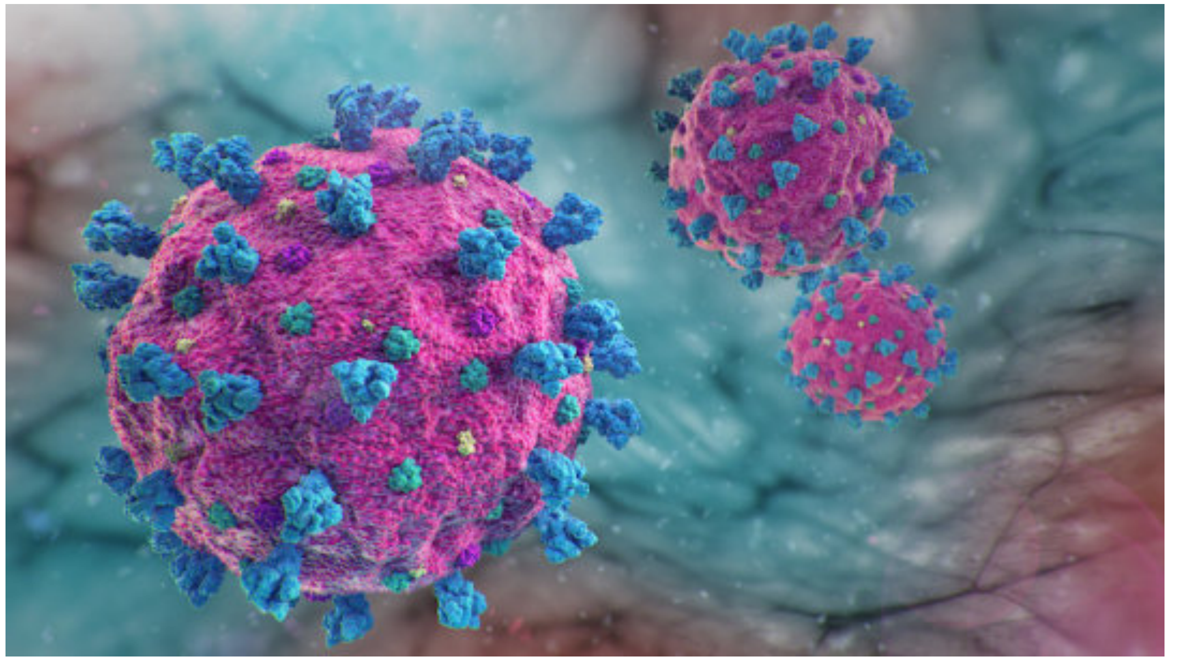
Viral Entry

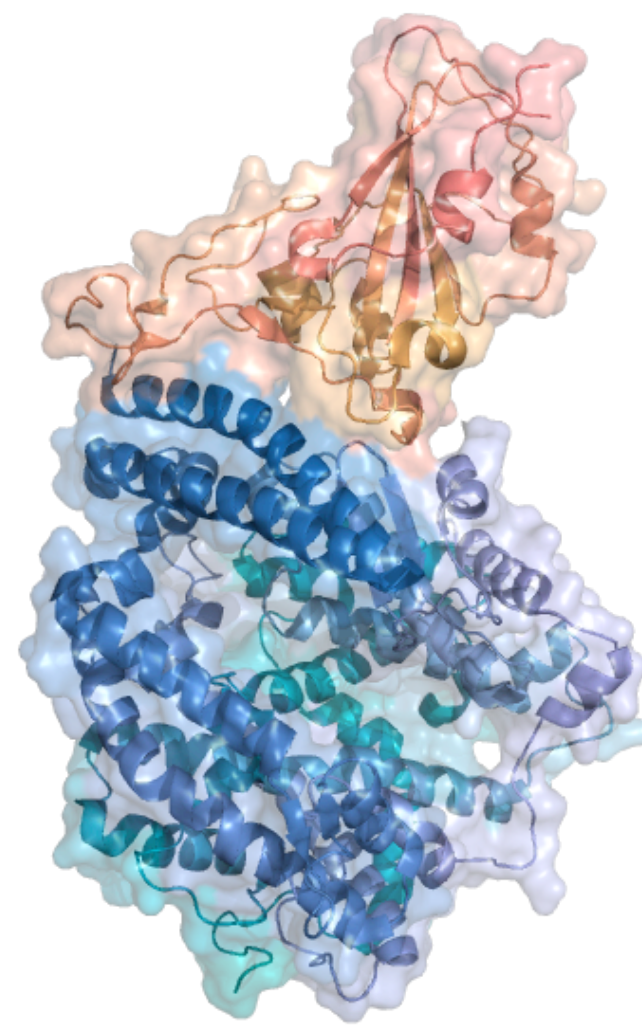


MacMillan Group Meeting
May 04, 2020

Daniel Kim

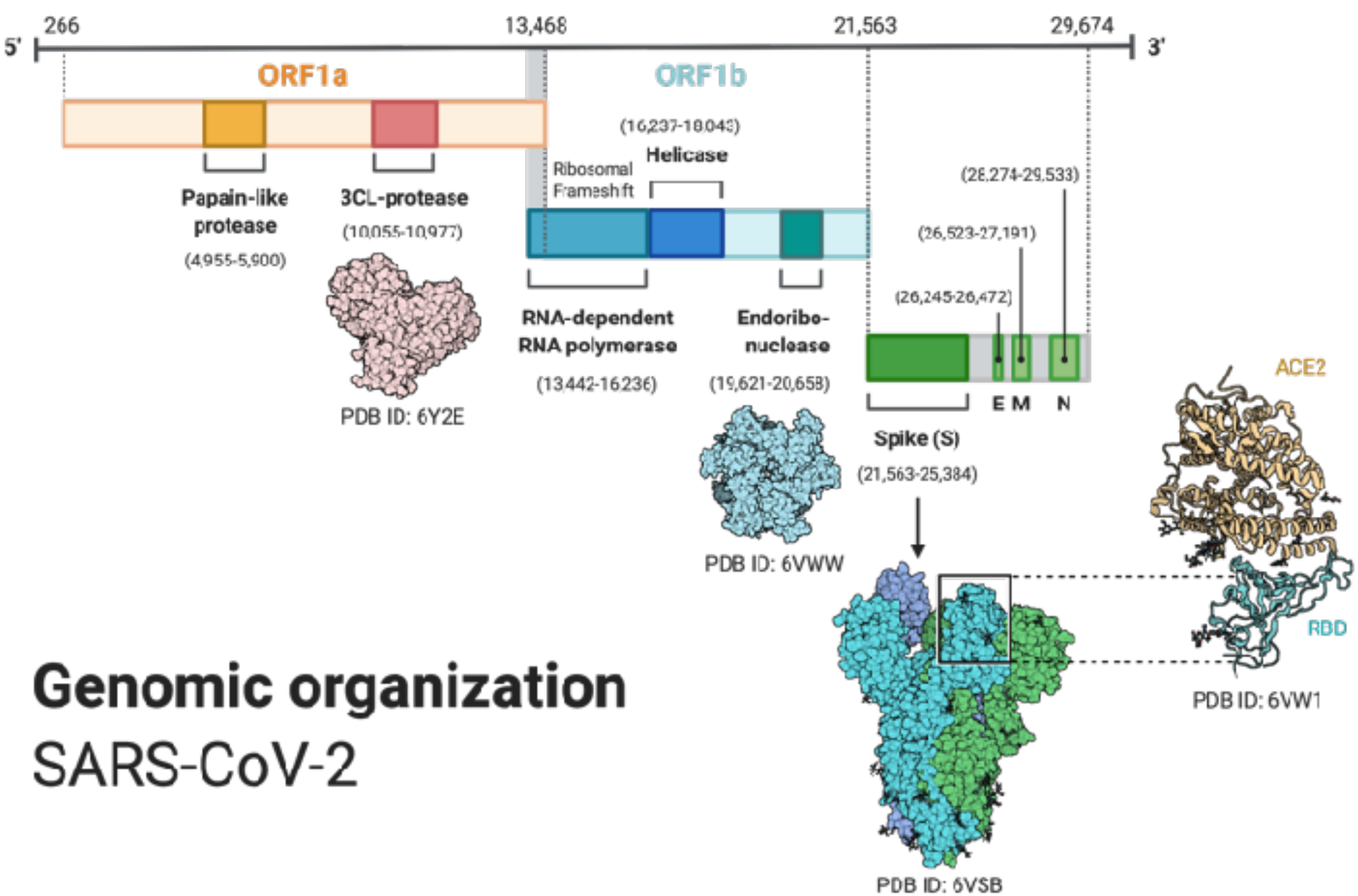
Outline

- Brief Overview of Viruses & Nomenclature
- General Mechanisms of Viral Entry
- Important Factors for Successful Viral Entry
    - Attachement
    - Signaling
    - Endocytosis
    - Penetration
    - Uncoating



- Details Associated Towards SARS-CoV-2 & Other Examples

Virus Overview



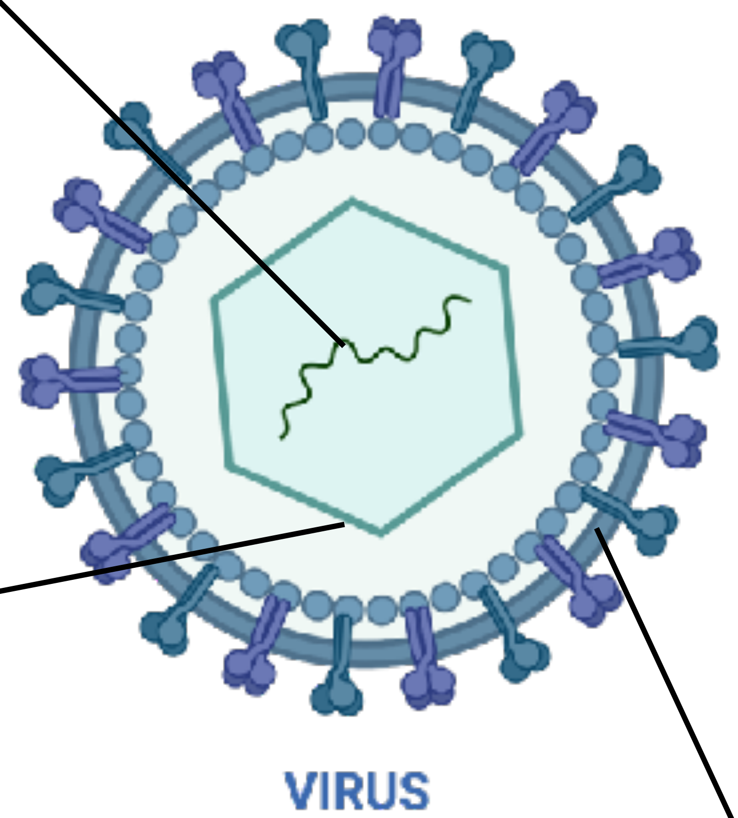
Virus Overview

DNA or RNA

the genetic information of the virus responsible for viral replication

Baltimore Classification System

simplified virus classification based on the viral use of nucleic acid



Capsid Shell

tightly packs nucleic information

Lipid Bilayer Membrane

found only in envelope viruses & contains additional virally encoded proteins

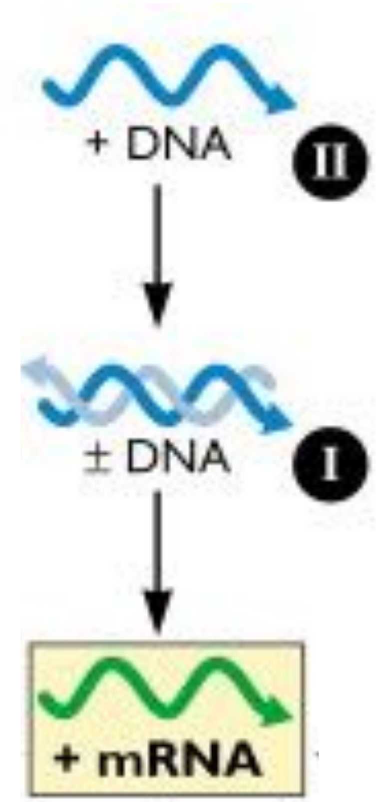
Virus Overview

Baltimore Classification System

mRNA encodes for proteins

Virus Overview

Baltimore Classification System



Transcription
RNA polymerase copies DNA sequence into RNA

Virus Overview

Baltimore Classification System

Class VI

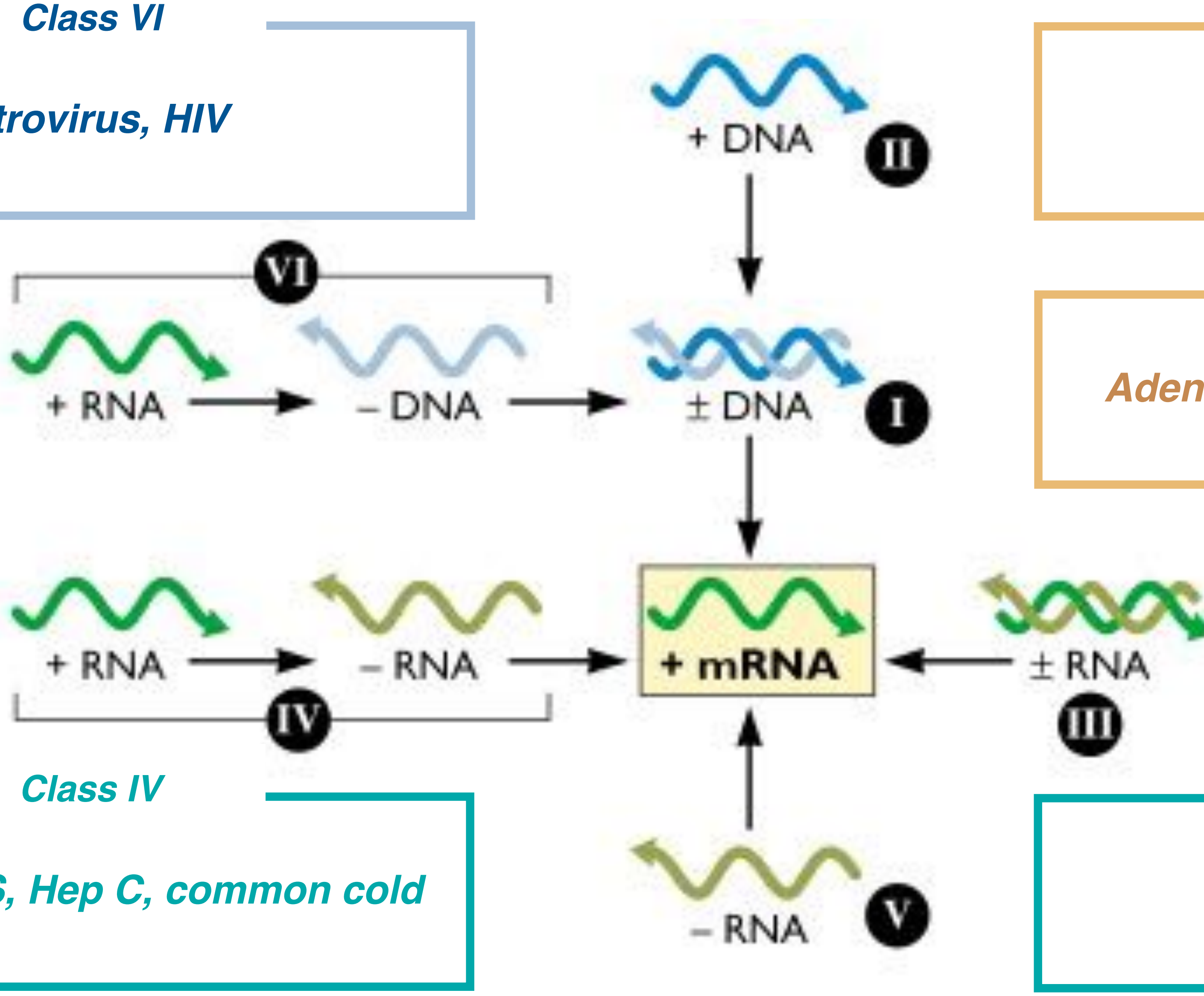
retrovirus, HIV

Class II

Parvoviruses

Class I

Adenovirus, Herpes & Small Pox



Class IV

SARS, MERS, Hep C, common cold

Class III

rotavirus

Class V

influenza, ebola, marburg

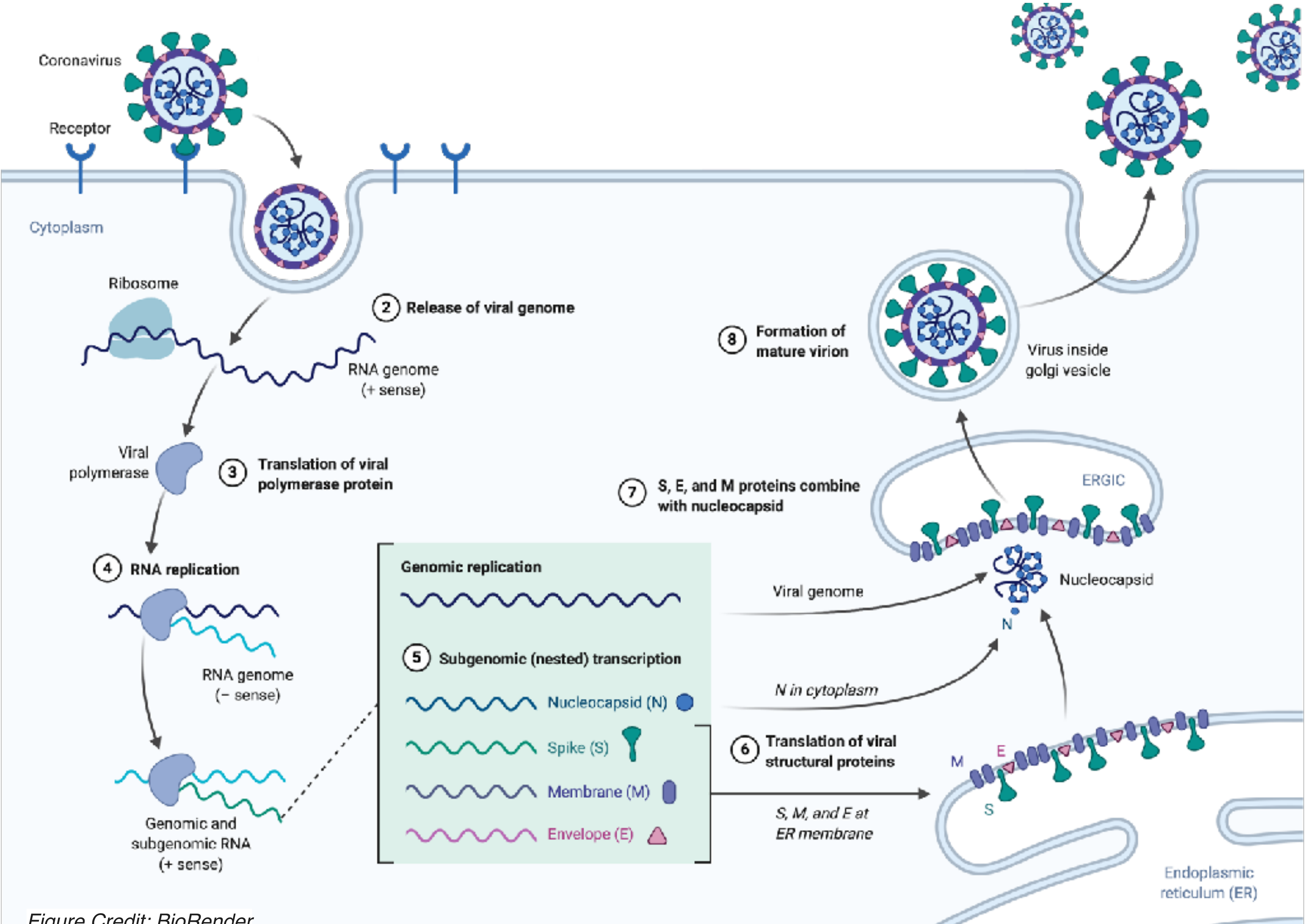
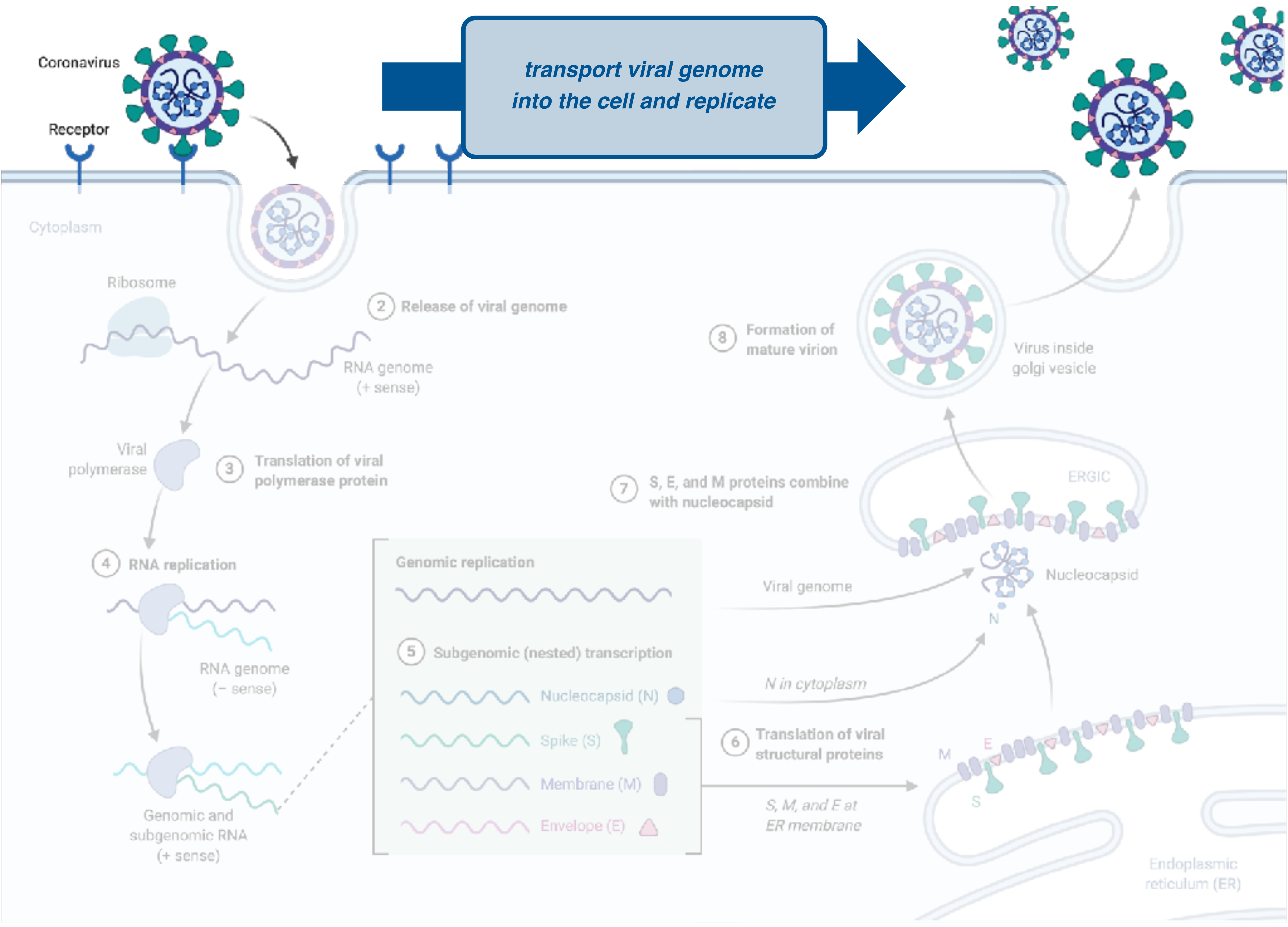
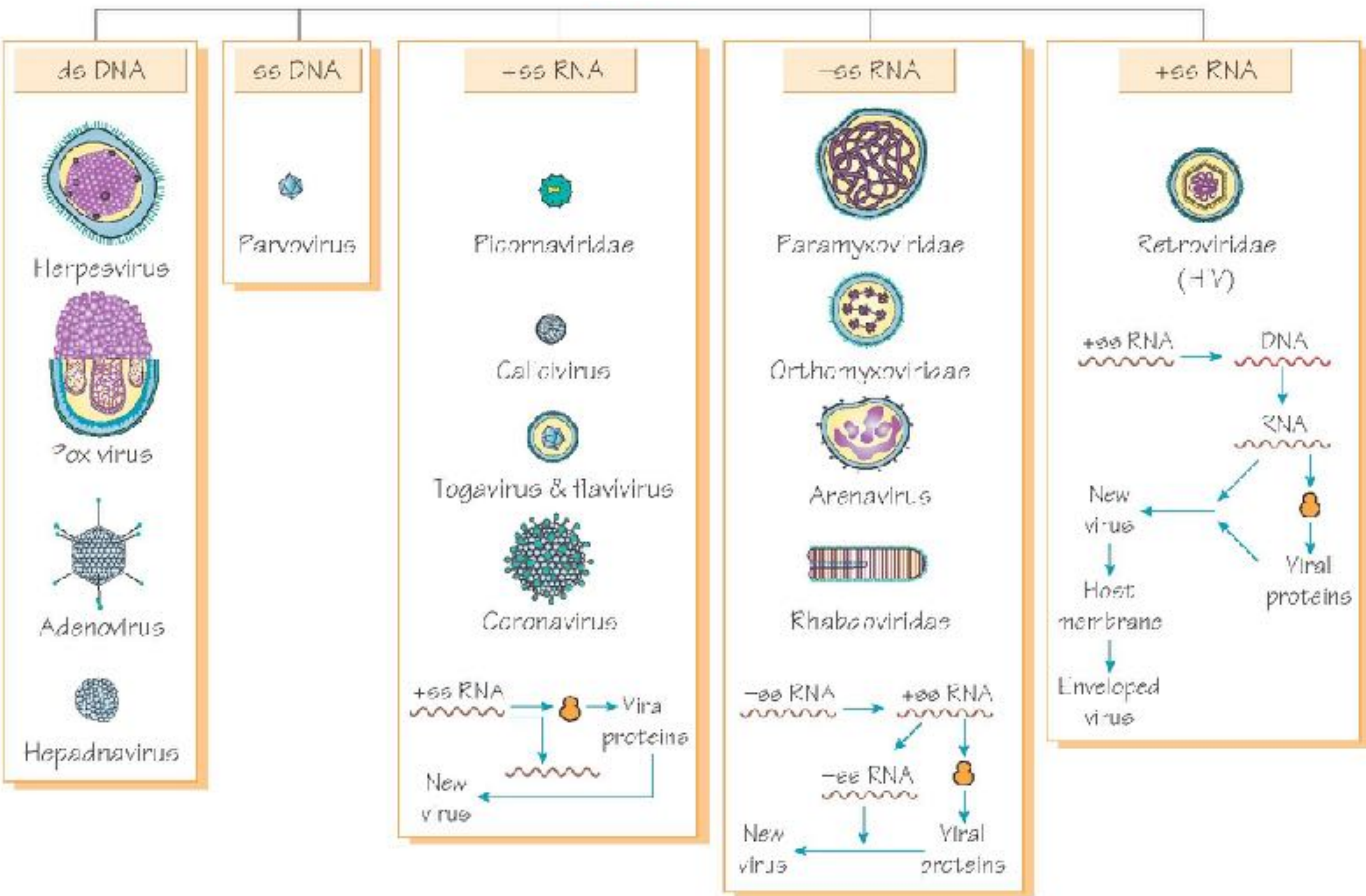


