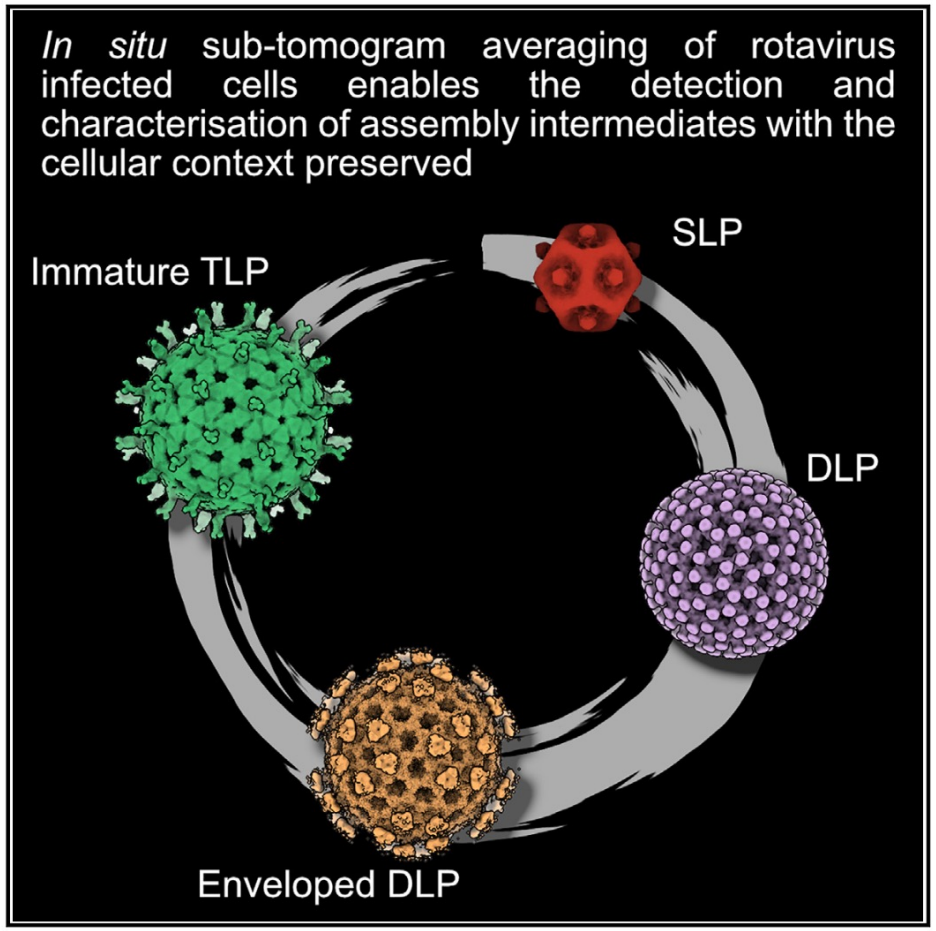
Cell Host & Microbe

Characterization of the rotavirus assembly pathway *in situ* using cryoelectron tomography

Graphical abstract



Authors

Pranav N.M. Shah, James B. Gilchrist, Björn O. Forsberg, ..., Geoff Sutton, David I. Stuart, Mark Boyce

Correspondence

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In brief

Shah et al. used cryo-tomography to characterize the assembly pathway of rotavirus inside infected cells. The use of flash frozen samples preserved the assembly stages in their native state and relative proportions, and subtomogram averaging revealed their molecular structures, to near atomic detail in the best case.

Author

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Research / Stuart Group: Structural Virology

Stuart Group: Structural Virology

Viruses are attractive targets for study at the molecular level, since they are sufficiently simple that we may hope to achieve a rather complete understanding of their biology. In practice although their genomes are compact they display astonishing diversity, both in structure and function. Our attempts to relate structure to function have benefited from the developments in X-ray crystallographic methods that have brought very complex structures within reach of description in atomic detail. Our targets range from picornaviruses, small ssRNA viruses, which include a number of important animal and human pathogens, to the larger dsRNA viruses. At both ends of this spectrum (from less than 10,000,000 to about 100,000,000 Daltons) we now have representative atomic structures.

Our efforts are particularly focused on virus-receptor interactions and basic

OUR TEAM

Mohammad Bahar
Post Doctoral Research Associate

Mark Boyce
Senior fellow

John Clarke
Student

Elizabeth Fry
Post Doctoral Research Associate

SELECTED PUBLICATIONS

Hand-foot-and-mouth disease virus receptor KREMEN1 binds the canyon of Coxsackie Virus A10

Journal article

Zhao Y. et al, (2020), Nature Communications, 11

Multiple liquid crystalline geometries of highly compacted nucleic acid in a dsRNA virus

Journal article

Ilca SL. et al, (2019), Nature, 570, 252 - 256

The structure of a prokaryotic viral envelope protein expands the landscape of membrane fusion proteins

Journal article

El Omari K. et al, (2019), Nature Communications, 10

Machining protein microcrystals for structure

Author

PRANAV SHAH

CONTACT INFORMATION

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RESEARCH GROUPS

Stuart Group: Structural Virology

Pranav Shah

POST DOCTORAL RESEARCH ASSOCIATE

RECENT PUBLICATIONS

Characterization of the rotavirus assembly pathway in situ using cryoelectron tomography.

Journal article

Shah PNM. et al, (2023), Cell Host Microbe, 31, 604 - 615.e4

A robust normalized local filter to estimate occupancy directly from cryo-EM maps

Preprint

Forsberg BO. et al, (2023)

Delineating organizational principles of the endogenous L-A virus by cryo-EM and computational analysis of native cell extracts

Preprint

Schmidt L. et al, (2022)

