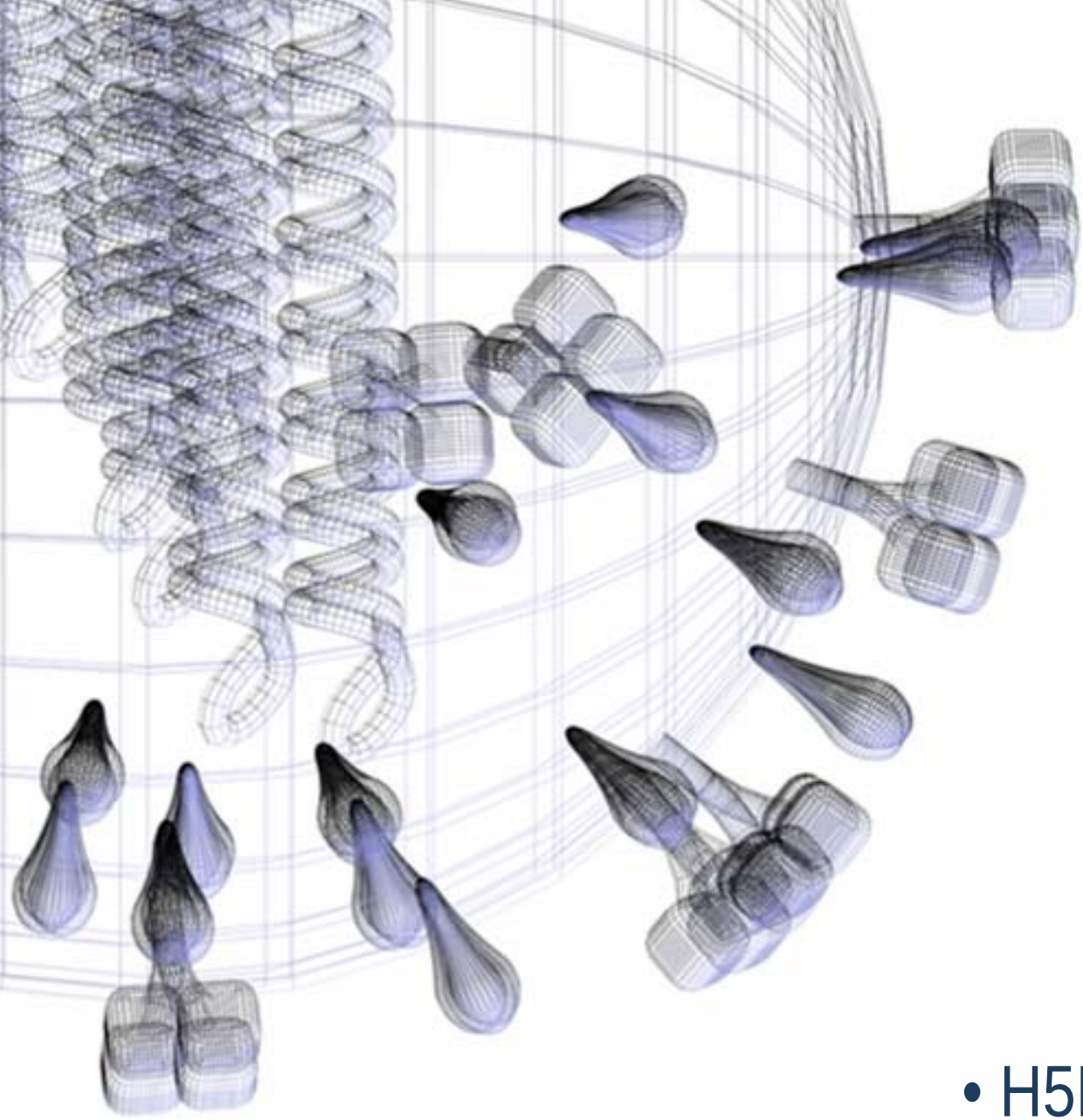
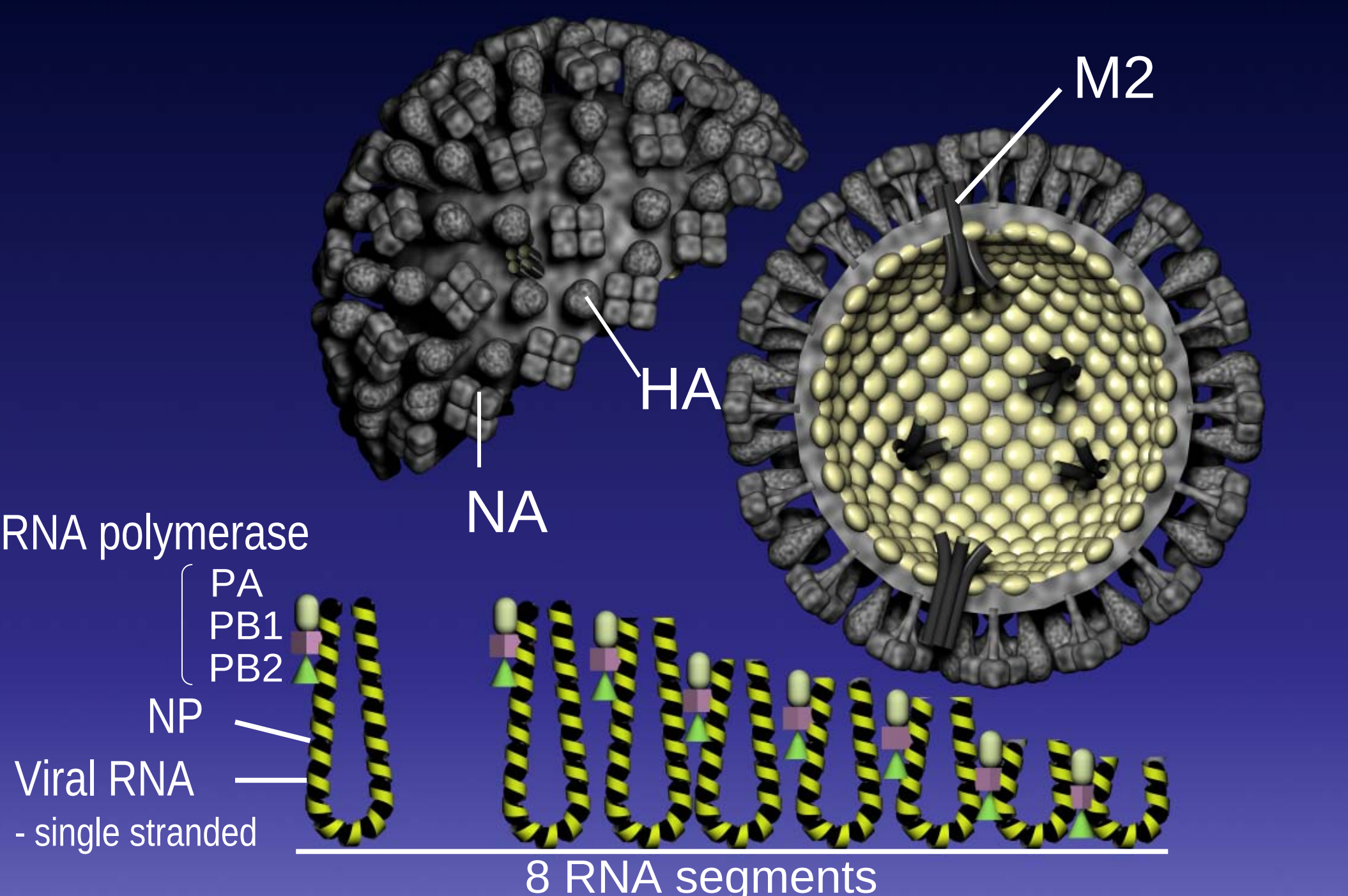
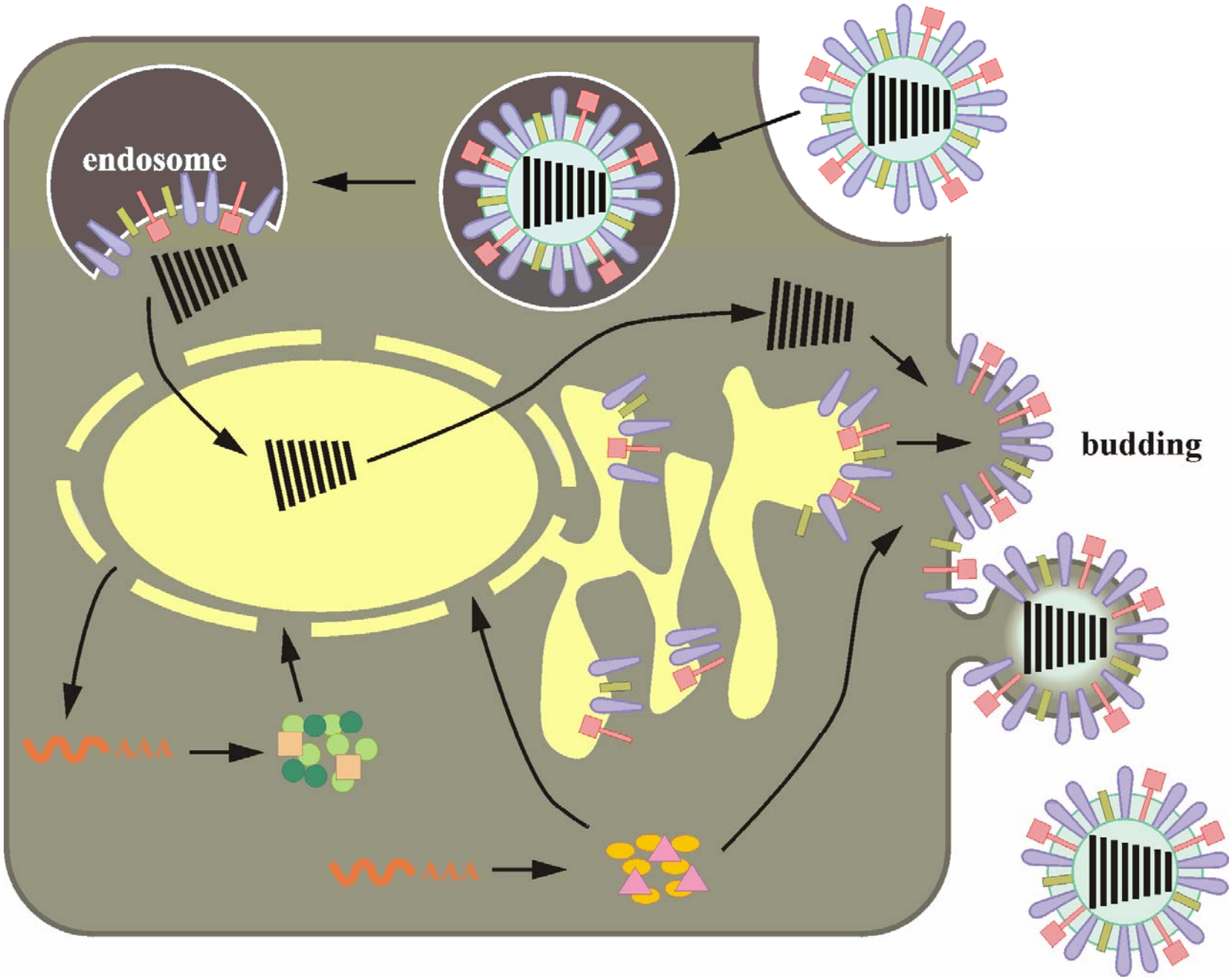
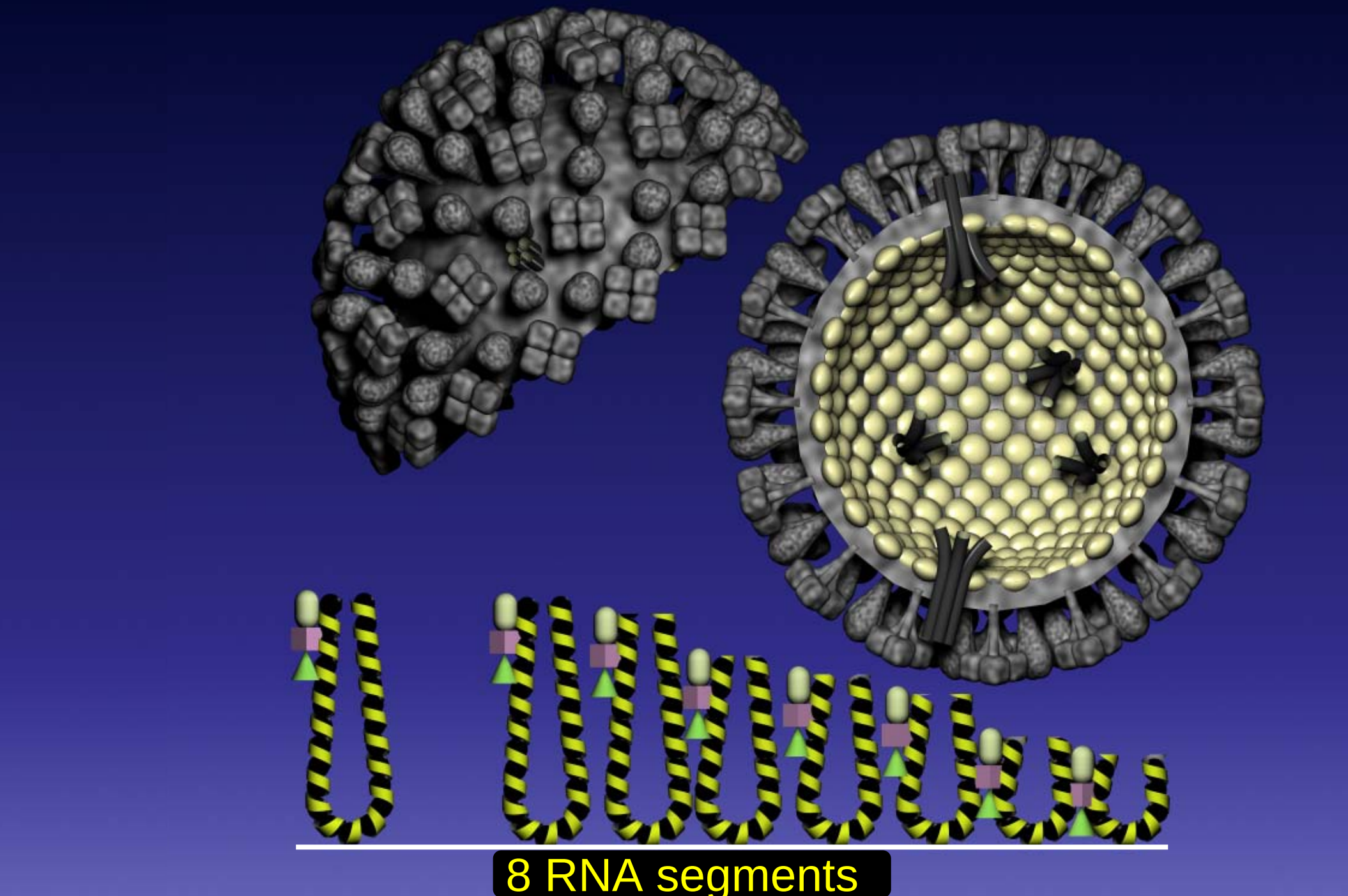


