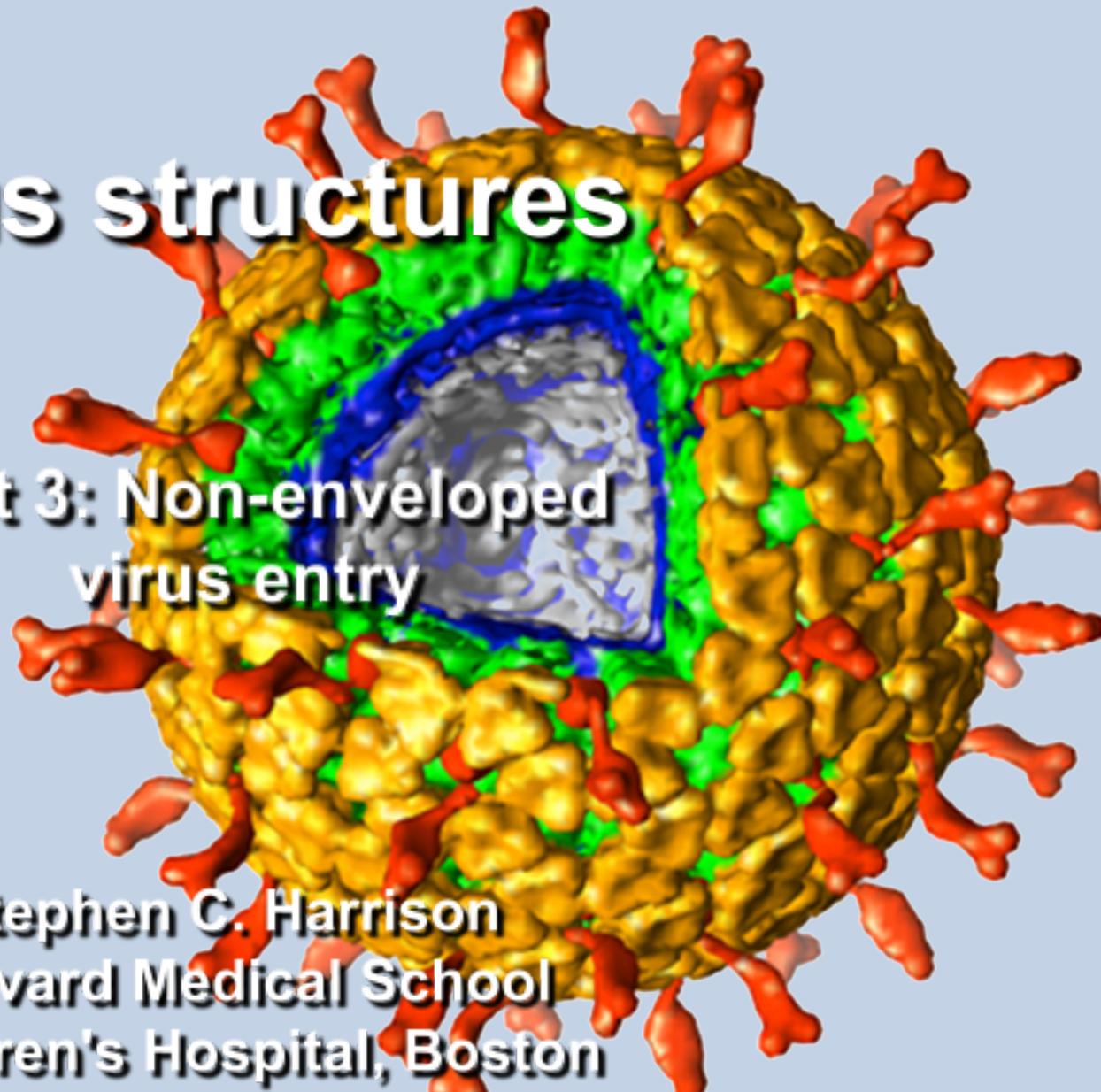


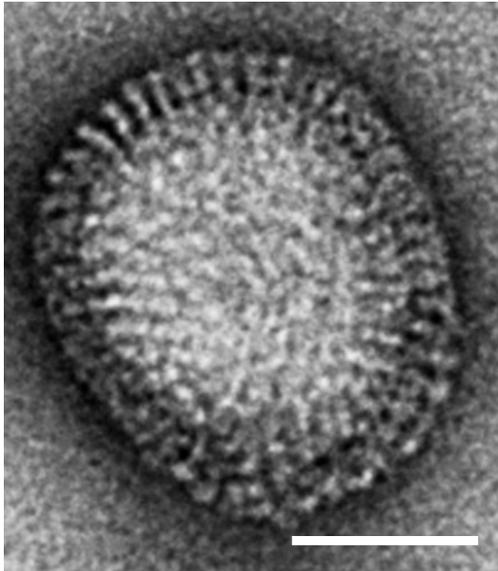
Virus structures

Part 3: Non-enveloped virus entry



Stephen C. Harrison
Harvard Medical School
Children's Hospital, Boston
Howard Hughes Medical Institute