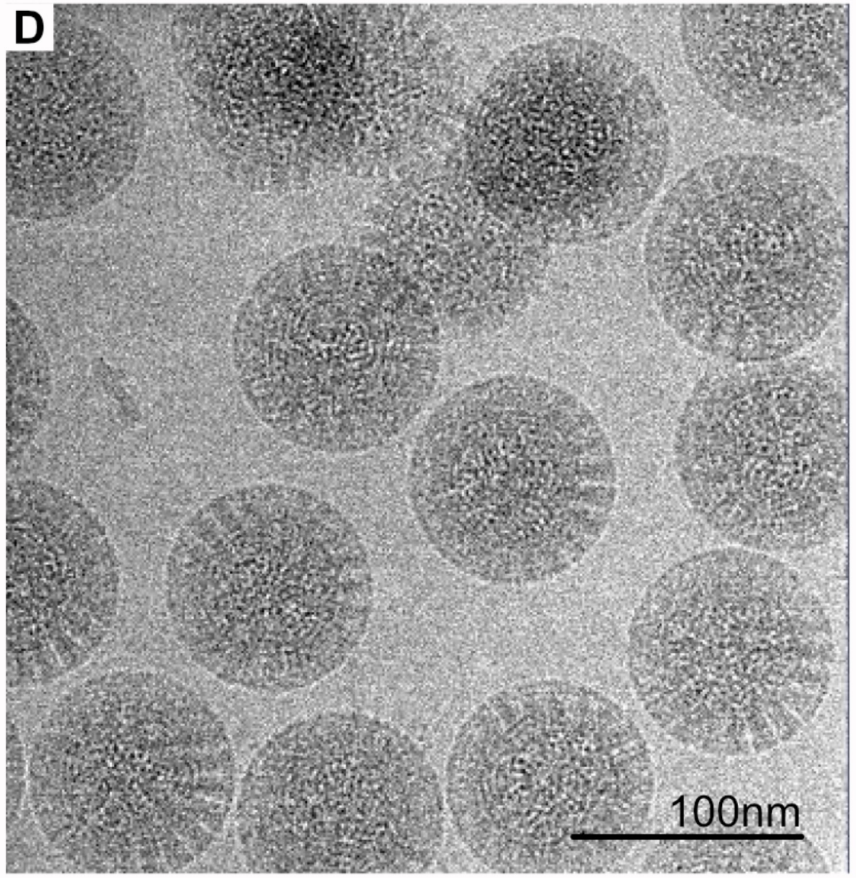
Purification of African Swine Fever Virus.

Journal article

Shimmon GL. et al, (2022), Methods in molecular biology (Clifton, N.J.), 2503, 179 - 186

Author Correction: Neutralizing nanobodies bind SARS-CoV-2 spike RBD and block interaction with ACE2

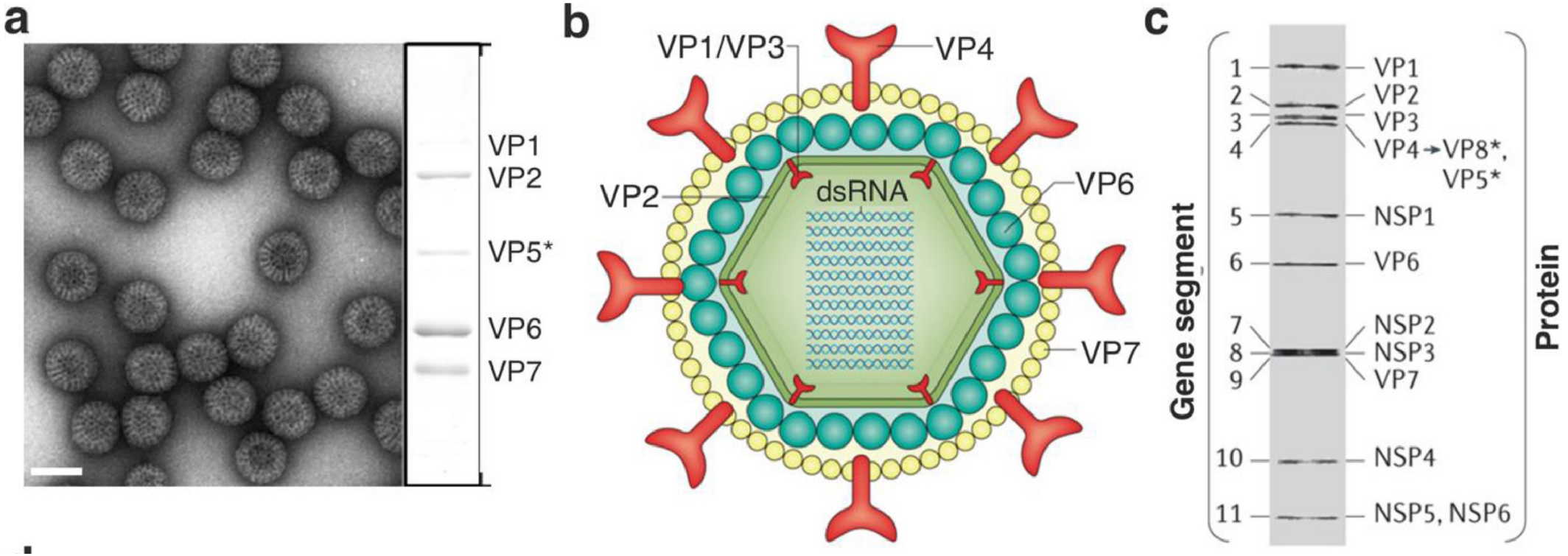
Background



Reoviridae，or Respiratory Enteric Orphan viruses呼肠孤病毒，是双链RNA病毒的一个科

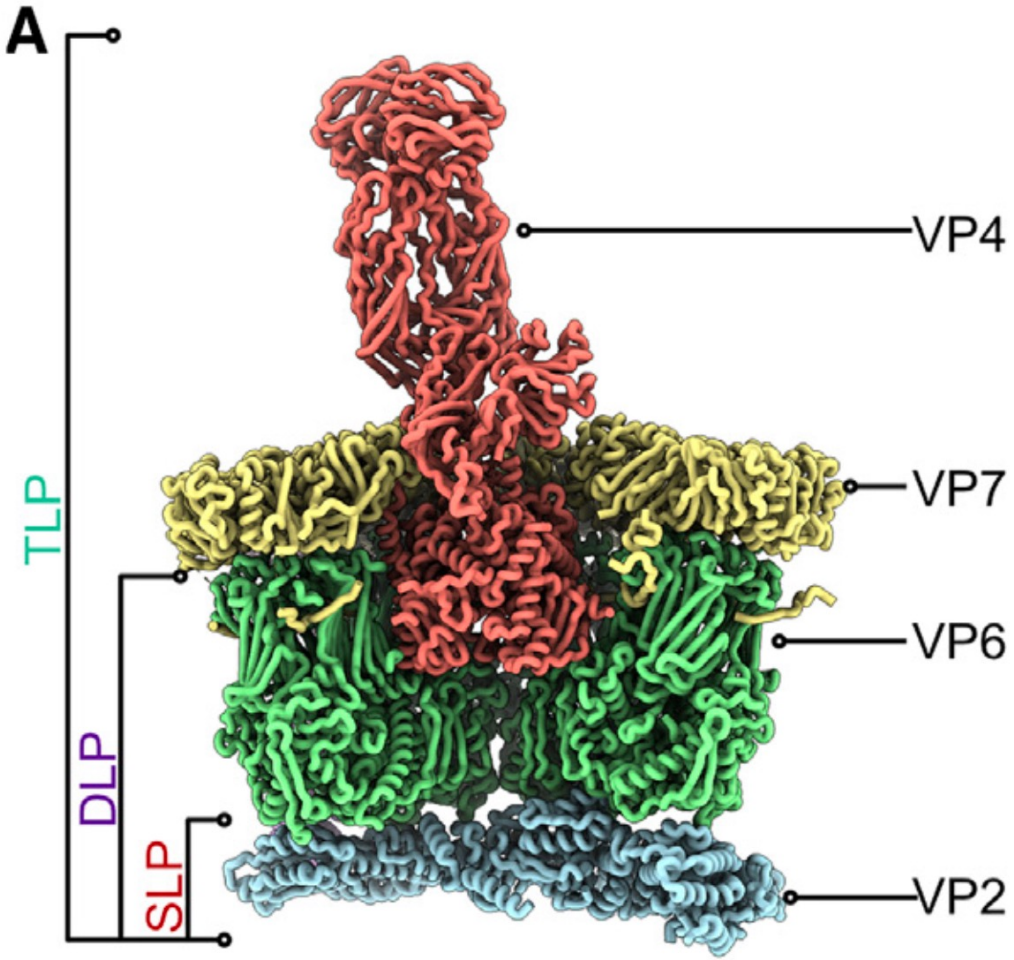
- Rotaviruses, part of the Reovirus family, are a major cause of infant deaths in the developing world, killing over 800,000 children under the age of 2 each year.
- viral gastroenteritis病毒性肠胃炎