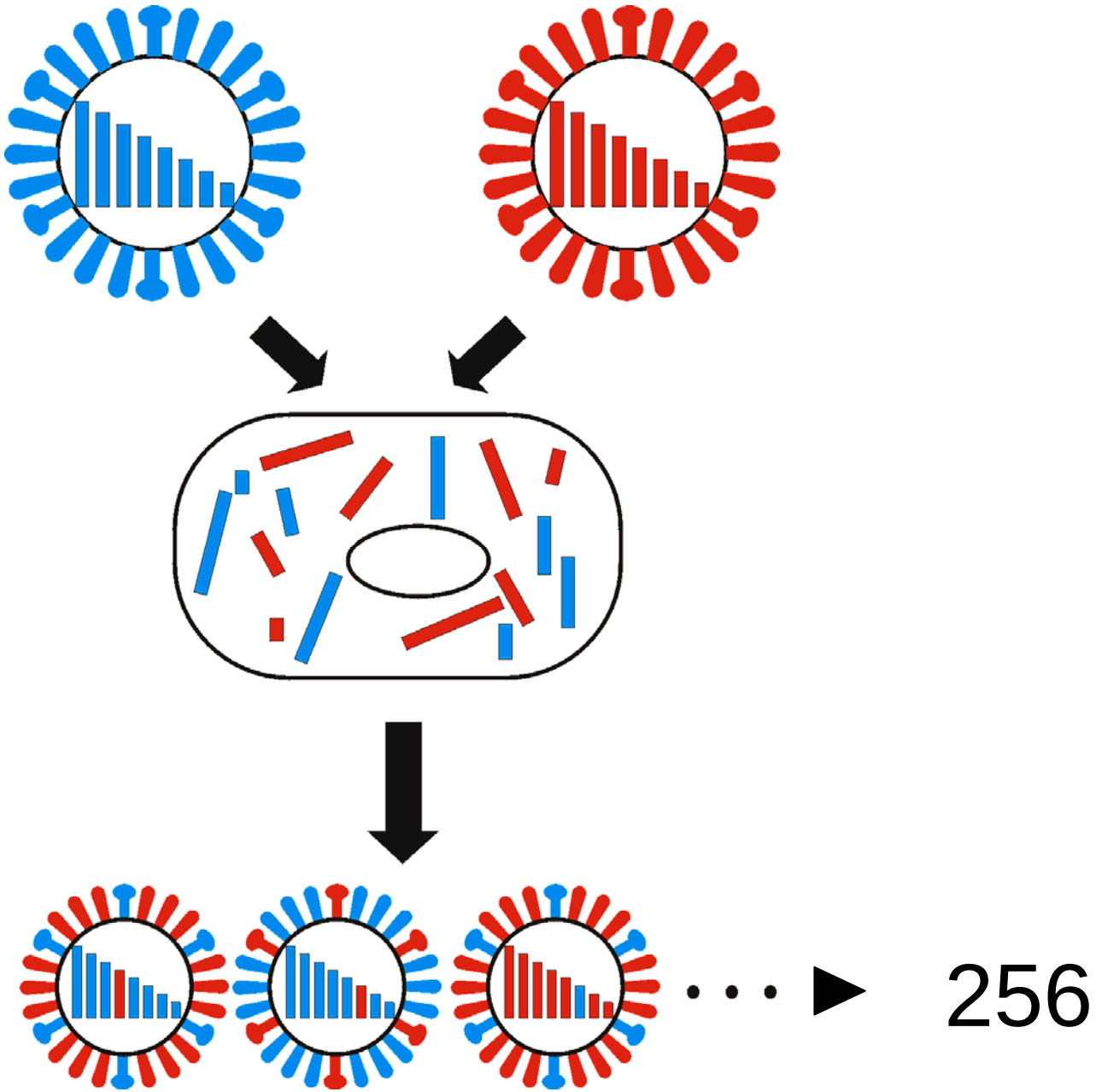
University of Wisconsin
Yoshihiro Kawaoka