Figure Credit: BioRender

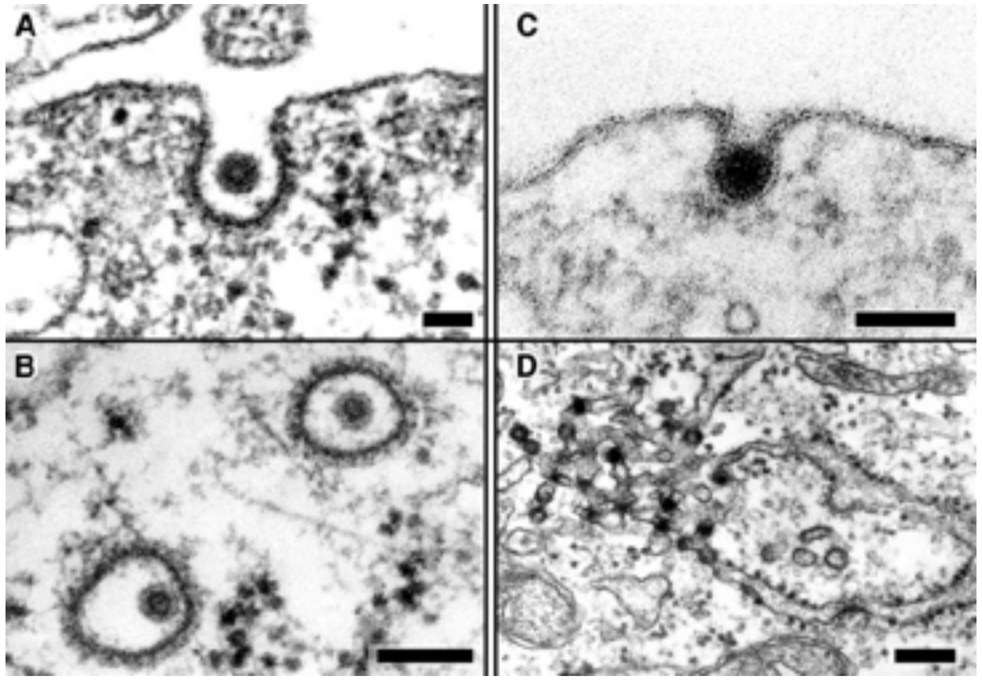


transport viral genome
into the cell and replicate
Coronavirus
Receptor
Cytoplasm
Ribosome
2 Release of viral genome
RNA genome
(+ sense)
Viral
polymerase
3 Translation of viral
polymerase protein
4 RNA replication
RNA genome
(− sense)
Genomic and
subgenomic RNA
(+ sense)
Genomic replication
5 Subgenomic (nested) transcription
Nucleocapsid (N)
Spike (S)
Membrane (M)
Envelope (E)
6 Translation of viral
structural proteins
S, M, and E at
ER membrane
N in cytoplasm
Viral genome
Nucleocapsid
N
M
E
S
7 S, E, and M proteins combine
with nucleocapsid
ERGIC
8 Formation of
mature virion
Virus inside
golgi vesicle
Endoplasmic
reticulum (ER)

Examples of Different Classes of Viruses



Challenges of Understanding Viral Entry



Understanding Size

× 1,000,000 size magnification

virus

orange

animal cell

circus tent

Techniques

light & electron microscopy

in vitro modeling

perterbations via

chemical inhibitors
mutant cells or viruses
siRNA silencing

Expertise

virology

cell & molecular biology

biochemistry & biophysics

systems biology

computer science

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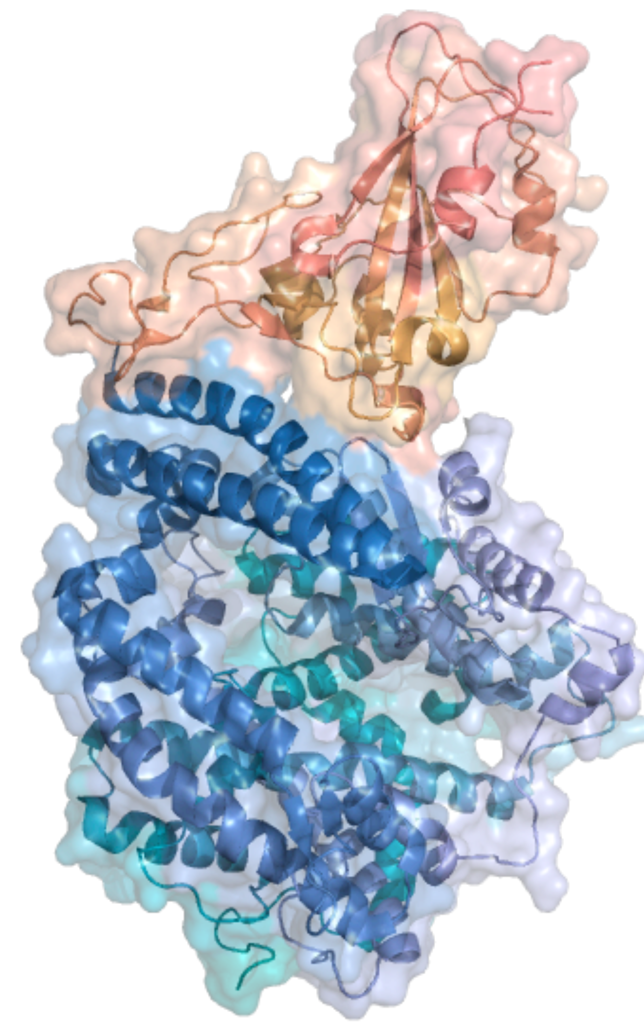
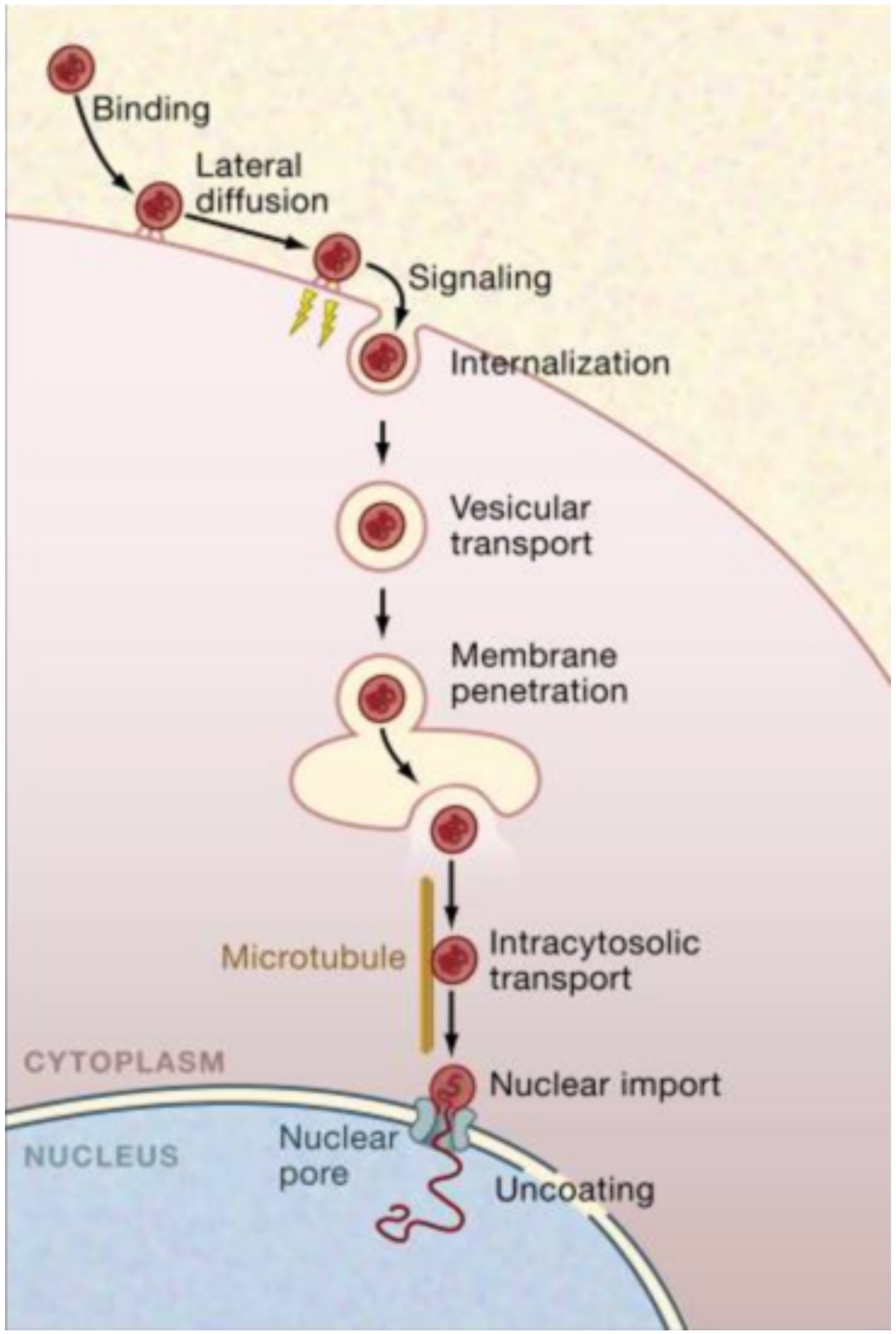


Viral Entry Overview



General Lessons Learned

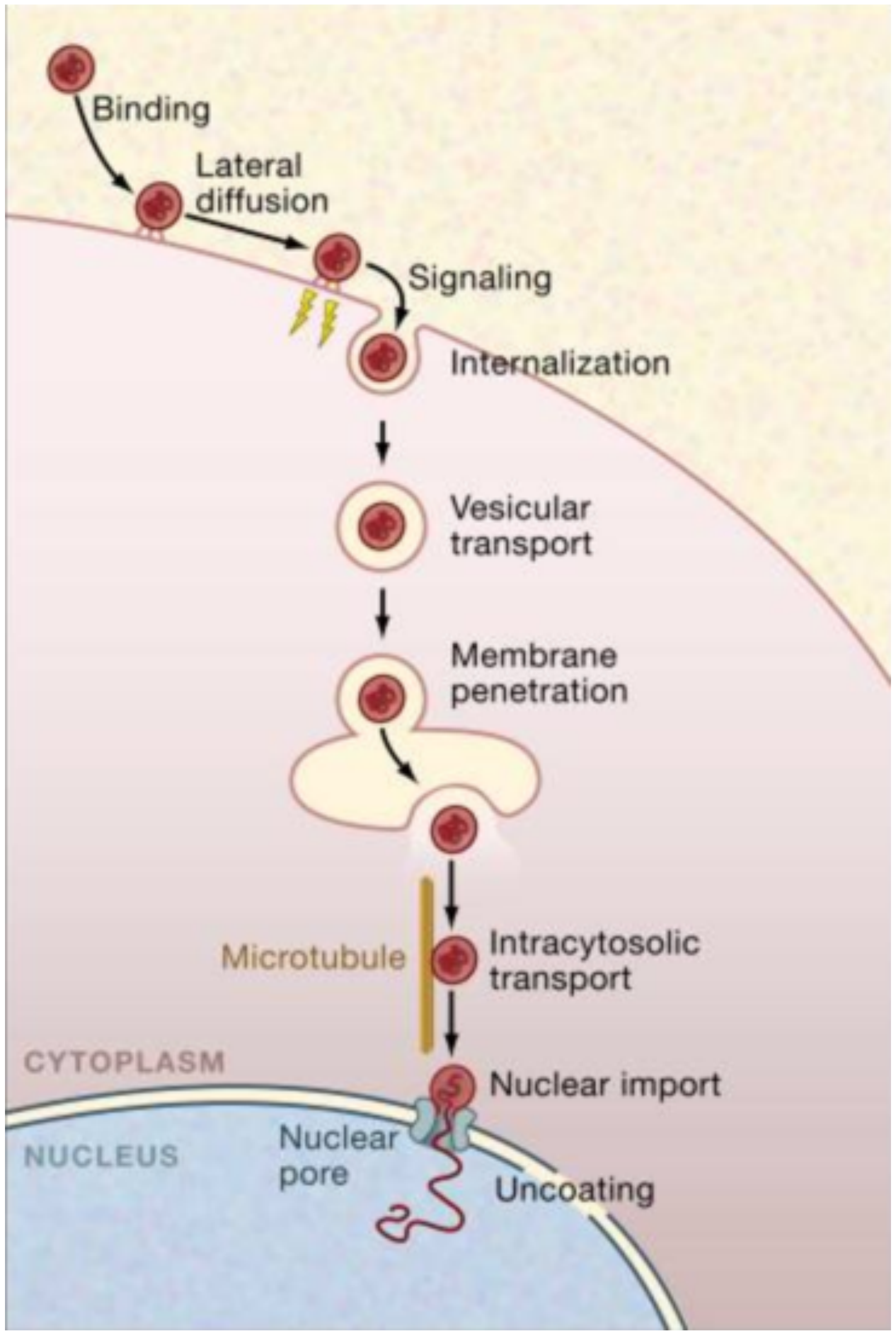
entry into the cell is a process of multiple steps
not simple, very complex system

uncoating: built-in program (passive)

virus proteins are meta-stable

proteins respond to cellular cues

Viral Entry Overview



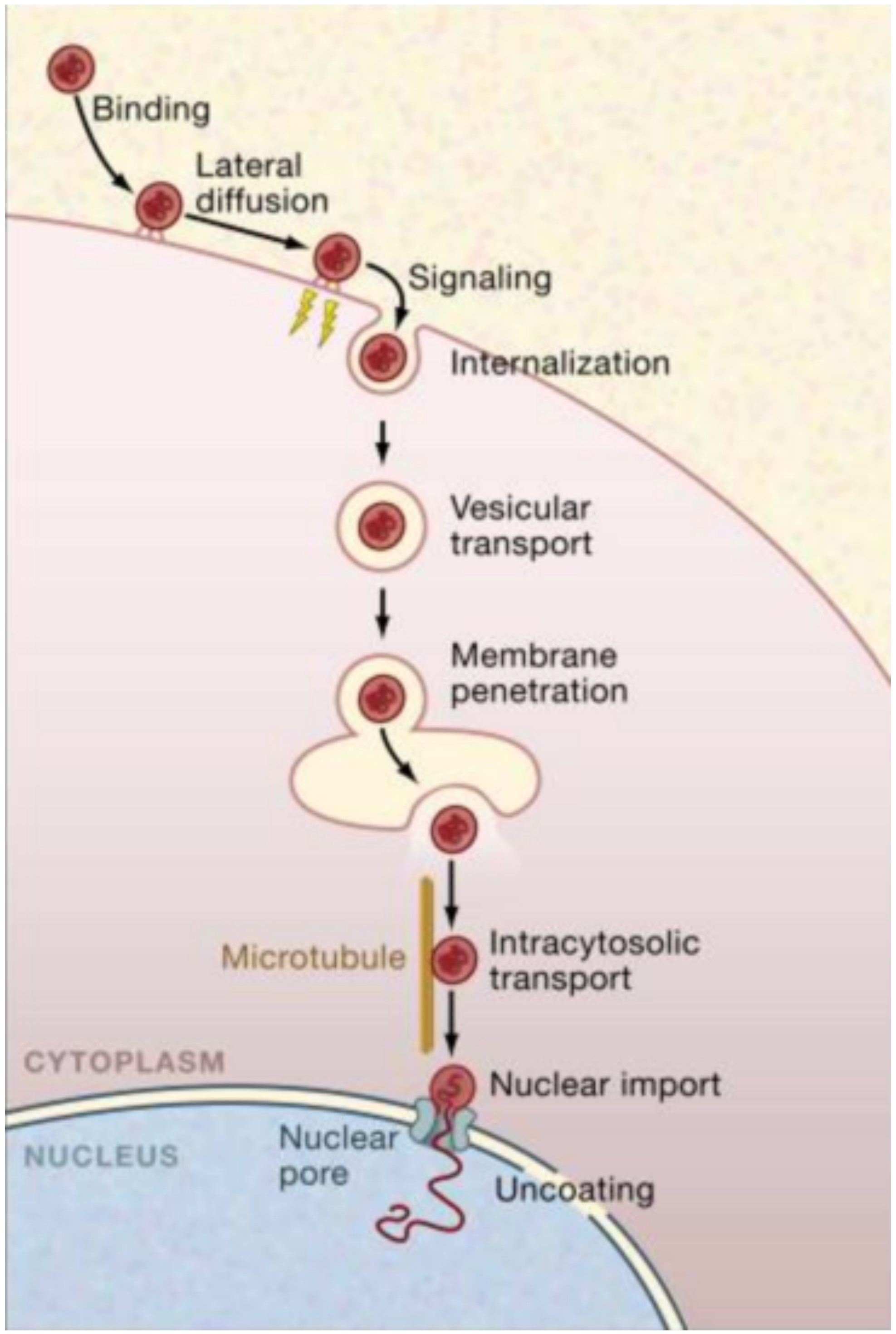
General Lessons Learned

cellular processes & cellular factors are critical components

viruses activate cellular signaling pathways

viruses 'speak' the language of the cell

Cellular Cues for Viral Entry



what leads to stepwise mechanisms?