Background



(Sarah Caddy, et al. Virus Research, 2021)

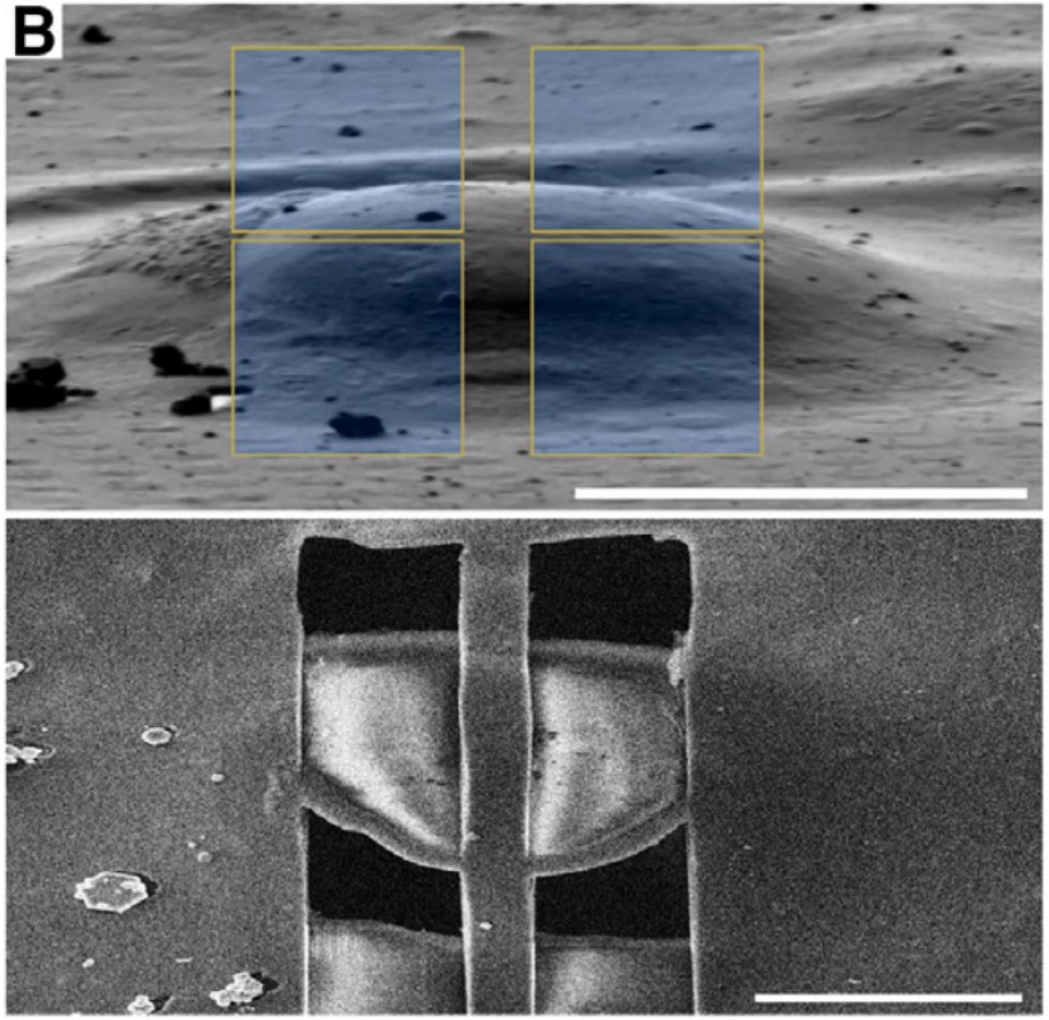
Background

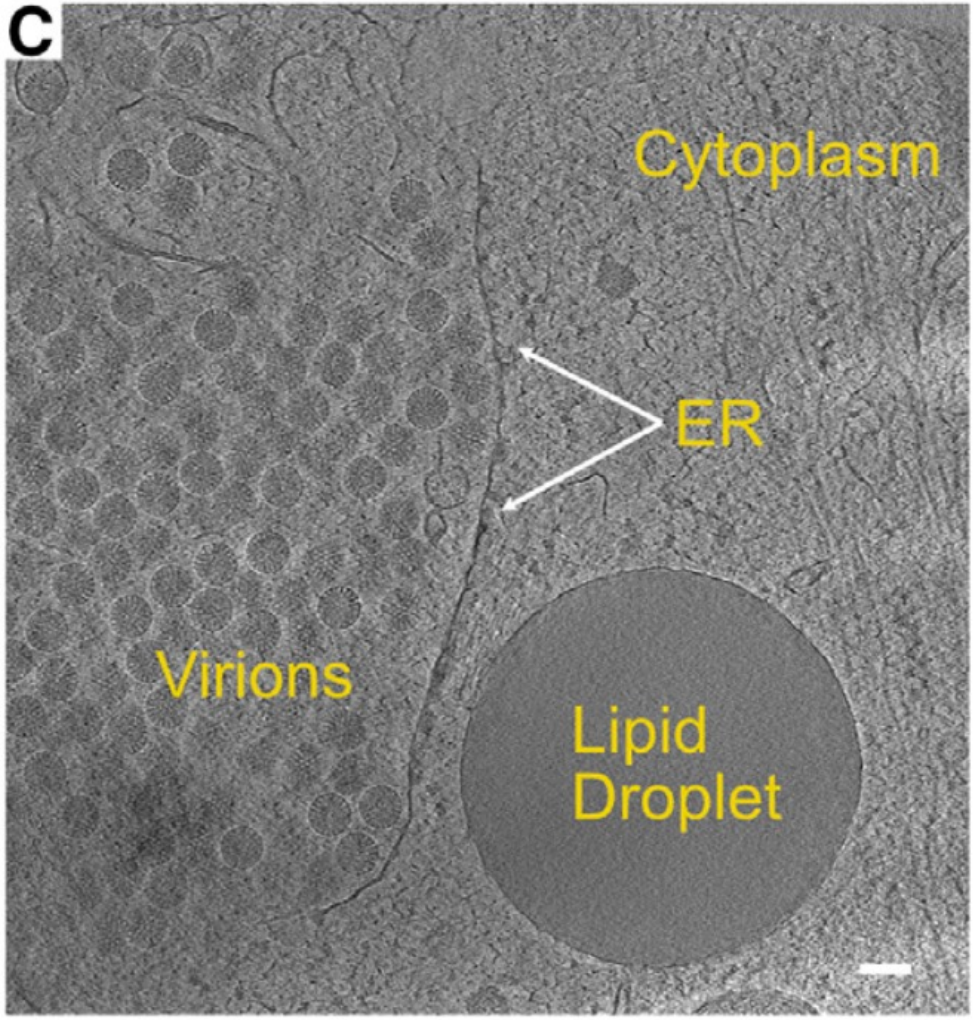


- Multi-layered
- Icosahedral shell:
  - 120 VP2, 11 VP1
  - 260 VP6
  - 260 VP7
  - 60 VP4

  } TLP
- dsRNA