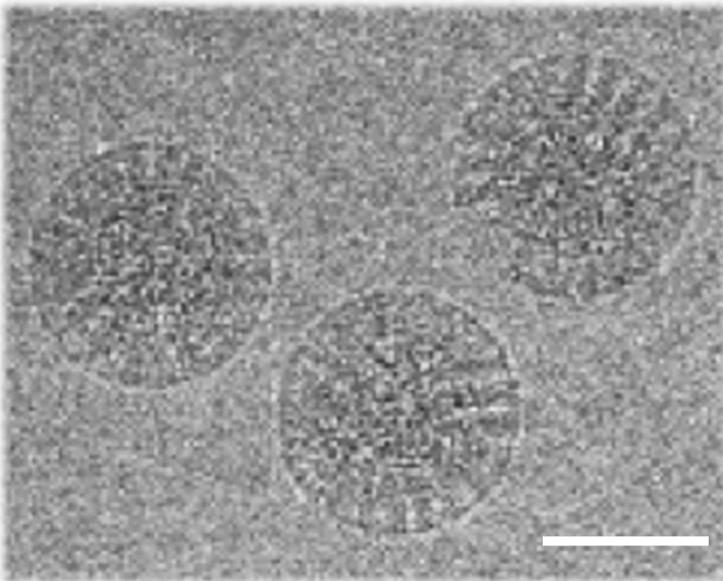
"Enveloped" and "non-enveloped" viruses



influenza virus

membrane fusion

negative-stain
contrast

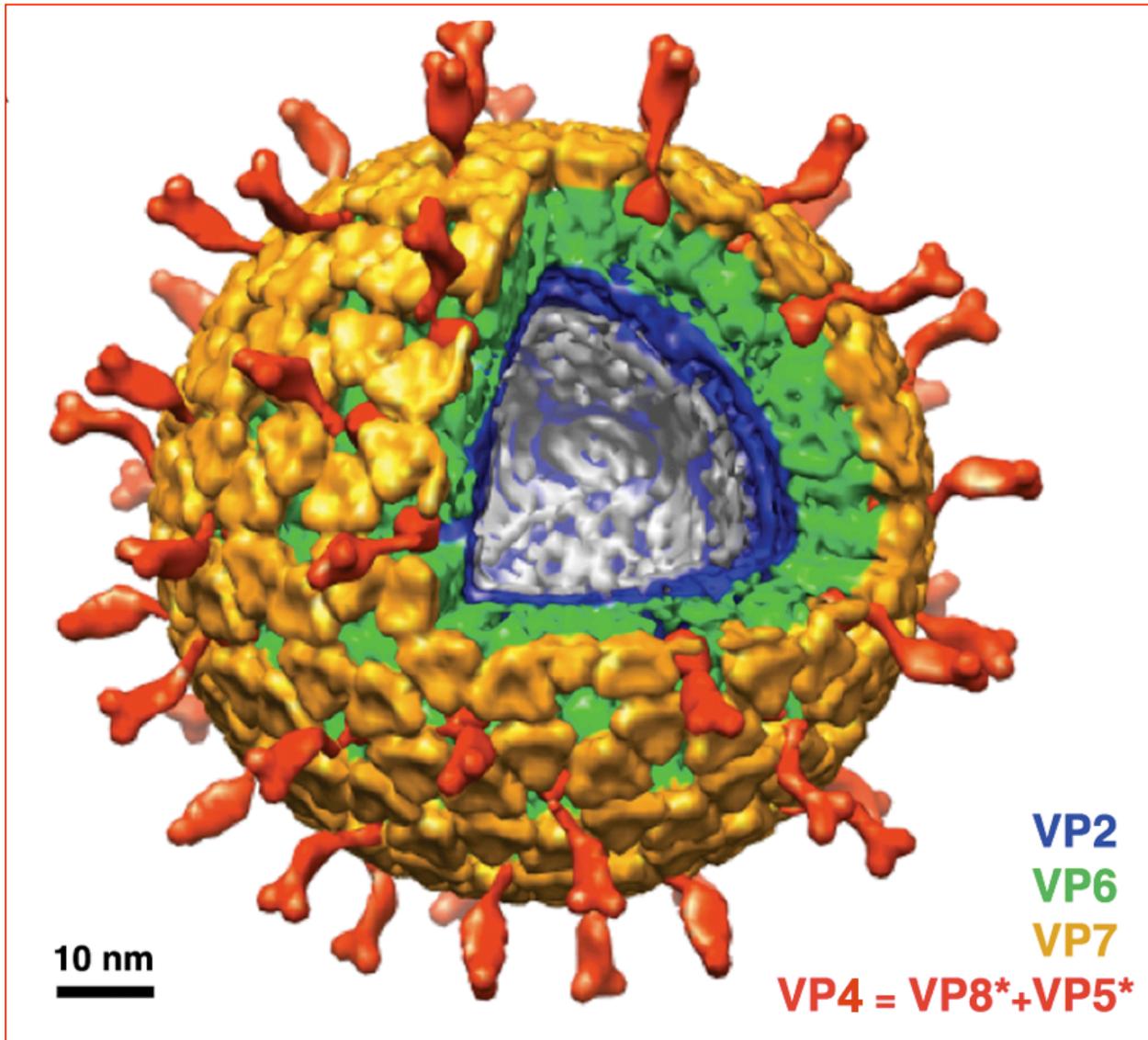


rotavirus

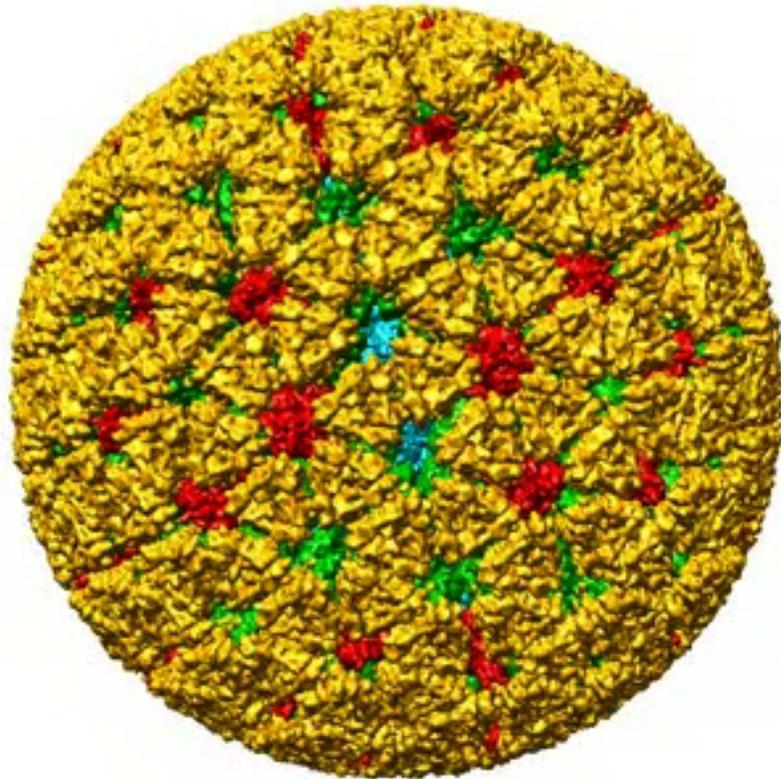
membrane
perforation

cryo specimen
(no stain)

Rotavirus



TLP



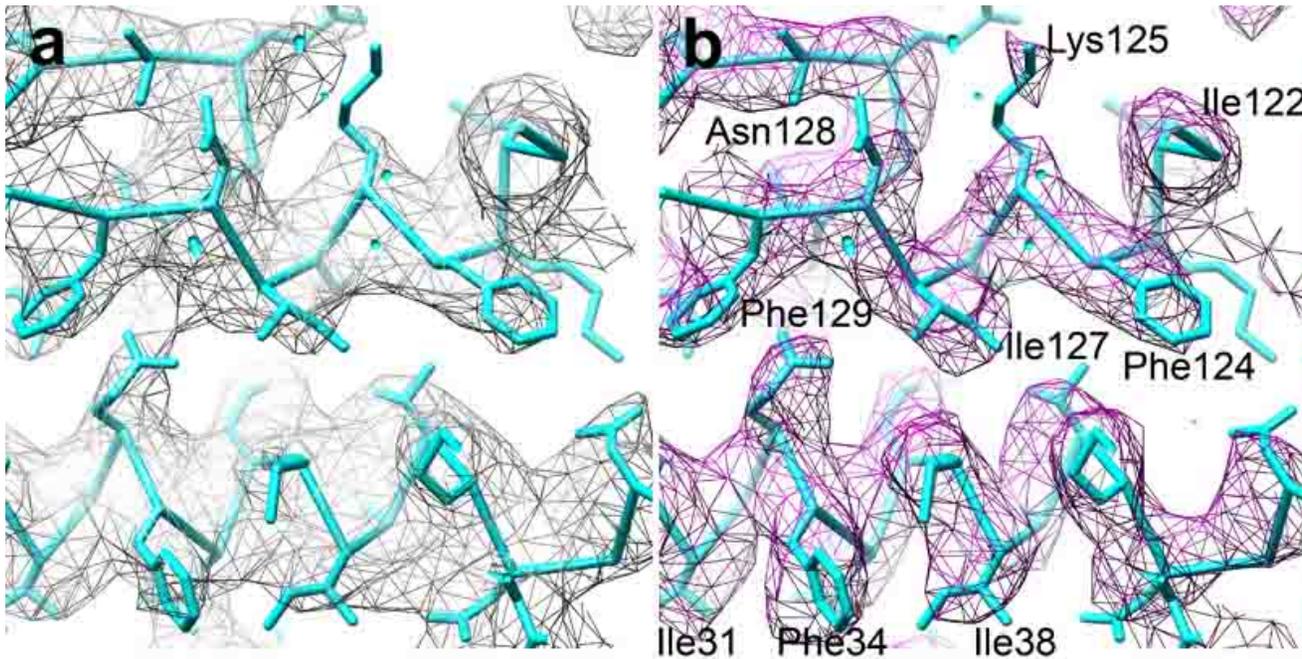
Structural data used for animation:

X-ray crystal structures of various proteins and fragments: VP8*, VP5*CT, VP7, (VP6), VP1

X-ray crystal structure of DLP

Image reconstruction from electron cryomicroscopy (cryoEM) of DLP, DLP recoated with VP7, & virion

Image reconstruction from electron
cryomicroscopy
(cryoEM) of DLP, DLP recoated with
VP7, & virion



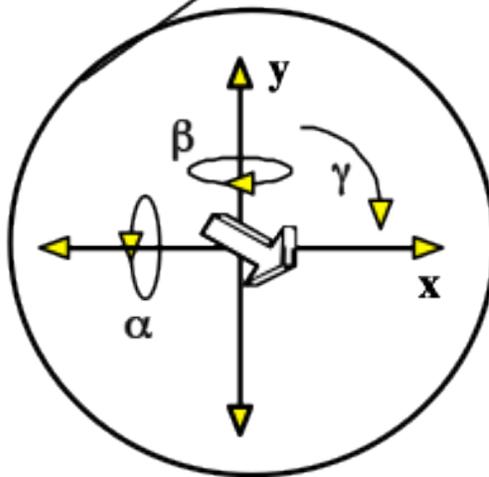
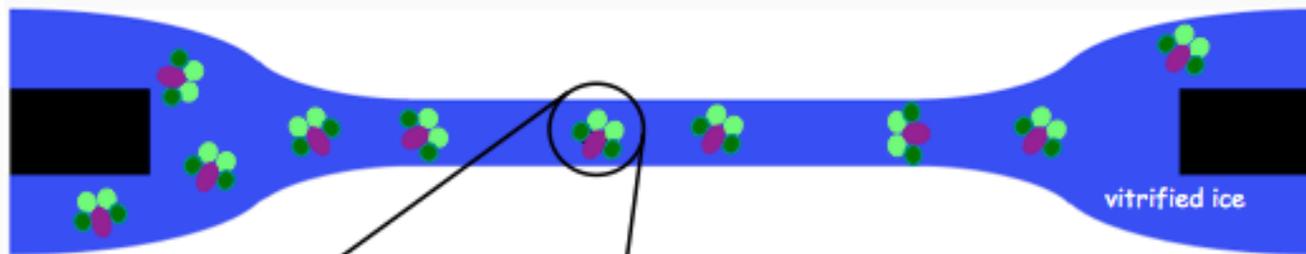
x-ray

McClain, Settembre, Bellamy

cryoEM

Zhang, Settembre, Bellamy, Grigorieff

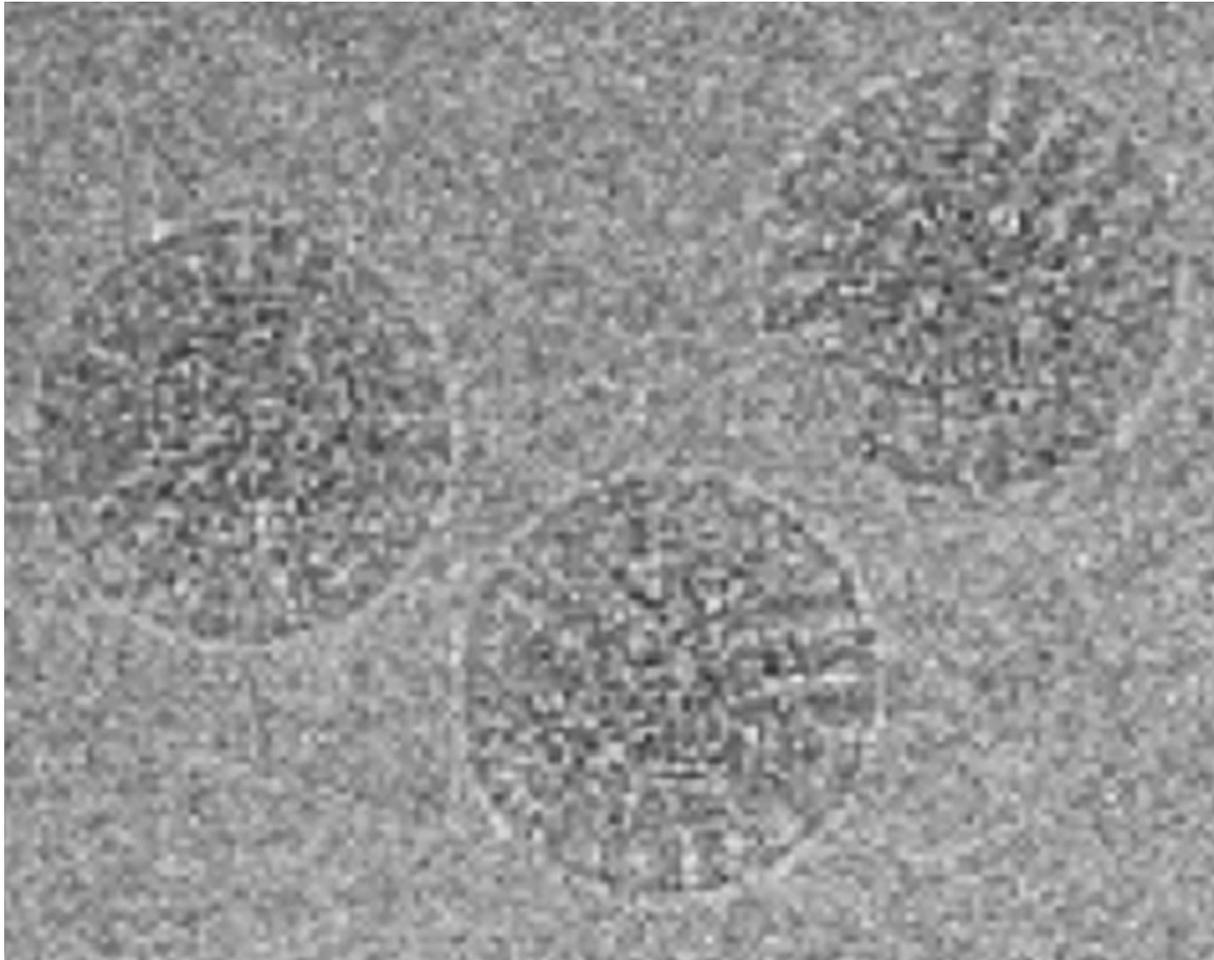
"Single-particle" structure analysis by electron cryomicroscopy