- H5N1 avian influenza viruses
- Viruses possessing the 1918 virus HA and NA

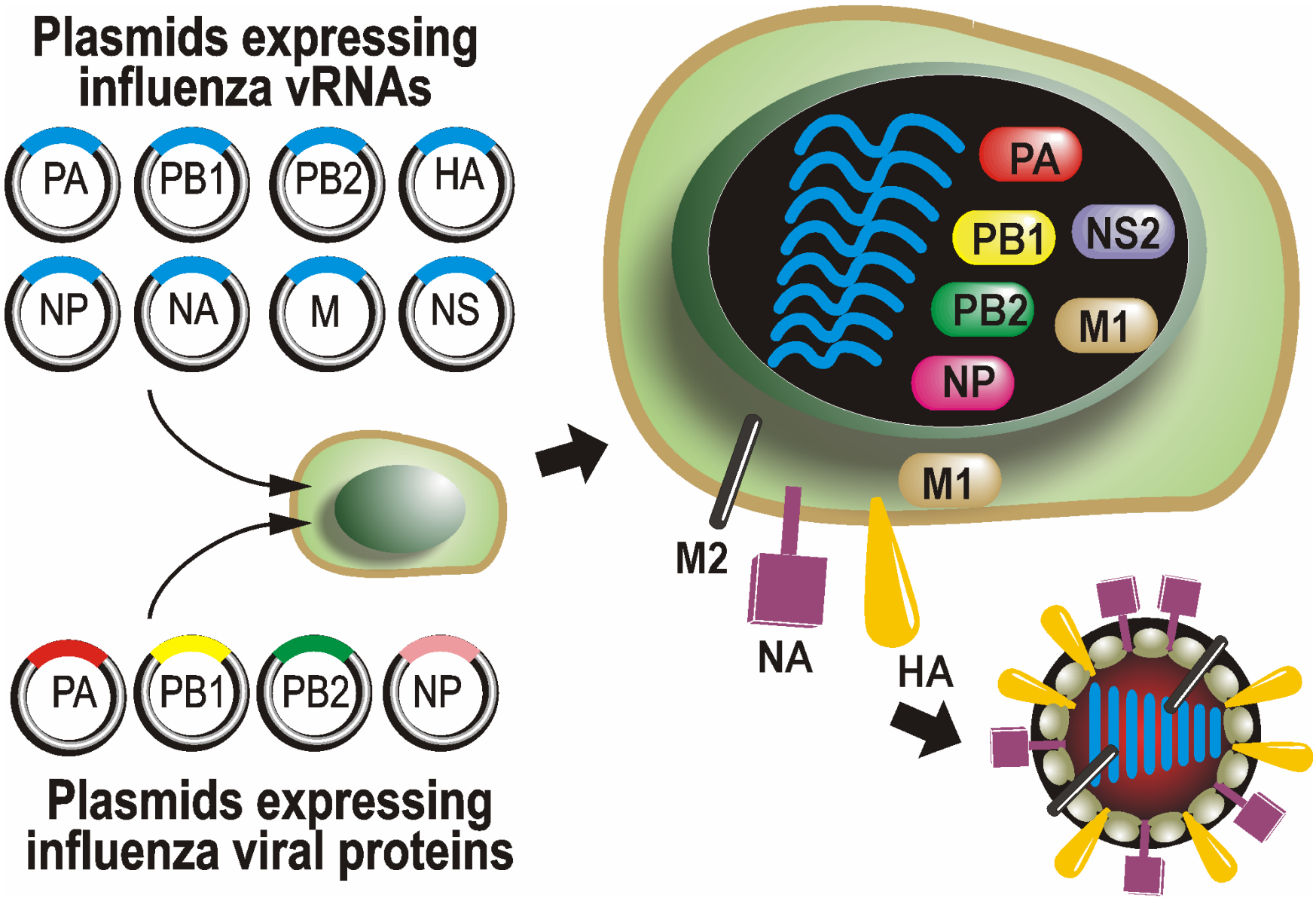

M2
HA
NA
RNA polymerase
PA
PB1
PB2
NP
Viral RNA
- single stranded
8 RNA segments


endosome
budding
AAA
AAA

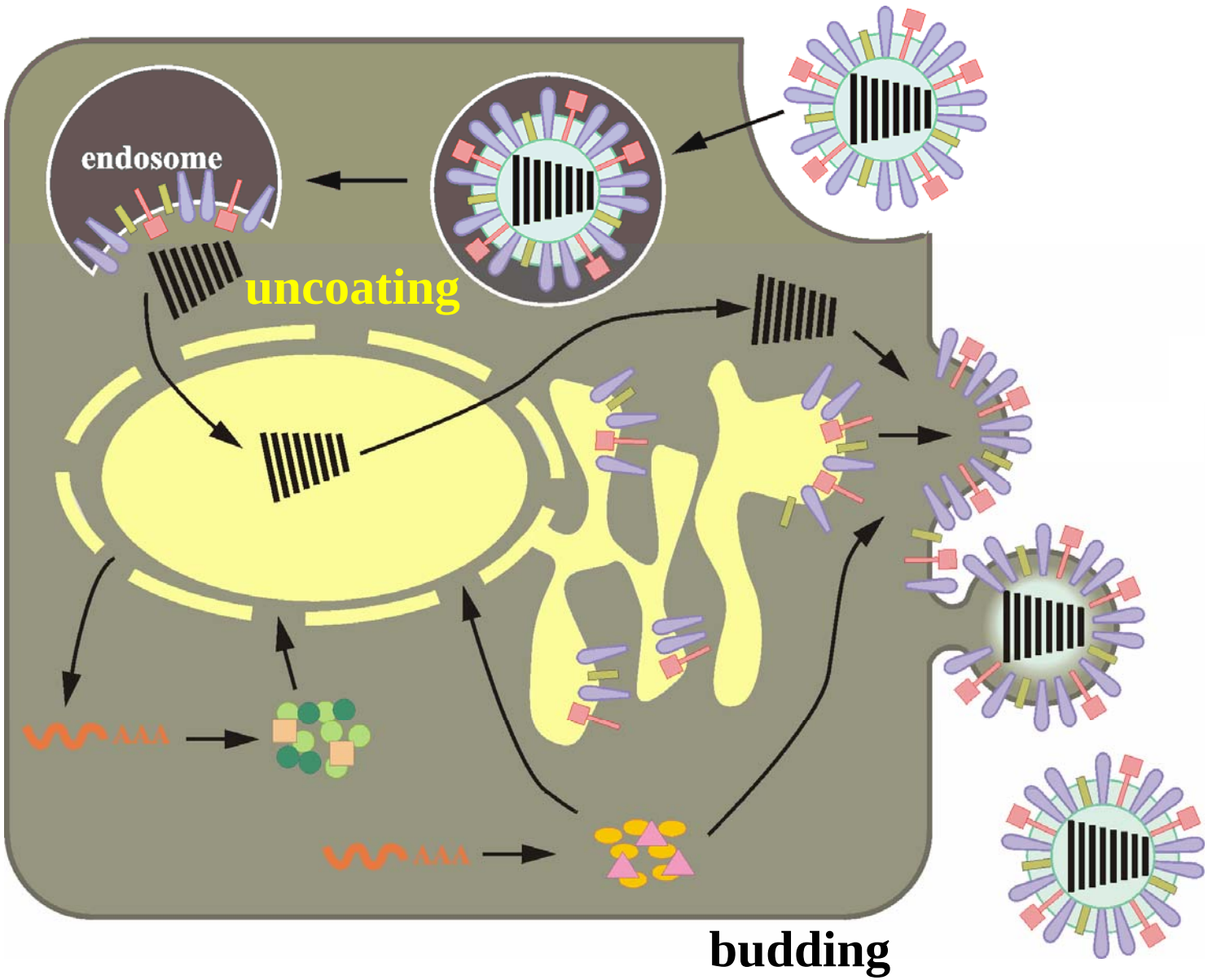

8 RNA segments


256

Reverse genetics

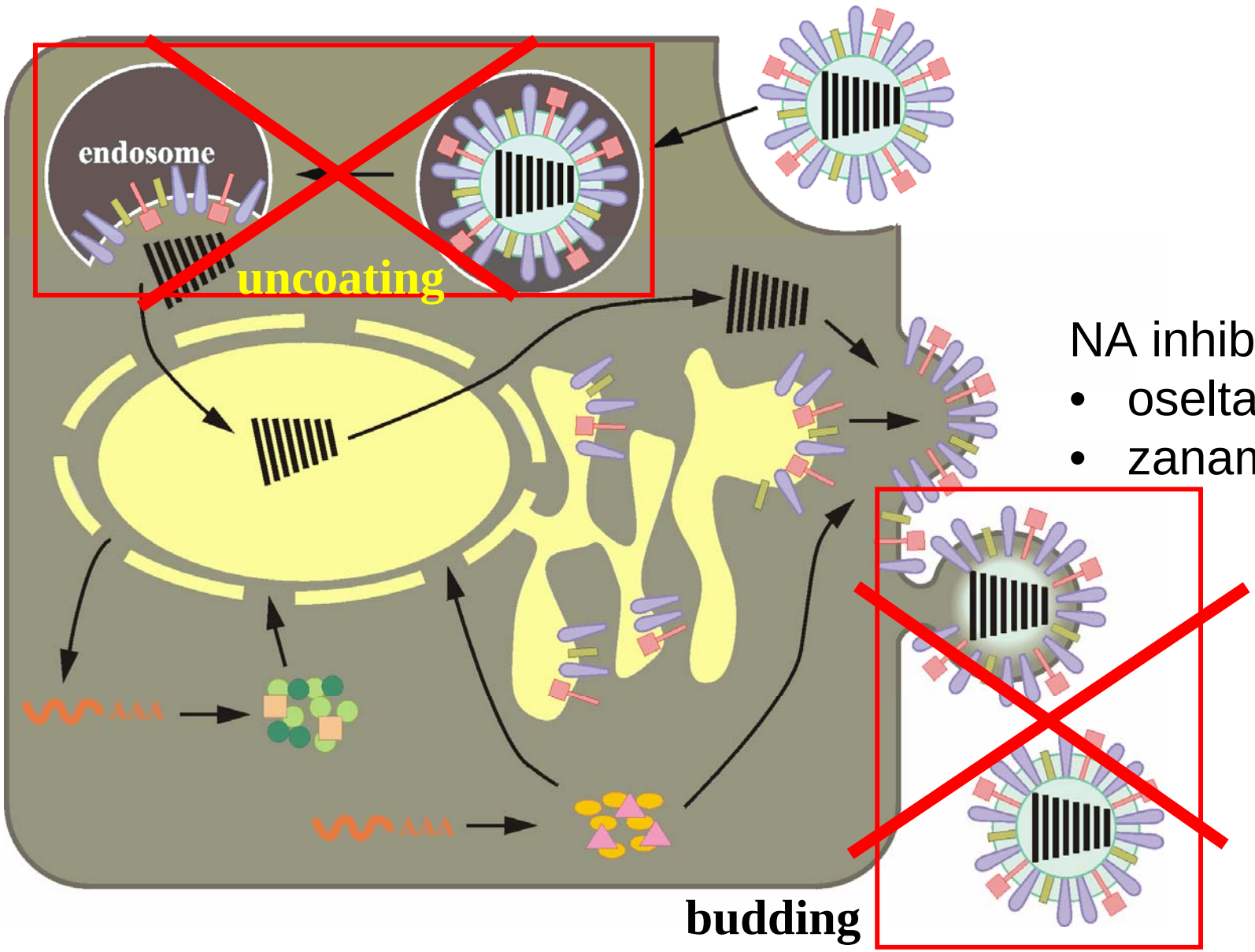


Anti-influenza drugs



Anti-influenza drugs

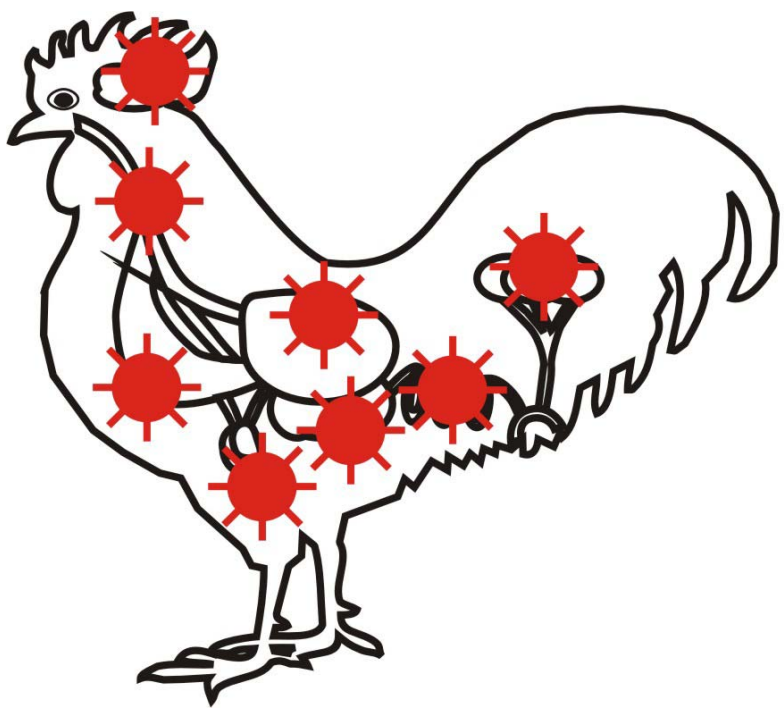
M2 inhibitors
- amantadine and rimantadine



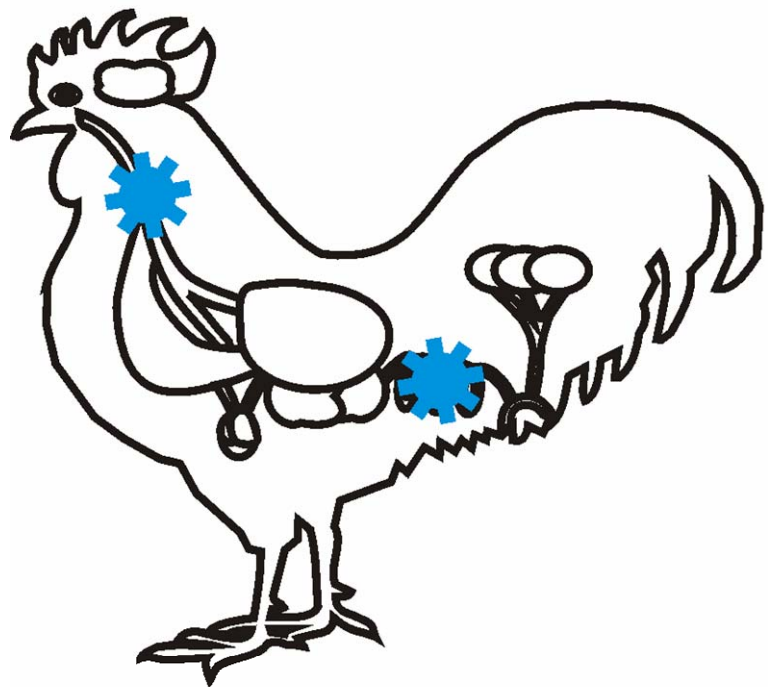
NA inhibitors
- oseltamivir
- zanamivir

H5N1 avian influenza virus

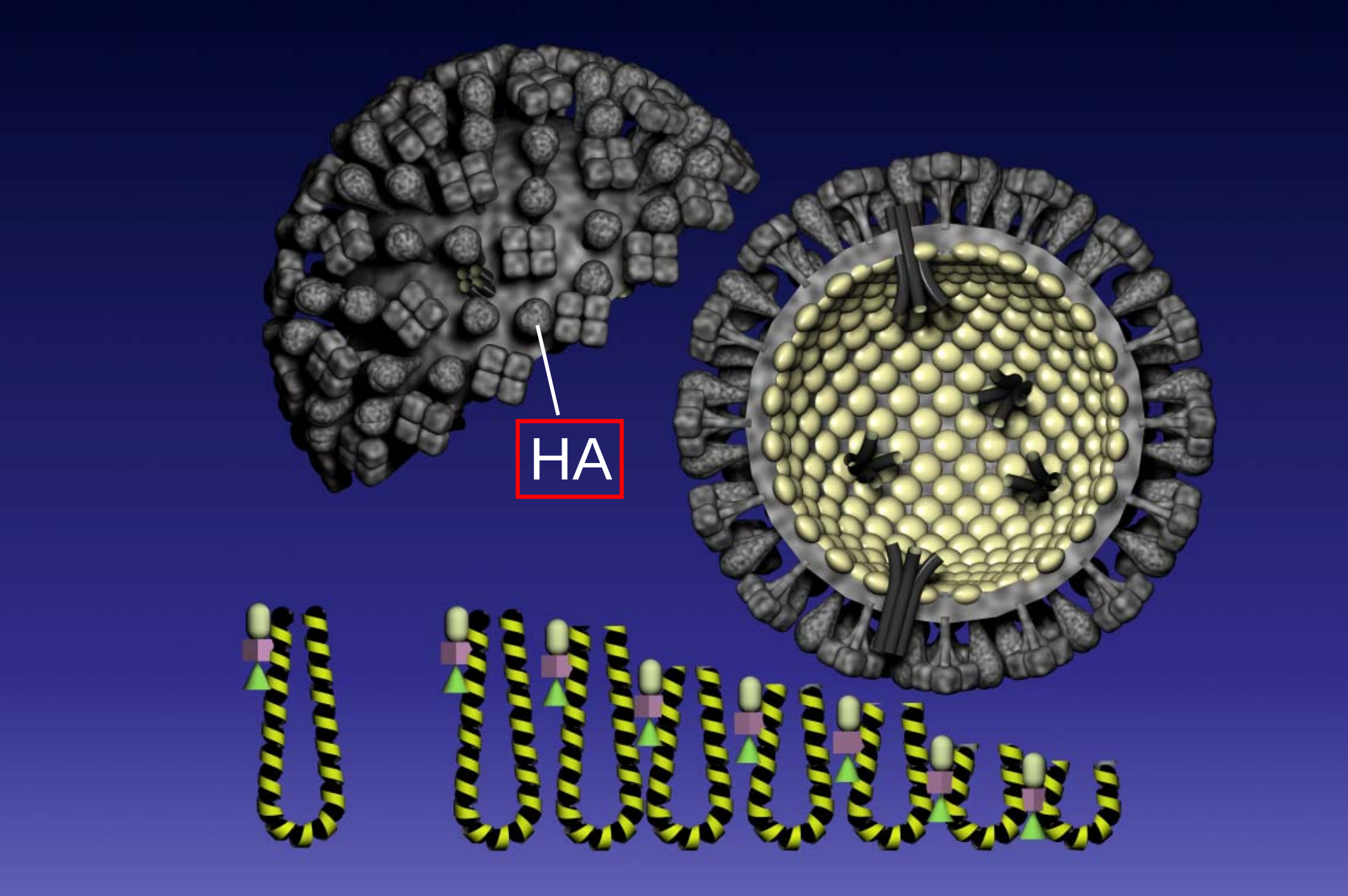

South Korea
People's Republic of China
Japan
Taiwan
Thailand
Viet Nam
Cambodia
Malaysia
Indonesia