Result 1: Intermediates of assembly





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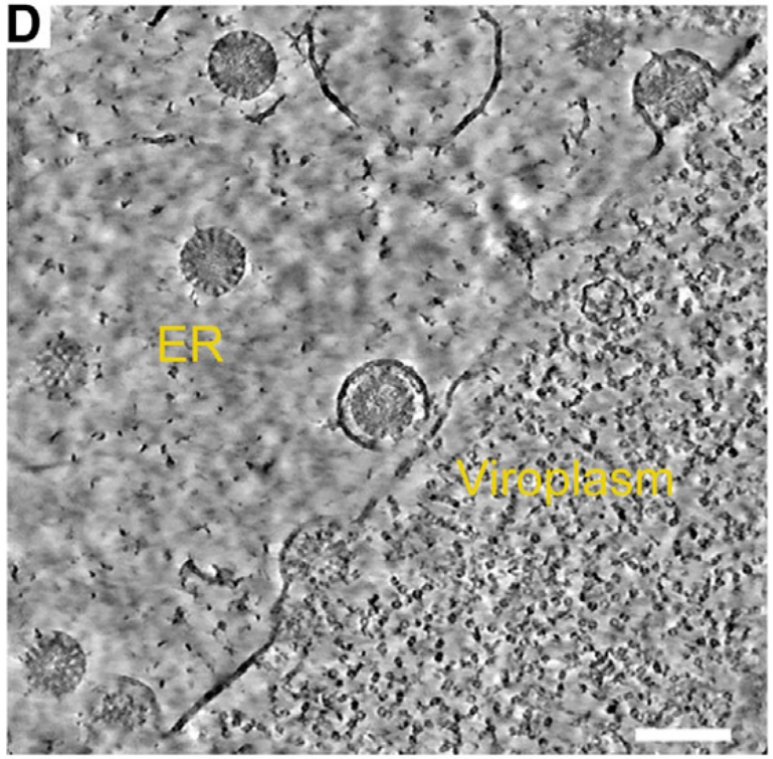
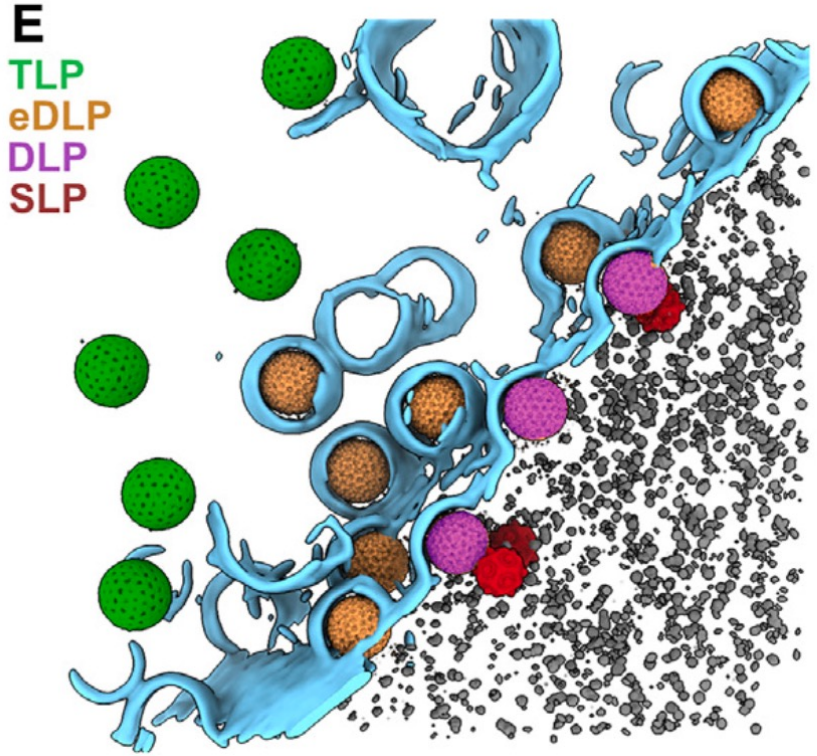
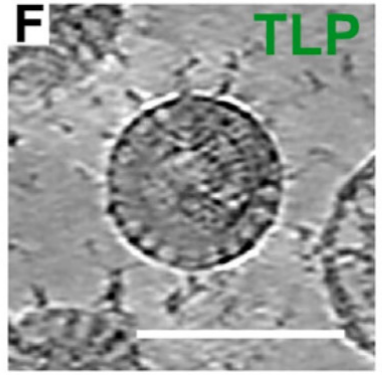
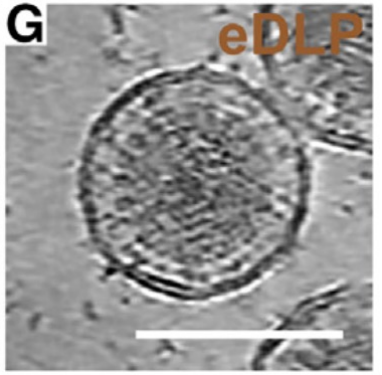
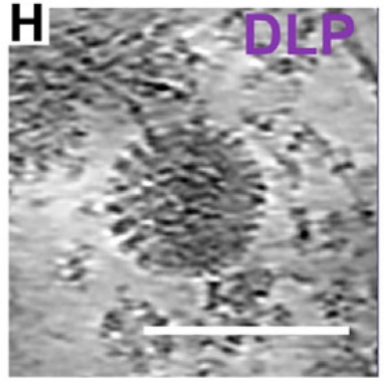