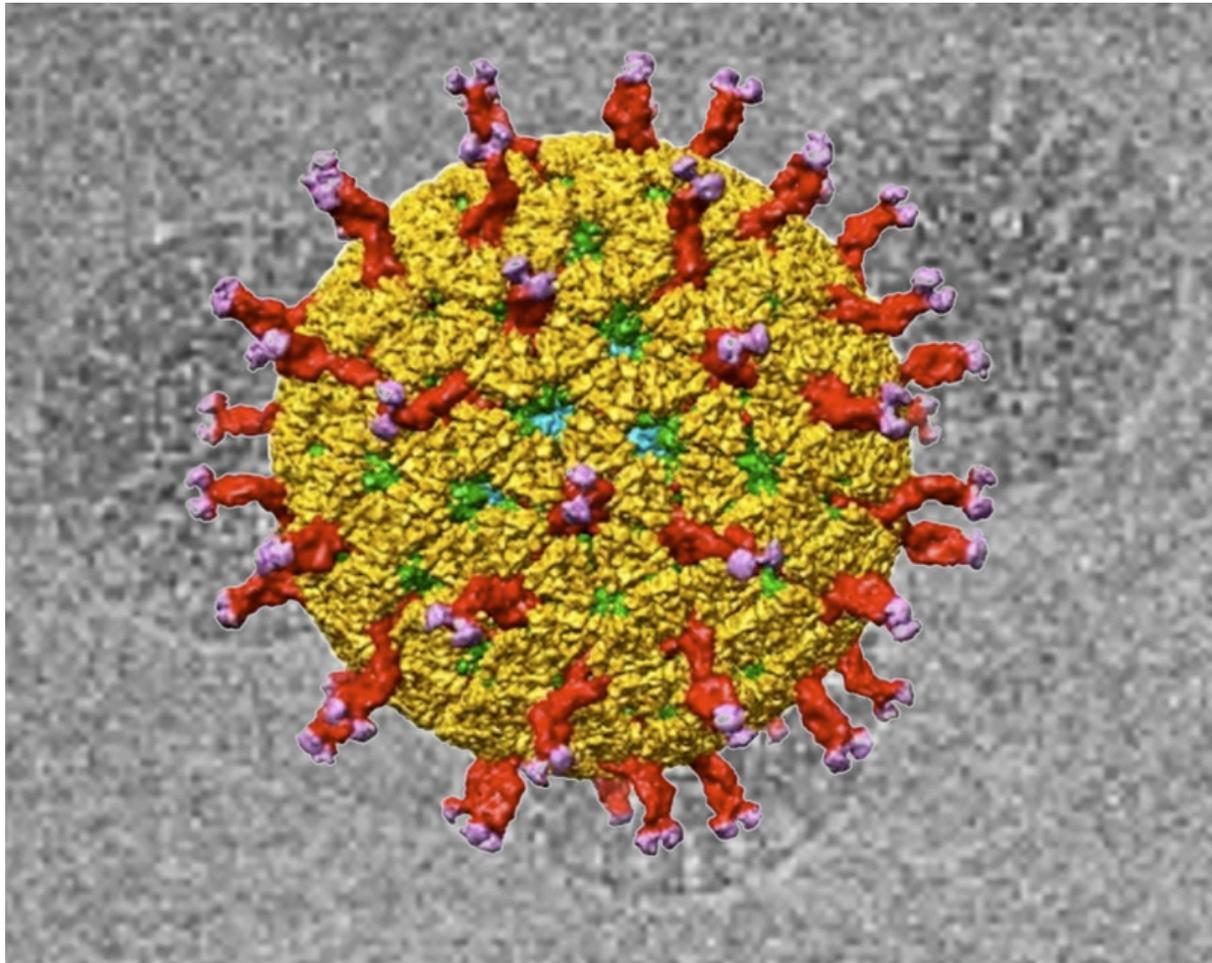
The geometry of each particle is determined by 5 parameters: three Euler angles and two in-plane shifts.

**Single particles are randomly oriented
in vitreous ice**

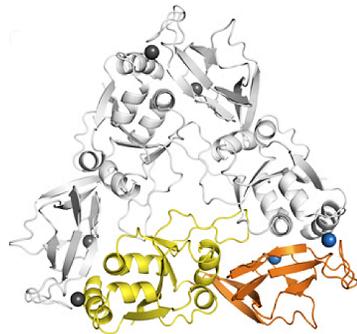
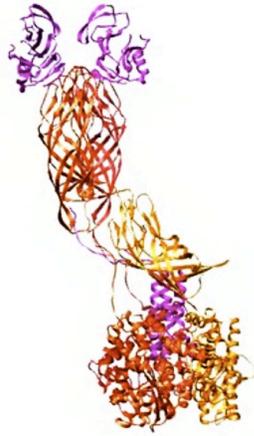
CryoEM images of biological structures are noisy because of very low electron dose required to avoid specimen damage ...



... but uniformity and high symmetry of viruses make it possible to reach molecular resolution by combining data from thousands of images



Outer layer proteins deliver the
DLP into the cell



VP7

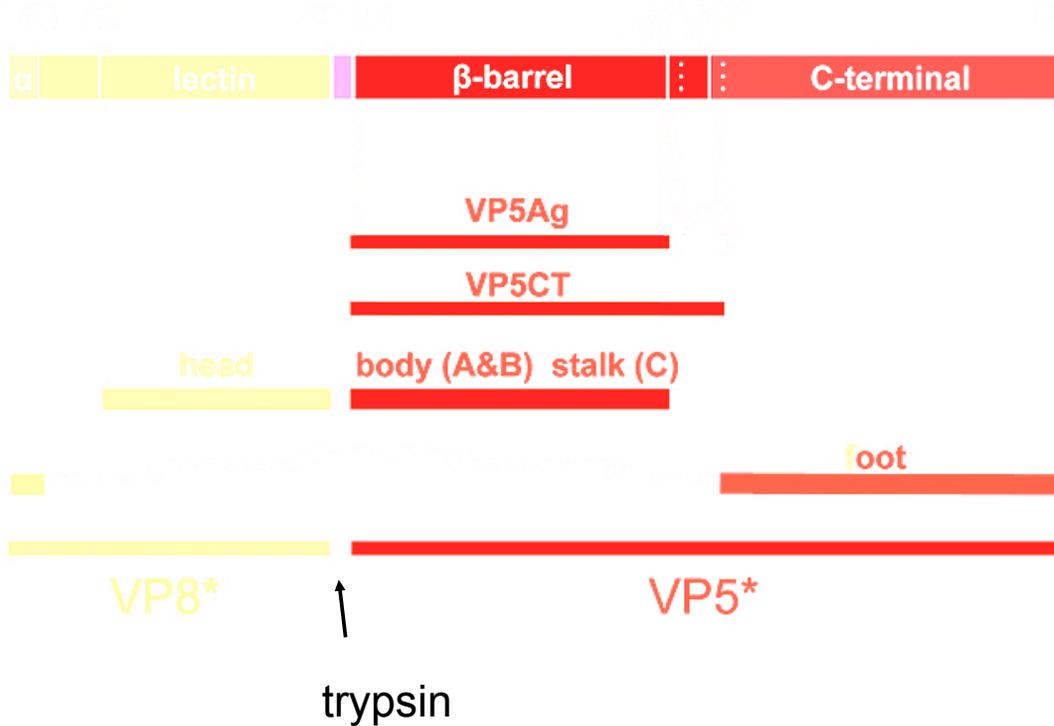
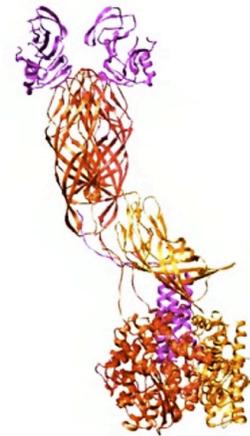
"Spike" protein VP4