Systemic infection

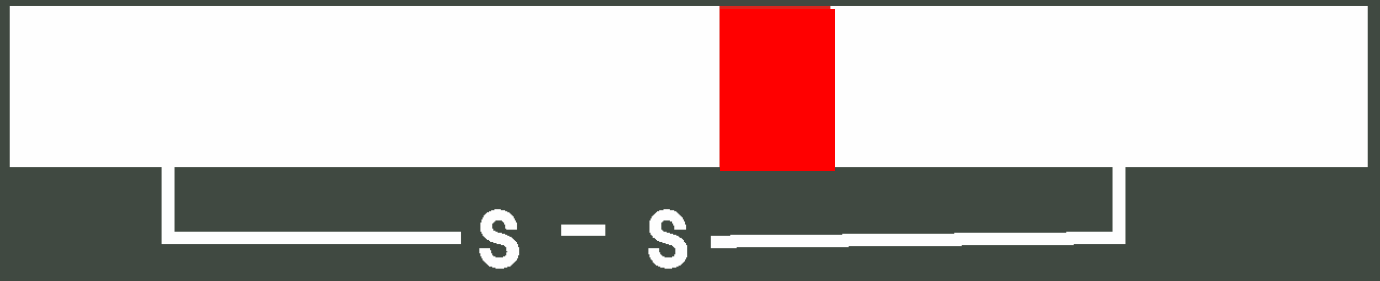


Localized infection


HA

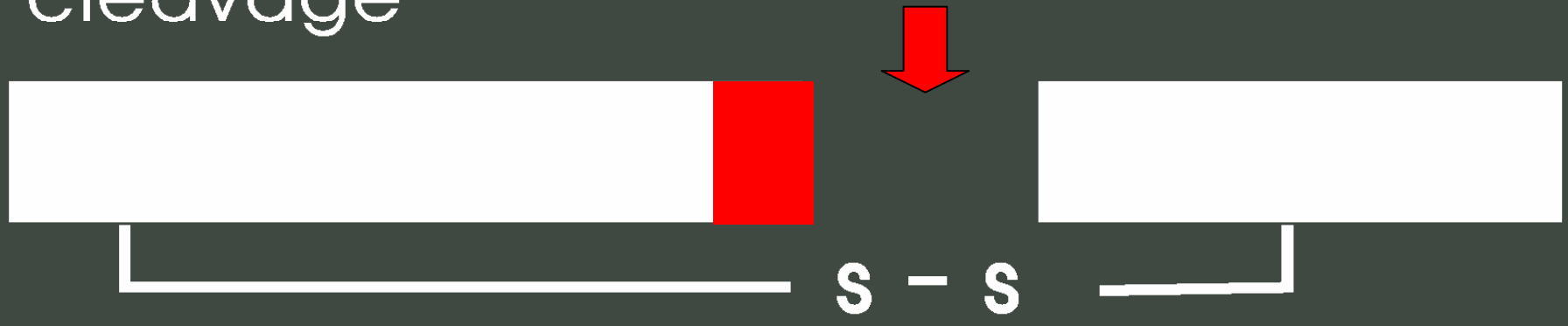
Cleavage of HA

Before cleavage

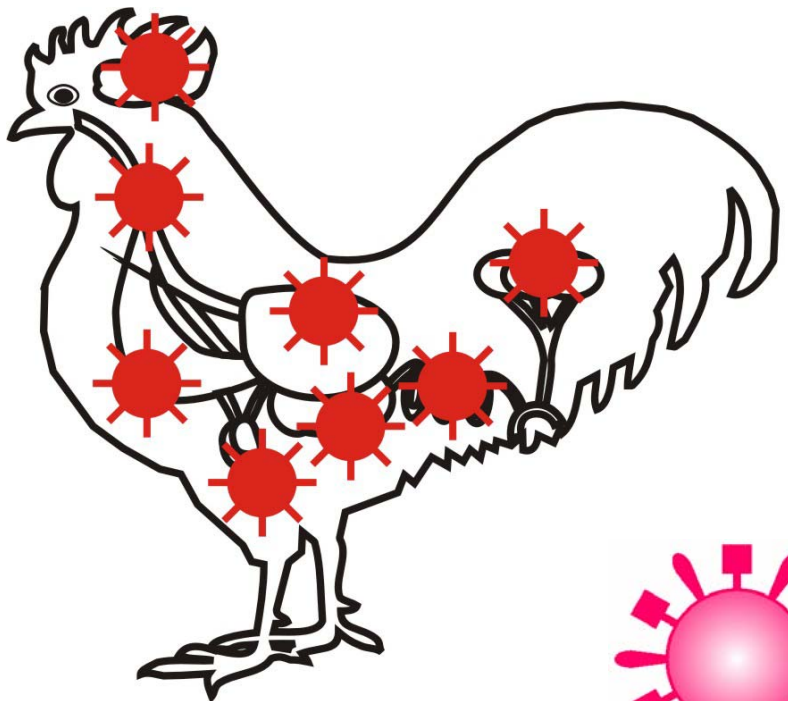


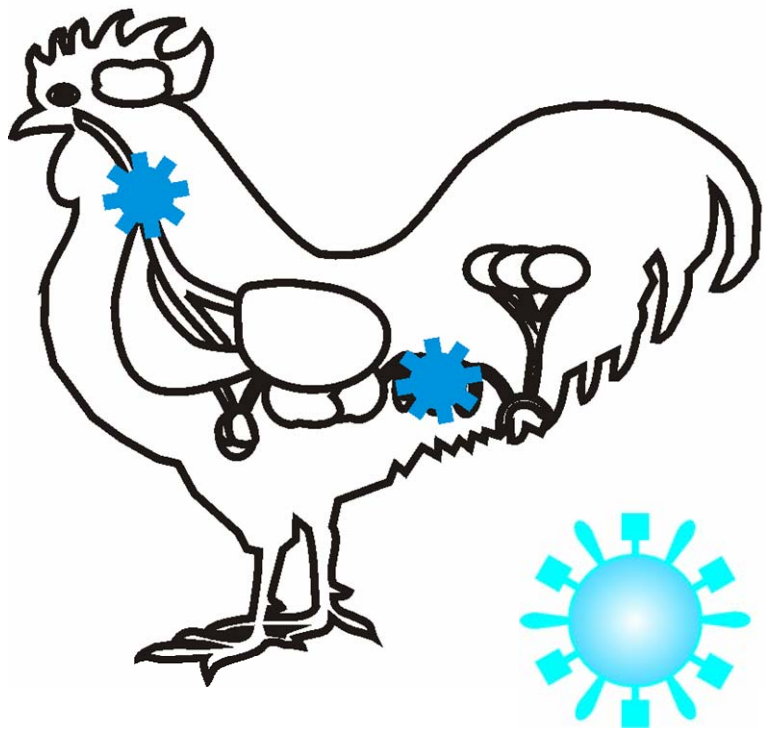
Host protease

After cleavage



HA cleavage is essential for viral infectivity.





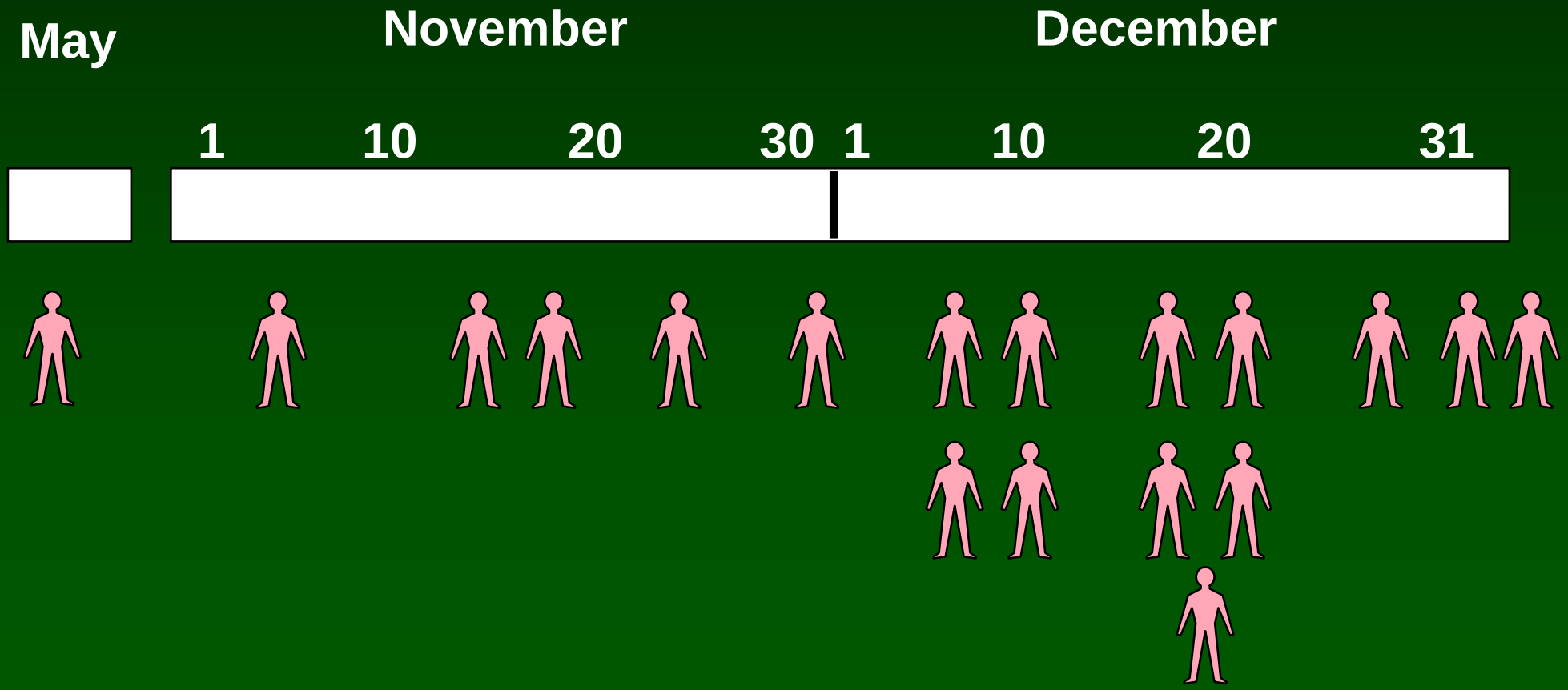
REERRRKKRR

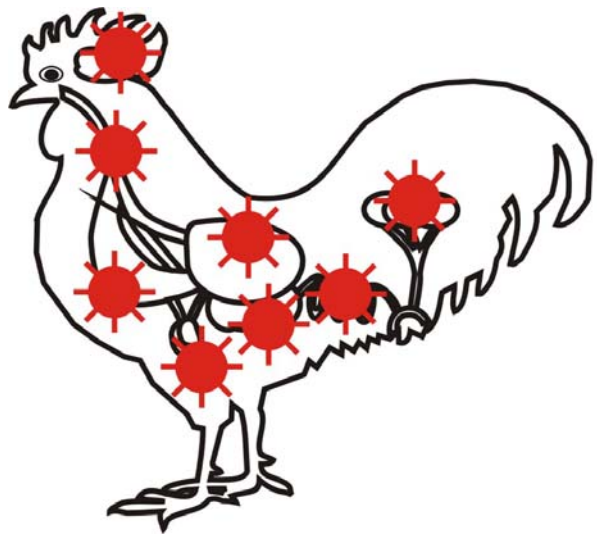
- - - - -

RXXR

Representative experiments with H5N1 viruses
- Determinants affecting pathogenicity of influenza virus -