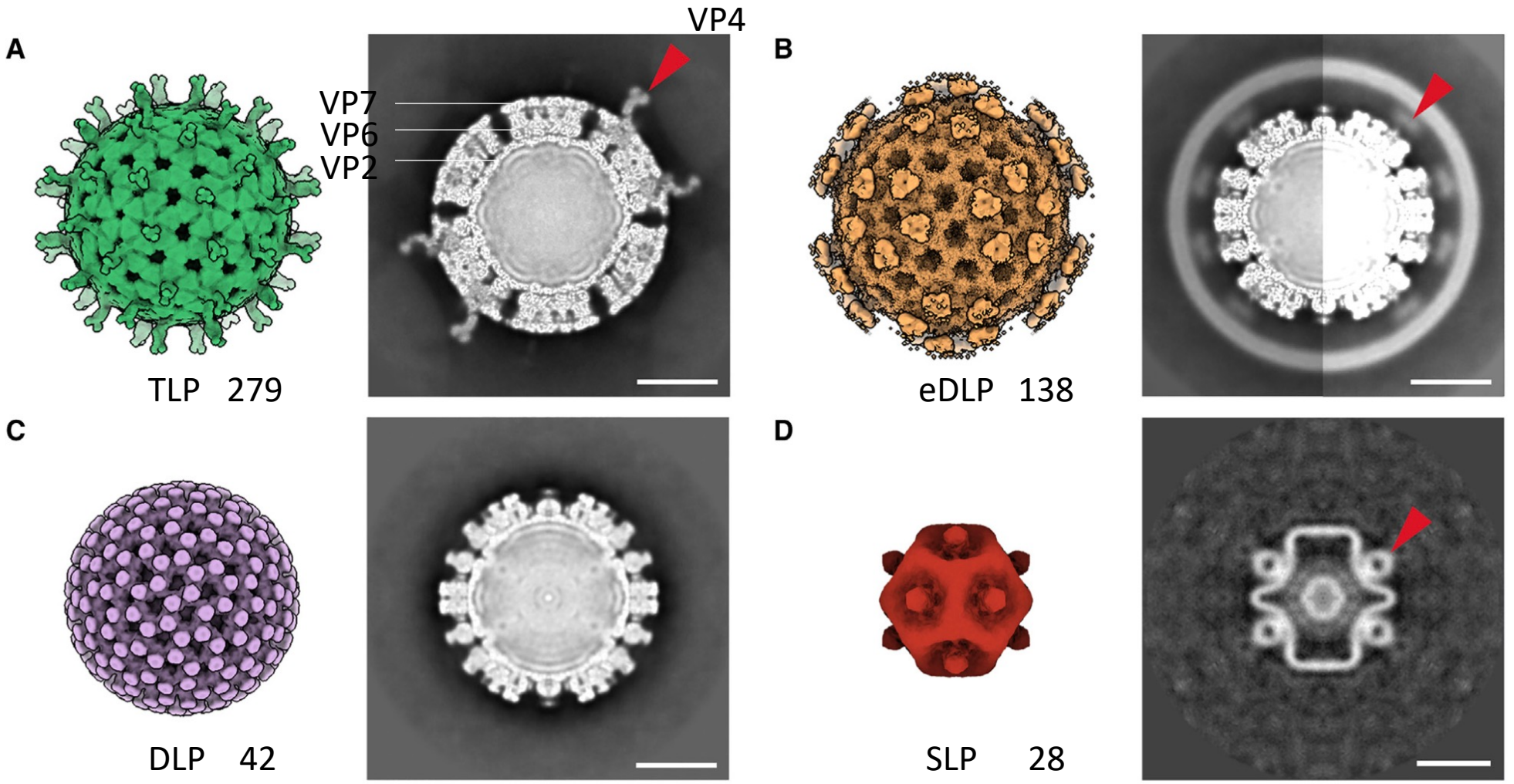








Result2: High-resolution structures using STA



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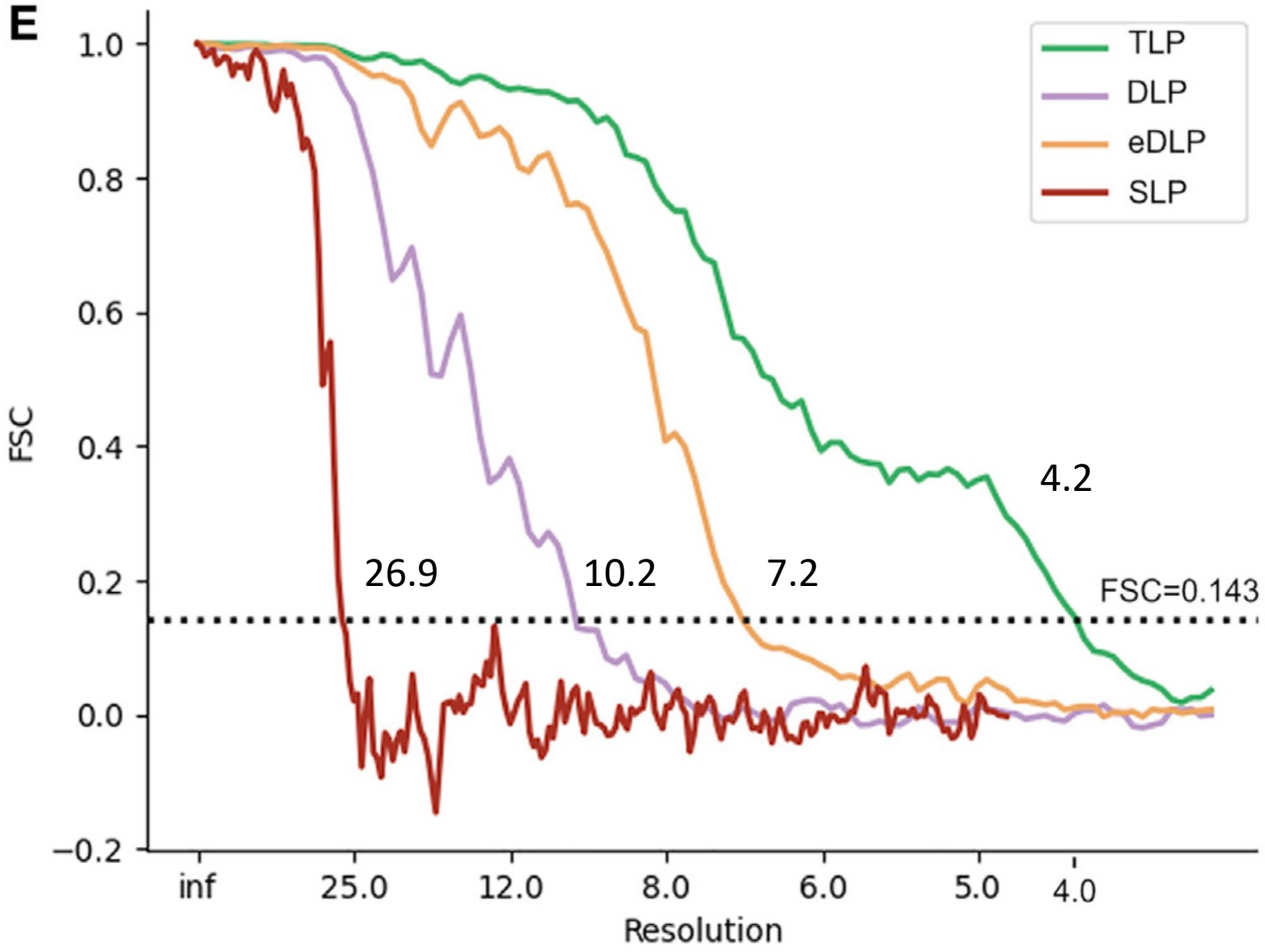