VP5* VP8*

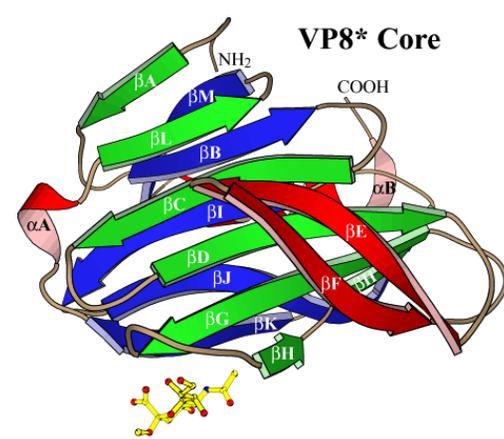
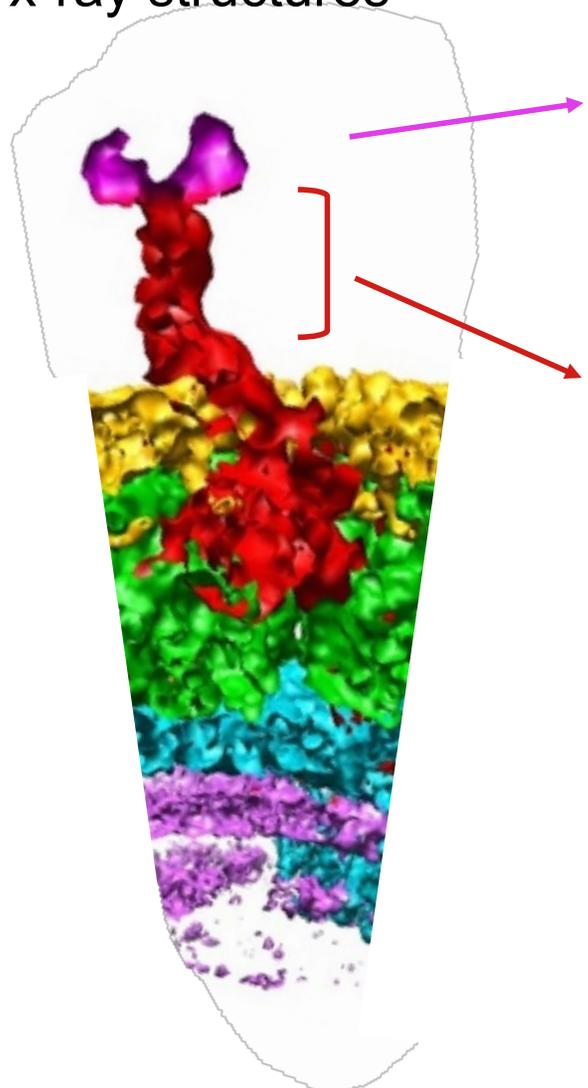