H5N1 Influenza Chronology in 1997

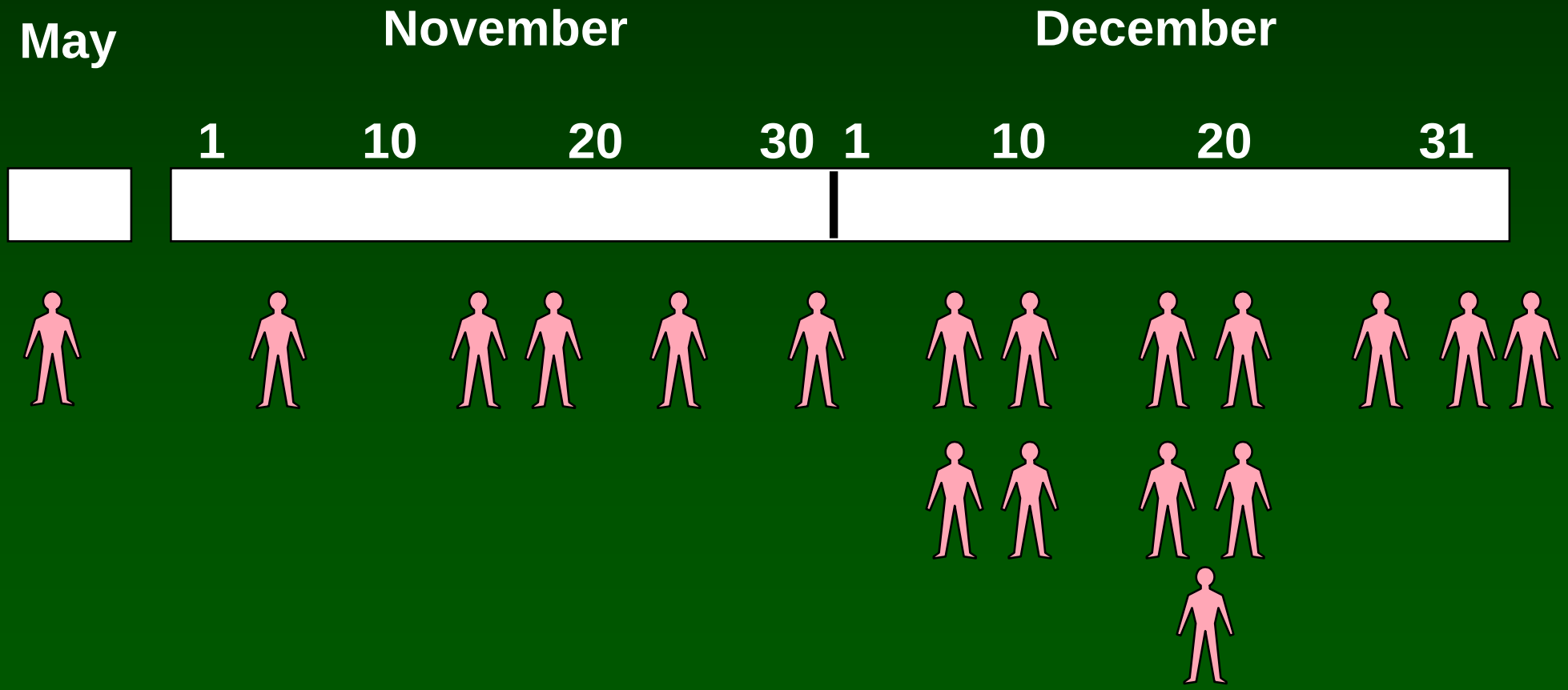


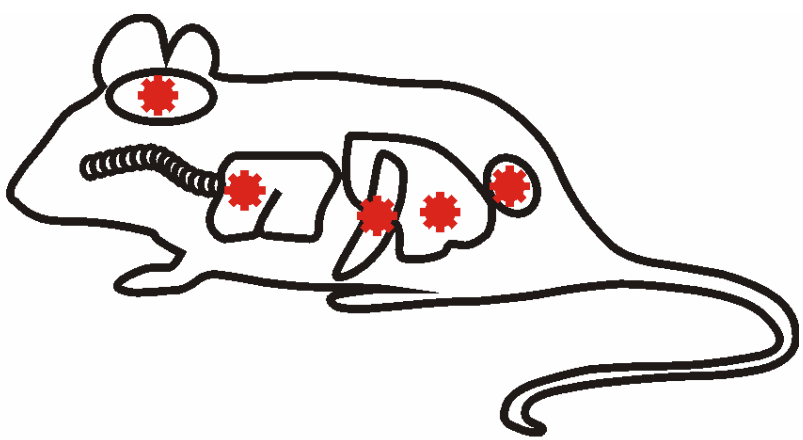


H5N1 Hong Kong virus
HA cleavage site

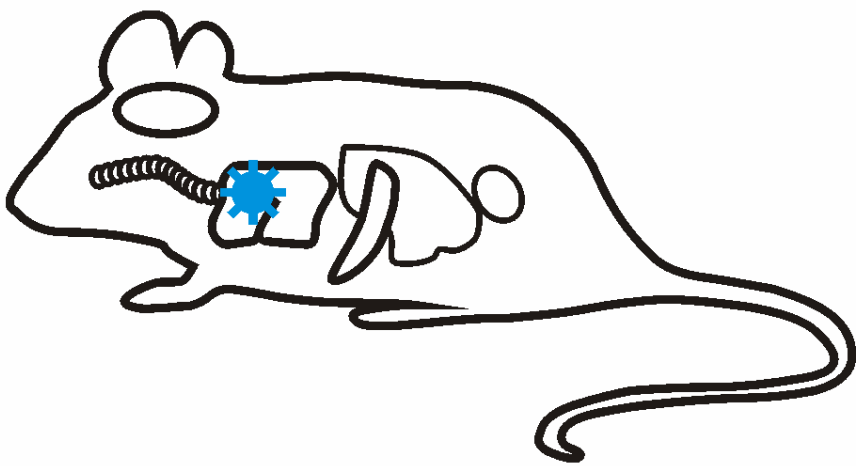
RERRRKKRR

H5N1 Influenza Chronology in 1997





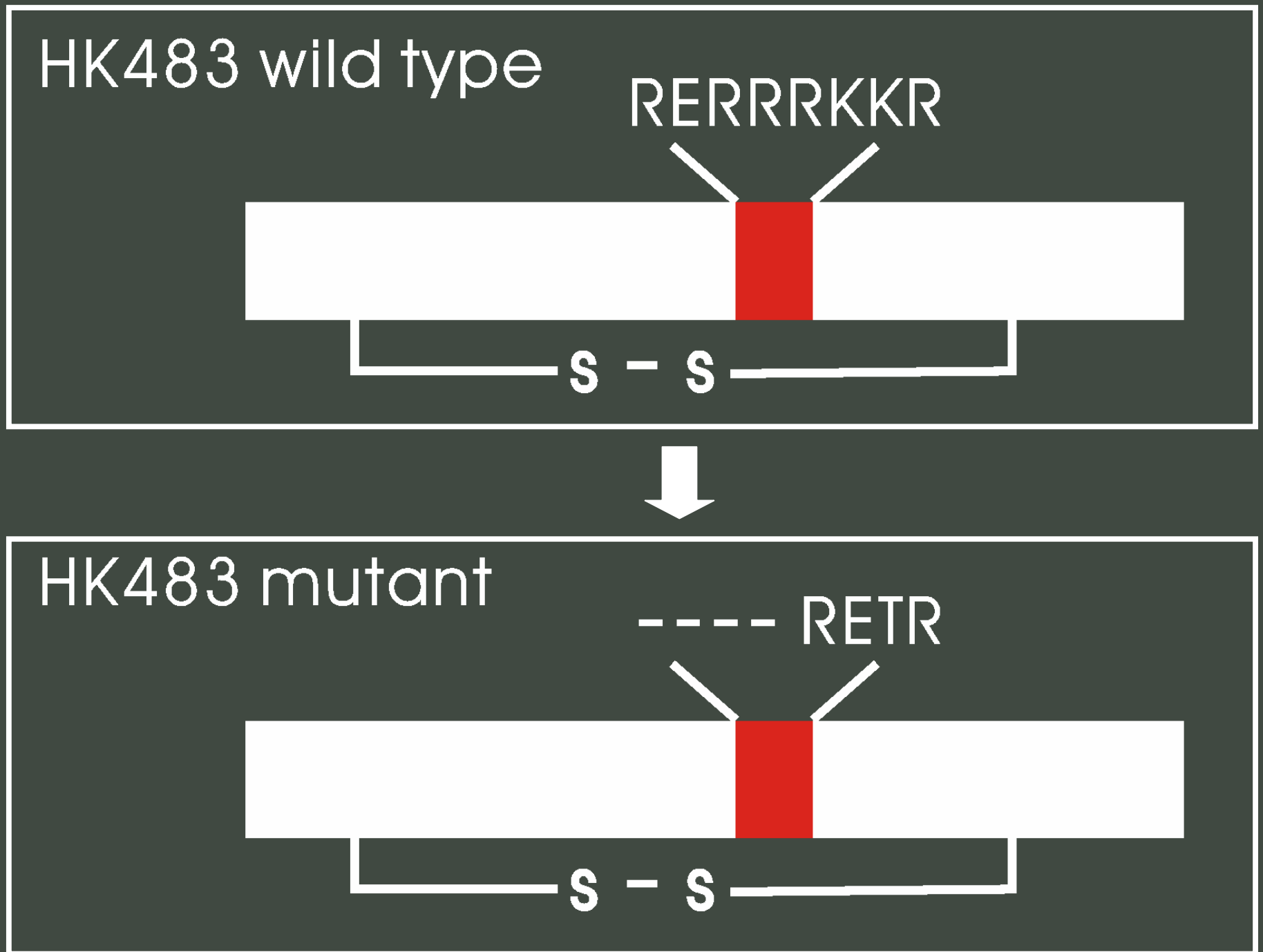
Systemic Infection



Local Infection

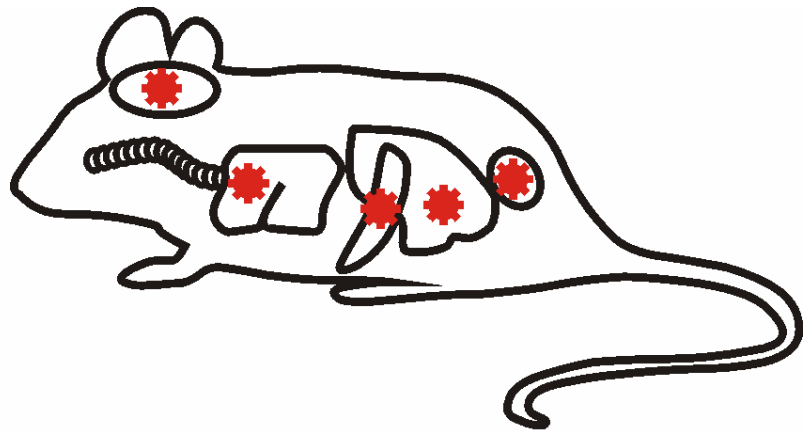
- Importance of high HA cleavability for pathogenicity of the Hong Kong virus in mice

- Molecular basis for the difference in mouse pathogenicity among the Hong Kong viruses


HK483 wild type
RERRRKKR
S – S
HK483 mutant
---- RETR
S – S

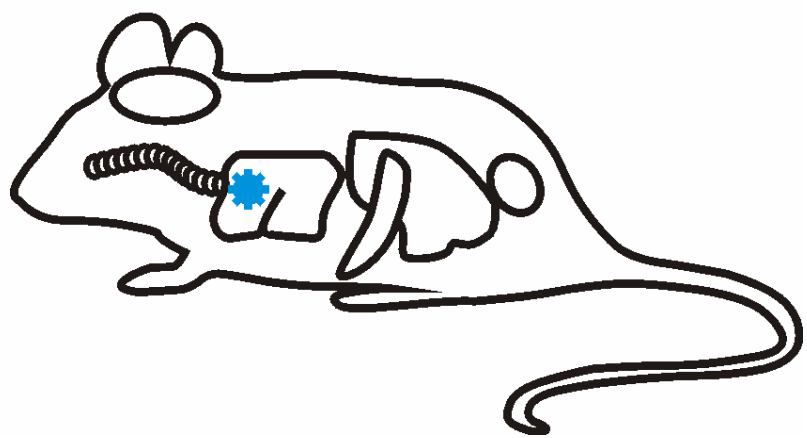
HK483 (R E R R R K K R)

LD_{50} 1.7 PFU



HK483 (– – – – R E T R)

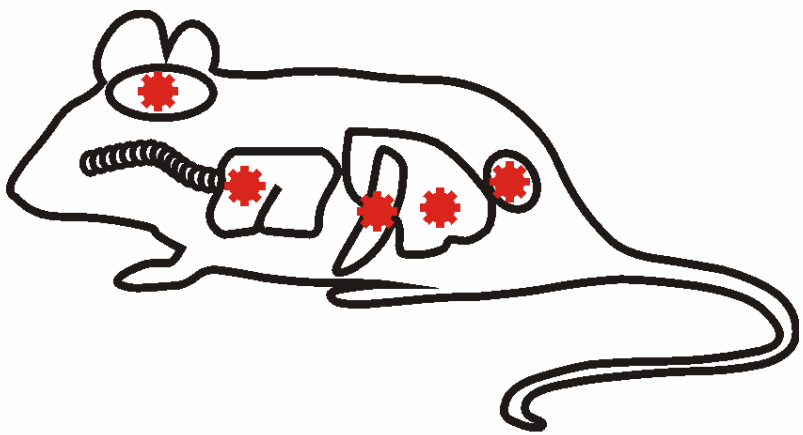
LD_{50} >1 x 10^3 PFU



- Importance of high HA cleavability for pathogenicity of the Hong Kong virus in mice

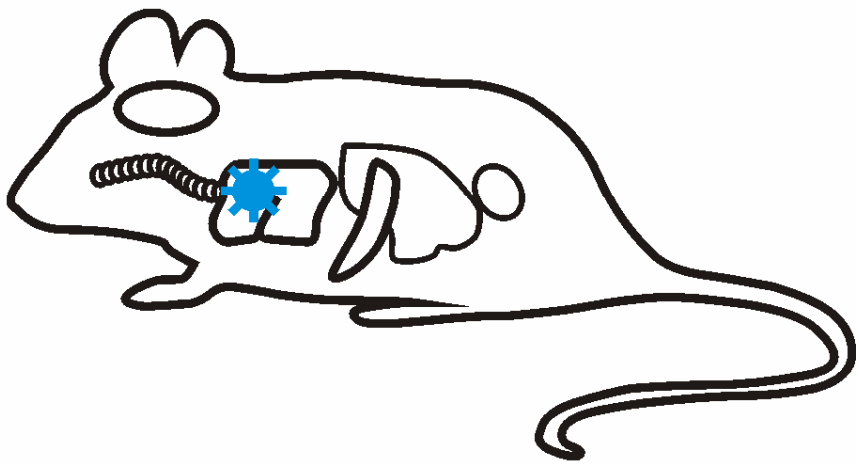
- Molecular basis for the difference in mouse pathogenicity among the Hong Kong viruses