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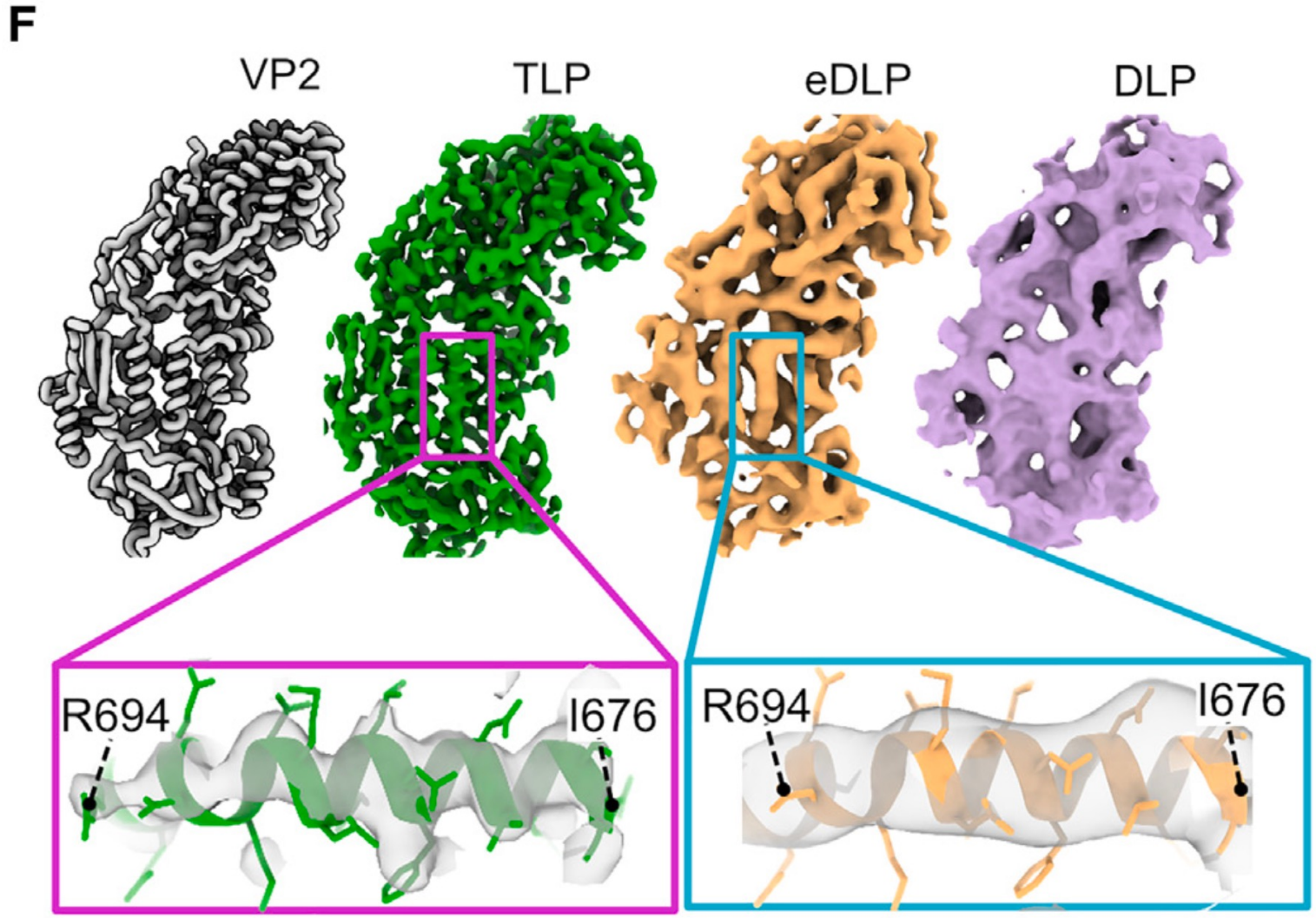
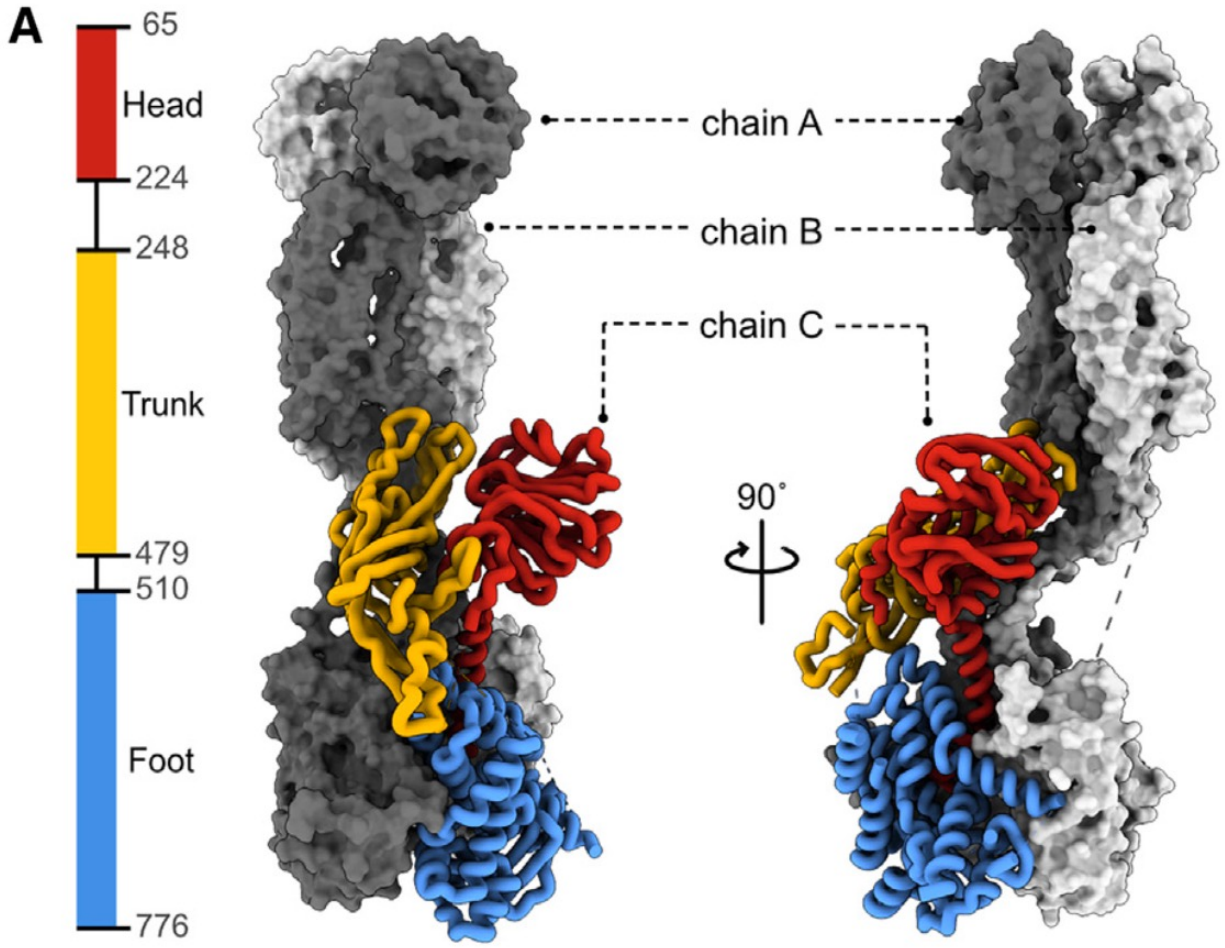