"Spike" protein VP4

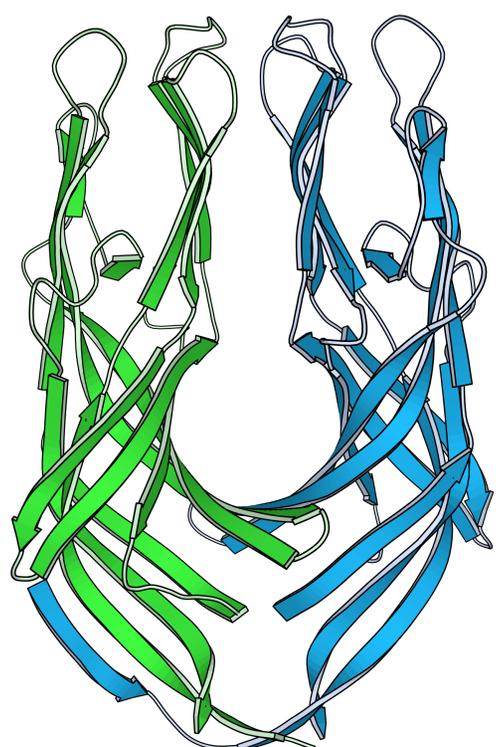
VP5* VP8*



Comparing known x-ray structures

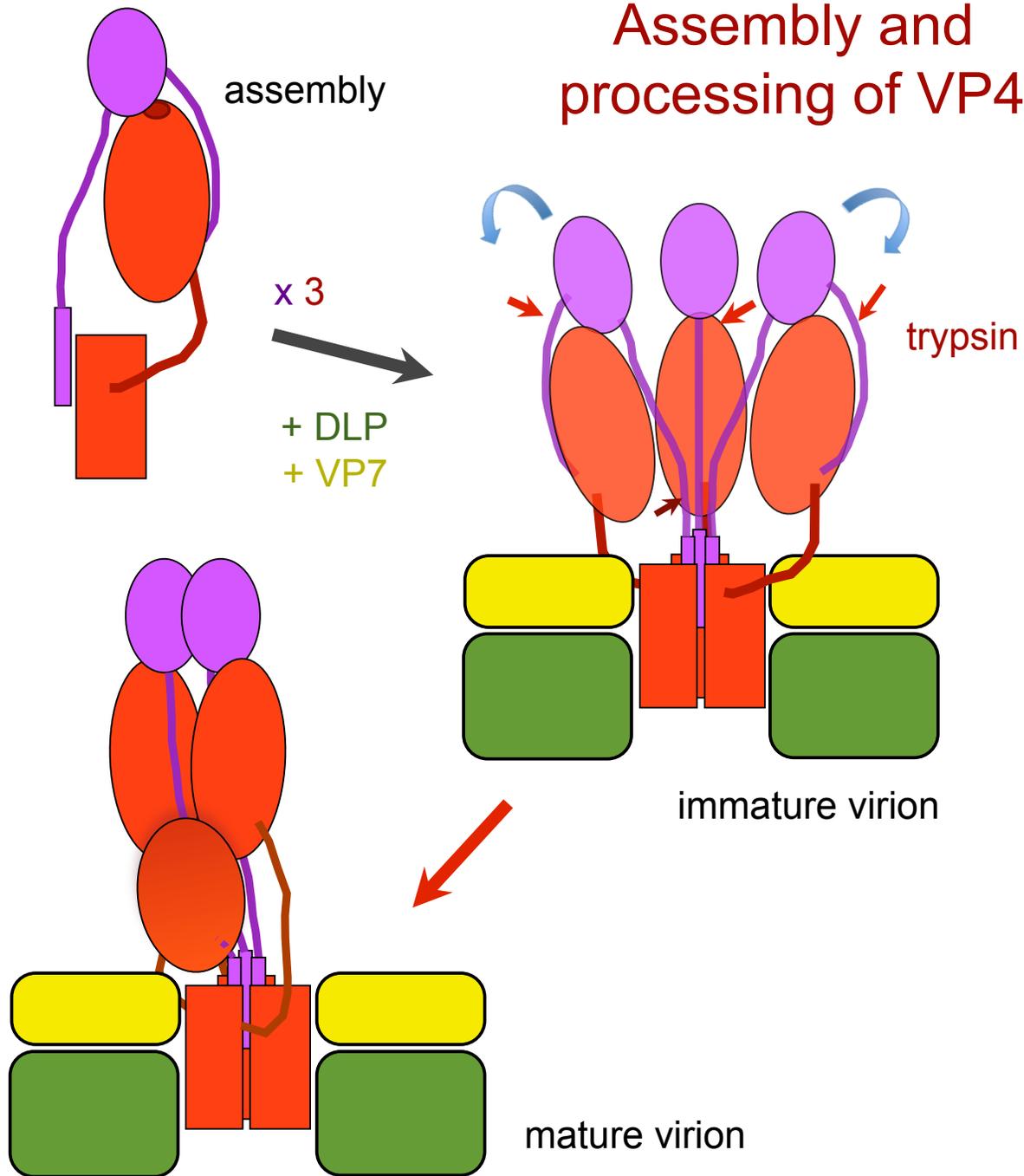


hydrophobic loops

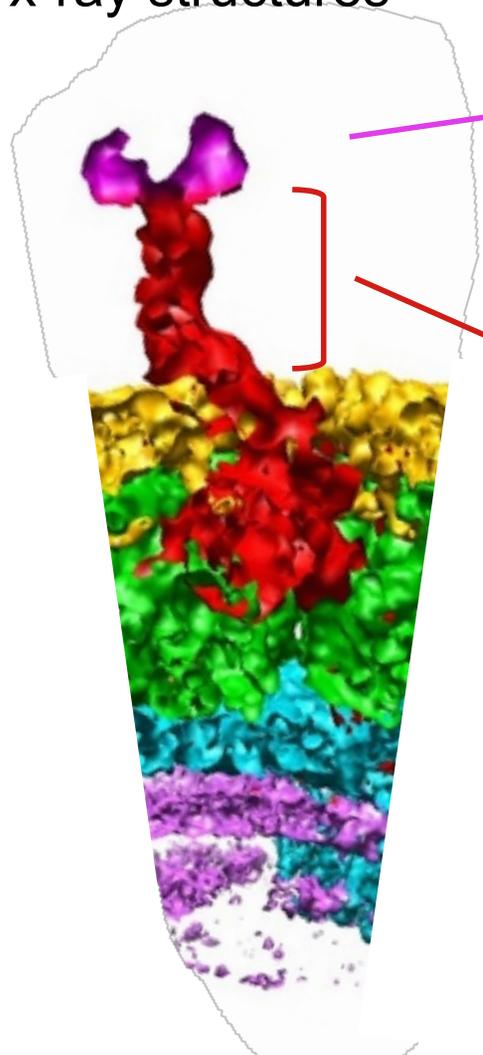


Yoder & Dormitzer, 2006
Yoder et al, 2009

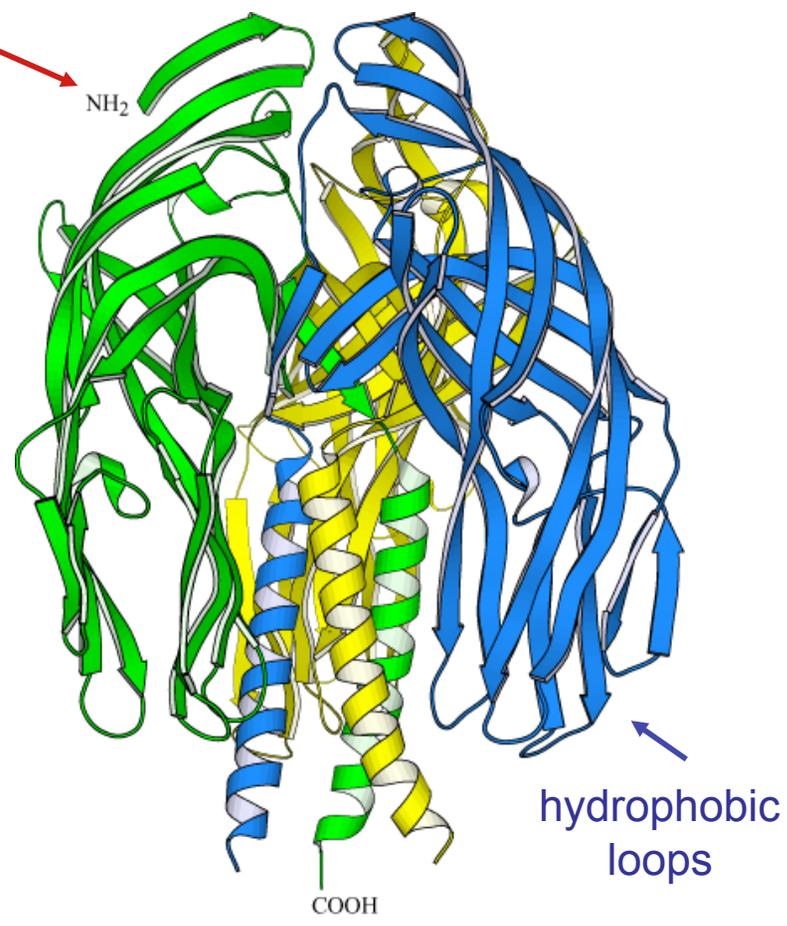
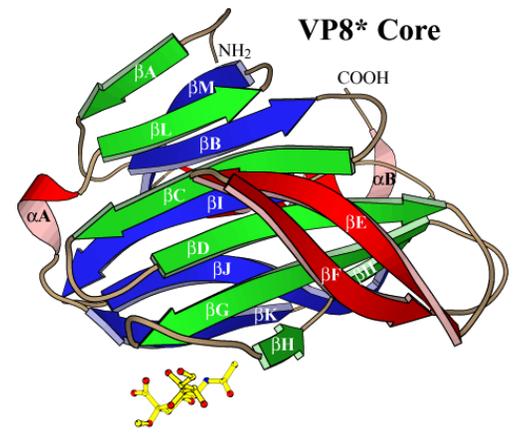
Assembly and processing of VP4