HK483

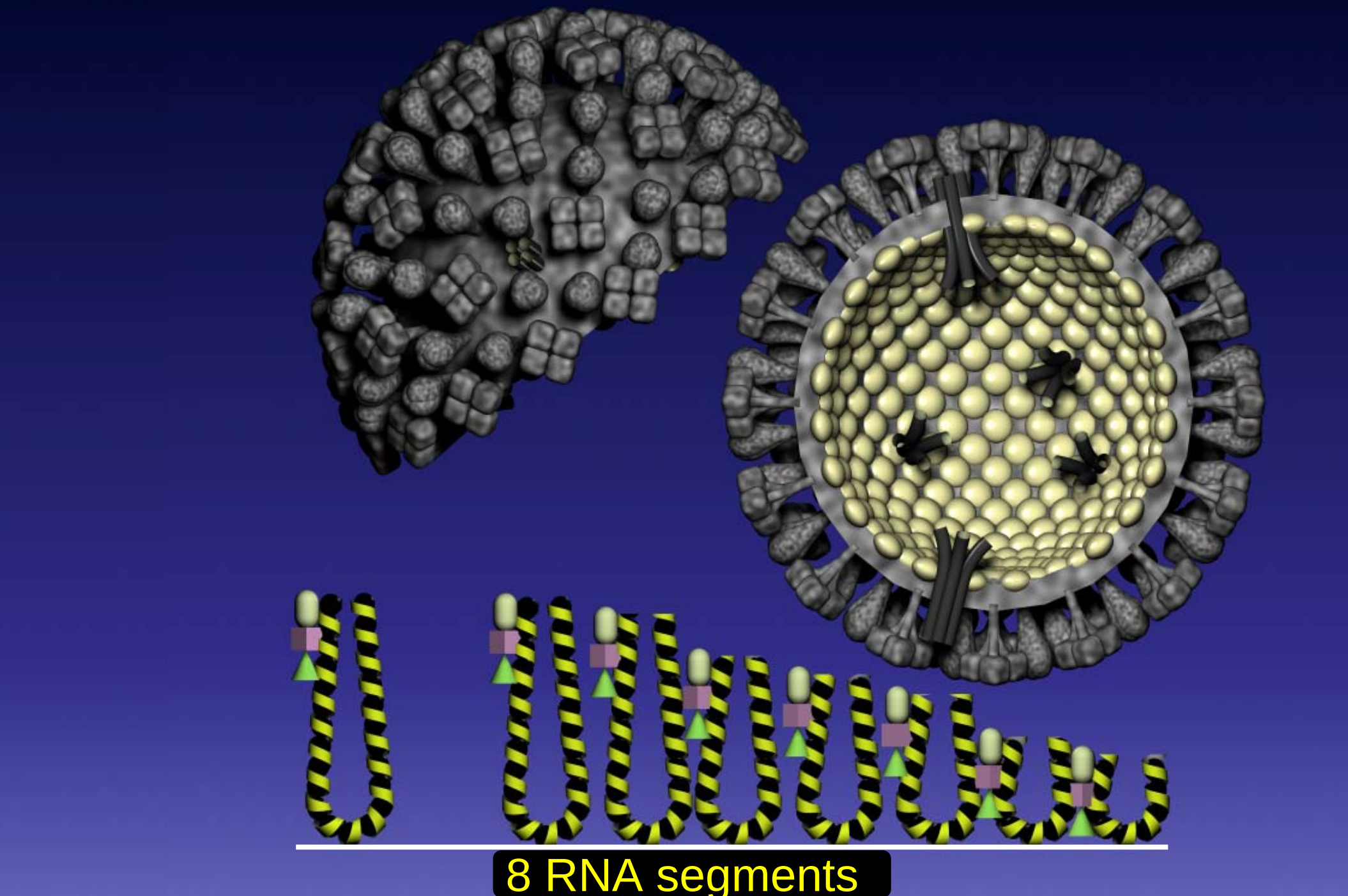


Systemic Infection

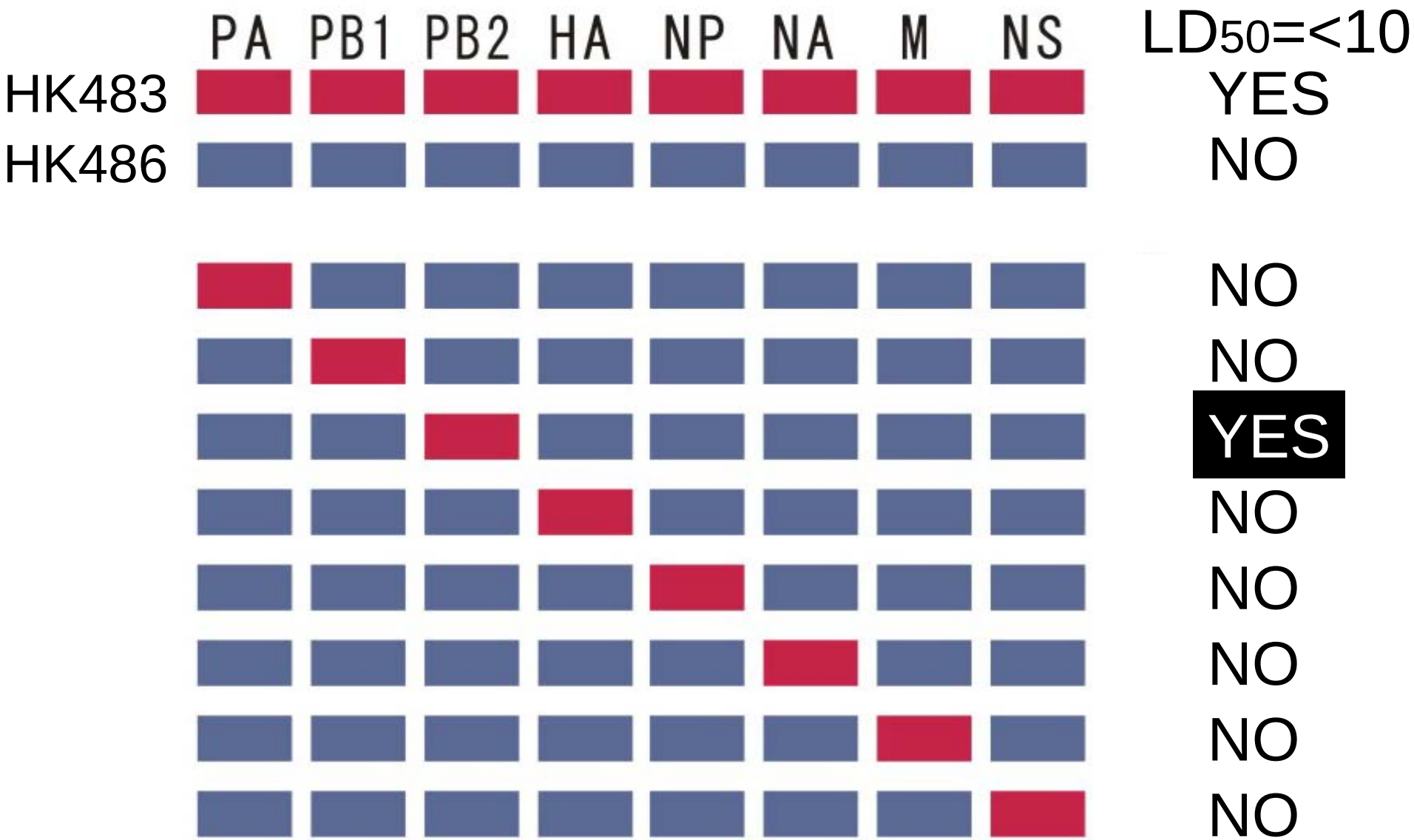
HK486



Local Infection


8 RNA segments

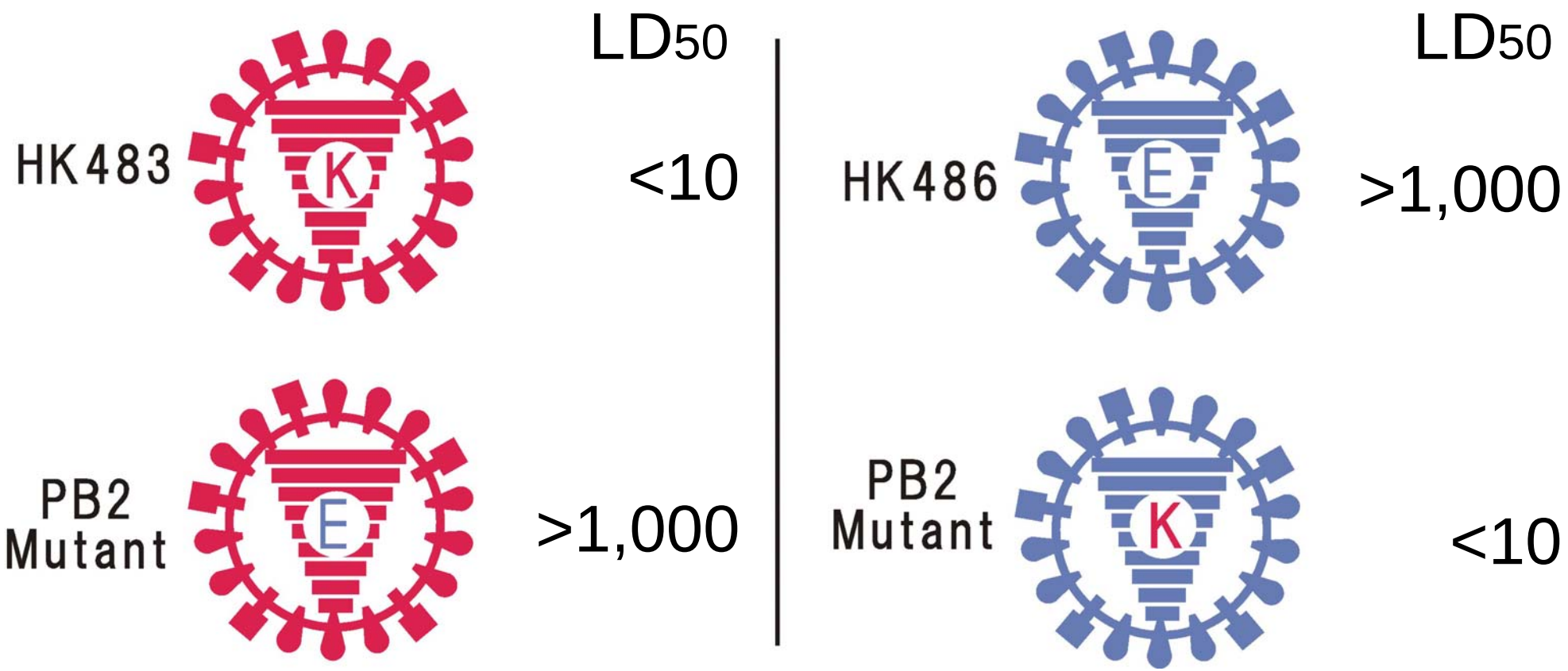
Reassortants between HK483 and HK486 viruses



Difference between HK483 and HK486

- Amino acid alteration from glutamic acid to lysine at position 627 of PB2 enhances the pathogenicity of an H5N1 virus in mice.

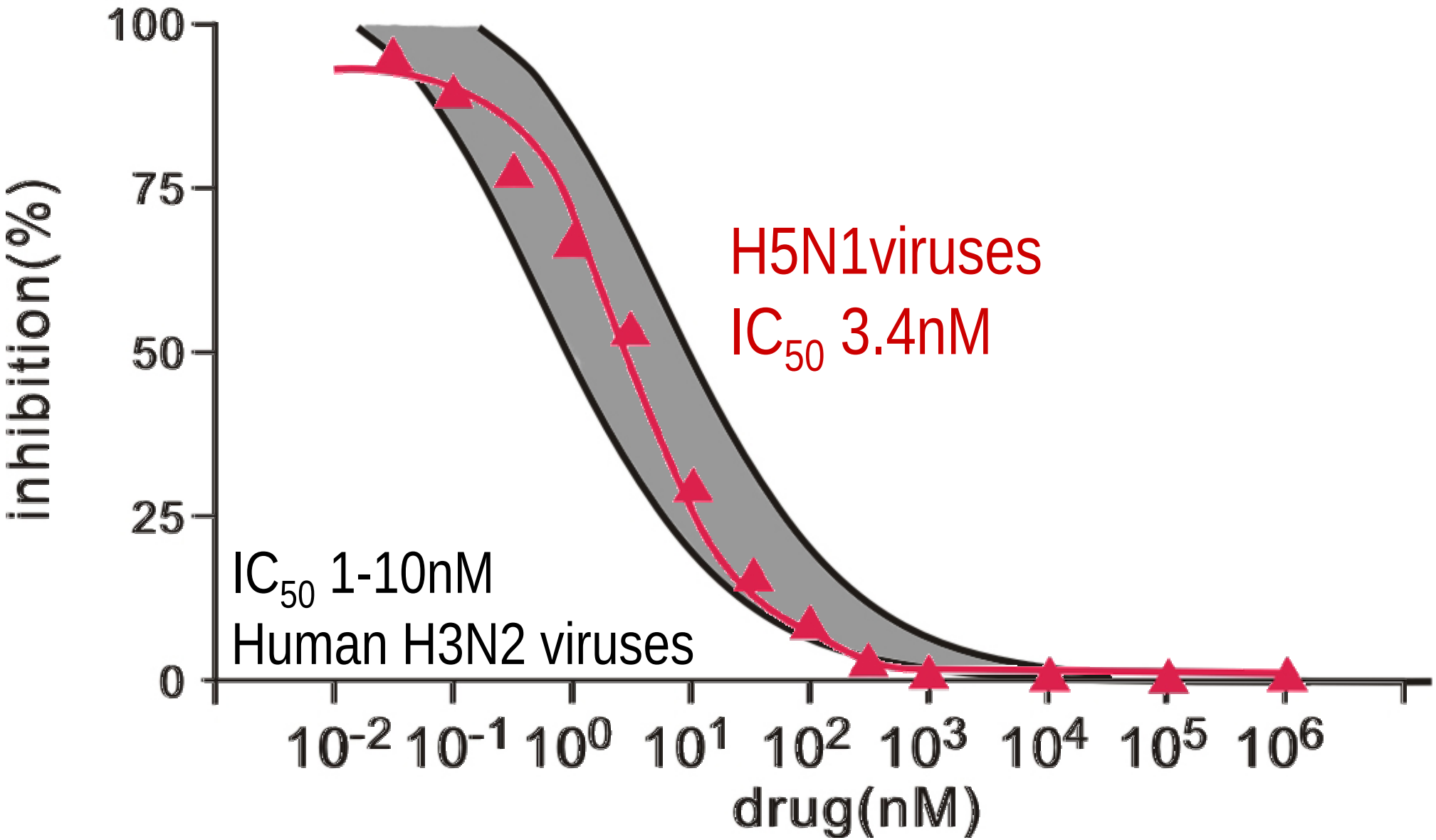
- All human virus PB2 proteins possess lysine at this position.



Determinants affecting pathogenicity of influenza virus

- HA cleavability
- PB2 amino acid at position 627 and others
- NS1 protein

Sensitivity of H5N1 viruses to oseltamivir



H5N1 viruses isolated since 1997 in Asia

Molecular cloning (genetic material)

RISK ASSESSMENT

- Noninfectious
- Inability to insert into human genome

➡ RISK GROUP 1

NIH Guidelines[1], App. B.1; 9 CFR[2] 121.3f.2

BIOSAFETY LEVEL

BSL-2

[1] NIH Guidelines, NIH Guidelines for Research Involving Recombinant DNA Molecules
[2] 9 CFR 121, Title 9 Code of Federal Regulations

H5N1 viruses isolated since 1997 in Asia

Virus generation, cell culture, experimental infection (mice, ferrets, chickens)

RISK ASSESSMENT

- Known to cause lethal infection in avian species and humans
- Aerosol transmission
- Prophylaxis available
- low human-to-human transmission

➡ RISK GROUP 3

NIH Guidelines, Sect. IIA; 9 CFR 121.3d

➡

BIOSAFETY LEVEL

BSL-3
ABSL-3

H5N1 viruses isolated since 1997 in Asia

Virus generation, cell culture, experimental infection (mice, ferrets, chickens)

SPECIFIC PRACTICES

1) Annual vaccination required
2) Prophylaxis when aerosols likely
3) PAPRs with face shields
4) Shower out
5) Susceptibility testing of agents