binding to cell surface receptors

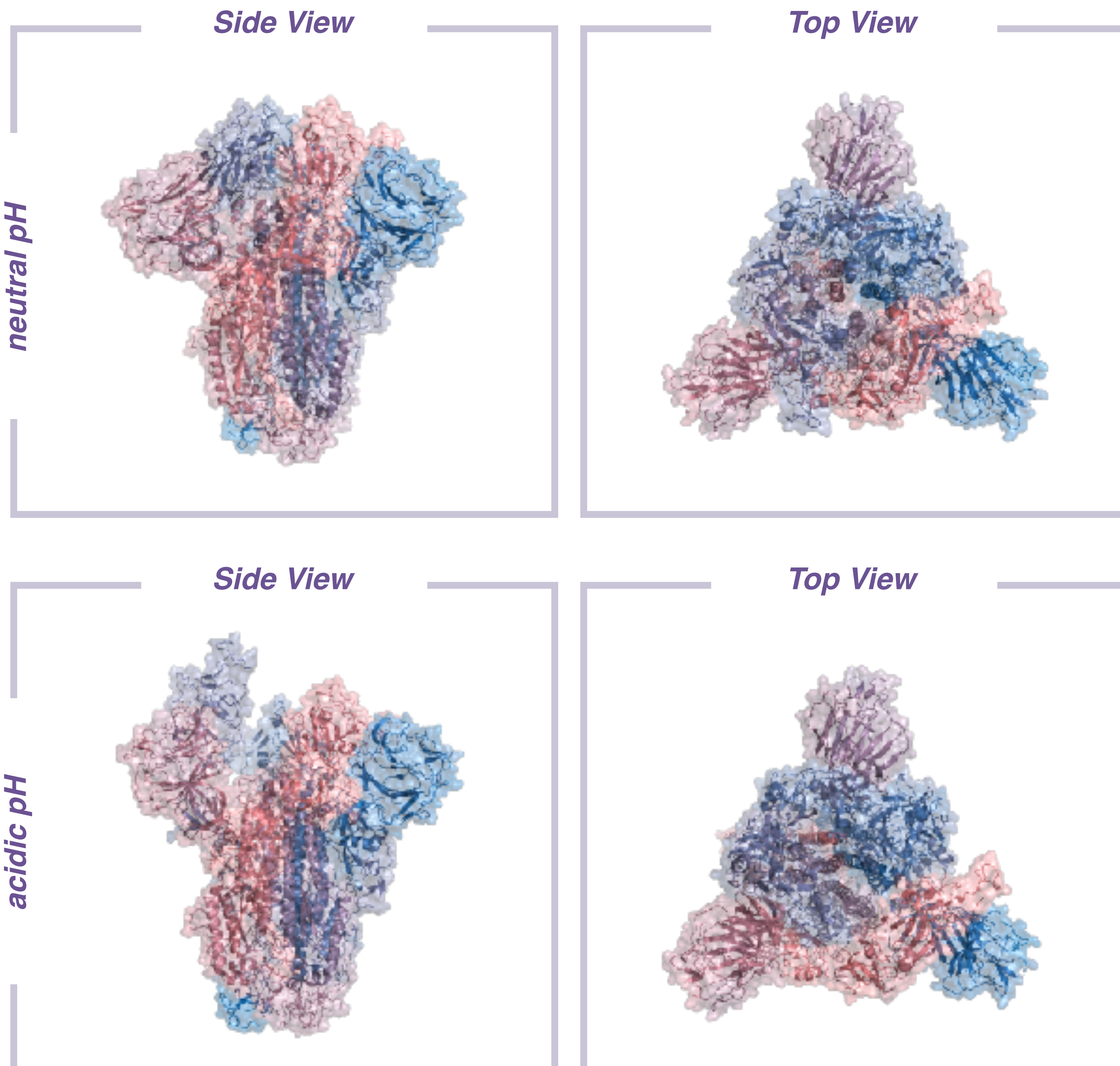
exposure to lower pH

cleavage by cellular proteases

re-entry into a reducing environment

exposure to other enzymes *(thiol-oxidoreductases)*

Example of pH Response in SARS-CoV-2 Spike Protein



spike protein in novel 2019 SARS-CoV-2 virus

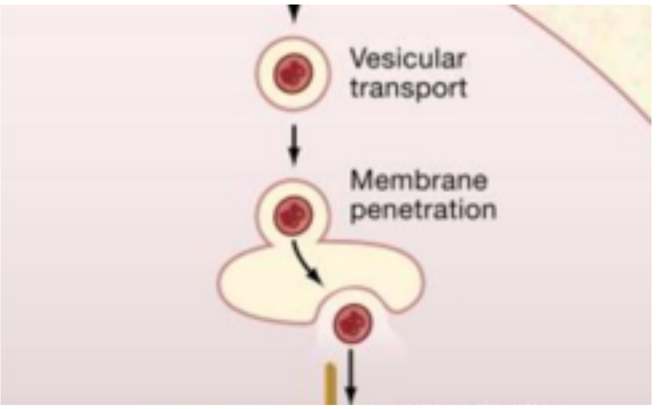
PDB: 6ACC & 6ACD

endocytosis changes viral surroundings to a more acidic (lower pH) environment

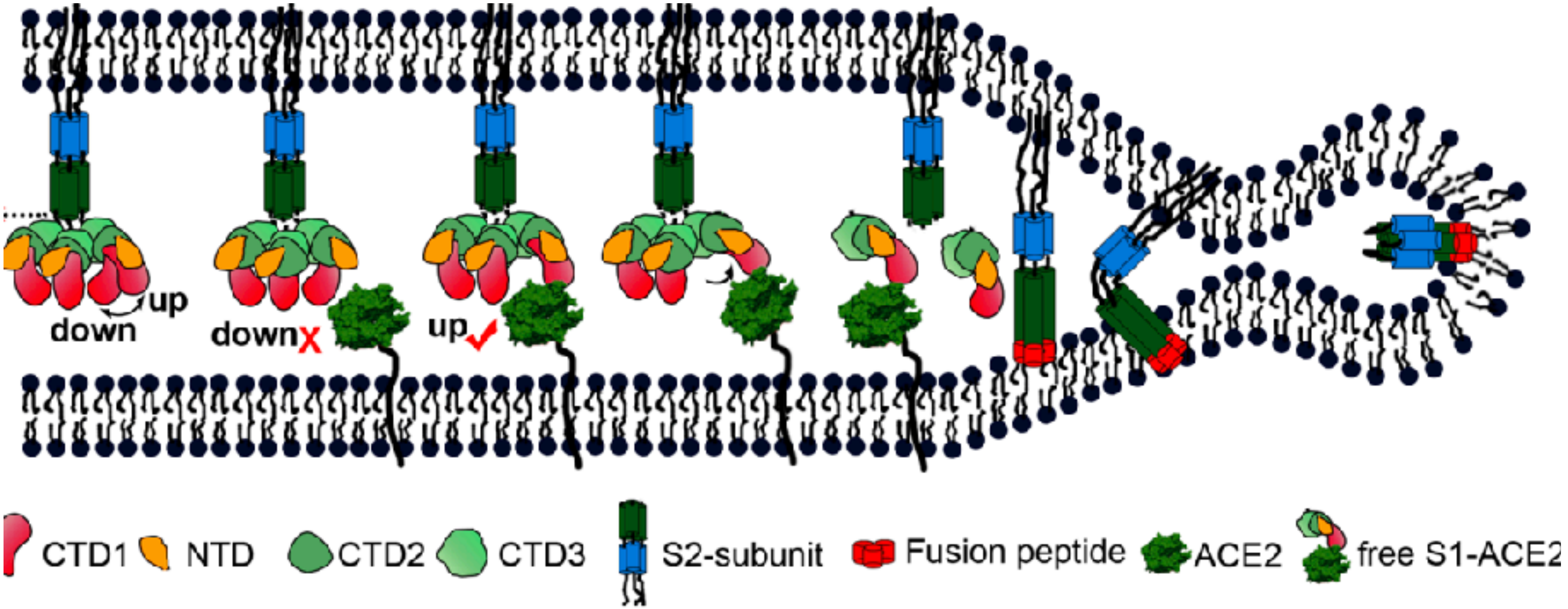
viral proteins exhibit meta-stable state

stepwise process for viral entry

Example of pH Response in SARS-CoV-2 Spike Protein

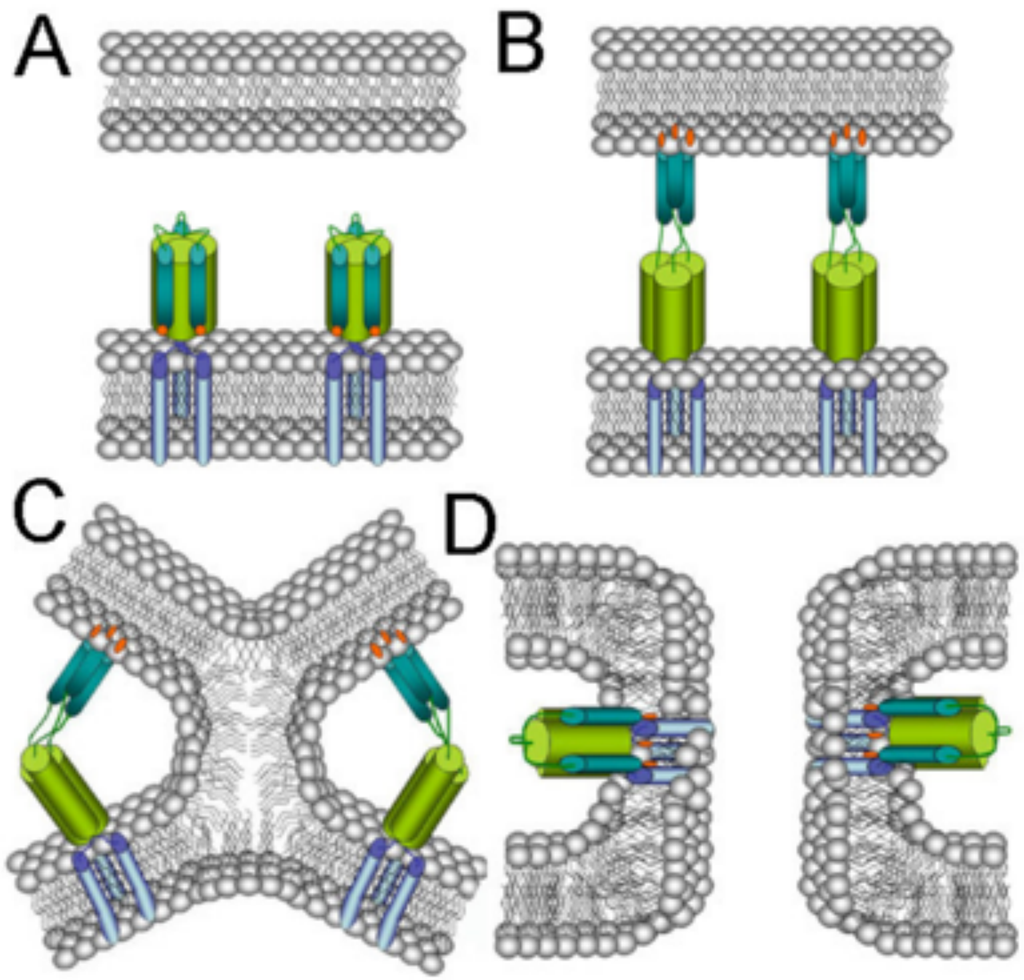


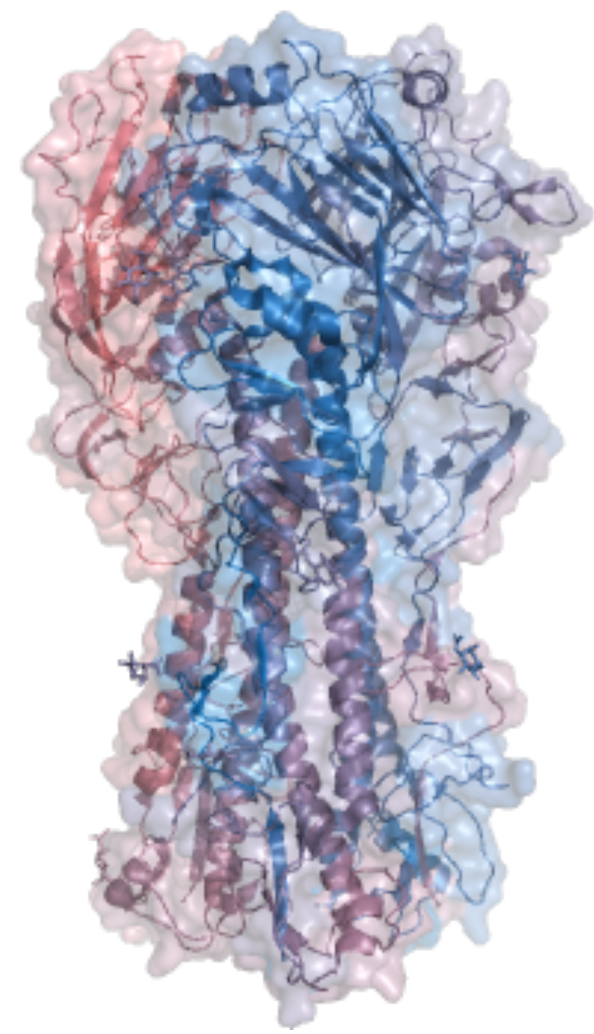
large conformational changes in response to pH facilitates endocytosis



Song, W.; Gui, M.; Wang, X.; Xiang, Y. Cryo-EM structure of the SARS coronavirus spike glycoprotein in complex with its host cell receptor ACE2. DOI: 10.1371.journal.ppat.1007236.

Example of pH Response in Influenza HA Protein





lipid bilayer glycoprotein
***hemagglutinin** PDB: 1ruz*

Wessels, L. & Weninger, K. (2009). Physical Aspects of Viral Membrane Fusion. TheScientificWorldJournal. 9. 764-80. 10.1100/tsw.2009.76.

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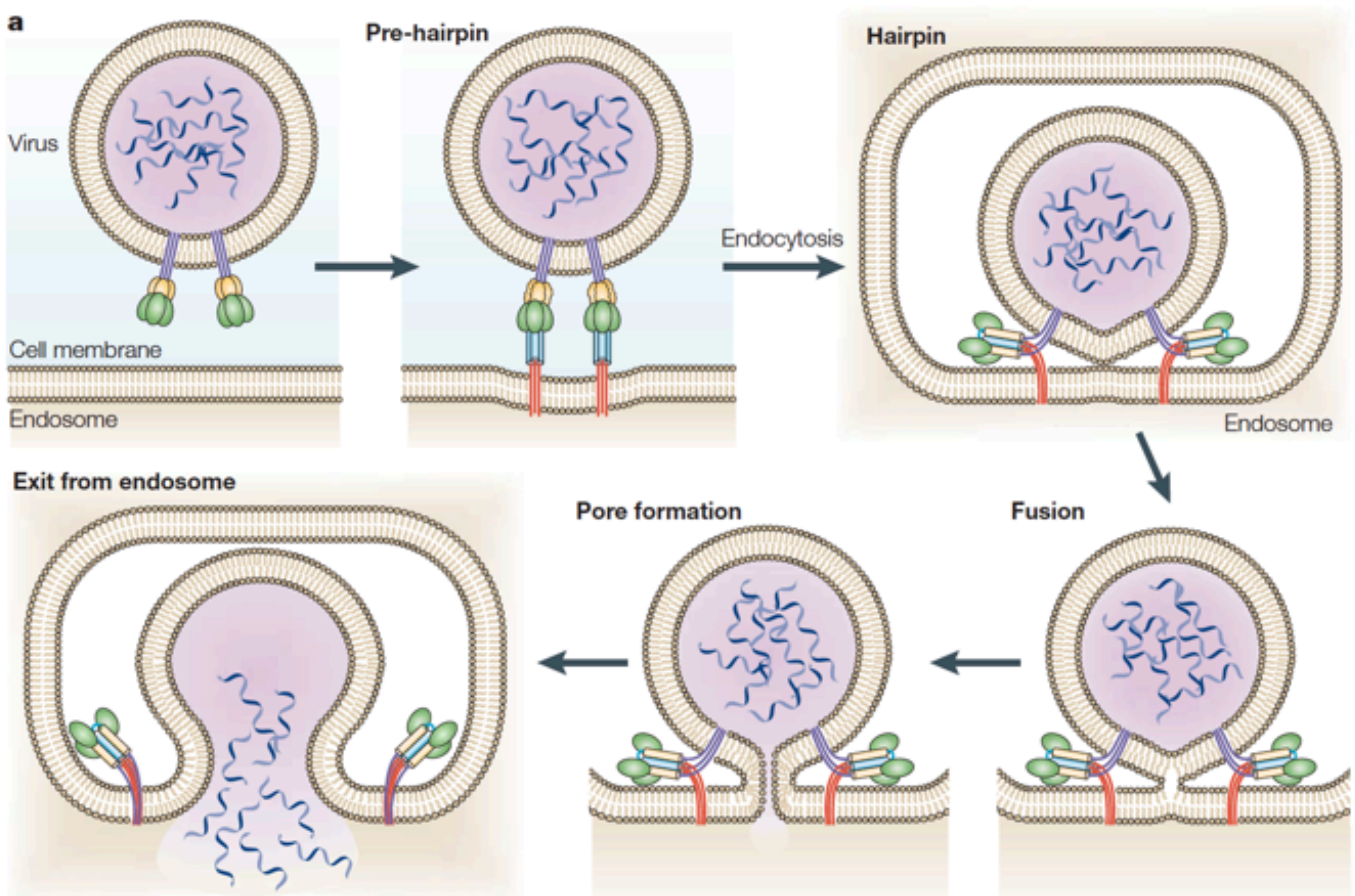
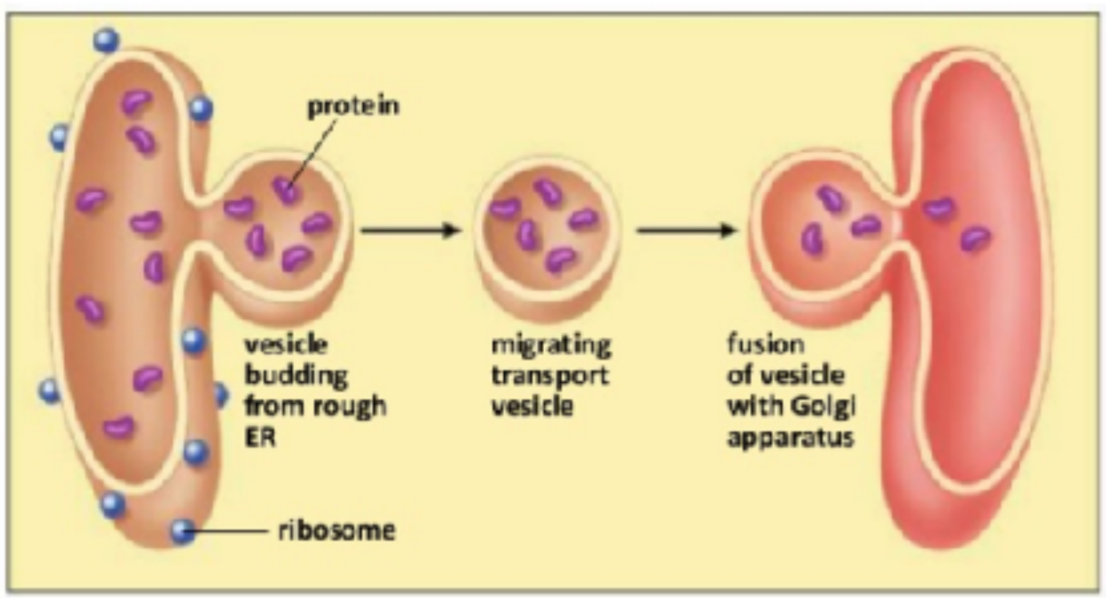
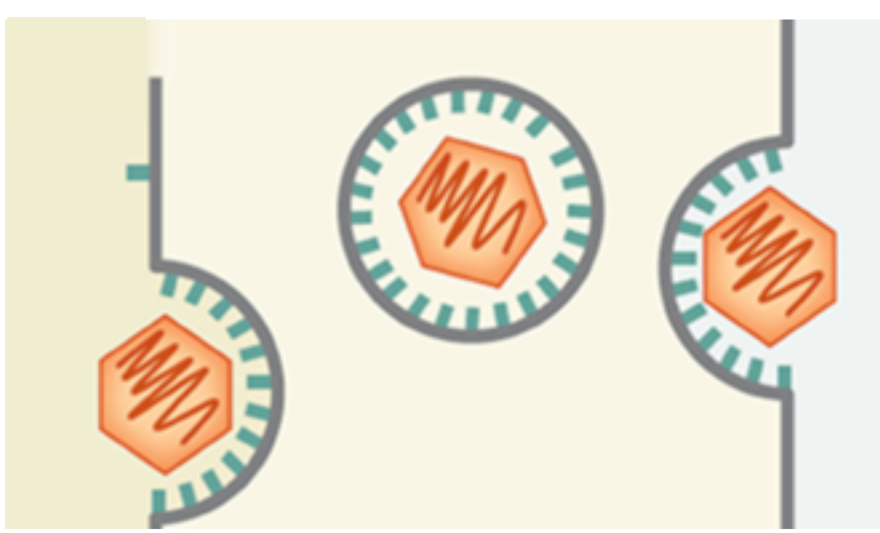


Wessels, L. & Weninger, K. (2009). Physical Aspects of Viral Membrane Fusion. TheScientificWorldJournal. 9. 764-80. 10.1100/tsw.2009.76.

Enveloped vs Non-Enveloped Viruses

Vesicle transport





Infected Cell

Uninfected Cell

budding

fusion

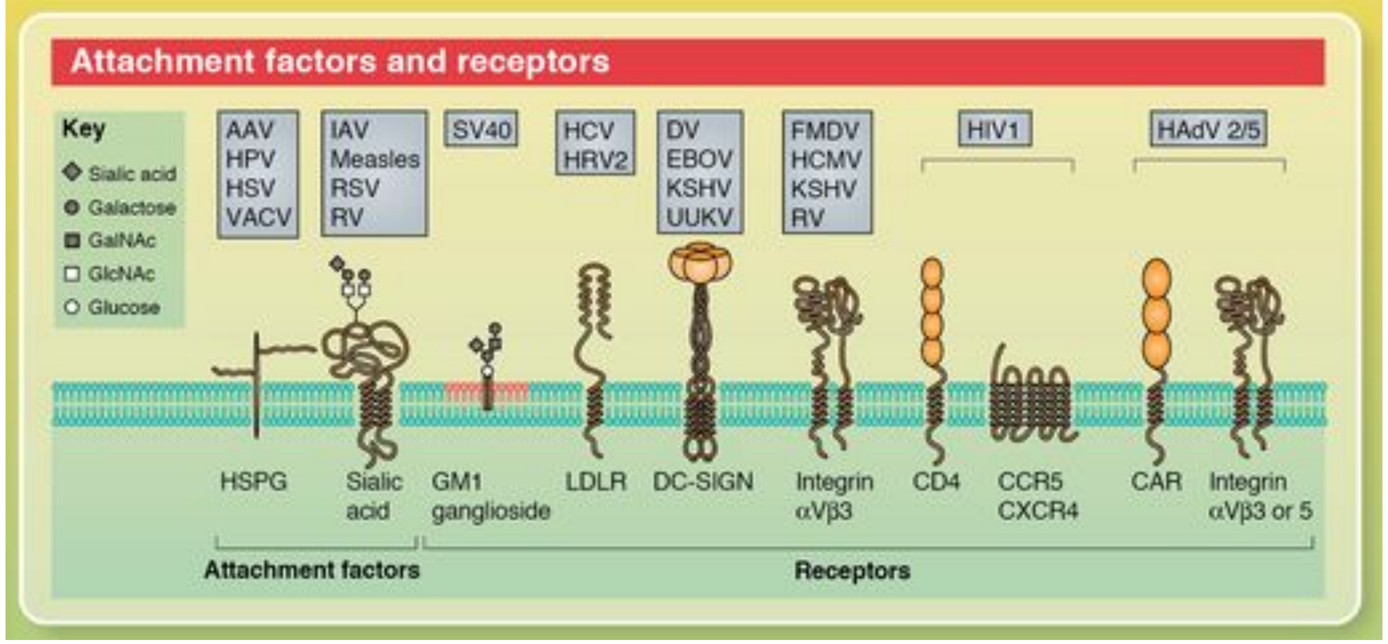
enveloped viruses use lipid bilayer

similar mechanism as a vesicle

mechanism to transport from infected cell to uninfected cell

Binding: Viral Proteins & Cellular Receptors

a virus cannot infect a cell it cannot bind to



attachement factors: simply bind the virus

(to increase concentration of viral particles on cell surface)

Binding: Viral Proteins & Cellular Receptors

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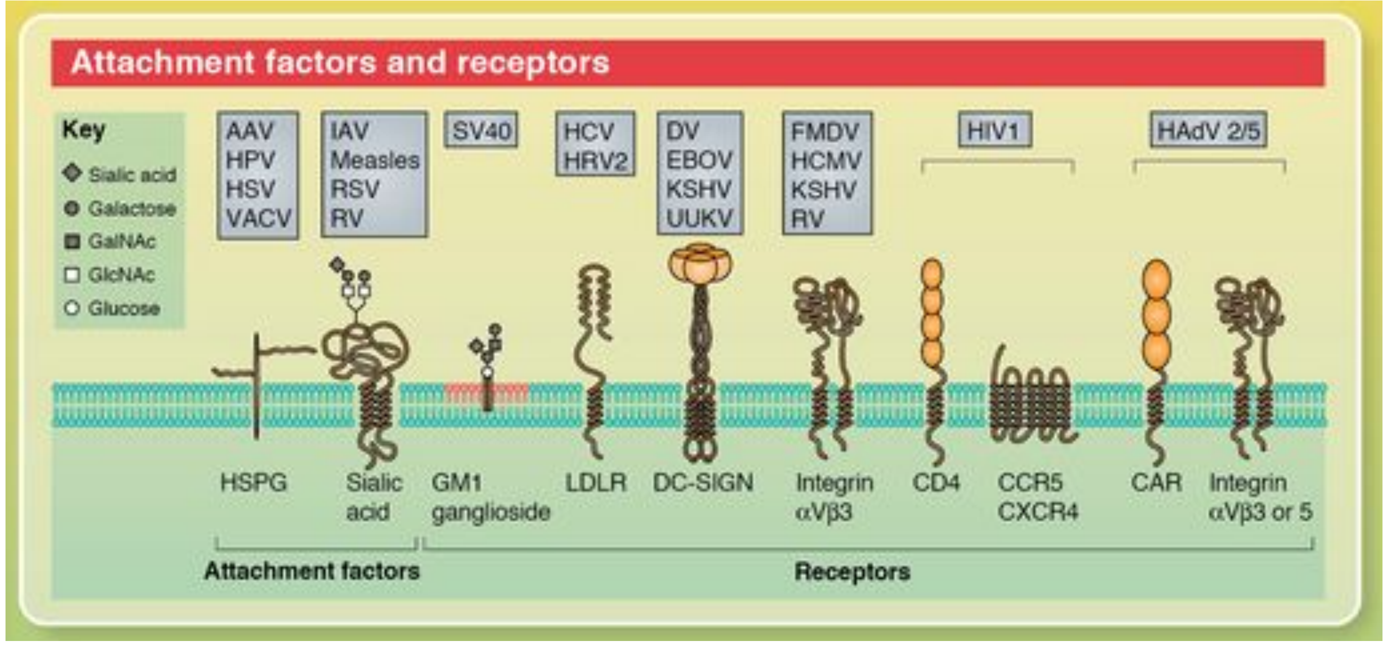

receptors: bind virus & provides additional signaling or viral conformational changes
(mechanisms vary, but can also start the endocytosis process)