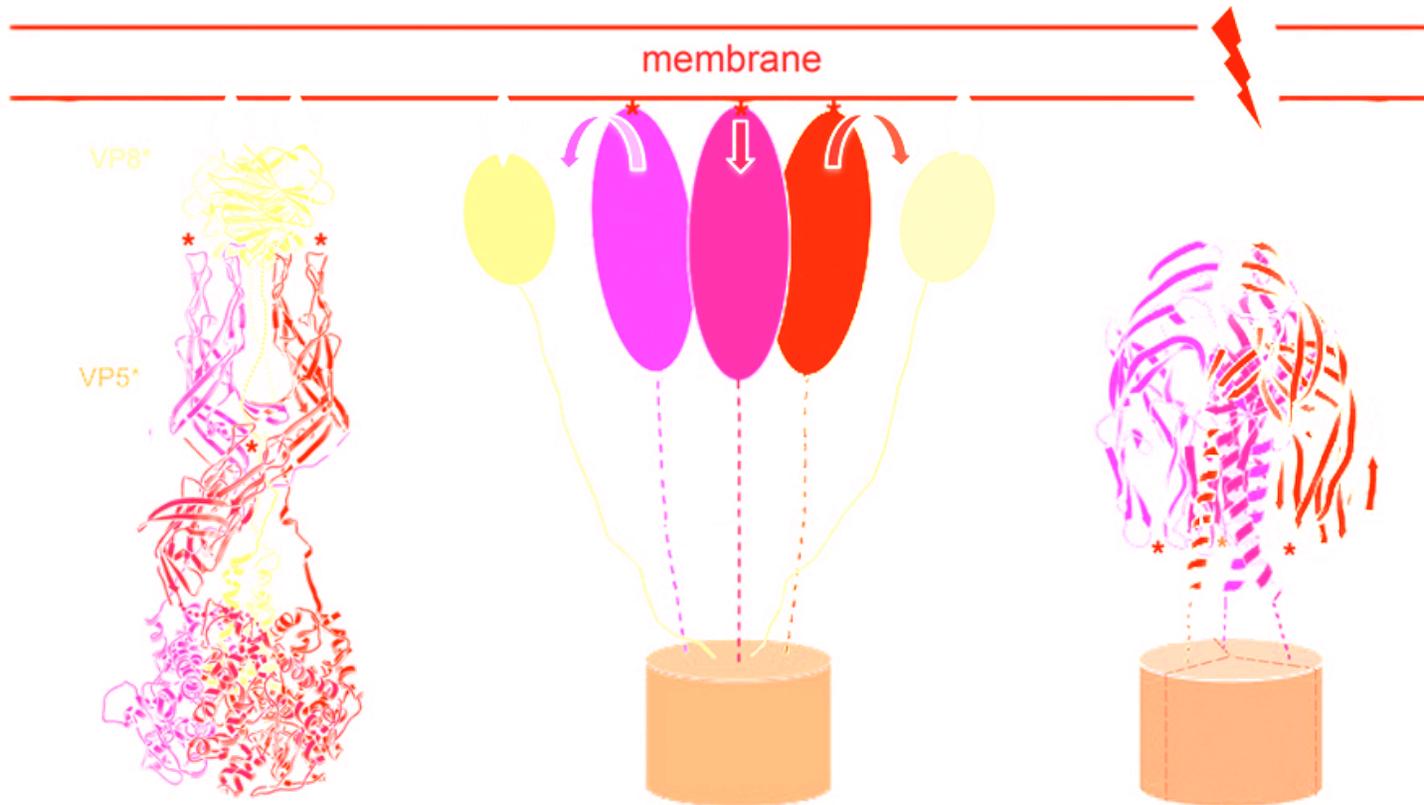
Comparing known x-ray structures



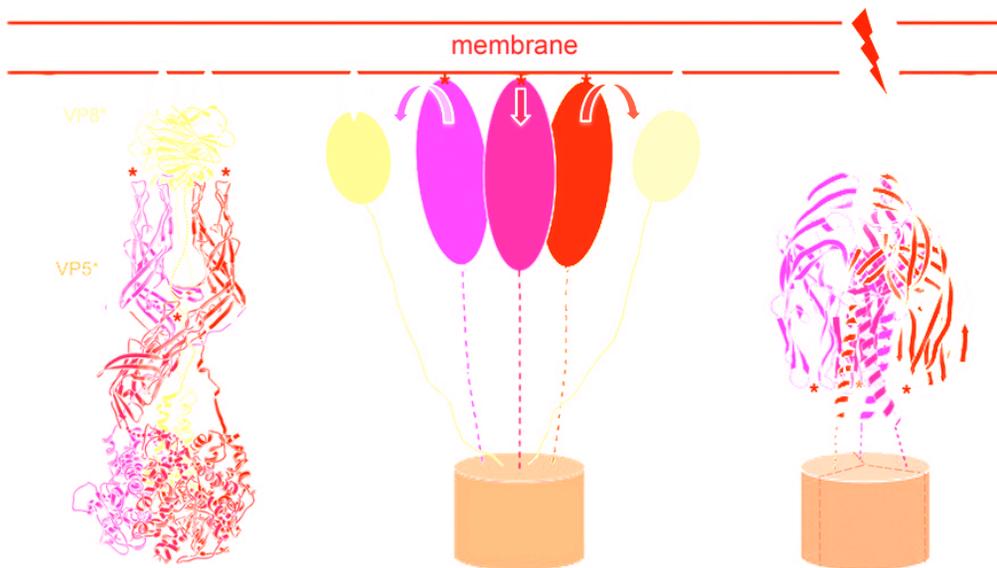
Dormitzer et al, 2004



Model for rotavirus perforation mechanism

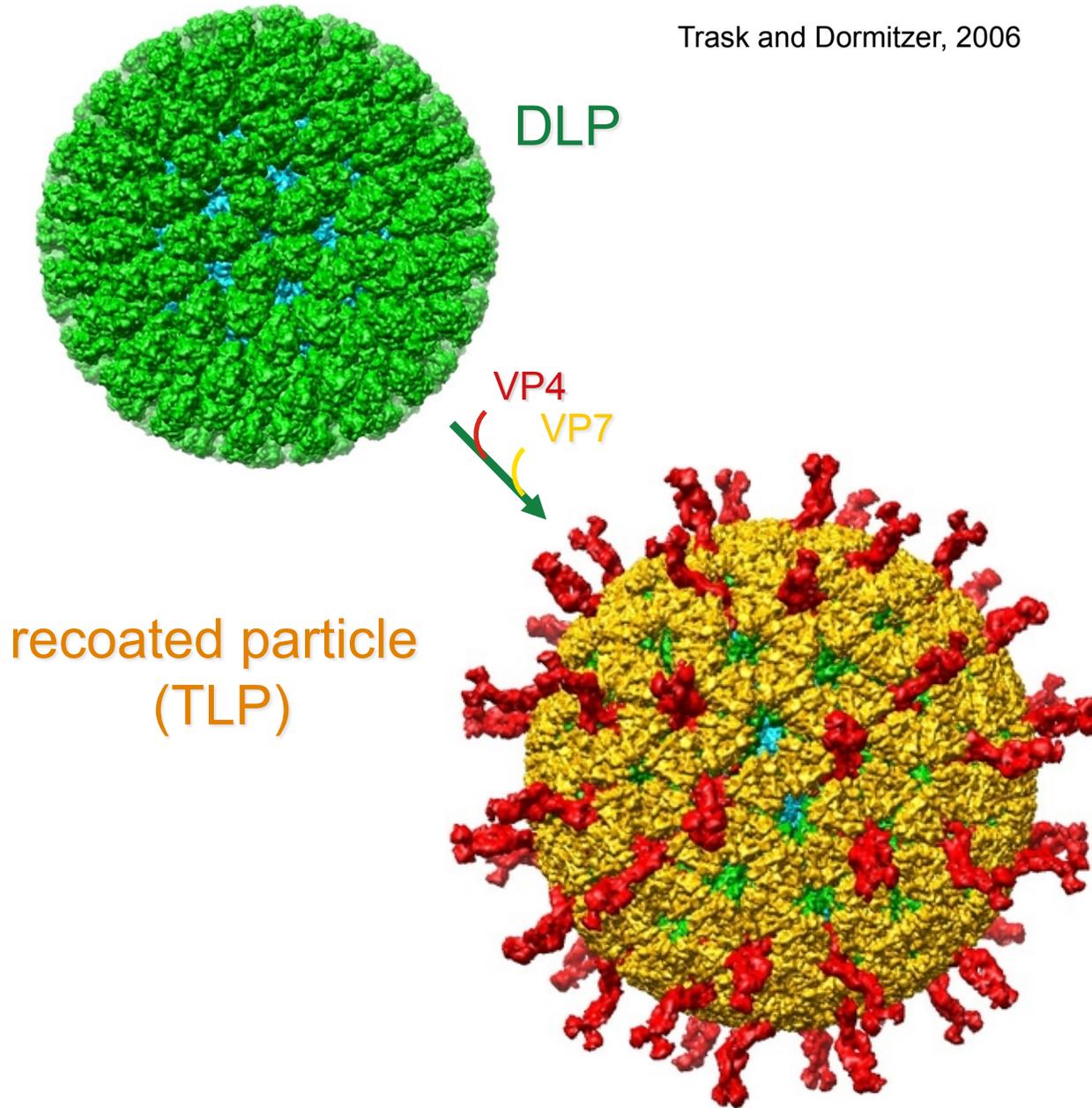


1. **Extended intermediate** forms
2. **Hydrophobic loops** contact membrane
3. **VP5*** folds back to "umbrella" conformation
4. **Coupling** of fold-back and membrane contact **perforates** bilayer