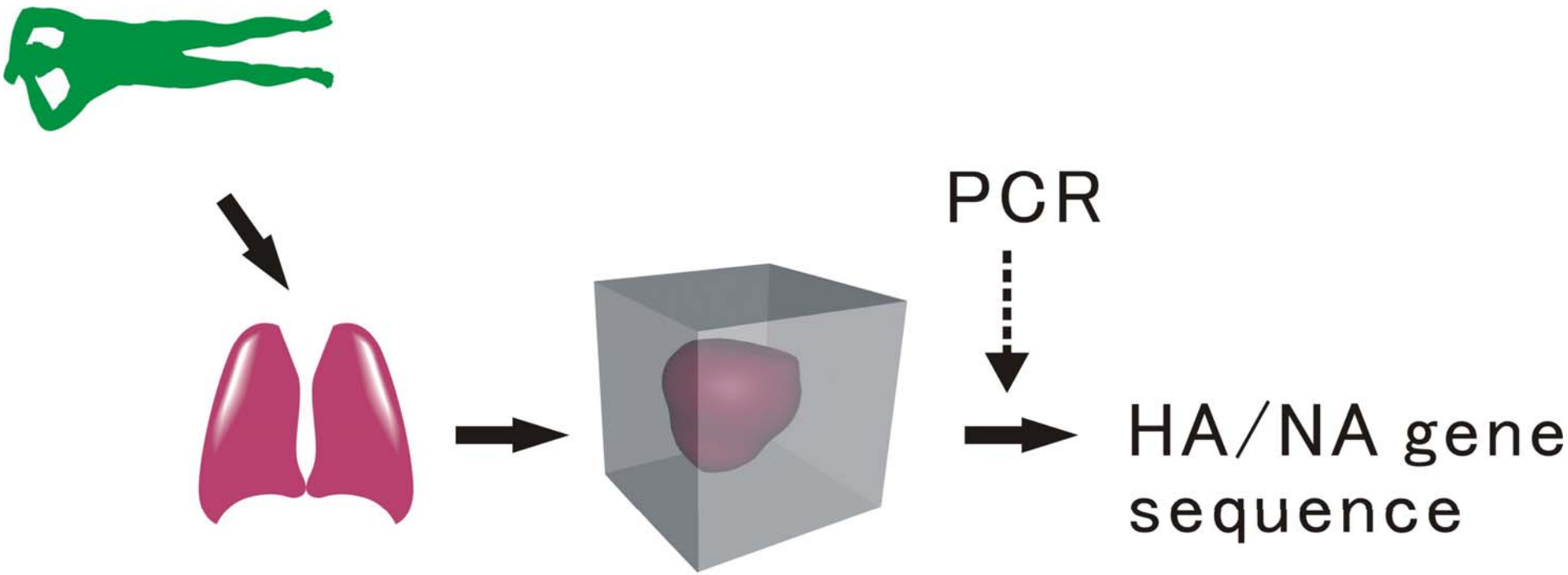
PAPRs: Powered air-purifying respirators

Additional features/procedures in BSL-3 at UW

1) Entry/exit through change and shower rooms
2) Double-door autoclave
3) Shower facilities
4) Daily decontamination of work surfaces
5) All personal clothing removed in outer change rooms
6) Established system for reporting and treating exposures
7) Annual inspection by federal regulatory agencies
8) Contact with non-experimental mice, ferrets, and chickens is prohibited within one week of contact with experimentally infected animals

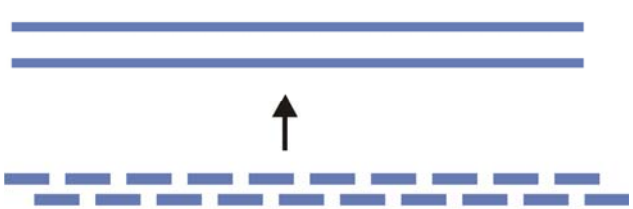
Experiments with viruses possessing the 1918 virus genes

The Spanish influenza virus does not exist.

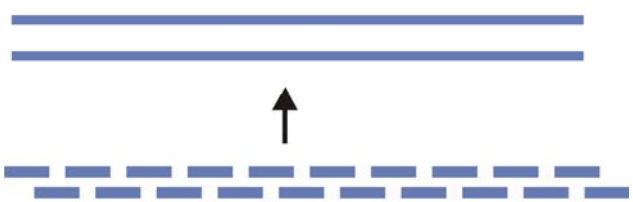


Reid AH et al. PNAS (1999)
Reid AH et al. PNAS (2000)

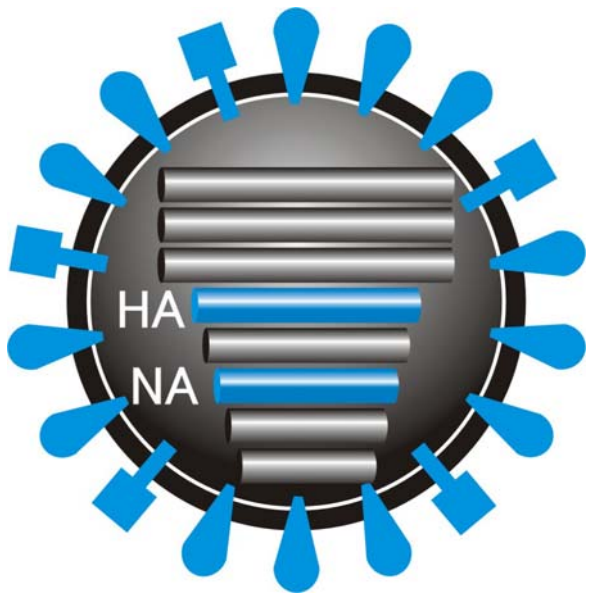
HA



NA



1918 virus



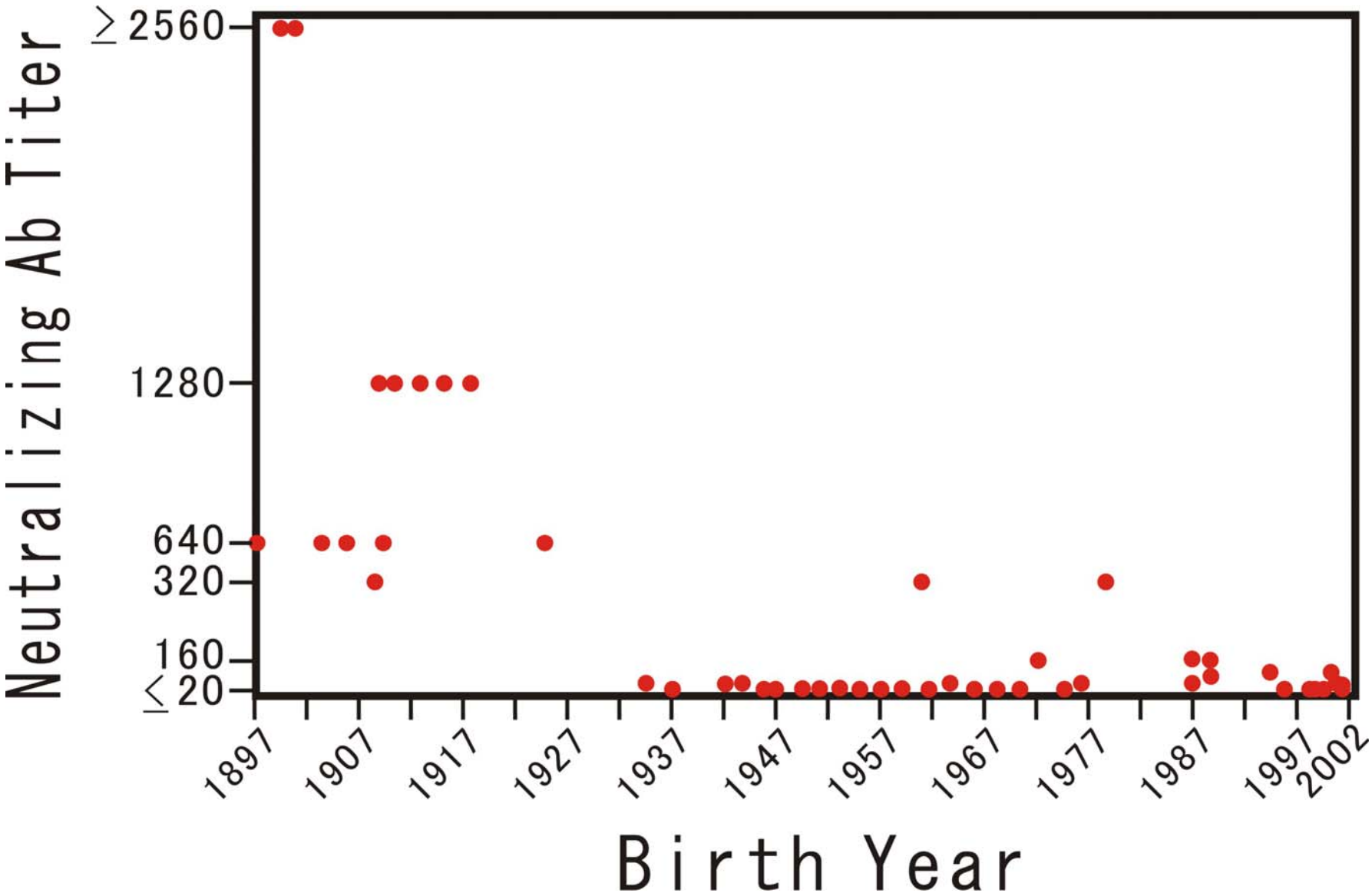
Properties of viruses with the 1918 virus HA and/or NA in mice

Virus	Origin of Genes			LD50 (Log_{10} PFU)	Virus titer in lung (Log_{10} PFU)
	HA	NA	Others		
WSN (H1N1)	WSN	WSN	WSN	3.3	5.0 ± 0.3
WSN/HspNsp	1918	1918	WSN	3.0	5.0 ± 0.1
M88 (H3N2)	M88	M88	M88	>6.2	2.9 ± 0.2
M88/Hsp	1918	K173	M88	4.4	5.1 ± 0.1
M88/HspNsp	1918	1918	M88	5.2	4.7 ± 0.2
K173 (H1N1)	K173	K173	K173	>7.4	3.5 ± 0.3
K173/Hsp	1918	K173	K173	5.2	4.8 ± 0.2
K173/HspNsp	1918	1918	K173	6.9	ND

Detection of viral neutralizing antibodies

Virus	avirus with the 1□□□virus HA and NA
Sera	1 year old (born in 2001□ ⟷ 102 years old (born in 1897□ 

Neutralizing antibodies to a virus with the 1918 virus HA and NA



Viruses with the 1918 virus HA and NA genes

Molecular cloning (genetic material)

RISK ASSESSMENT

- Noninfectious
- Inability to insert into human genome

➡ RISK GROUP 1

NIH Guidelines[1], App. B.1; 9 CFR[2] 121.3f.2

➡

BIOSAFETY LEVEL

BSL-2

[1] NIH Guidelines, NIH Guidelines for Research Involving Recombinant DNA Molecules
[2] 9 CFR 121, Title 9 Code of Federal Regulations

Viruses with the 1918 virus HA and NA genes

Virus generation, cell culture, experimental infection (mice)

RISK ASSESSMENT

- Potential risk for human infection
- Increased pathogenicity
- Human population may be susceptible
- Aerosol transmission
- Potential for human-to-human transmission
- Protection with current vaccines ???
- Prophylaxis ???

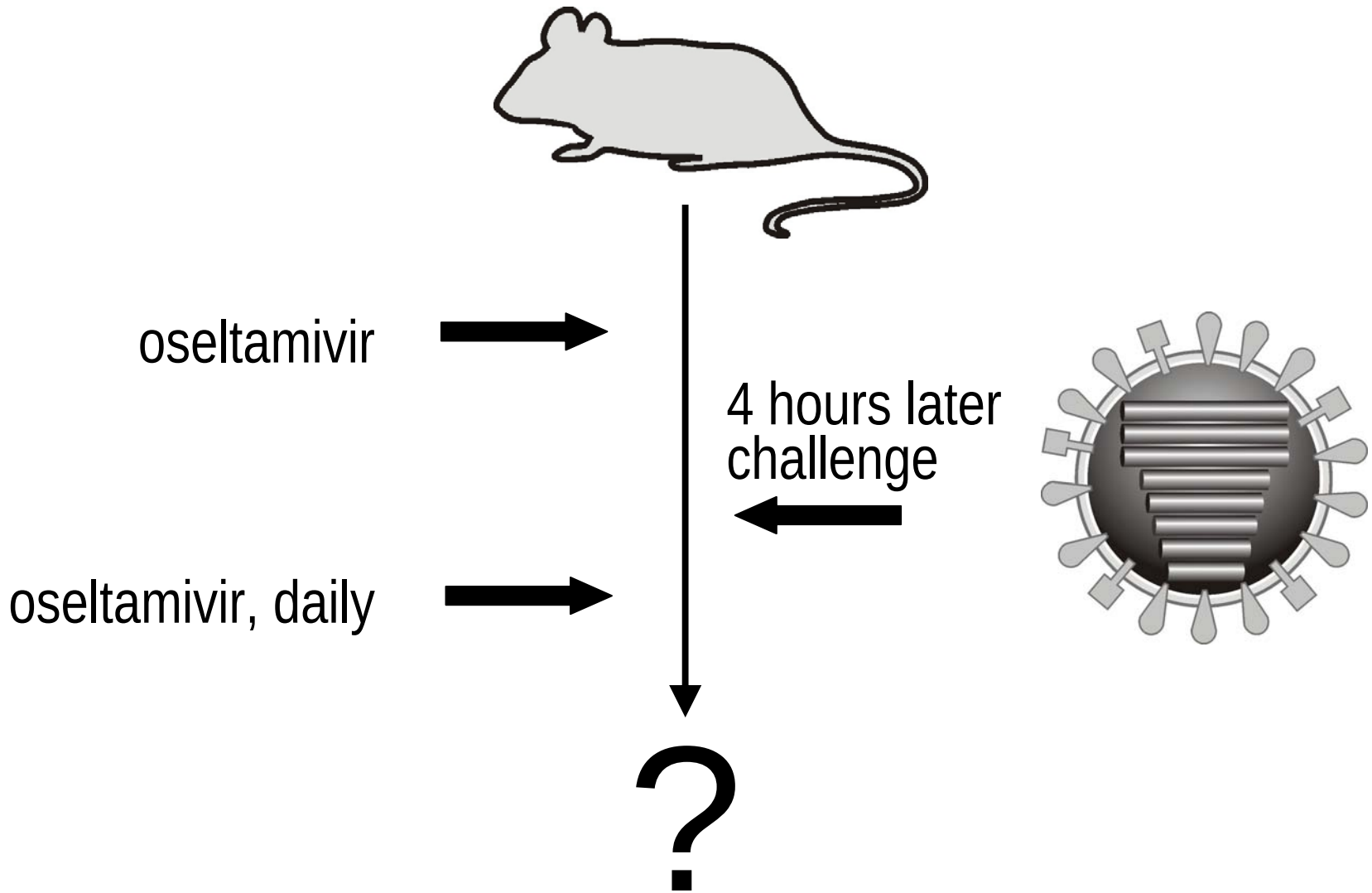
➡ RISK GROUP 3 or 4 ?