Binding: Viral Proteins & Cellular Receptors

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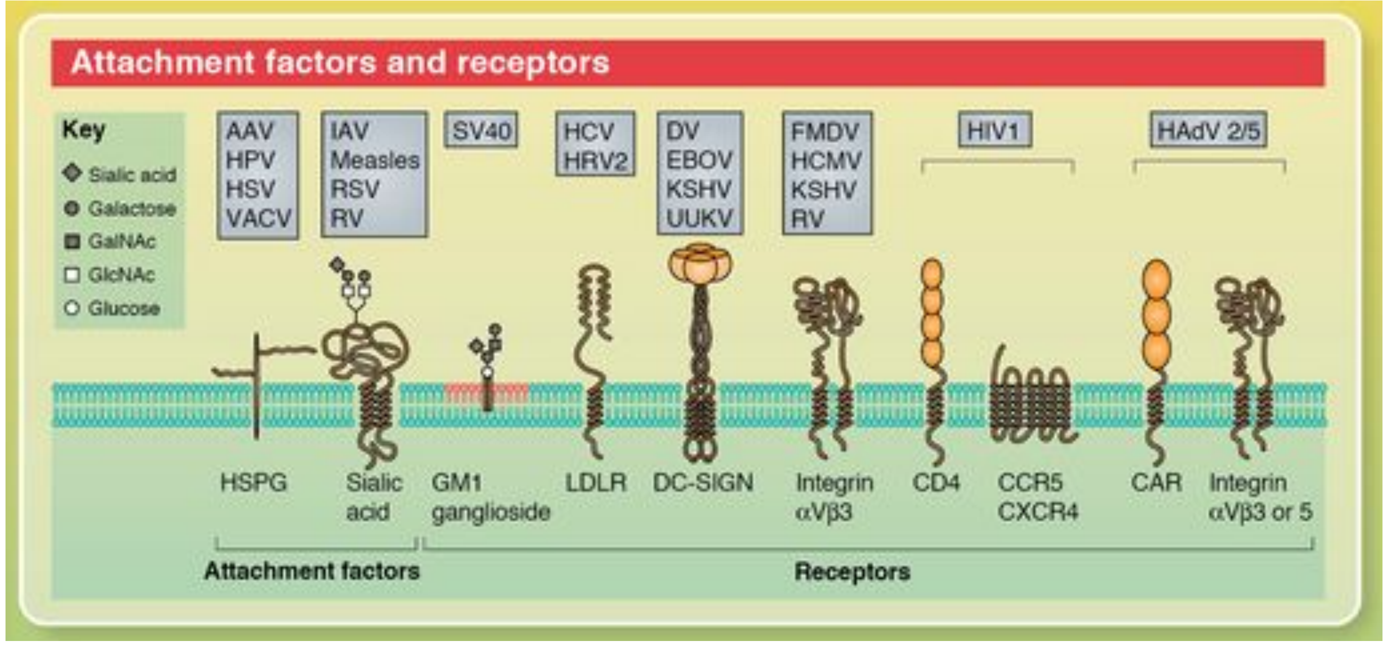
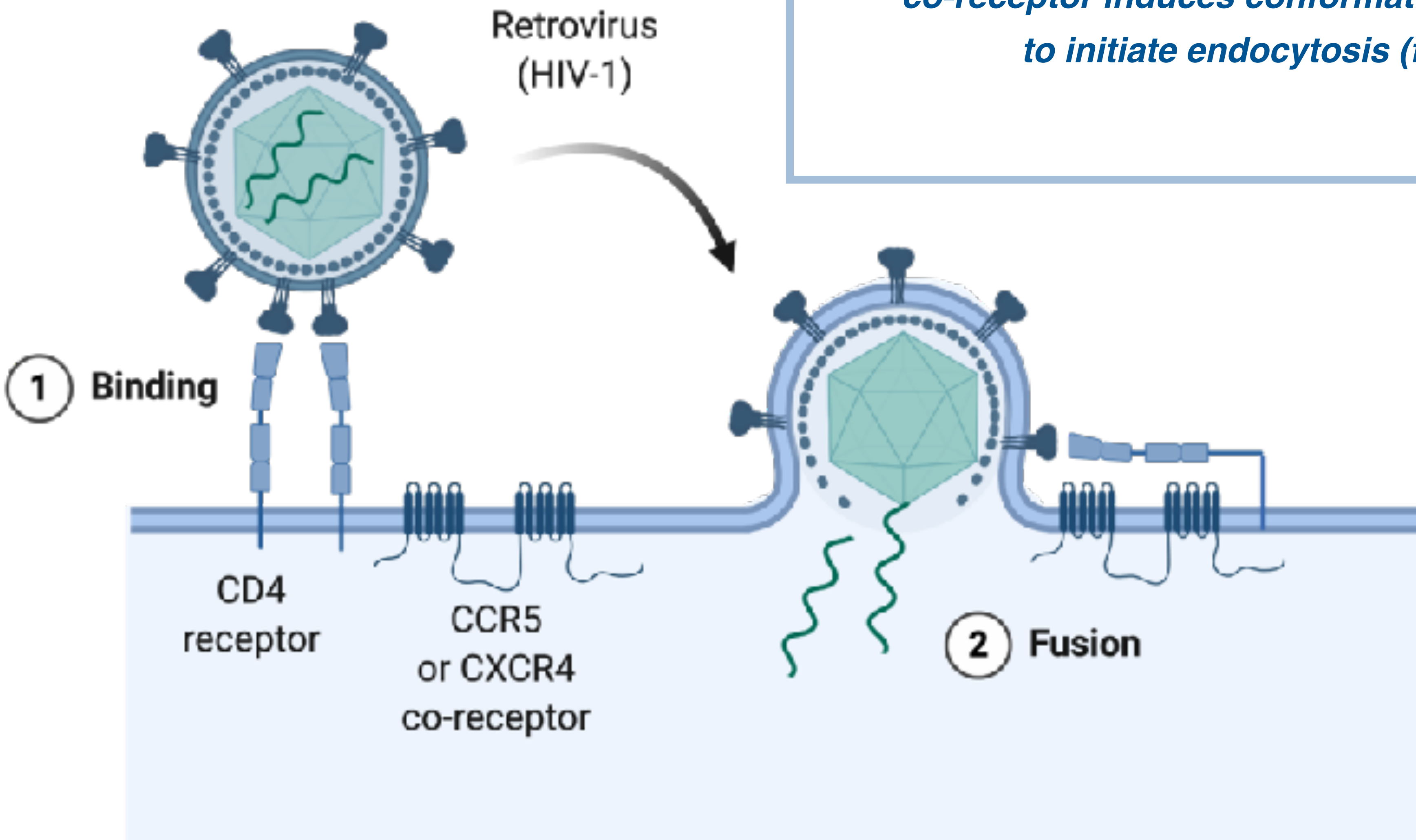


important note: most viruses are known to have more than one type of receptor binding

*they can also be **multivalent** (binding to multiple receptor or attachement factors at the same time)*

HIV-1 is Multivalent & Uses Multiple Receptors

co-receptor induces conformational changes to initiate endocytosis (fusion)



Examples of Viruses & Their Target Receptors

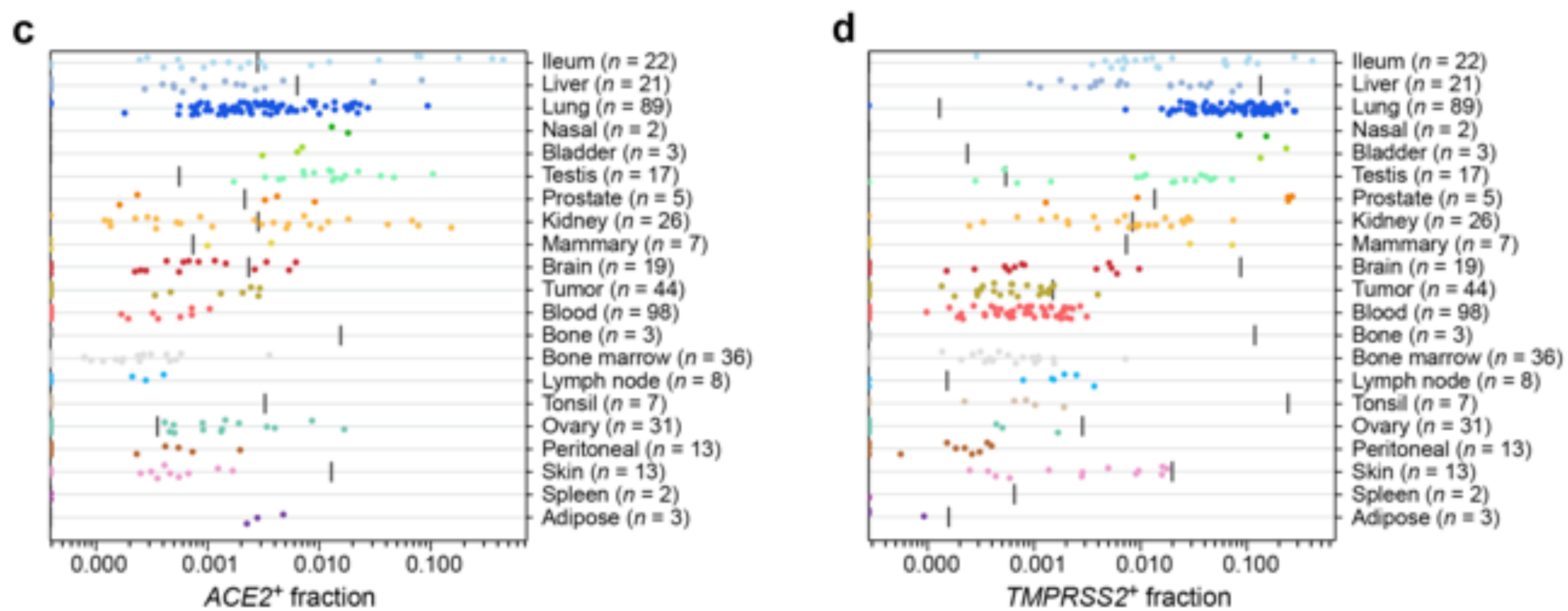
Virus	Entry protein*	Receptor‡	Co-receptor	Alternative receptor§	Notes
Influenza A	Haemagglutinin	Sialic acid (mM)	Unknown	unknown	There are no indications that influenza needs co-receptor(s) for entry
HIV-1	gp160 (gp120)	CD4 (nM)	CCR5, CXCR4, other (nM–μM)	Galactosyl ceramide (μM)	Some HIV-1 isolates are CD4-independent and can use CCR5 or CXCR4 as receptors; affinities for co-receptors are higher in the presence of CD4; entry in the absence of CD4 is typically much less efficient.
SARS-CoV	S (S1)	ACE2 (nM)	Unknown	Unknown	
Herpes simplex virus 1 (HSV-1)	Glycoprotein D (gD)	HveA (μM)	Unknown	Unknown	Several other viral (gB, gC, and the heterodimer gH/gL) and cellular (heparin sulphate, nectin-1) receptors are implicated in the complex entry mechanism; a truncated form of gD exhibits 100-fold higher affinity for HveA
Poliovirus 1	Capsid shell (VP1, VP2, VP3)	CD155 (nM–μM)	Unknown	Unknown	CD155 is the receptor for all three serotypes; affinities forcell surface receptors significantly differ from those for soluble receptors and are also temperature-dependent.
Rhinovirus 3 (HRV3)	Capsid shell (VP1, VP2, VP3)	ICAM-1 (μM range)	Unknown	Unknown	Minor-group human rhinoviruses use VLDL-R as a receptor; there are structural similarities between ICAM-1 binding to capsid shell and CD4 binding to gp120.
Adenovirus 2	Fibre, penton base	CAR (nM)	αv integrins	Sialic acid and heparin sulphate proteoglycans	Adenovirus fibre attaches to the CAR and integrins interact with the penton base, leading to internalization.
Reovirus 1	σ1	JAM-1 (nM)	Unknown	Sialic acid	There are structural similarities between adenovirus fibre and σ1, and between CAR and JAM-1. All serotypes bind JAM-1.

Dimitrov, D. Virus entry: molecular mechanisms and biomedical applications. *Nat Rev Microbiol* **2,** 109–122 (2004).

Hoffmann, M. *et. al.* SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell **181**,* 271–280 (2020).

Binding: Viral Proteins & Cellular Receptors

the choice of the cell surface receptor determines
*which **cell types** and which **species** the virus can infect*



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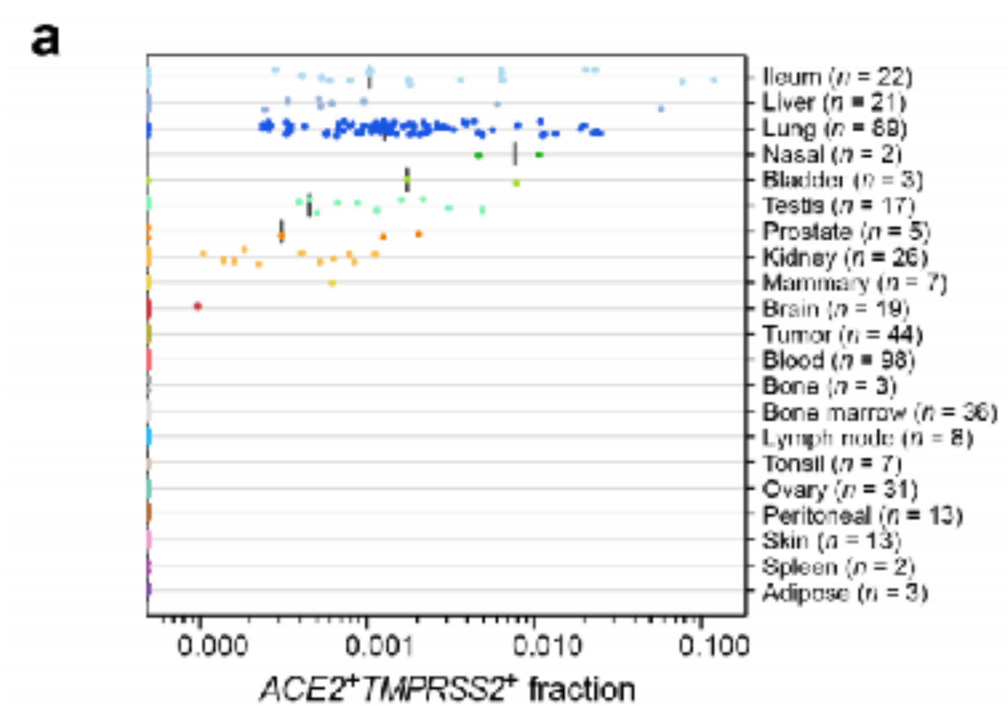

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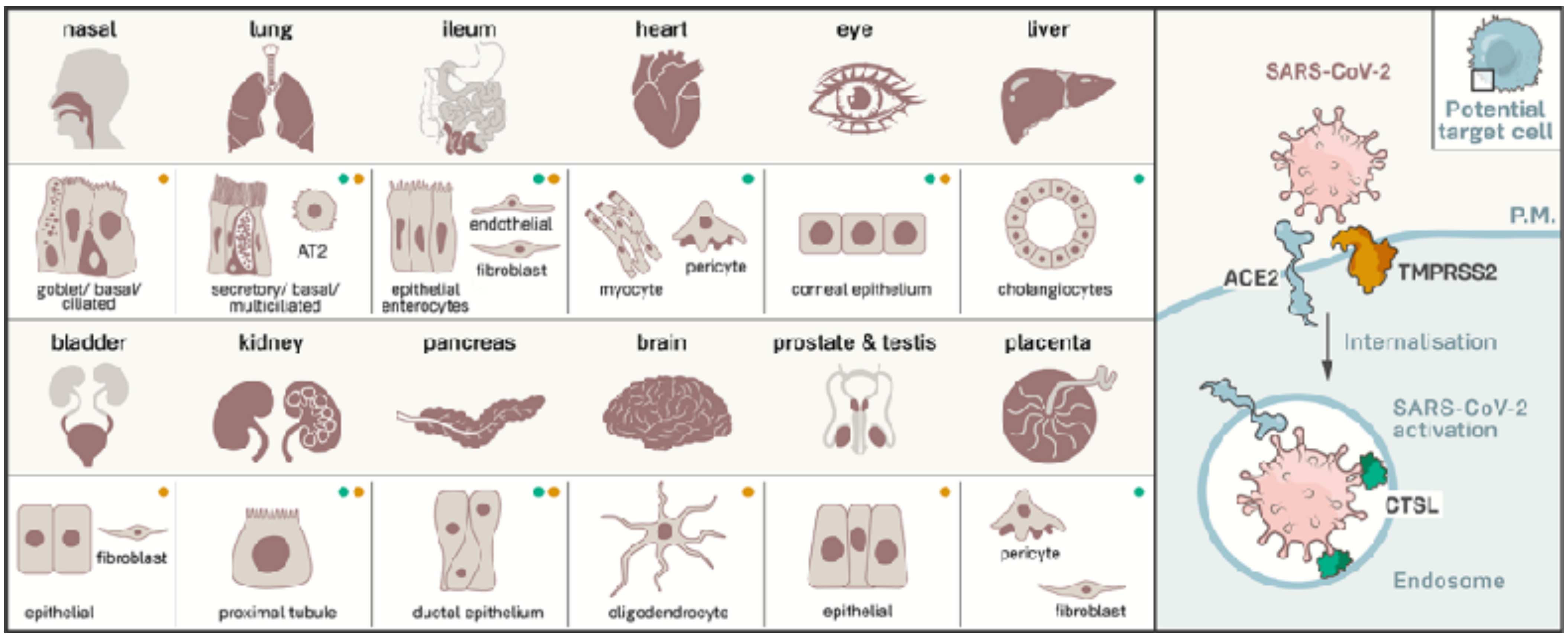
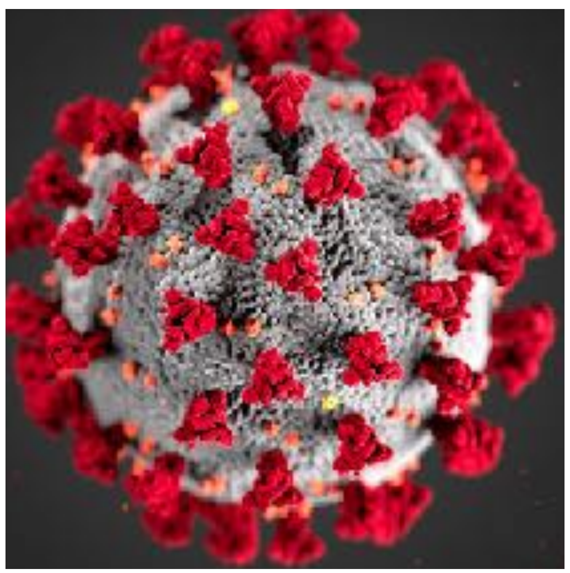


Muus, C. *et. al. BioRxiv April 21, 2020.* DOI: 10.1101/2020.04.19.049254.

Figure from *The Scientist*. Receptors for SARS-CoV-2 Present in Wide Variety of Human Cells.

What Binds to Receptors?

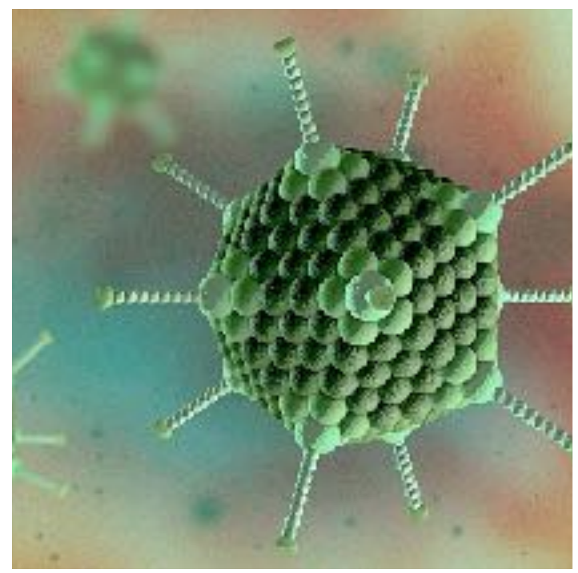
Enveloped



glycoproteins

coronavirus

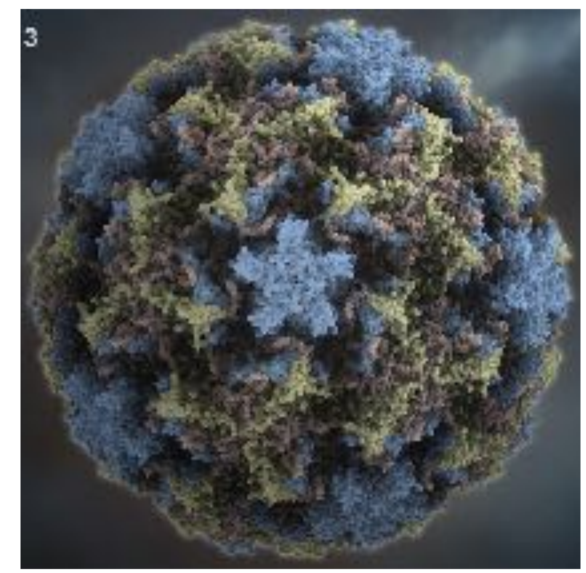
Non-Enveloped



fibers

adenovirus

Non-Enveloped



surface protrusions or indentations

rhinovirus

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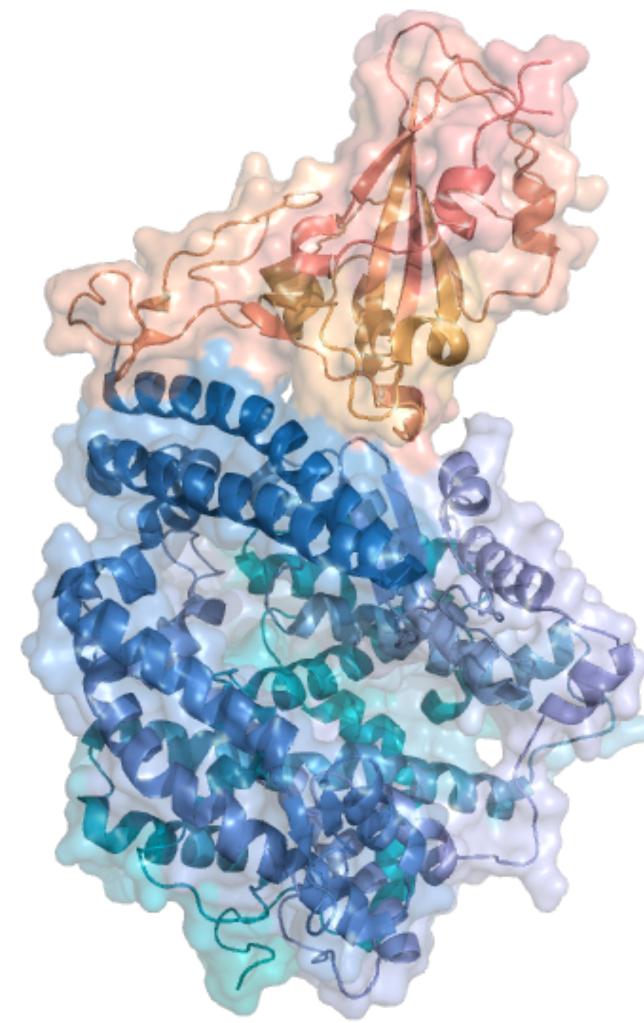
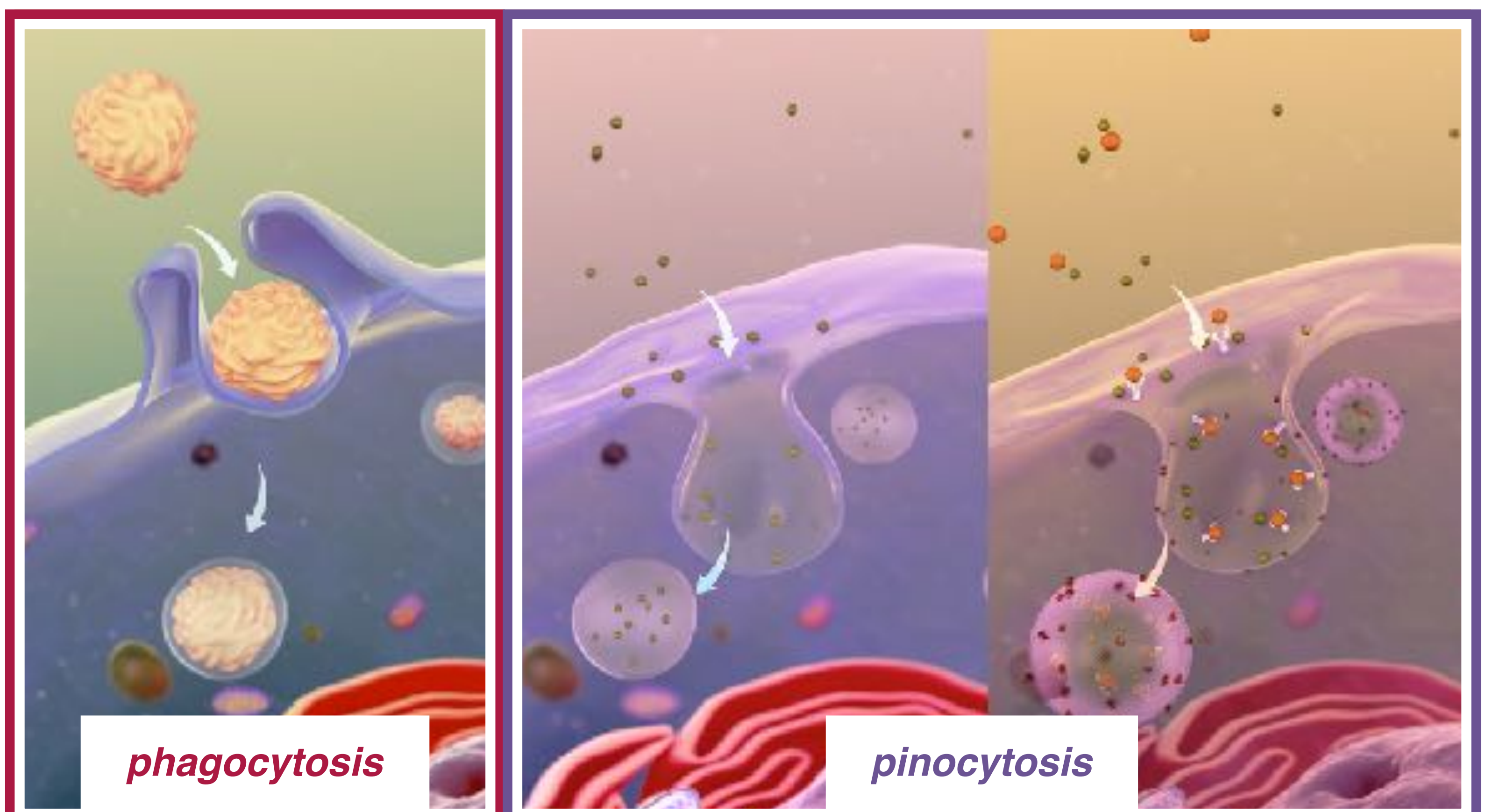