Result3: VP4 structure prior to exposure to trypsin



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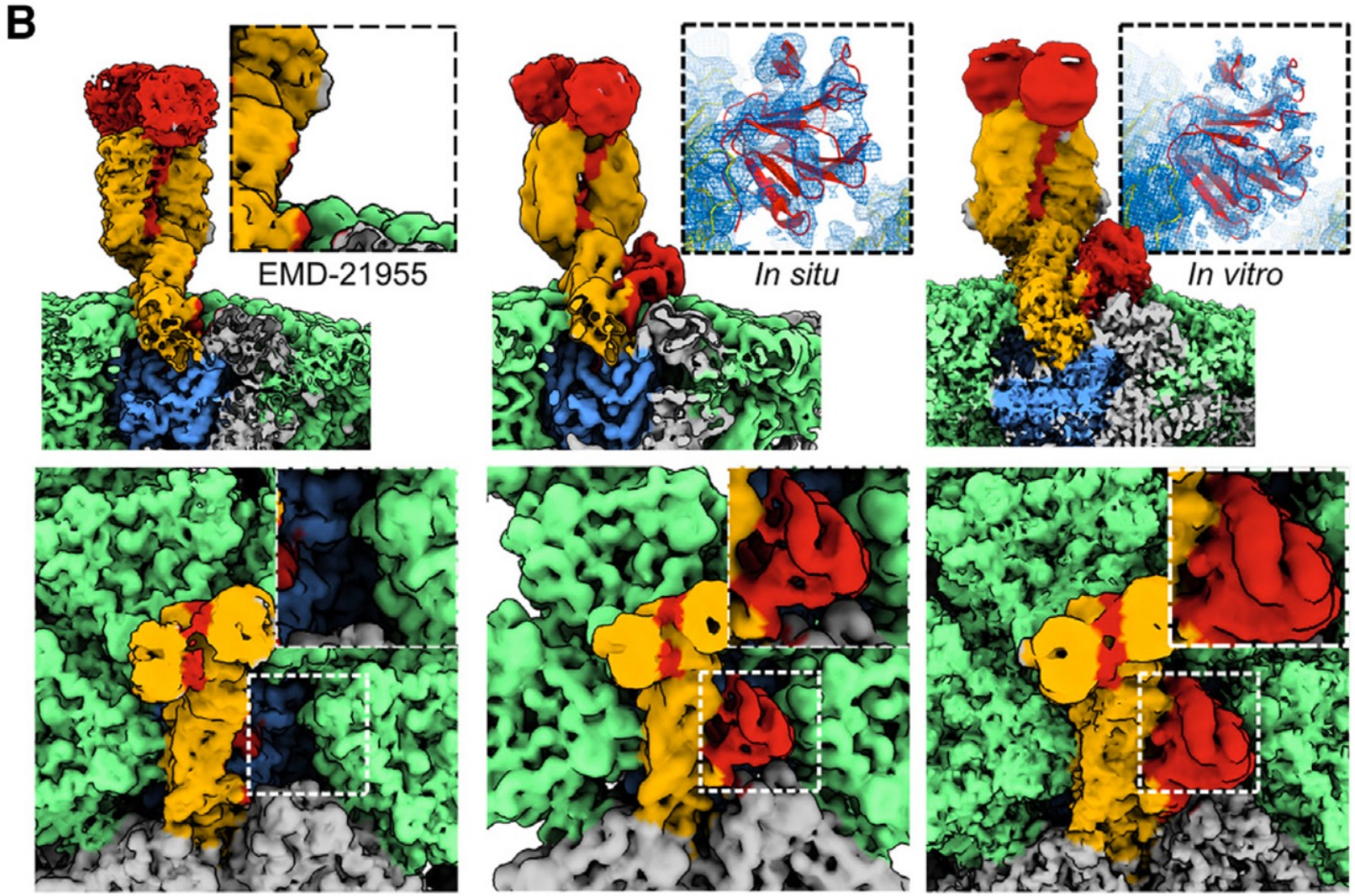