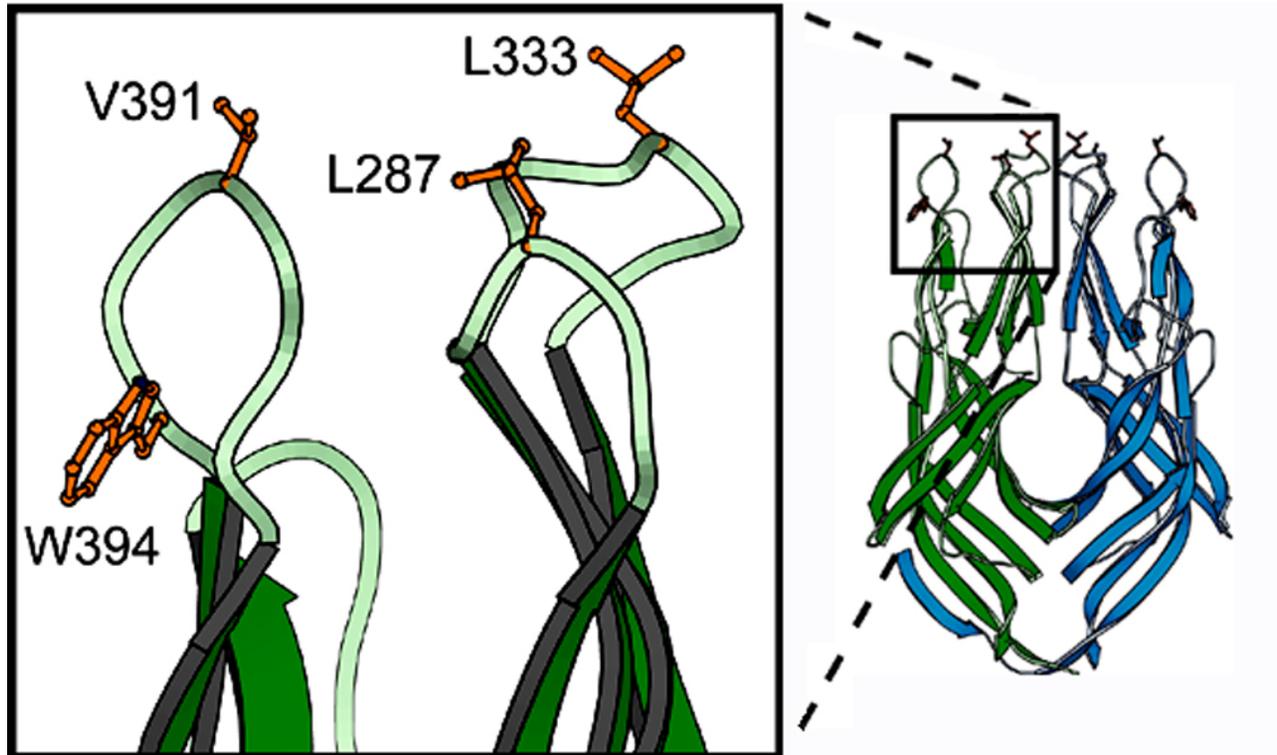


Functional recoating

Trask and Dormitzer, 2006



Mutate hydrophobic residues



1. Infectivity:

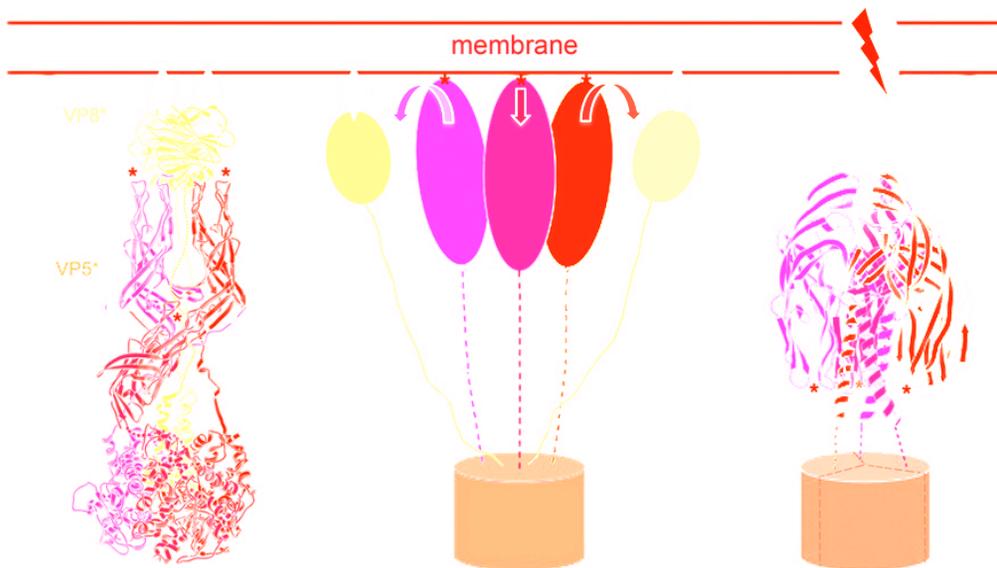
V391D $\sim 10^{-4}$

L333D $\sim 10^{-1}$ to 10^{-2}

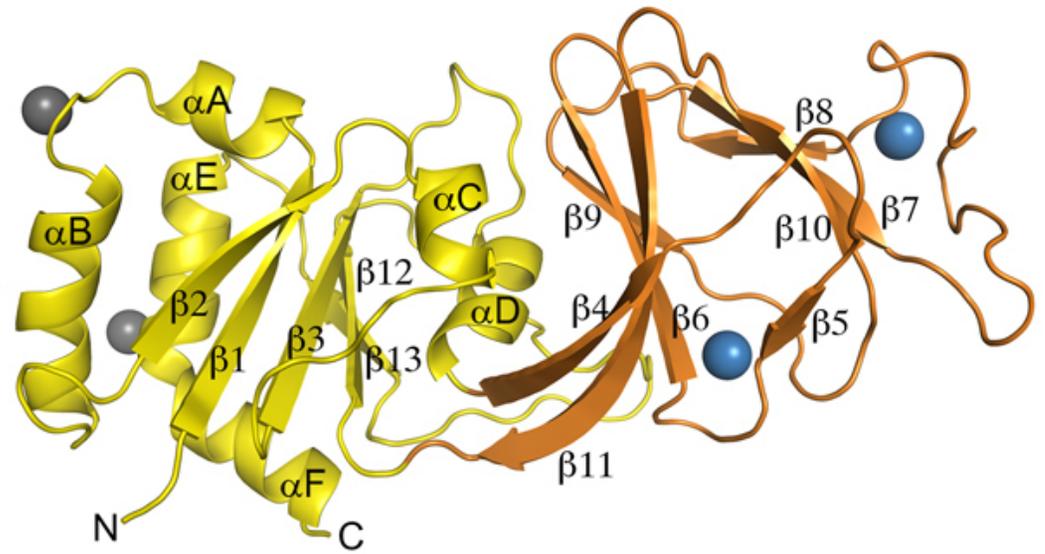
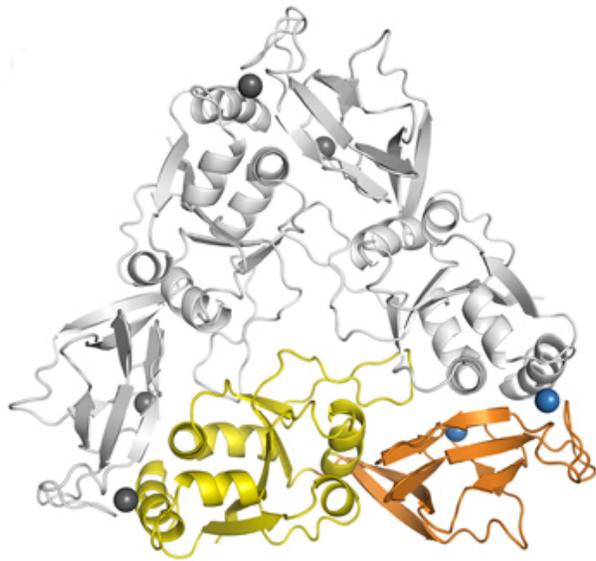
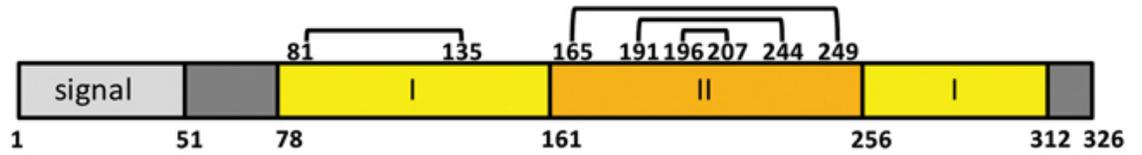
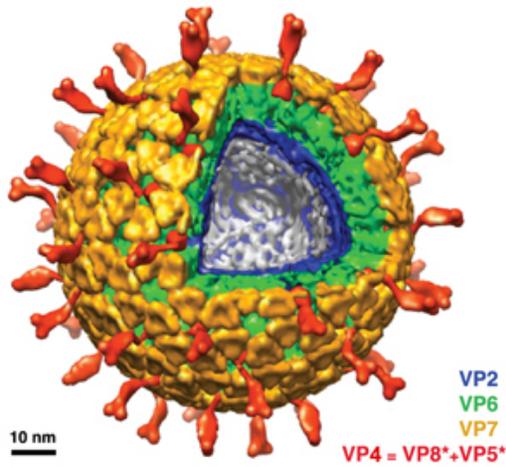
2. α -sarcin co-entry:

V391D does not mediate

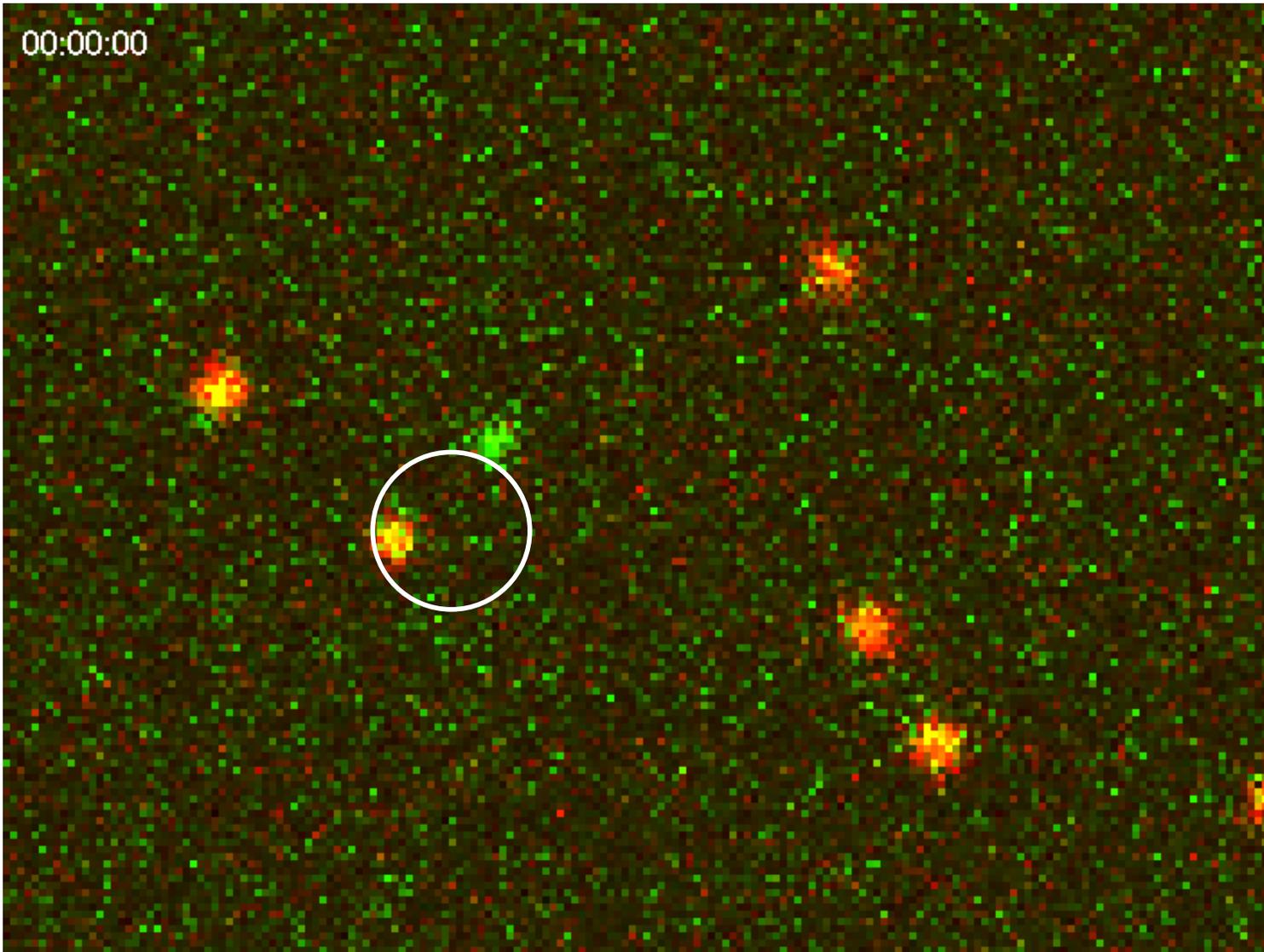
1. **Extended intermediate** forms
2. **Hydrophobic loops** contact membrane
3. **VP5*** folds back to "umbrella" conformation
4. **Coupling** of fold-back and membrane contact **perforates** bilayer