Endocytosis

endocytosis is the uptake of fluids, solutes, membrane, and particles by invagination of the plasma membrane and formation of cytoplasmic vesicles

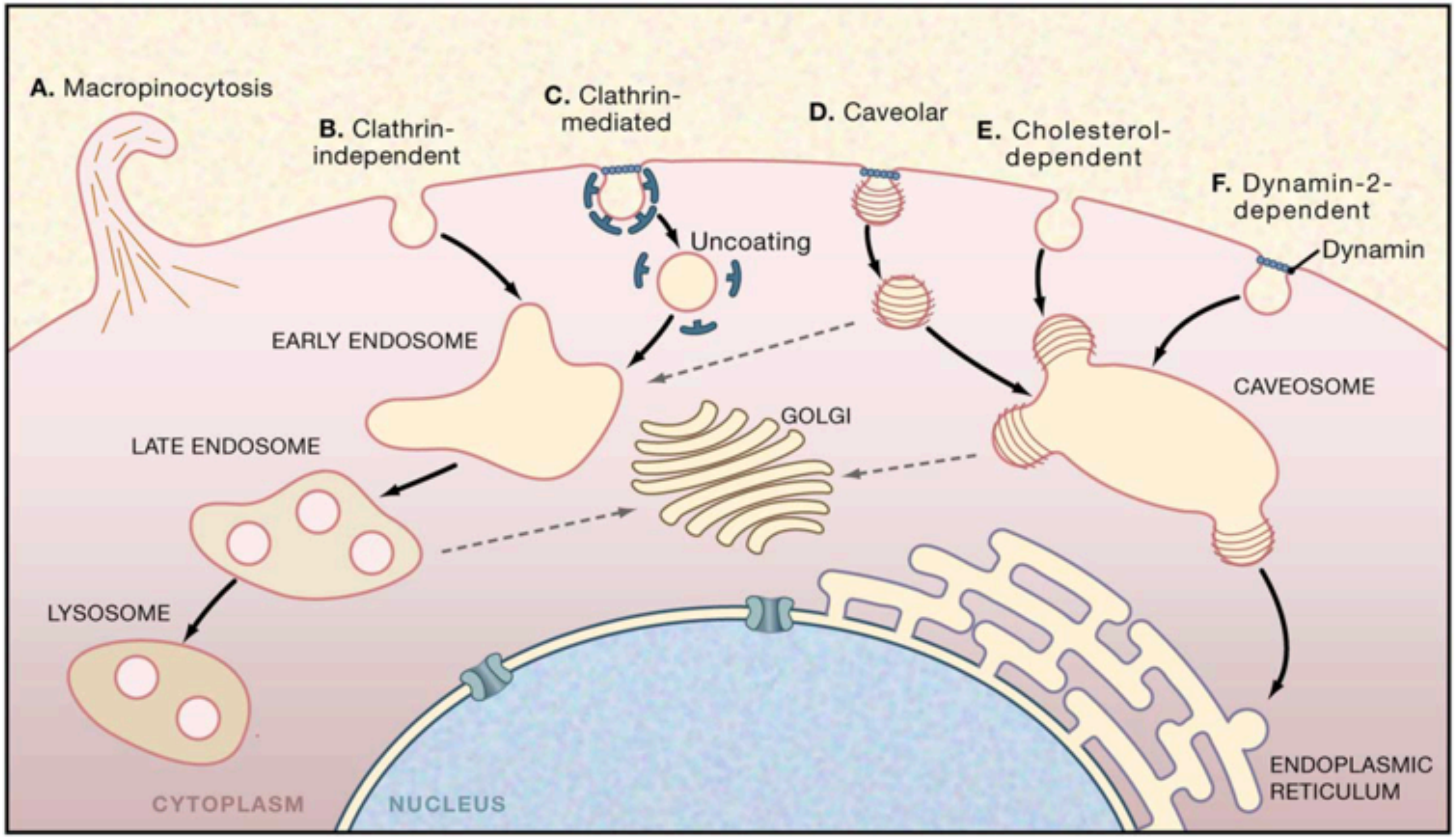
receptor-mediated endocytosis: uptake depends on its binding to a receptor



Endocytosis

dynamin vs dynamin-independent processes

dynamin is a GTPase protein responsibile in the scission of newly formed vesicles

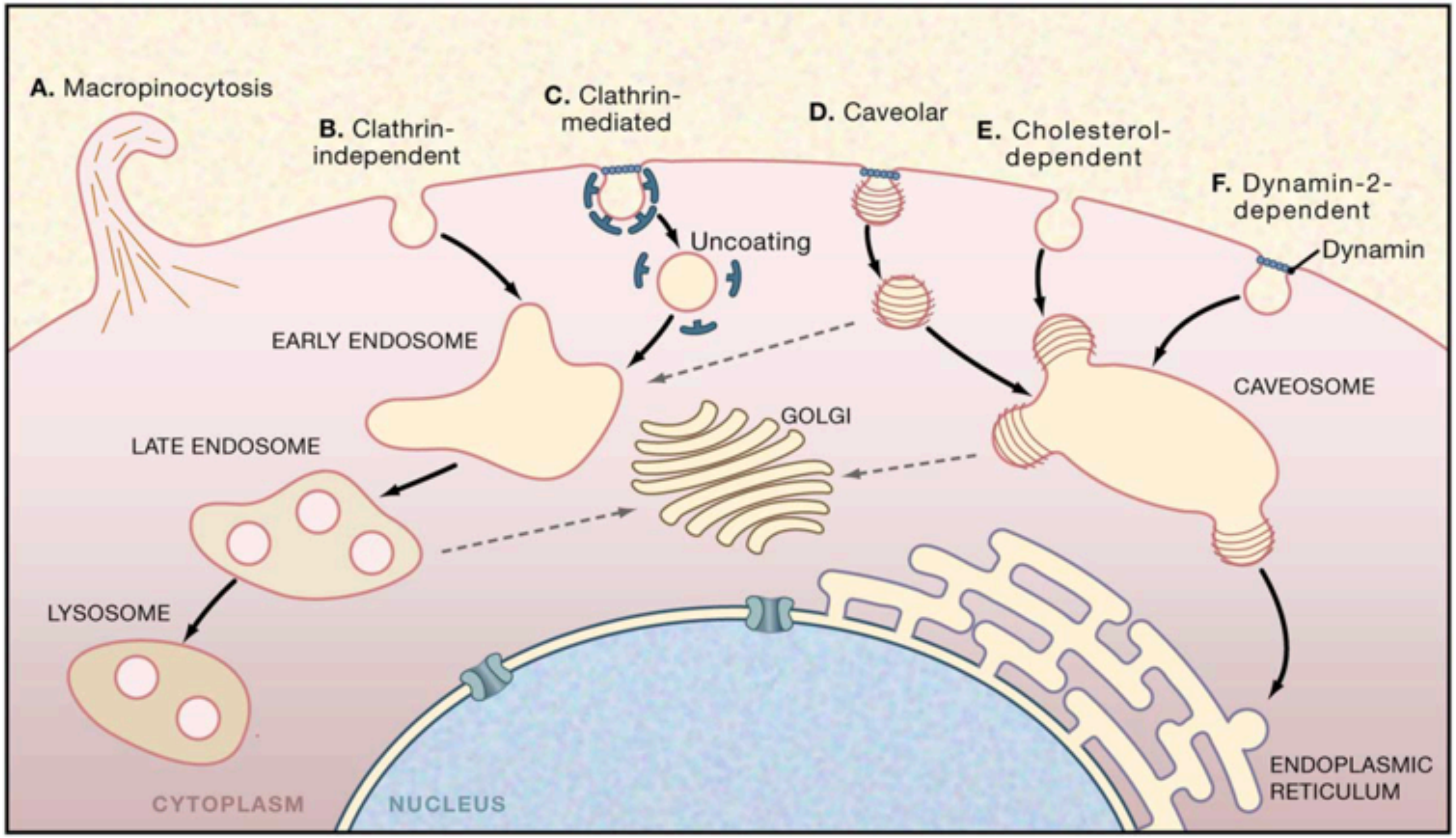


Marsh, M., Helenius, A. *Virus Entry: Open Sesame. Cell* **124,** 729–740 (2006).

Endocytosis

pH changes

◆ *surface* (7.0) ◆ *early endosome* (6.0–6.5) ◆ *late endosome* (5.0–5.5) ◆ *lysosome* (4.5–5)



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Endocytosis

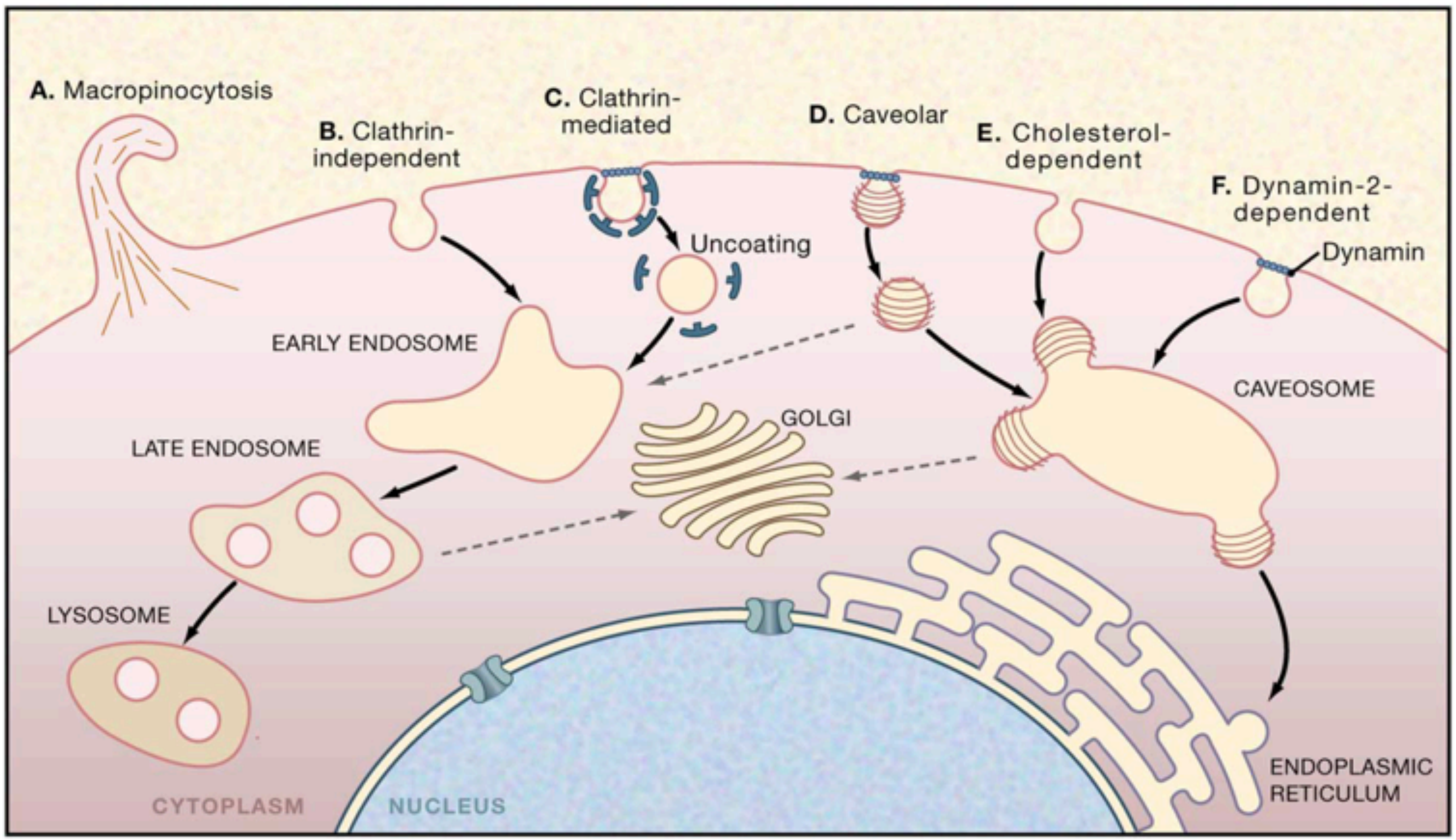
vaccinia
adeno B
HSV1

influenza
HPV16
LCMV

SFV
VSV
influenza
flavi
adeno 2/5

SV40
BK

viruses can have multiple endocytosis mechanisms



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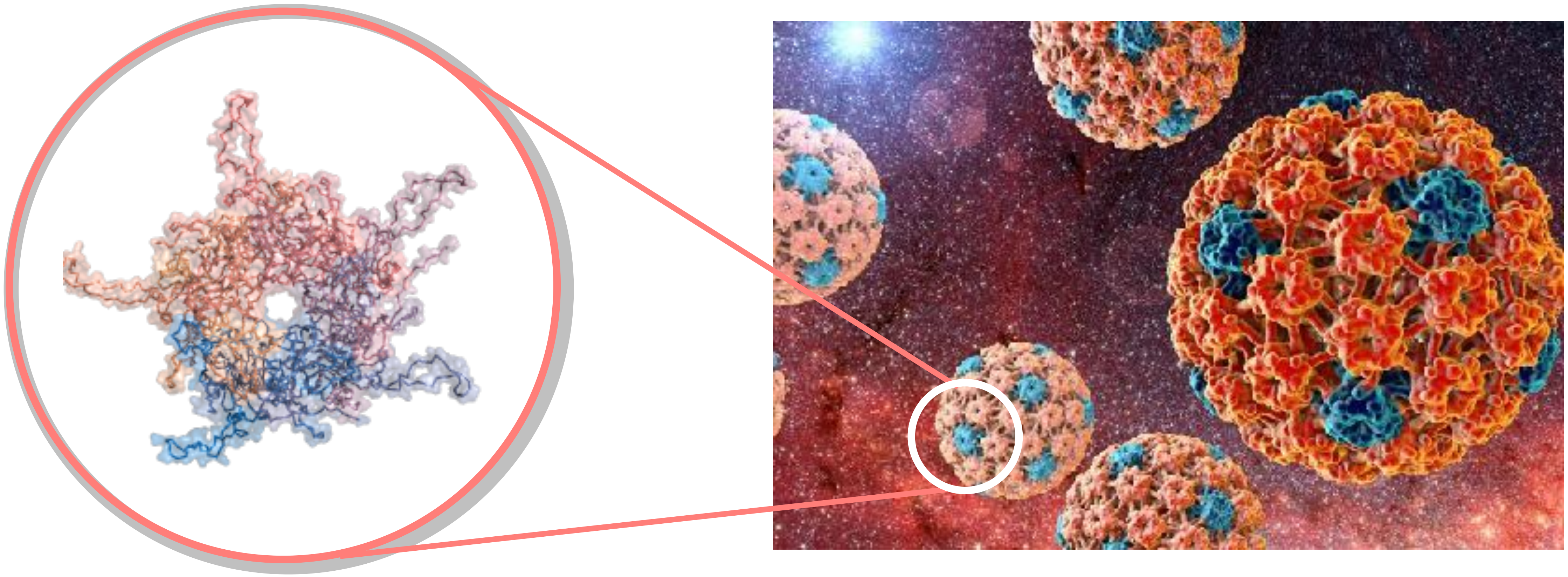
Endocytosis

endocytosis mechanism defines what cellular machinery is being used

- *clathrin, adapters, dynamin, caveolin*
- *cytoskeleton (actin, microtubule)*
- *signaling molecules (kinases)*
- *regulatory factors (Rho GTPases, Rabs, Arfs)*
- *ion channels (Na^+/H^+ exchangers, Ca^{+2} channels)*
- *acidification machinery (vATPases, CLICs)*
- *lipids (cholesterol, phosphatidylinosities)*

Marsh, M., Helenius, A. *Virus Entry: Open Sesame. Cell* **124,** 729–740 (2006).

Developing a Picture of Viral Entry: Human Papillomavirus 16



endocytosis to late endosome

noncoated pit (visually seen by microscopy)

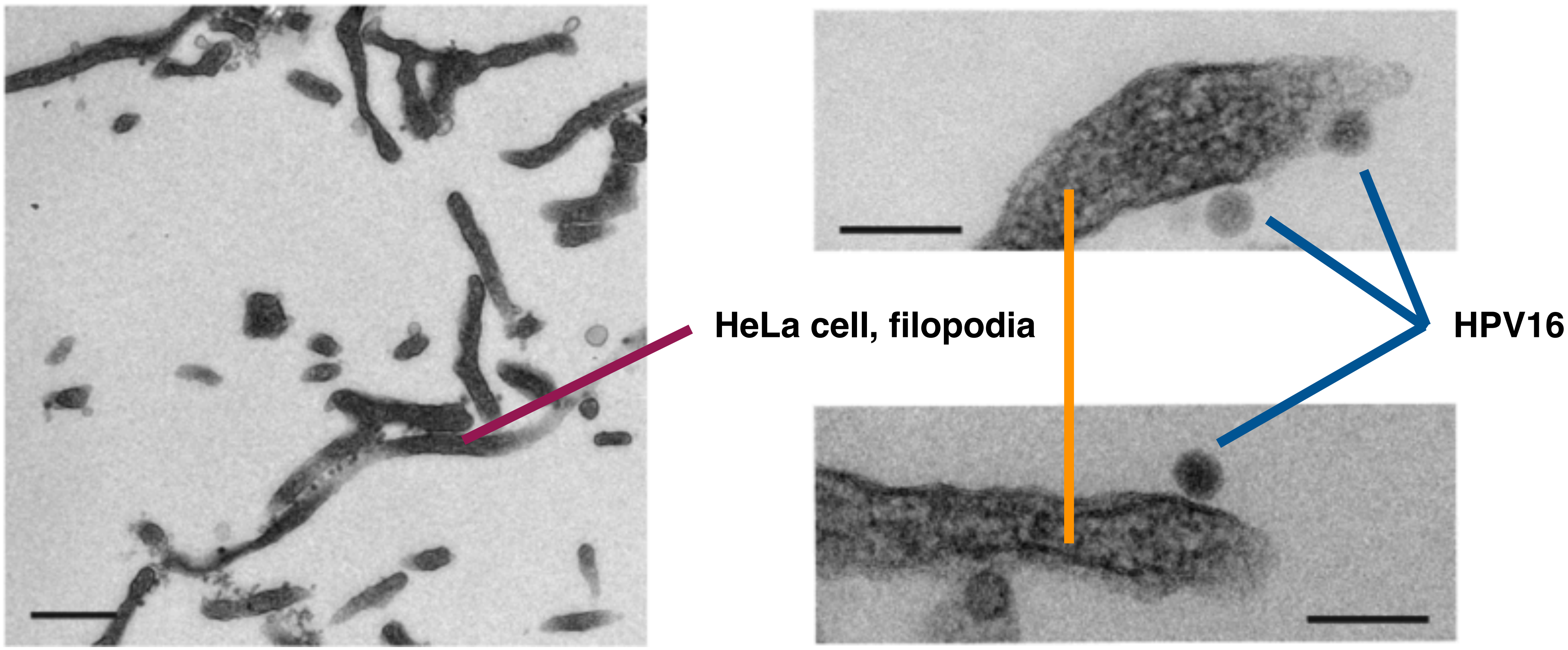
$t_{1/2}$ endocytosis = 3 hours

$t_{1/2}$ acid-activation = 10 hours

Developing a Picture of Viral Entry: Human Papillomavirus 16

not required	***required***
◆ *clathrin*	◆ *acidification*
◆ *AP2*	◆ *protein kinase C zeta*
◆ *dynamin-2*	◆ *Tyr-kinases & phosphatases*
◆ *Arf 6*	◆ *PI(3)kinase* ◆ *N-WASP*
◆ *cholesterols or lipid rafts*	◆ *Na^+/H^+ exchangers*
◆ *caveolin, flotillin*	◆ *actin & microtubules*
◆ *cdc42 or rac1*	◆ *Arf 1* ◆ *Rab5, Rab34*

Developing a Picture of Viral Entry: Human Papillomavirus 16



Schelhaas, M.; Ewers, H.; Rajamäki, M.-L.; Day, P. M.; Schiller, J. T.; Helenius, A. (**2008**) Human Papillomavirus Type 16 Entry: Retrograde Cell Surface Transport along Actin-Rich Protrusions. PLoS Pathog 4(9): e1000148. DOI: 10.1371/journal.ppat.1000148