NIH Guidelines, Sect. IIA; 9 CFR 121.3d

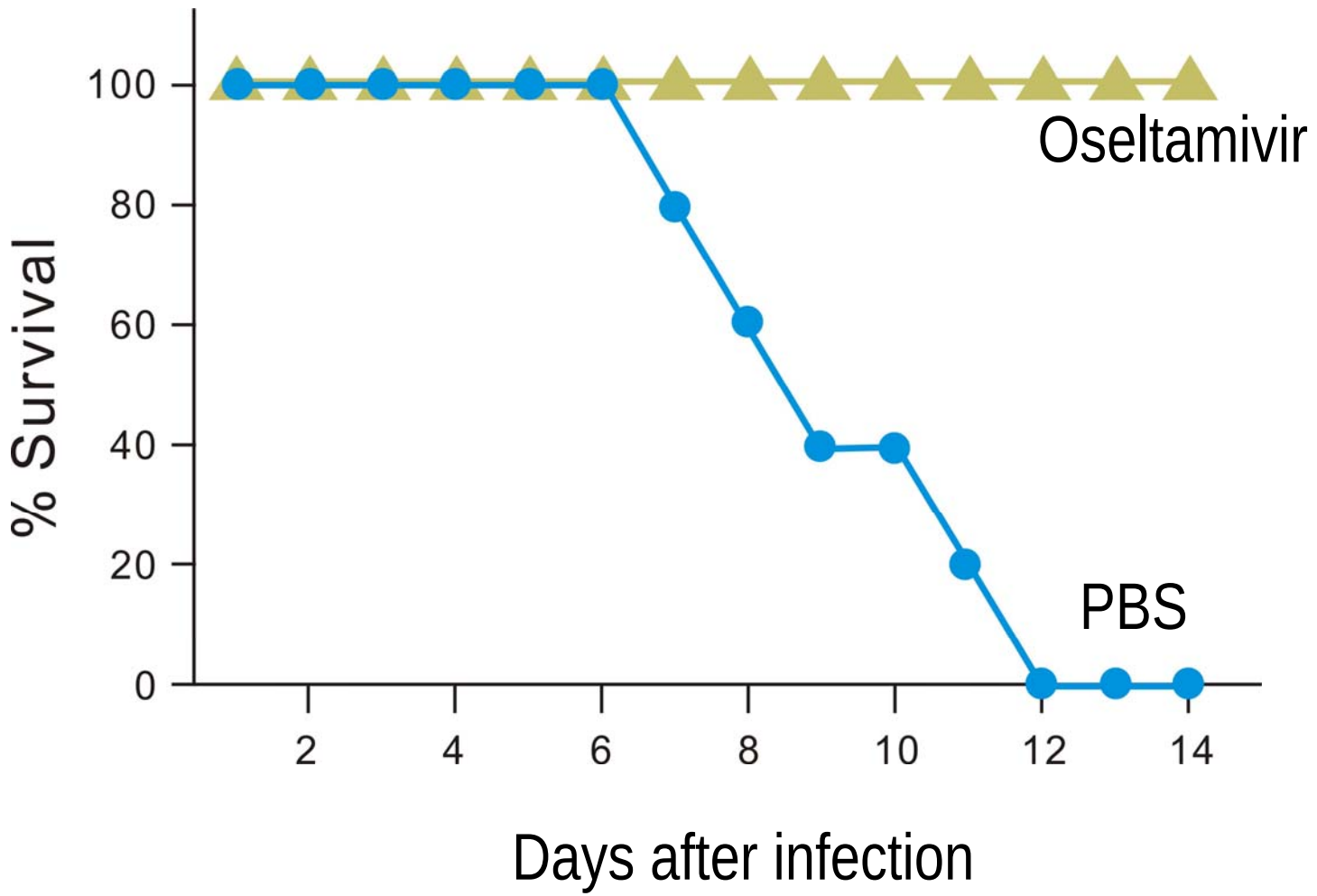
➡ BIOSAFETY LEVEL

BSL-4

Prophylaxis of a virus with the 1918 virus HA and NA with oseltamivir



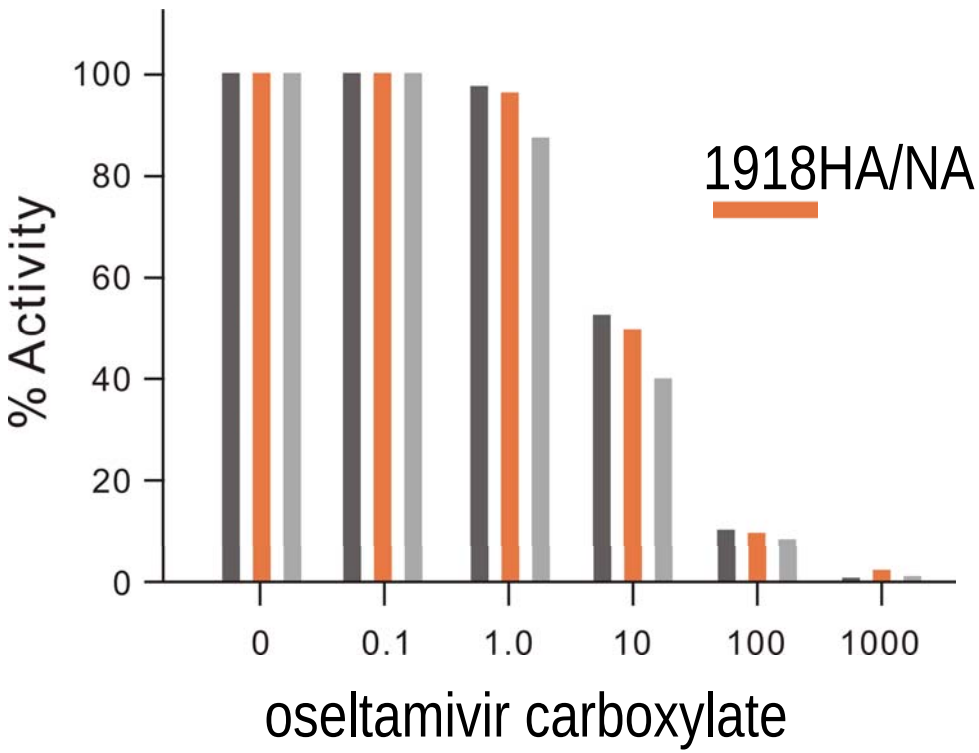
Oseltamivir protects mice from challenge with a virus possessing the 1918 virus HA and NA



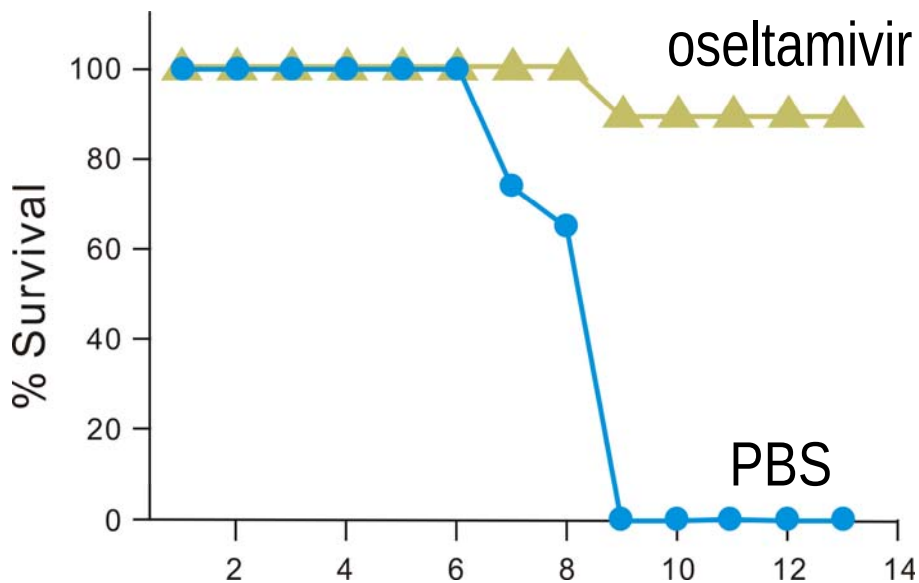
Antiviral

A virus with the 1918 virus NA is sensitive to oseltamivir carboxylate

In vitro



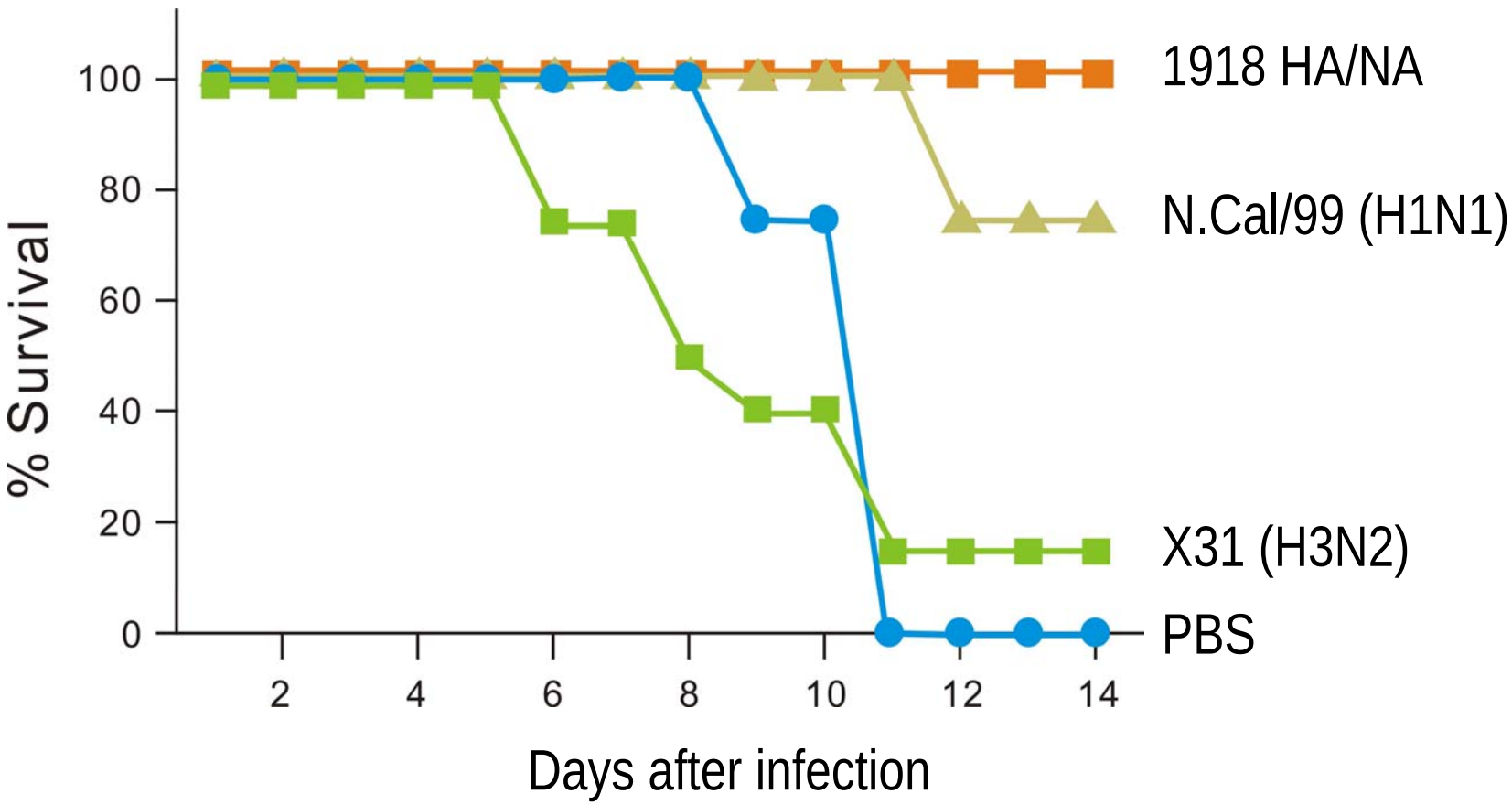
Mouse model



Tumpey et al. (2002) PNAS 99, 13849

Vaccine

Protection of mice against a virus with the 1918 HA and NA using inactivated vaccine made from a contemporary H1 strain



Tumpey et al. (2004) PNAS 101, 3166

Viruses with the 1918 virus HA and NA genes

Virus generation, cell culture, experimental infection (mice)

RISK ASSESSMENT

- Potential risk for human infection
- Likelihood of increased pathogenicity
- Human population may be susceptible
- Aerosol transmission
- Potential for human-to-human transmission
- Current vaccines may offer protection
- Prophylaxis available

➡ RISK GROUP 3

NIH Guidelines, Sect. IIA; 9 CFR 121.3d

BIOSAFETY LEVEL

BSL-3

Viruses with the 1918 virus HA and NA genes

Virus generation, cell culture, experimental infection (mice)

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