Outer-shell protein VP7

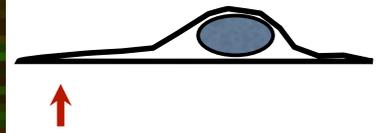


00:00:00

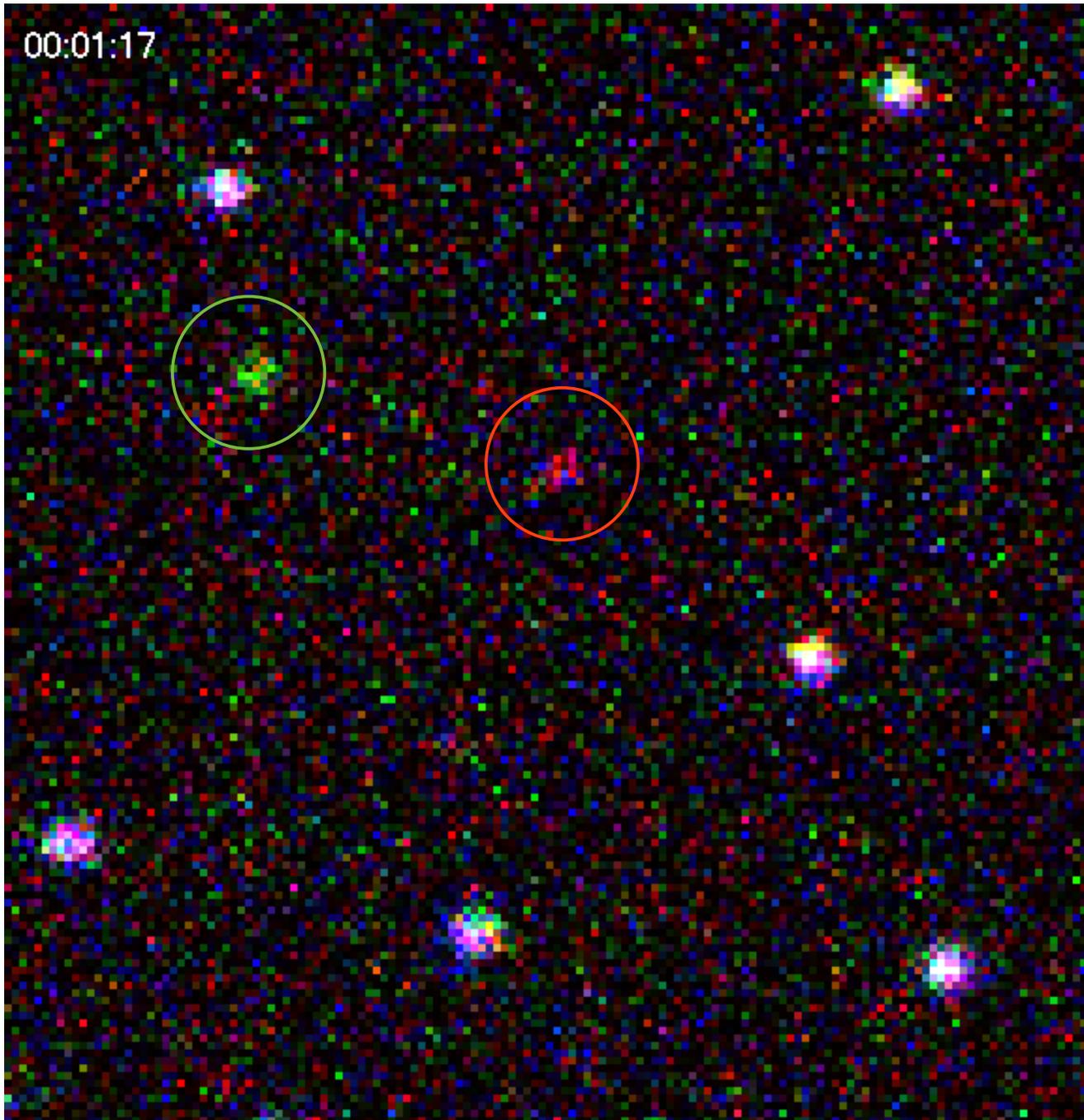


VP7
DLP

Bsc1 cells

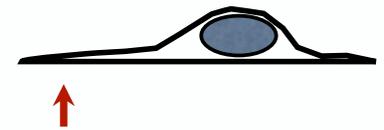


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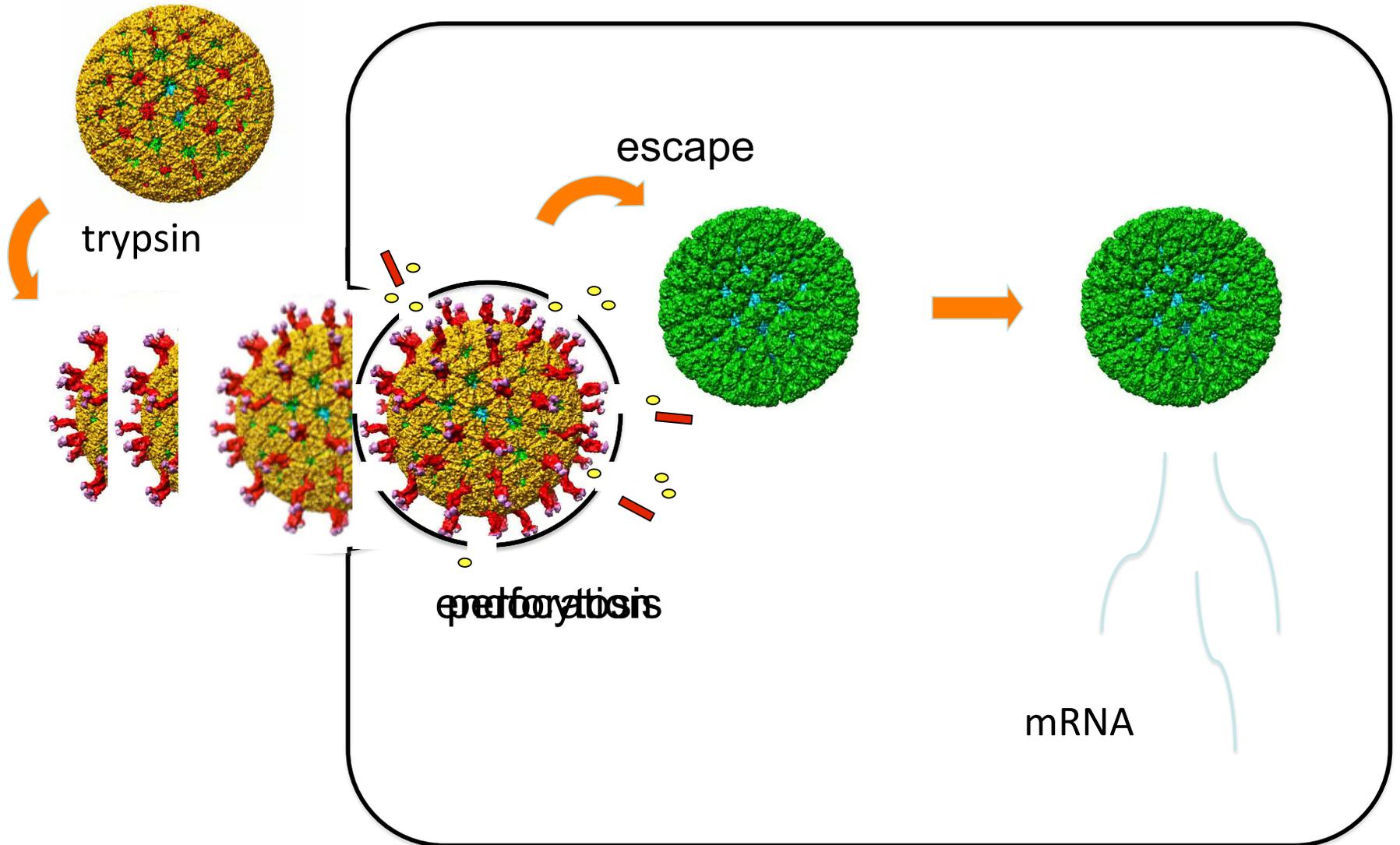


VP4
VP7
DLP

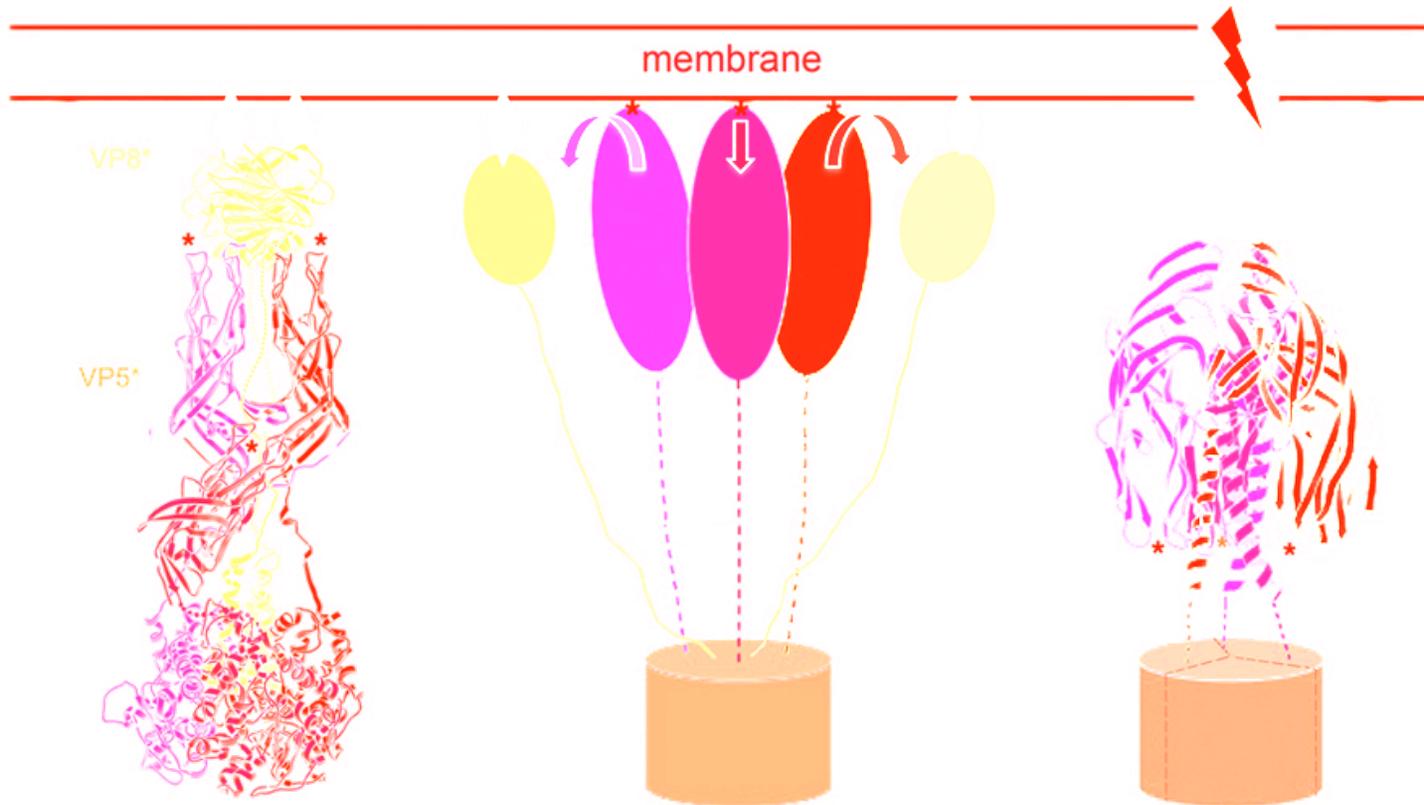
Bsc1 cells



ENTRY



Model for rotavirus perforation mechanism



Is engulfment simply an "autoendosome"?

Is membrane disruption by VP5* purely "mechanochemical"?

What is the structure of the VP5* extended intermediate?

Does VP5* remain anchored on the particle during membrane perforation?

What triggers the VP5* conformational change?

When does VP7 come off?

Rotavirus

Children's Hospital/HMS/HHMI

Aliaa Abdelhakim

Scott Aoki

Philip Dormitzer

Brian McClain

Irene Kim

Ethan Settembre

Shane Trask

Josh Yoder

Brandeis/HHMI

James Chen

Xing Zhang

Nikolaus Grigorieff

IDI/Children's/HMS

Comert Kural

T. Kirchhausen

Univ. Aukland

A. R. Bellamy