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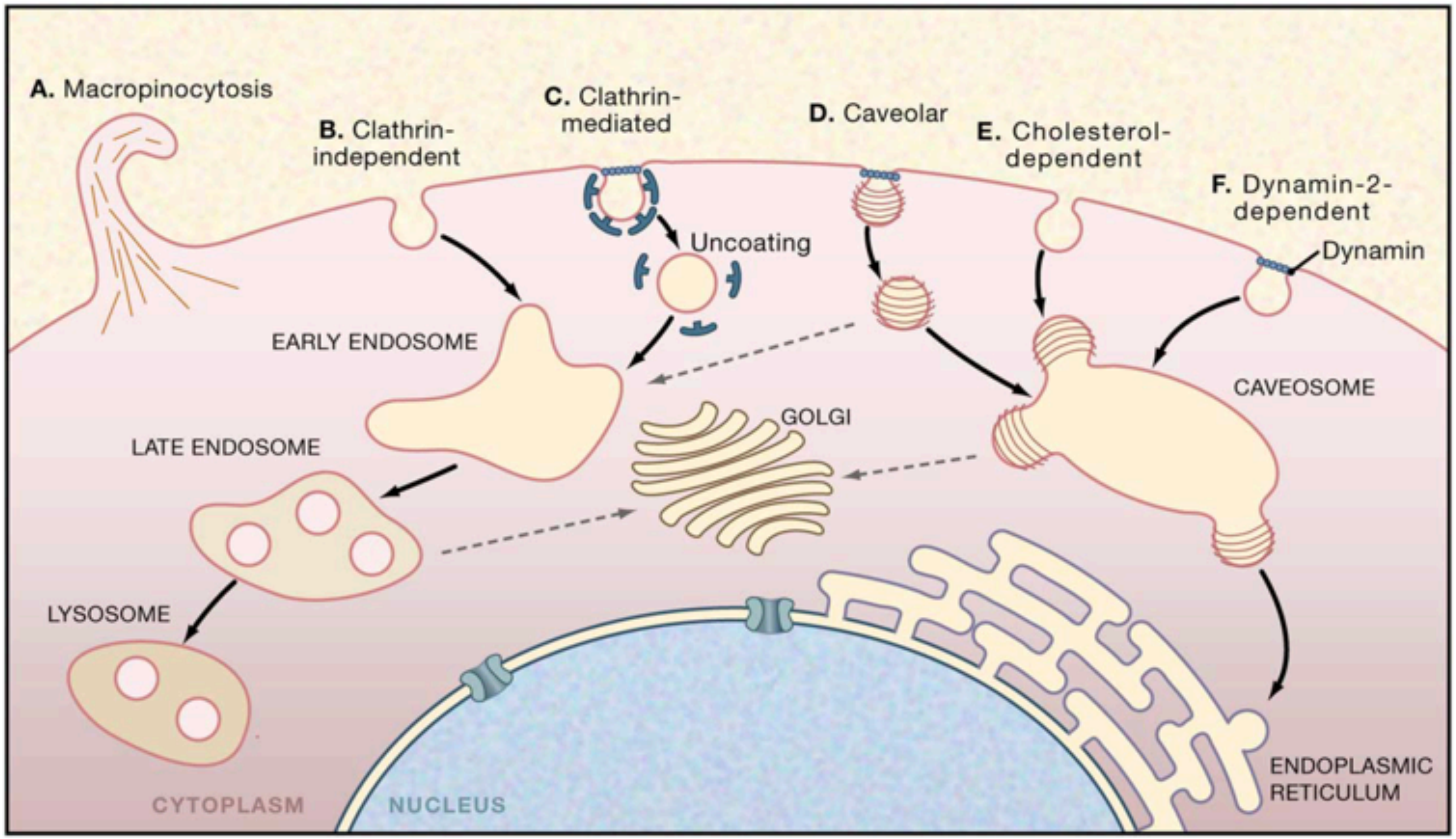

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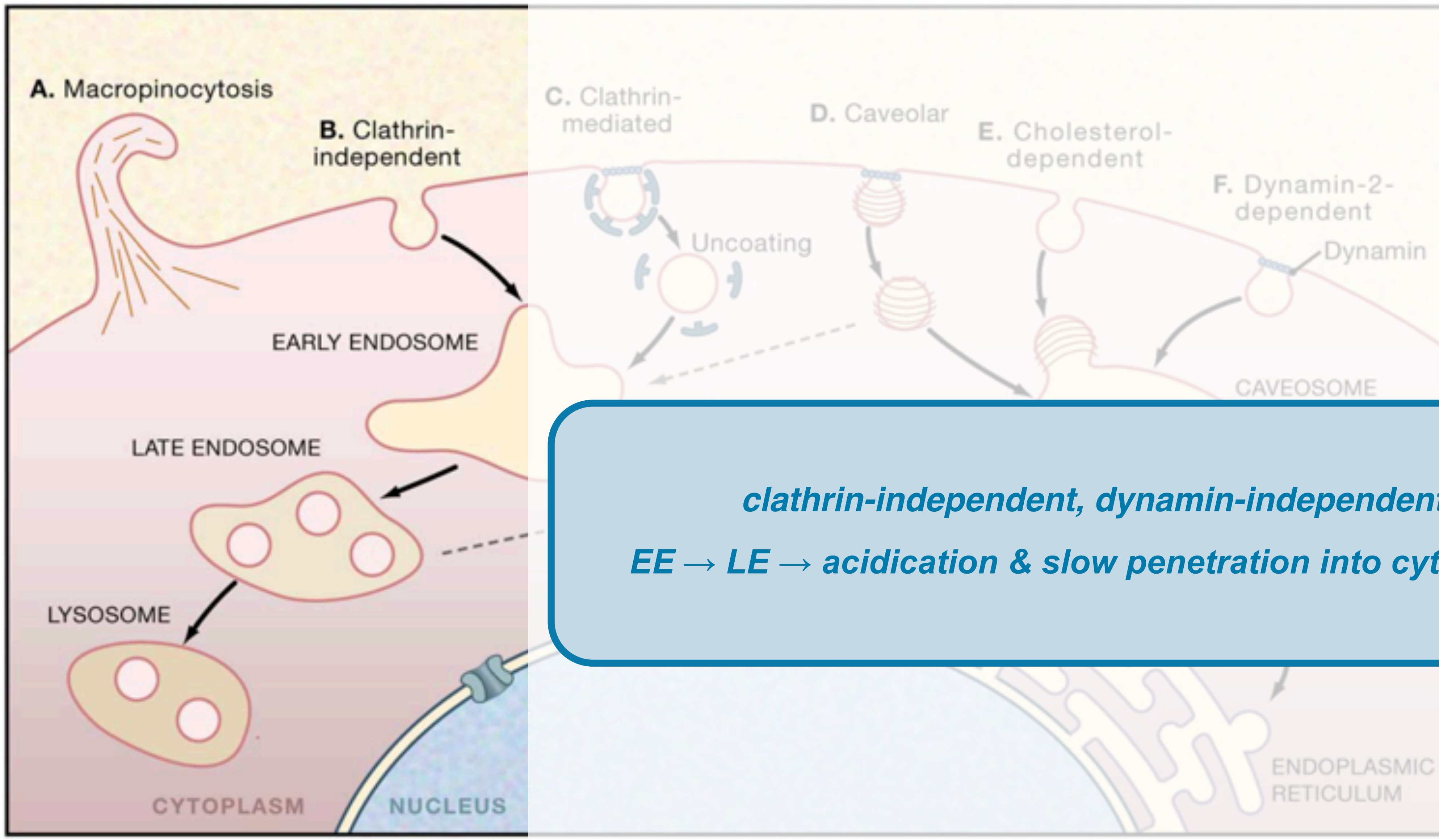
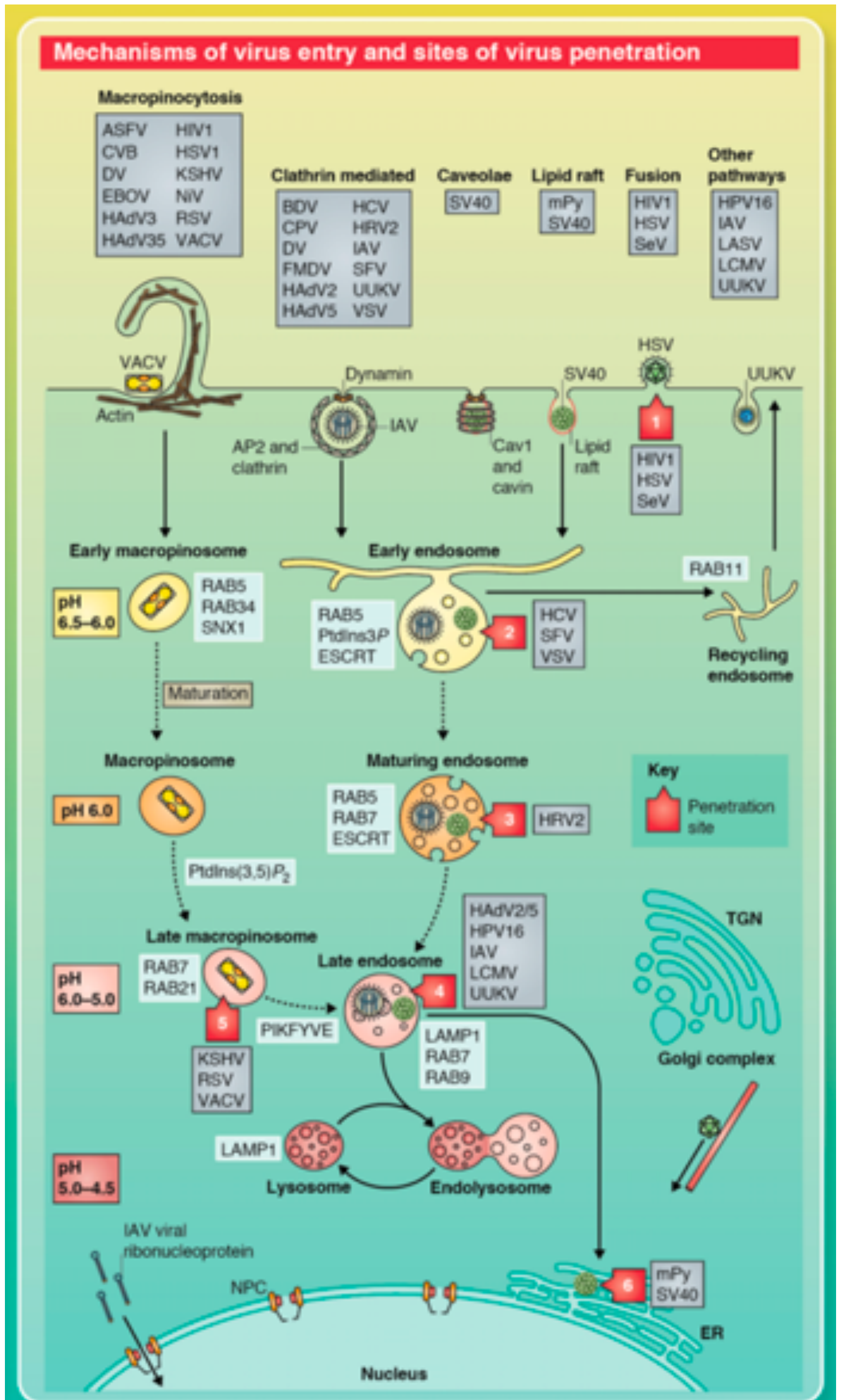


clathrin-independent, dynamin-independent

EE → LE → acidication & slow penetration into cytoplasm

Schelhaas, M.; Ewers, H.; Rajamäki, M.-L.; Day, P. M.; Schiller, J. T.; Helenius, A. (**2008**) Human Papillomavirus Type 16 Entry: Retrograde Cell Surface Transport along Actin-Rich Protrusions. PLoS Pathog 4(9): e1000148. DOI: 10.1371/journal.ppat.1000148

Cell Penetration



penetration: the event that allows the viral genome to move into the cytosol

during viral entry, virus is mostly passive

mostly responding to cellular cues & machinery

penetration requires the viral participation

penetration can occur anywhere along the endocytic route

cell membrane, EE, LE, Lysosome, ER

membrane fusion (enveloped virus)

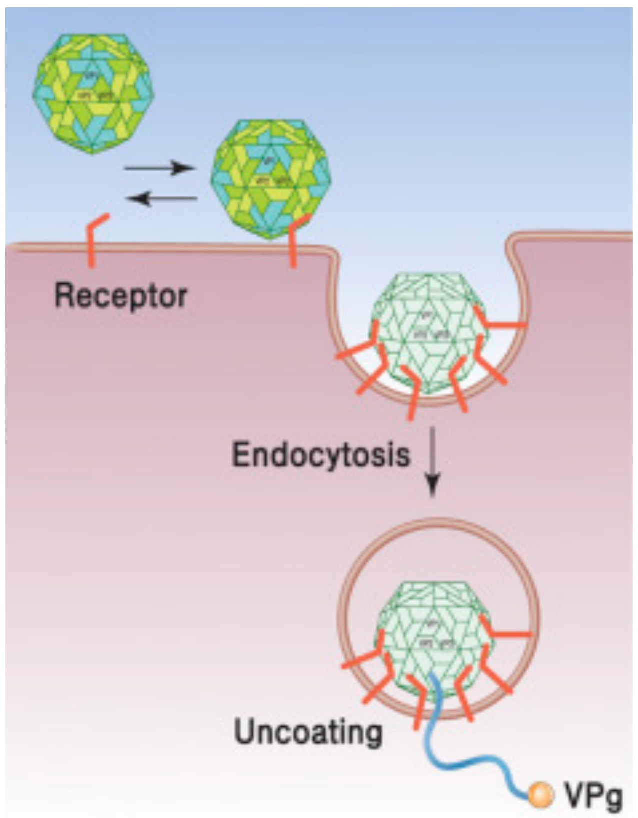
vacuole lysis

pore formation

ER associated degradation pathways

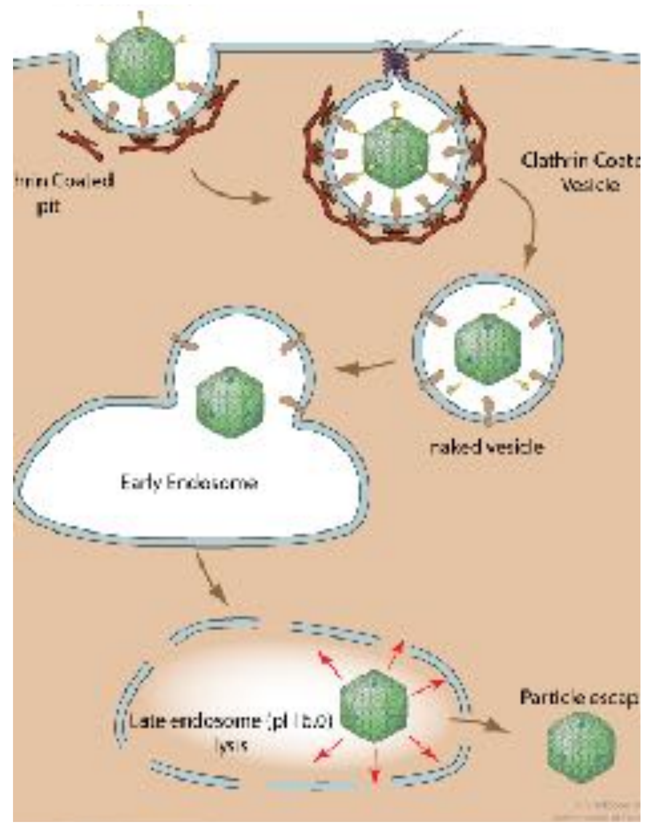
Examples of Viral Penetration

poliovirus



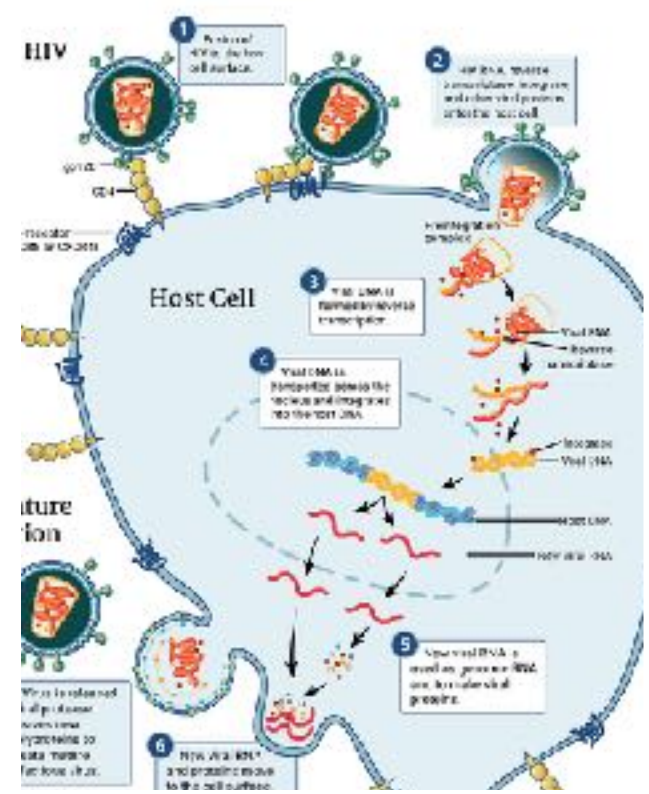
pores

adenovirus



vesicle lysis

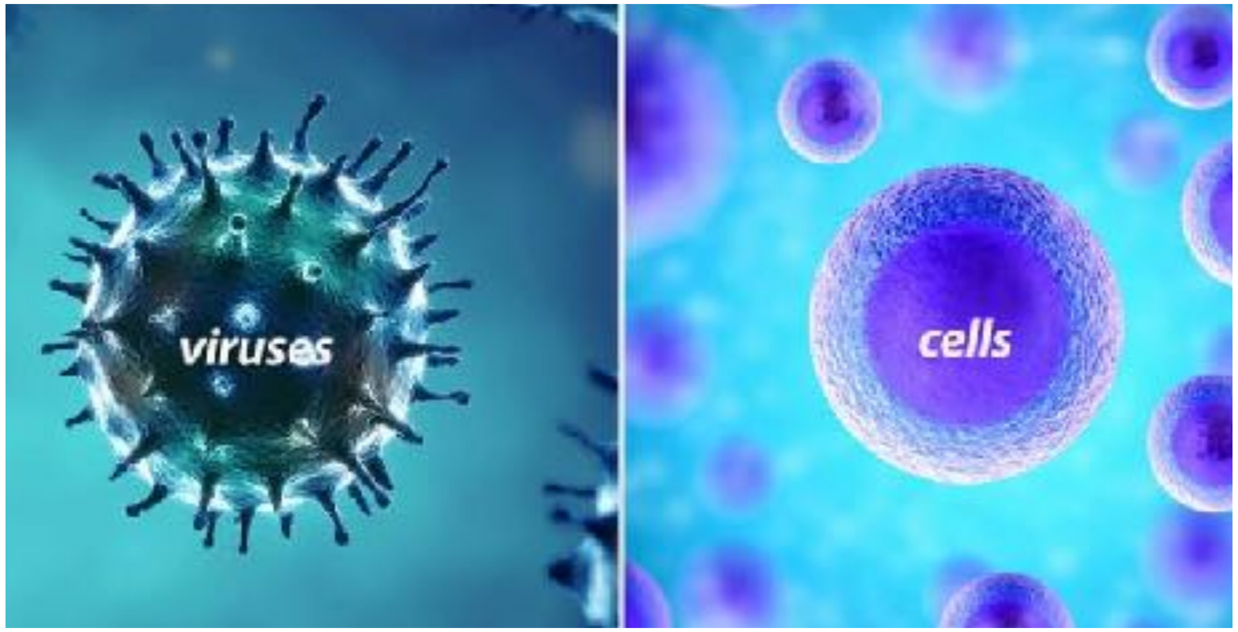
HIV-1



membrane fusion

A Thought

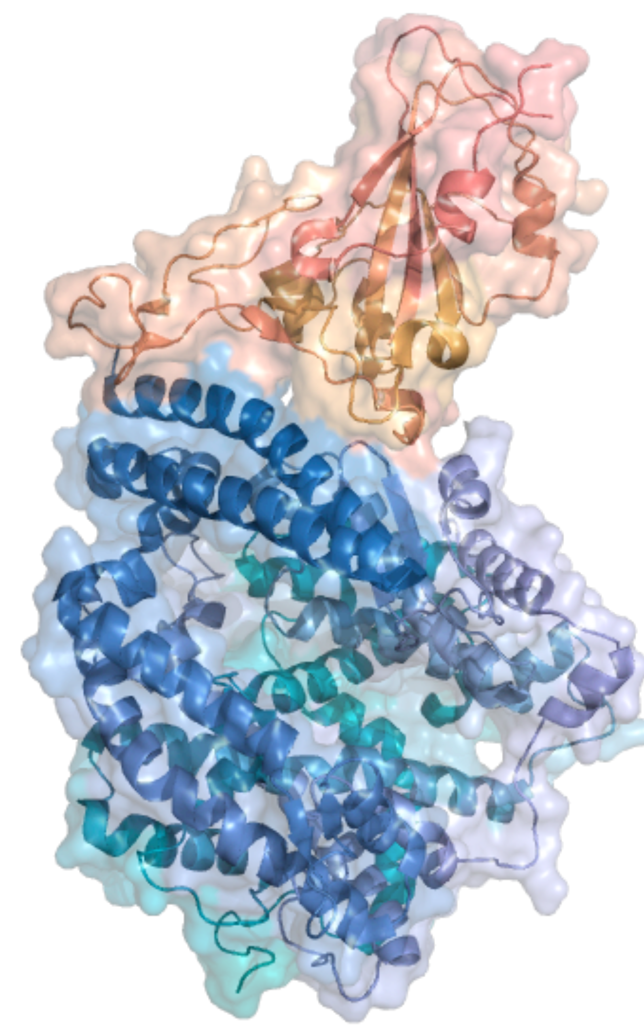
to understand a virus on must understand the cell that it infects



one can understand and learn about cells by studying viruses

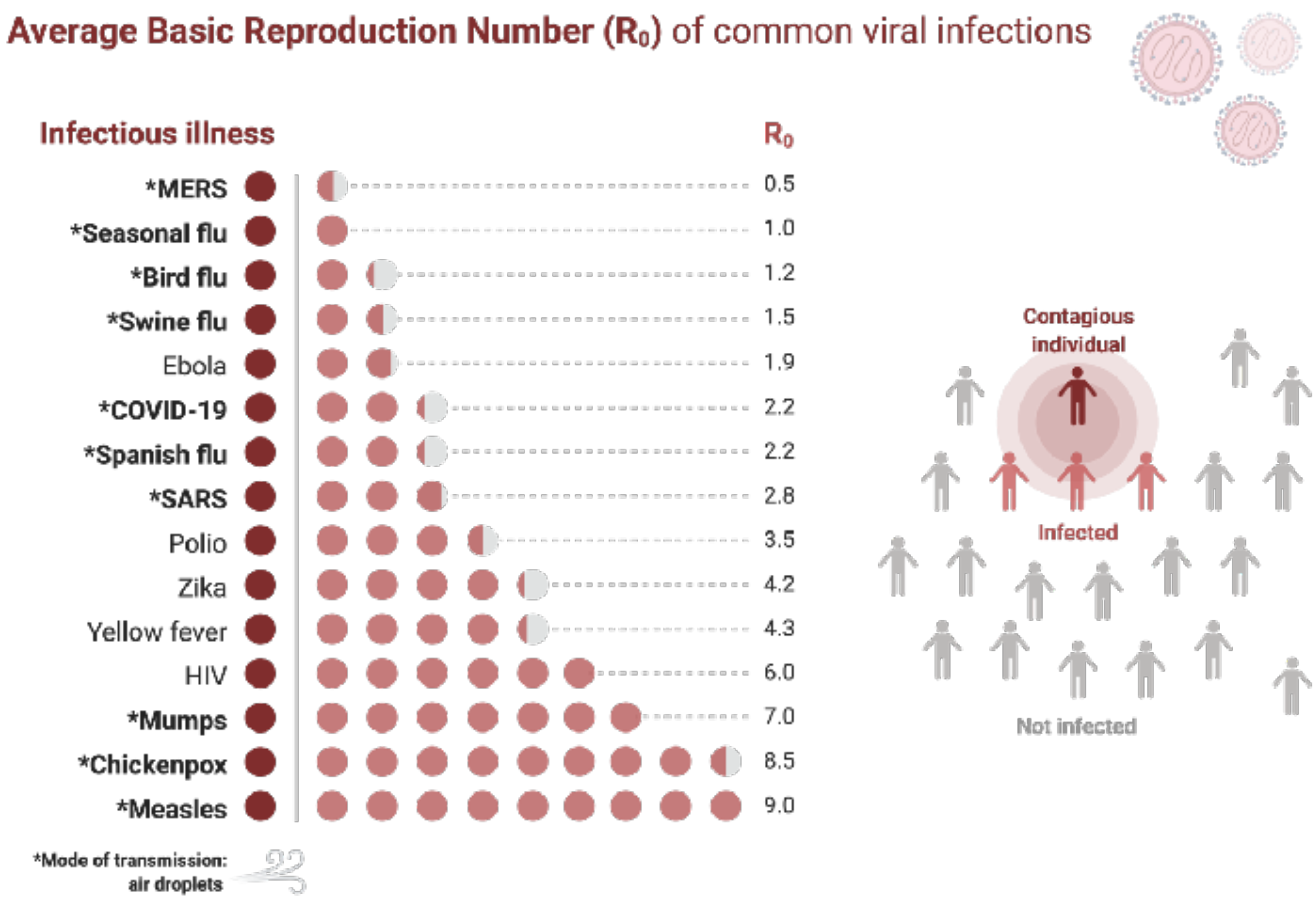
Outline

- Brief Overview of Viruses & Nomenclature
- General Mechanisms of Viral Entry
- Important Factors for Successful Viral Entry
    - Attachement
    - Signaling
    - Endocytosis
    - Penetration
    - Uncoating



- Details Associated Towards SARS-CoV-2 & Other Examples

SARS-CoV-2: A Quick Overview



The **average basic reproduction number (R_0)** is an epidemiologic metric that describes the transmissibility of infectious agents. R_0 measures the expected number of secondary infections produced by a single infectious individual in a susceptible population during the mean infectious period.

SARS-CoV-2: A Quick Overview

AIDS *(40 million HIV-1 infected, 25 million deaths)*

Hepatitis B Virus *(240 million chronically infected worldwide (2.2 million US)*
25% will succumb to liver disease or cancer)

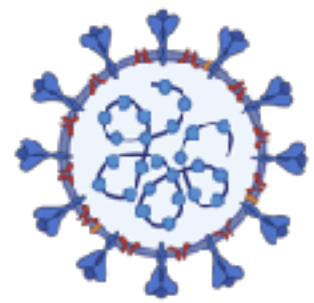
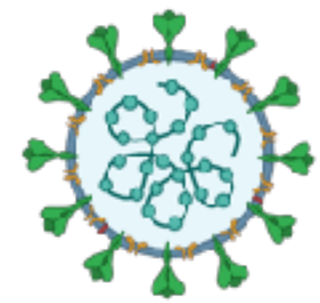
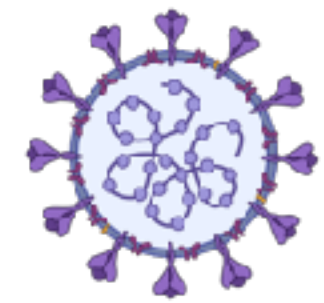
Rotavirus *(> 200,000 children deaths yearly)*

Influenza *(> 39 million deaths, 1918–19)*

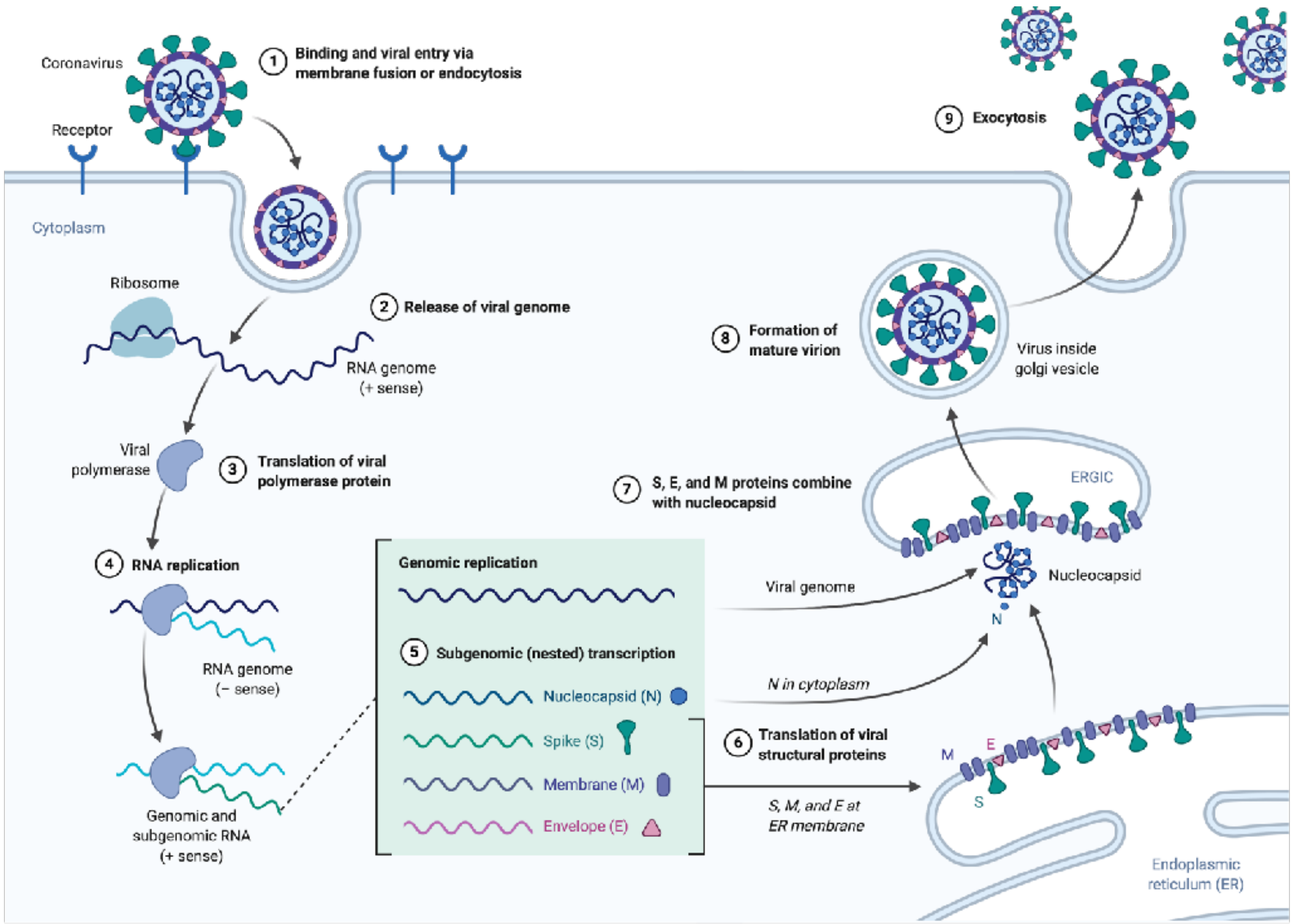
Avian Influenza (H5N1) *(pandemic threat in 2005)*

SARS-CoV-2 *(3.5 million infected, 250,000 deaths, declared pandemic in 2020)*

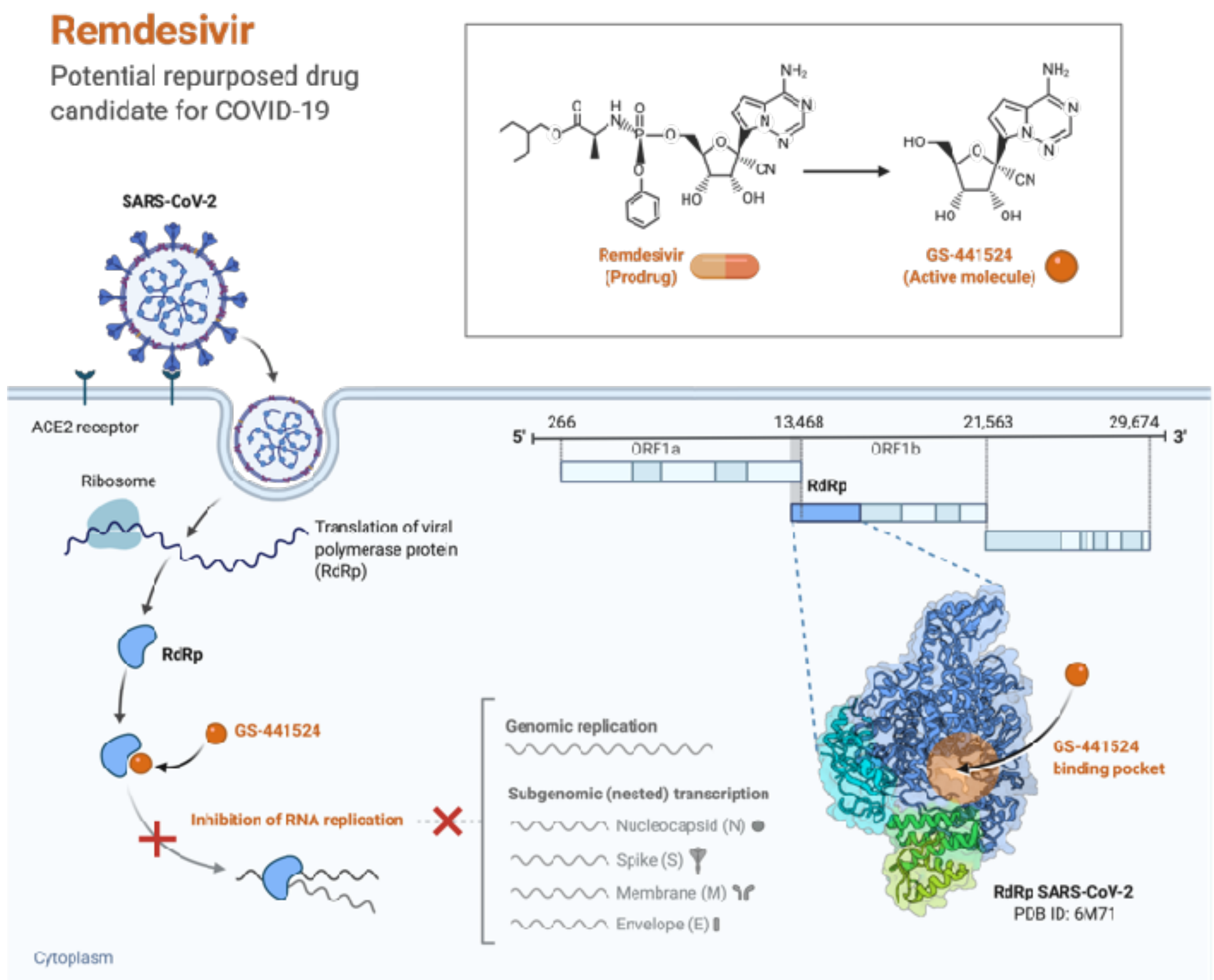
SARS-CoV-2: A Quick Overview

Disease	Flu	COVID-19	SARS	MERS
Disease Causing Pathogen	 Influenza virus	 SARS-CoV-2	 SARS-CoV	 MERS-CoV
R_0 Basic Reproductive Number	**1.3**	**2.0 - 2.5** *	**3**	**0.3 - 0.8**
CFR Case Fatality Rate	0.05 - 0.1%	~3.4% *	9.6 - 11%	34.4%
Incubation Time	1 - 4 days	4 - 14 days *	2 - 7 days	6 days
Hospitalization Rate	2%	~19% *	Most cases	Most cases
Community Attack Rate	10 - 20%	30 - 40% *	10 - 60%	4 - 13%
Annual Infected (global)	~ 1 billion	3.5 million (5/20)	8098 (in 2003)	420
Annual Infected (US)	10 - 45 million	1.18 million (5/20)	8 (in 2003)	2 (in 2014)
Annual Deaths (US)	10,000 - 61,000	68,000 (5/20)	None	None

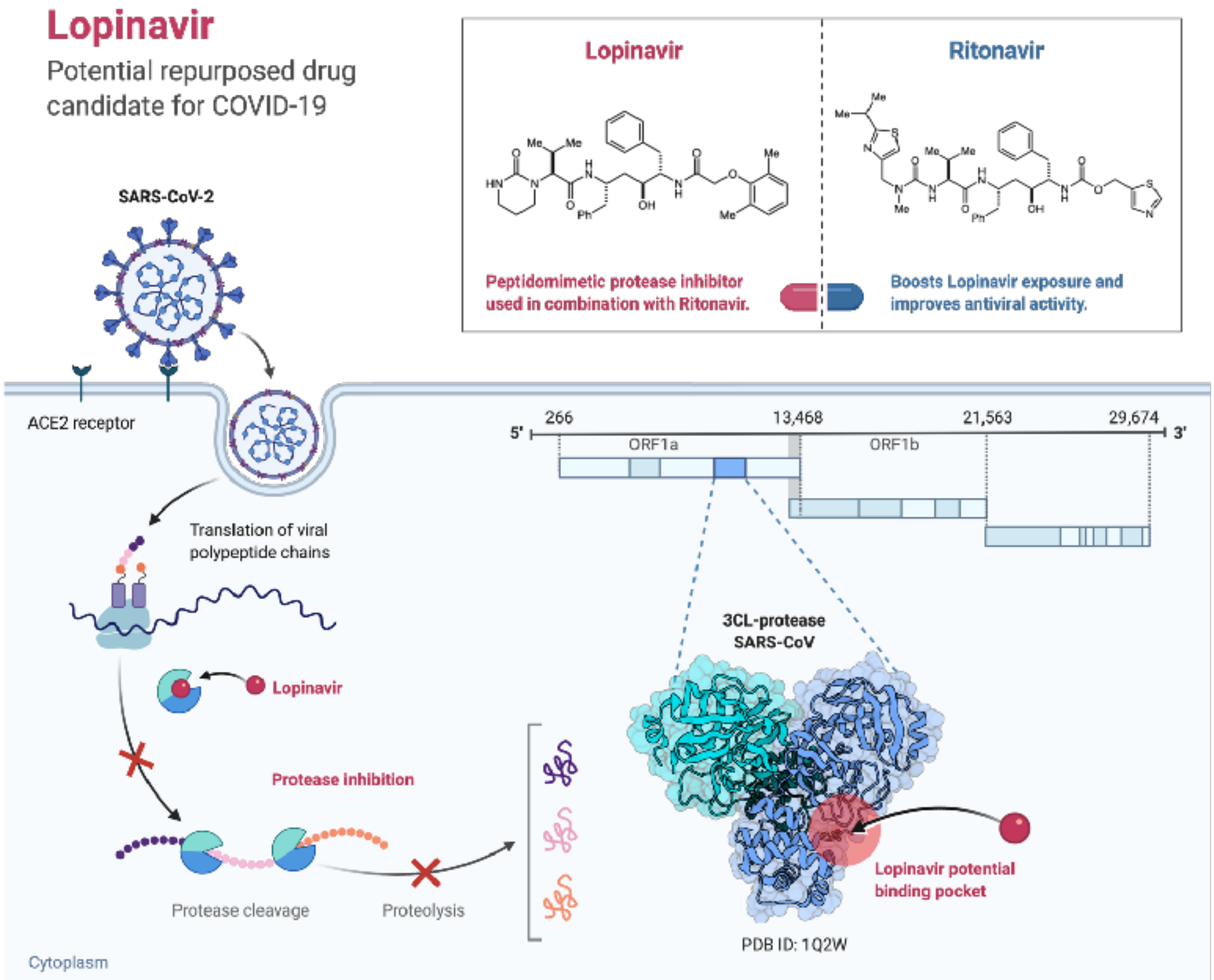
SARS-CoV-2: A Quick Overview



SARS-CoV-2: A Quick Overview



SARS-CoV-2: A Quick Overview



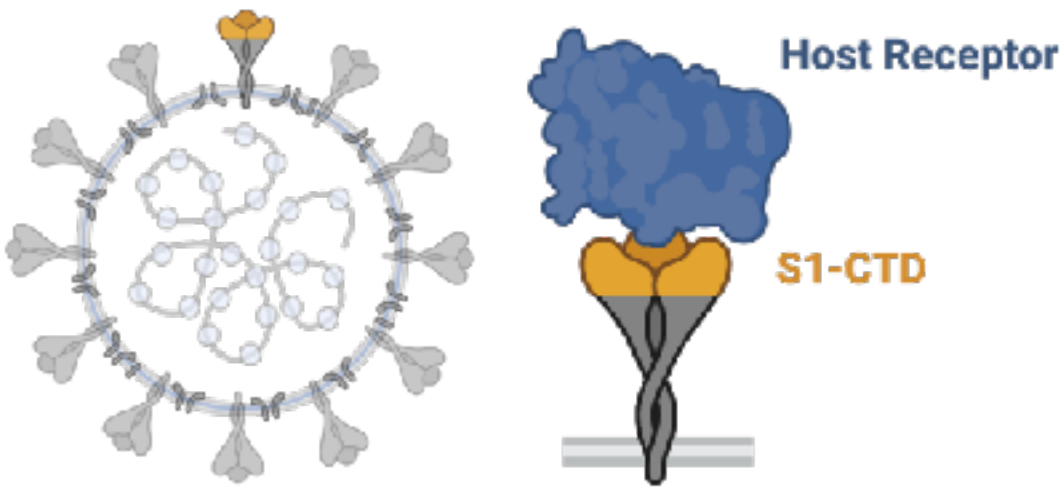
SARS-CoV-2: A Quick Overview

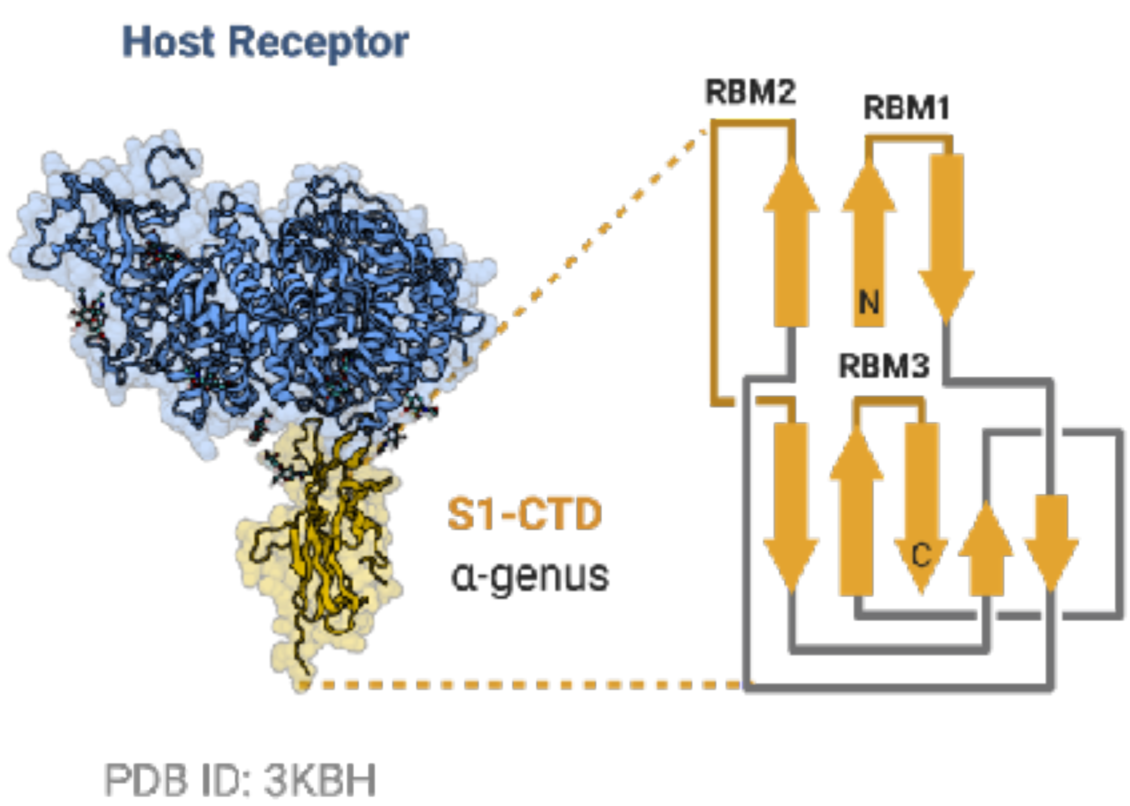
A SARS-CoV-2-Human Protein-Protein Interaction Map Reveals Drug Targets and Potential Drug-Repurposing

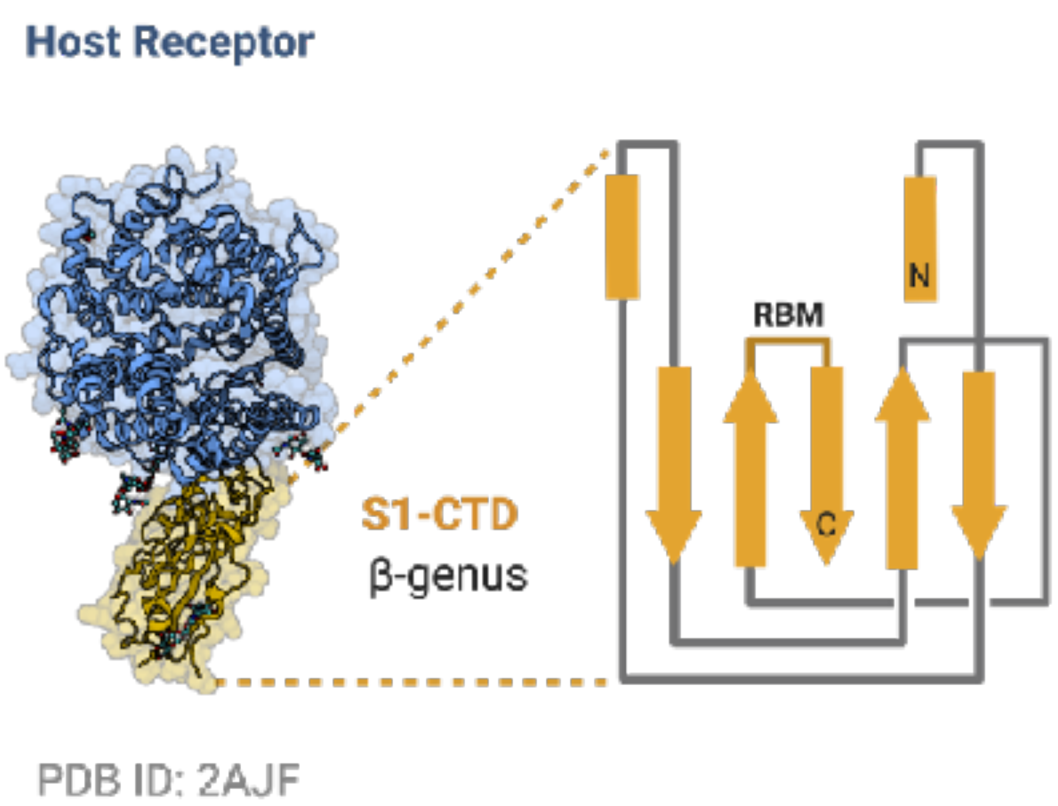
David E. Gordon[1,2,3,4], Gwendolyn M. Jang[1,2,3,4], Mehdi Bouhaddou[1,2,3,4], Jiewei Xu[1,2,3,4], Kirsten Obernier[1,2,3,4], Matthew J. O'Meara[5], Jeffrey Z. Guo[1,2,3,4], Danielle L. Swaney[1,2,3,4], Tia A. Tummino[1,2,6], Ruth Huettenhain[1,2,3,4], Robyn M. Kaake[1,2,3,4], Alicia L. Richards[1,2,3,4], Beril Tutuncuoglu[1,2,3,4], Helene Foussard[1,2,3,4], Jyoti Batra[1,2,3,4], Kelsey Haas[1,2,3,4], Maya Modak[1,2,3,4], Minkyu Kim[1,2,3,4], Paige Haas[1,2,3,4], Benjamin J. Polacco[1,2,3,4], Hannes Braberg[1,2,3,4], Jacqueline M. Fabius[1,2,3,4], Manon Eckhardt[1,2,3,4], Margaret Soucheray[1,2,3,4], Melanie J. Bennett[1,2,3,4], Merve Cakir[1,2,3,4], Michael J. McGregor[1,2,3,4], Qiongyu Li[1,2,3,4], Zun Zar Chi Naing[1,2,3,4], Yuan Zhou[1,2,3,4], Shiming Peng[1,2,6], Ilsa T. Kirby[1,4,7], James E. Melnyk[1,4,7], John S. Chorba[1,4,7], Kevin Lou[1,4,7], Shizhong A. Dai[1,4,7], Wenqi Shen[1,4,7], Ying Shi[1,4,7], Ziyang Zhang[1,4,7], Inigo Barrio-Hernandez[8], Danish Memon[8], Claudia Hernandez-Armenta[8], Christopher J.P. Mathy[1,9,10,2], Tina Perica[1,2,9], Kala B. Pilla[1,2,9], Sai J. Ganesan[1,2,9], Daniel J. Saltzberg[1,2,9], Rakesh Ramachandran[1,2,9], Xi Liu[1,2,6], Sara B. Rosenthal[11], Lorenzo Calviello[12], Srivats Venkataramanan[12], Jose Liboy-Lugo[12], Yizhu Lin[12], Stephanie A. Wankowicz[1,13,9], Markus Bohn[6], Phillip P. Sharp[4], Raphael Trenker[14], Janet M. Young[15], Devin A. Cavero[3], Joseph Hiatt[16,3], Theodore L. Roth[16,3], Ujjwal Rathore[3], Advait Subramanian[1,17], Julia Noack[1,17], Mathieu Hubert[18], Ferdinand Roesch[19], Thomas Vallet[19], Björn Meyer[19], Kris M. White[20], Lisa Miorin[20], Oren S. Rosenberg[21,22,23], Kliment A Verba[1,2,6], David Agard[1,24], Melanie Ott[3,21], Michael Emerman[25], Davide Ruggero[26,27,4], Adolfo García-Sastre[20], Natalia Jura[1,14,4], Mark von Zastrow[1,1,4,28], Jack Taunton[1,2,4], Alan Ashworth[1,27], Olivier Schwartz[18], Marco Vignuzzi[19], Christophe d'Enfert[29], Shaeri Mukherjee[1,17], Matt Jacobson[6], Harmit S. Malik[15], Danica G. Fujimori[1,4,6], Trey Ideker[30], Charles S. Craik[6,27], Stephen Floor[12,27], James S. Fraser[1,2,9], John Gross[1,2,6], Andrej Sali[1,2,6,9], Tanja Kortemme[1,9,10,2], Pedro Beltrao[8], Kevan Shokat[1,4,7], Brian K. Shoichet[1,2,6], Nevan J. Krogan[1,2,3,4]

Receptor Binding Motifs (RBM)

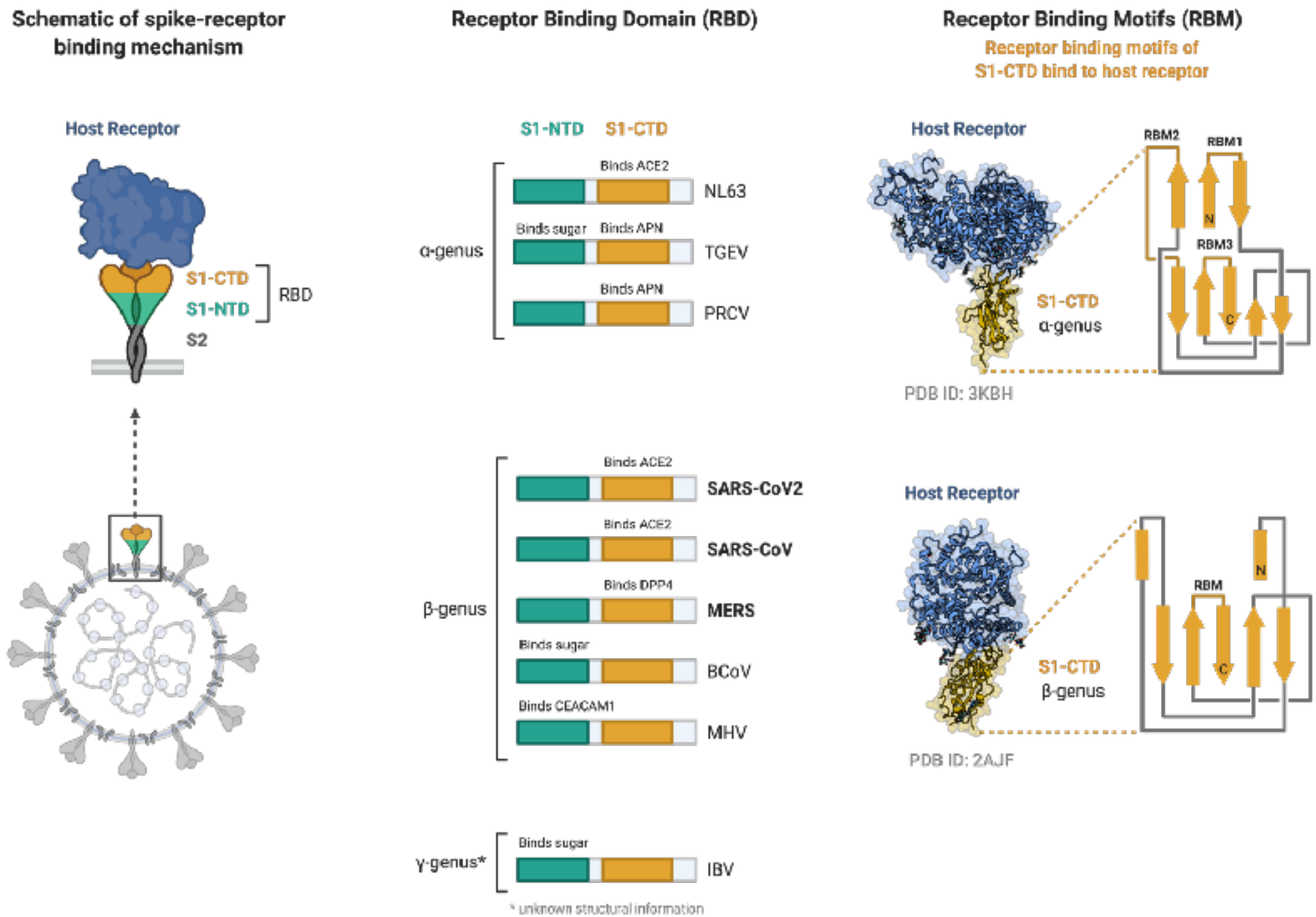
SARS-CoV-2 spike protein (S1-CTD)







SARS-CoV-2: A Quick Overview

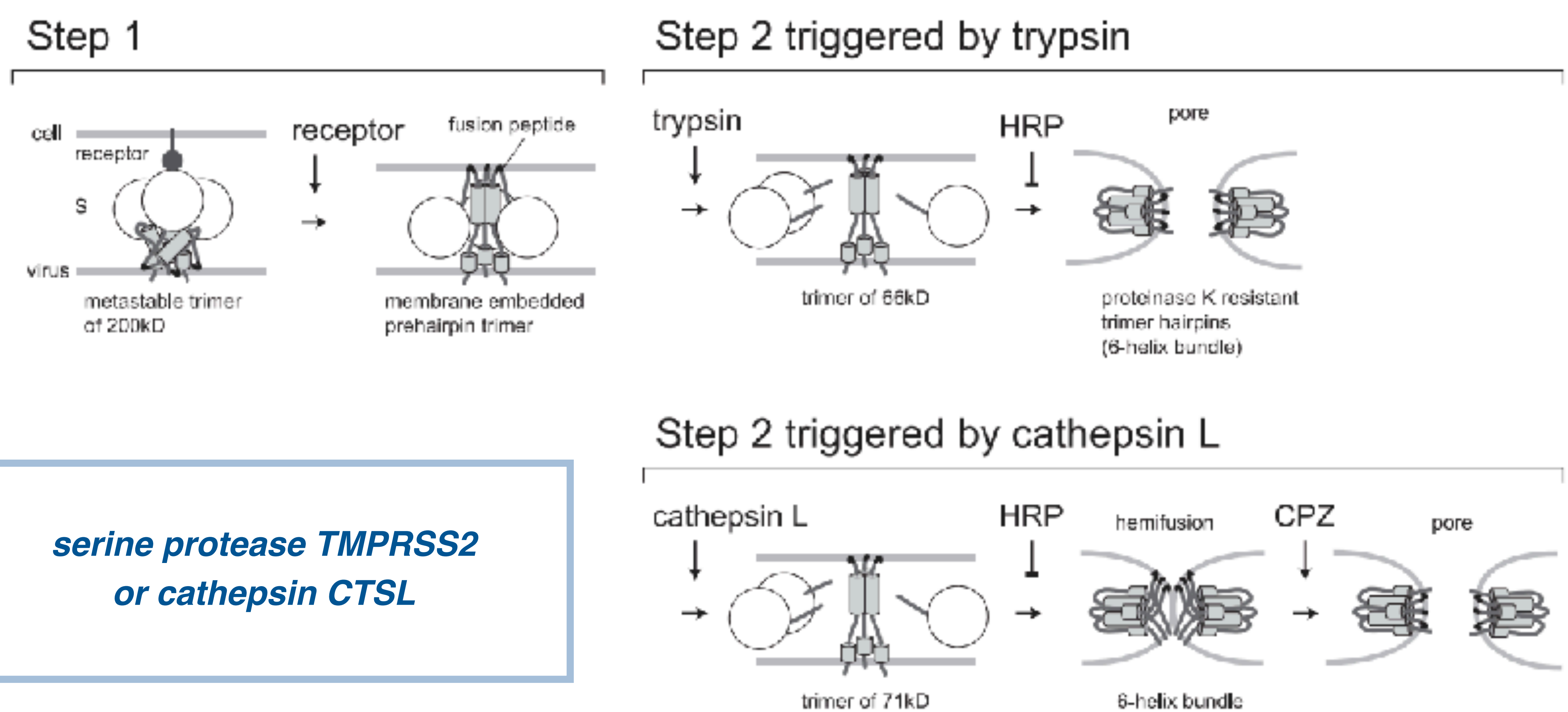


SARS-CoV-2: A Quick Overview

JOURNAL OF VIROLOGY, Nov. 2009, p. 11133–11141 Vol. 83, No. 21
0022-538X/09/$12.00 doi:10.1128/JVI.00959-09

Two-Step Conformational Changes in a Coronavirus Envelope Glycoprotein Mediated by Receptor Binding and Proteolysis∇

Shutoku Matsuyama* and Fumihiro Taguchi†



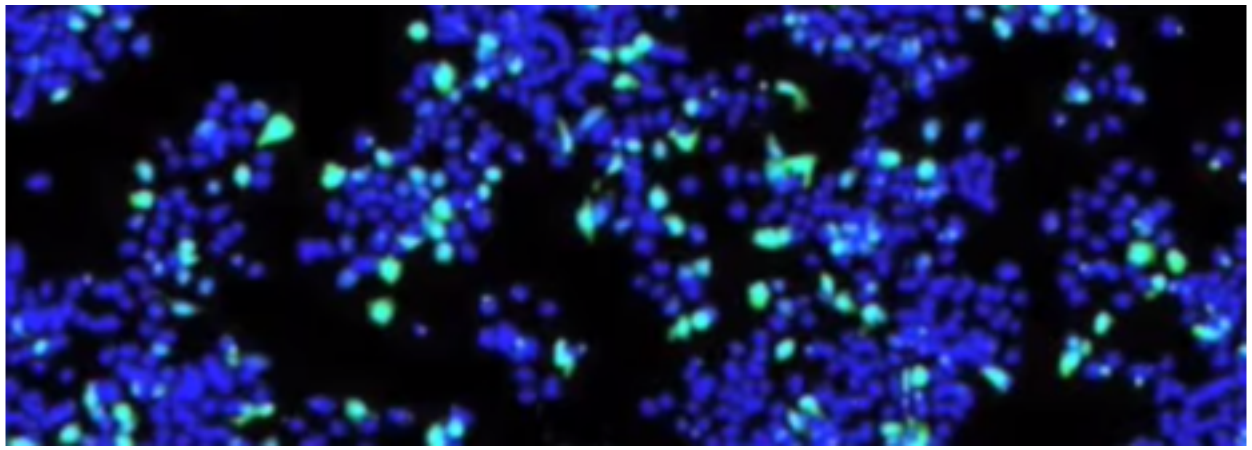
serine protease TMPRSS2
or cathepsin CTSL

Current State-of-the-Art for Understanding Systems Biology in Virology

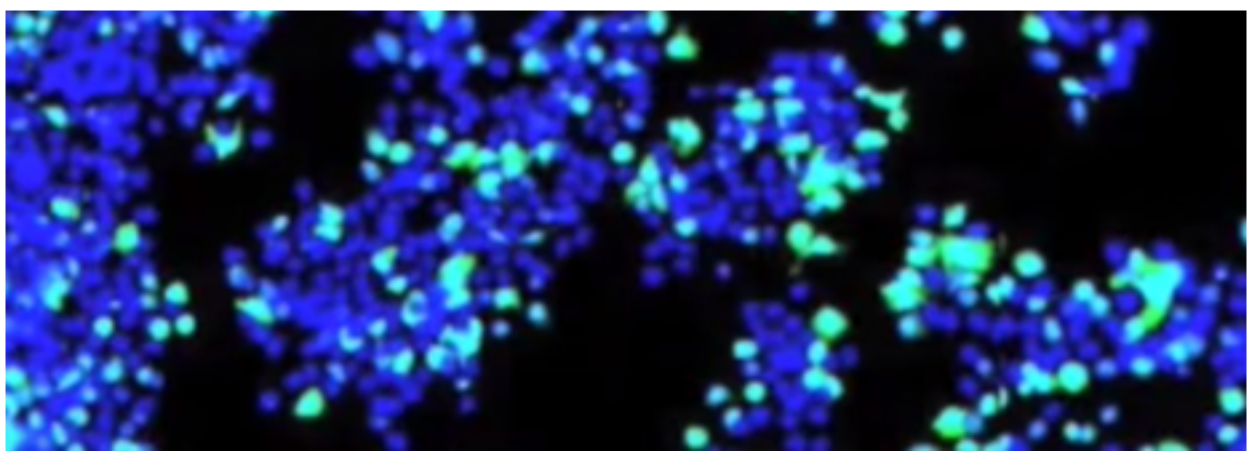
centered on siRNA silencing & high throughput experimentation

- **7000 druggable genes**
- **HTE using 384 well plates**
- **3 siRNA per gene (testing in triplicate)**
- **mature viruses (MV) expressing GFP (green fluorescent protein)**
- **automated microscopy (hits contain no green)**
- **Hit = 2/3 or 3/3 siRNA causing 50% or less infection compared to controls**

Current State-of-the-Art for Understanding Systems Biology in Virology



control
all normal human cell genes expressed



siRNA knockout of one gene
increased viral replication (rare)

siRNA knockout of one gene
decrease in viral replication

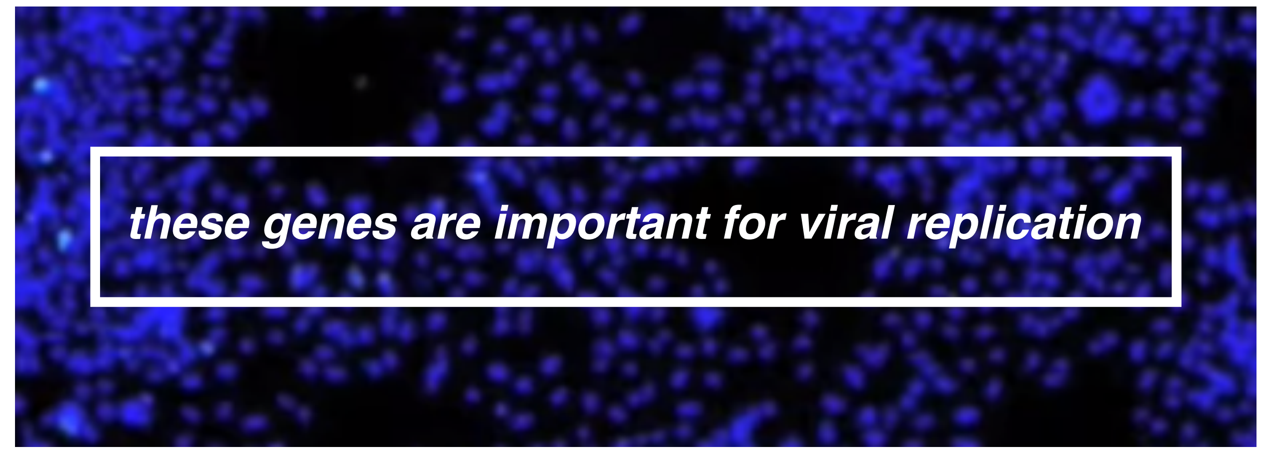
Current State-of-the-Art for Understanding Systems Biology in Virology

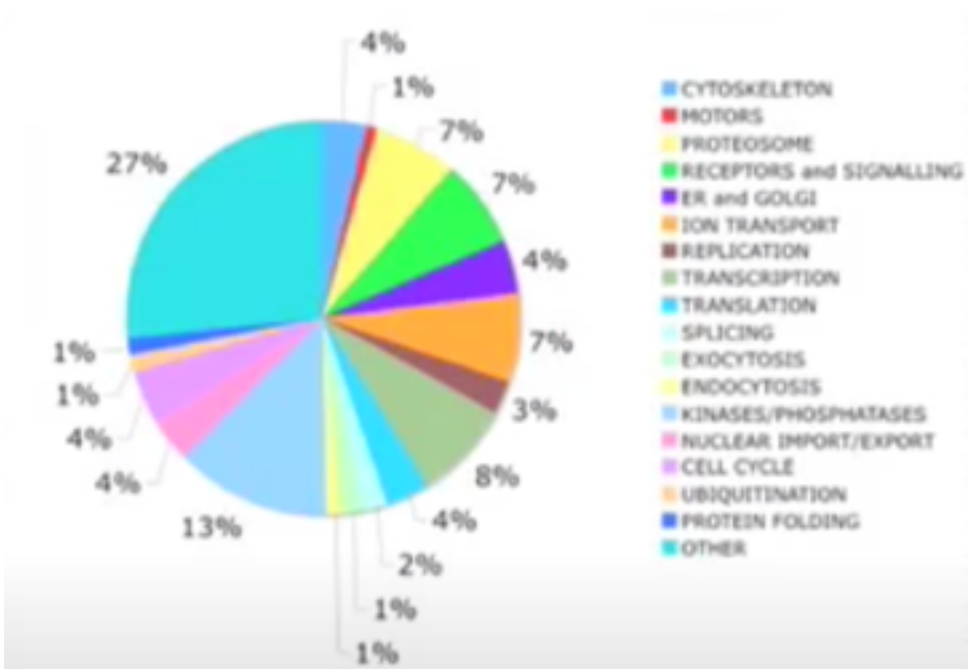
results

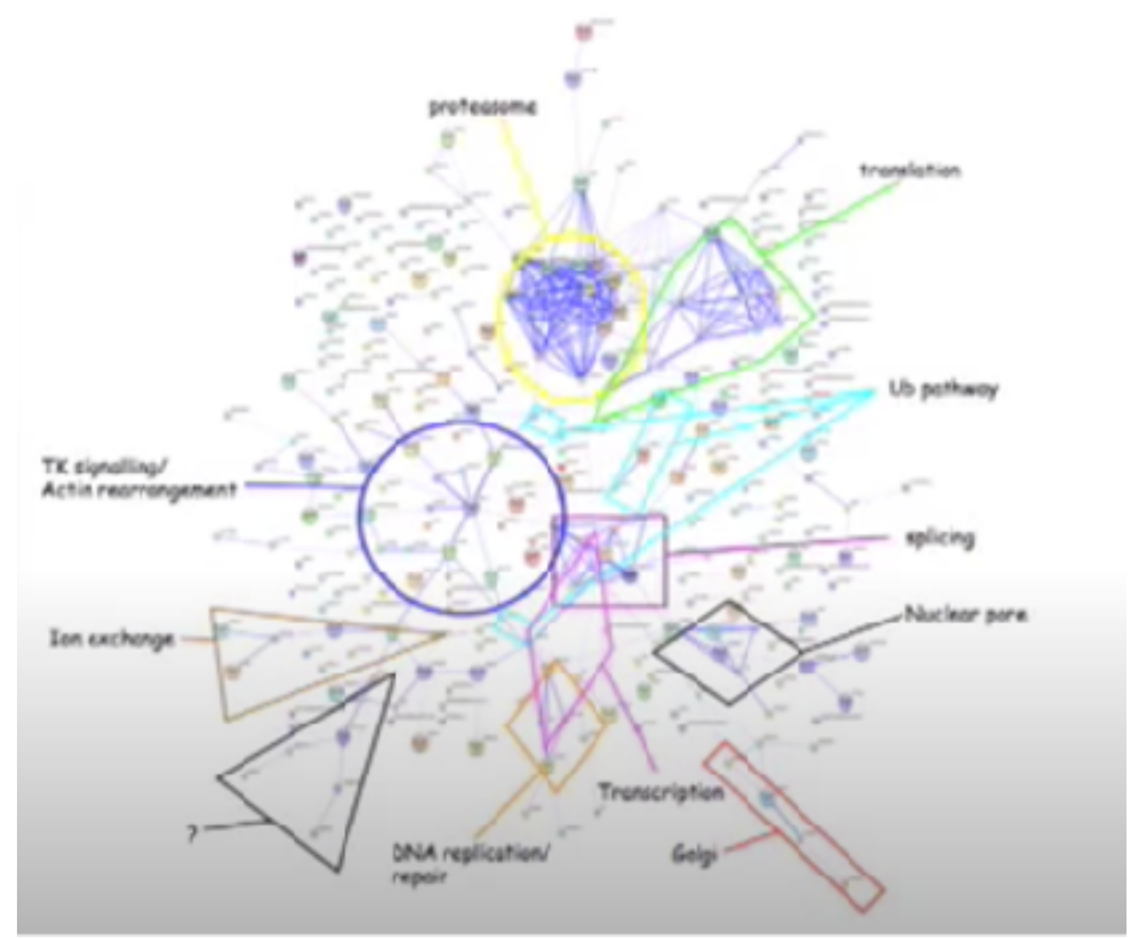
- *146 primary hits (3/3)*
- *158 secondary hits (2/3)*

total of 304 inhibit infection

- *4 increase infection*
- *string analysis of those genes*





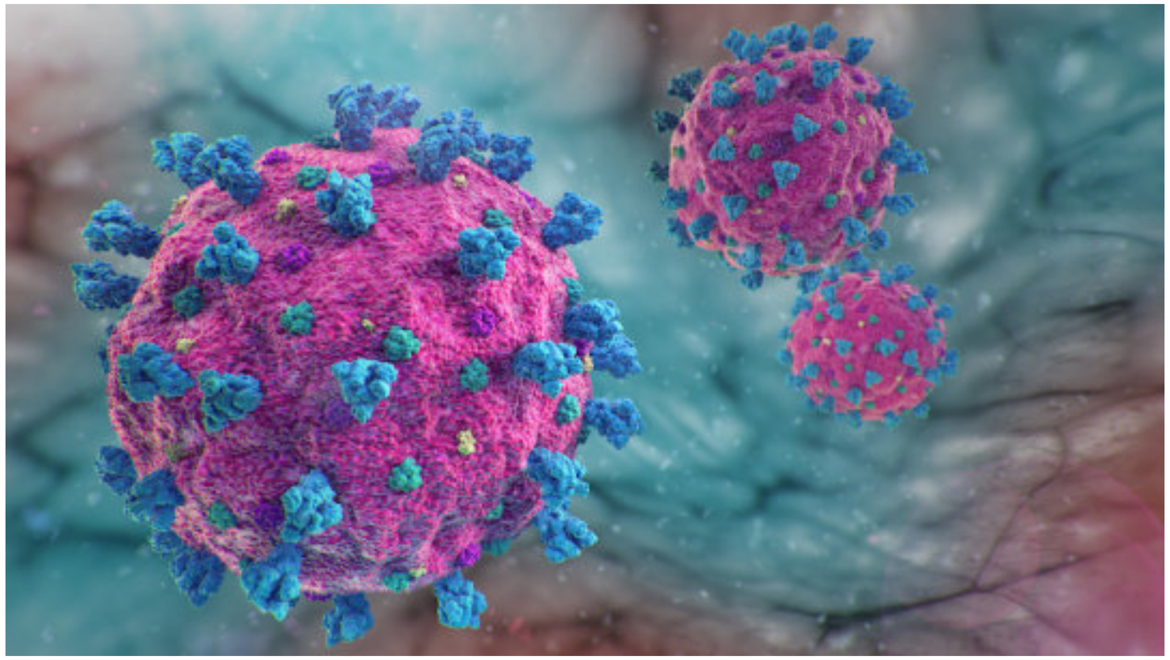


Mercer, J. *et. al. Cell Reports* **2**, 1036–1047 (2012).

Additional Resources

- **iBiology (video lecture series on many biology topics)**
    - **Virus Entry (Ari Helenius, ETH Zurich)**
    - **Virus Ecology & Evolution (Paul Turner, Yale)**
    - **Danger from the Wild: HIV (David Baltimore, Caltech)**
    - **Discovering Reverse Transcriptase (David Balitmore, Caltech)**
    - **Studying Coronavirus (Tracey Goldstein & Koen Van Rompay, UCD)**
- **BioRender ("ChemDraw" for biomolecules, Princeton License)**

Viral Entry



MacMillan Group Meeting
May 04, 2020

Daniel Kim