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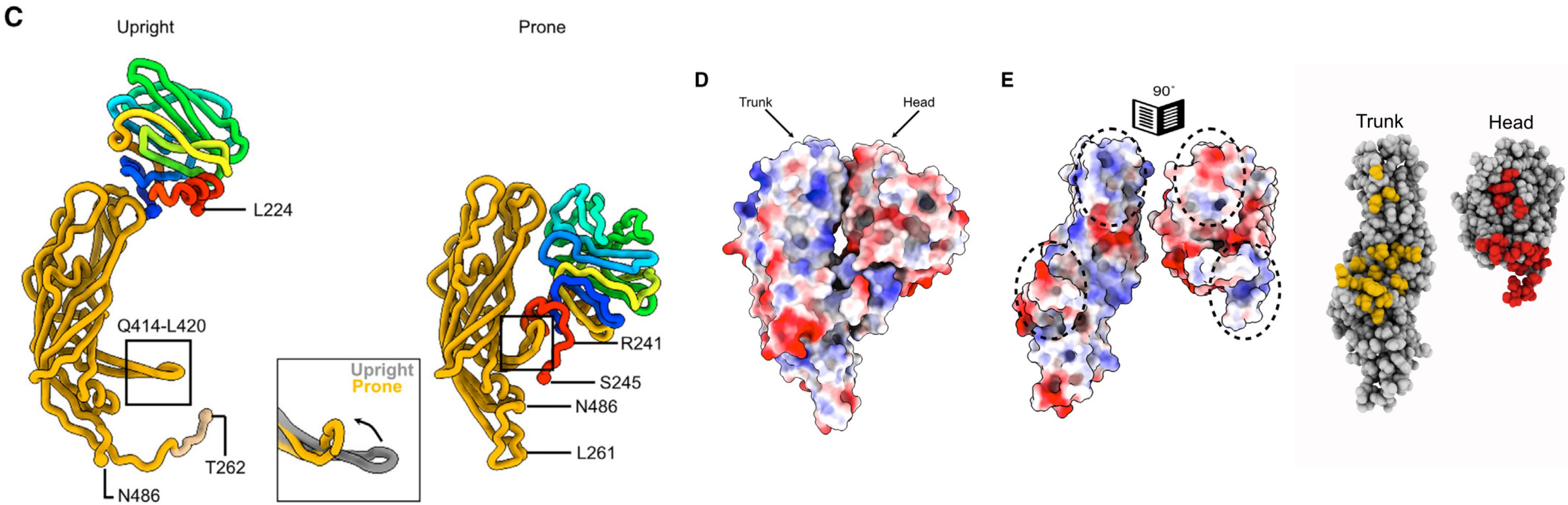
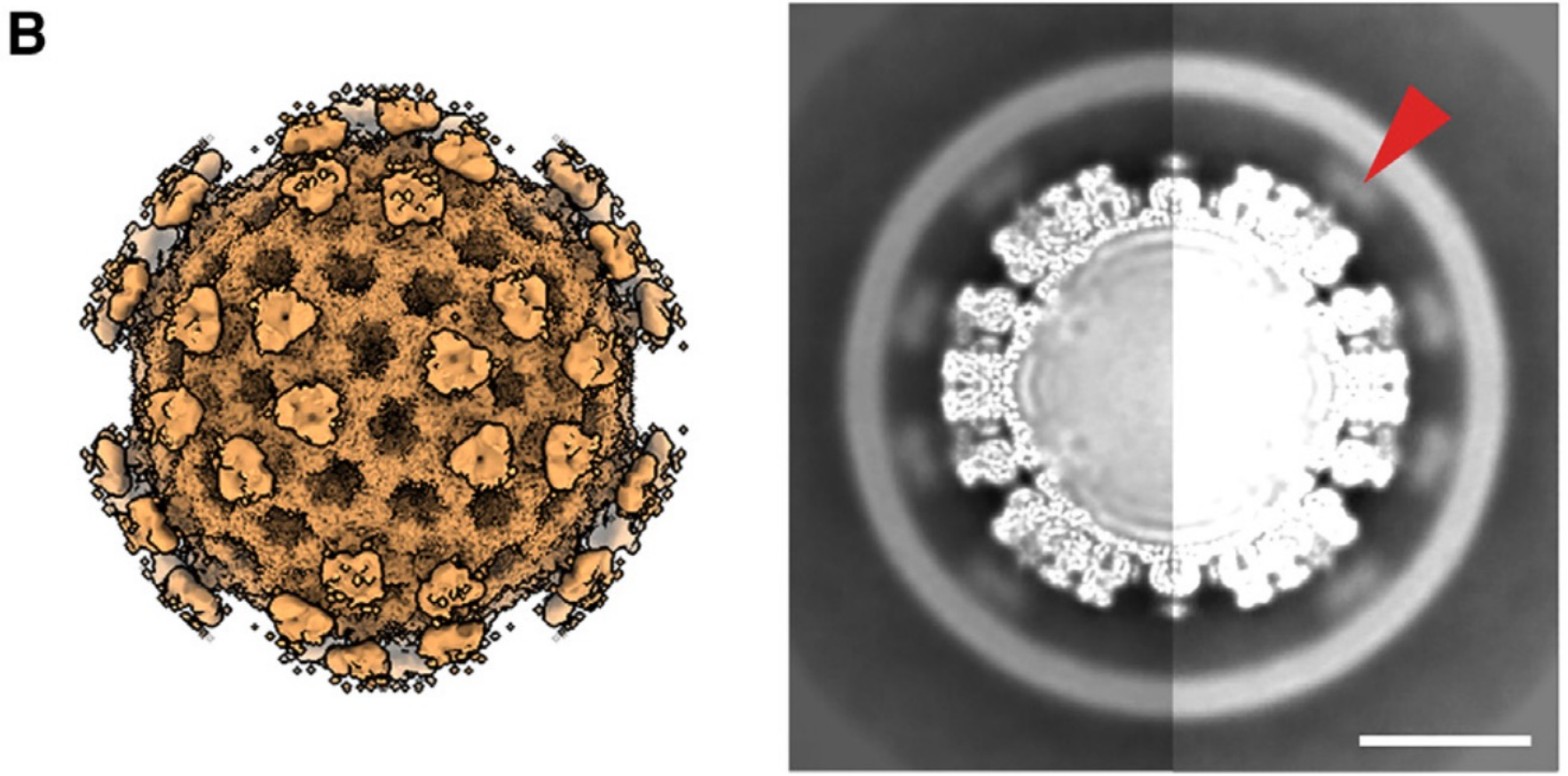


Result4: a trimeric conformation of VP4 in eDLP



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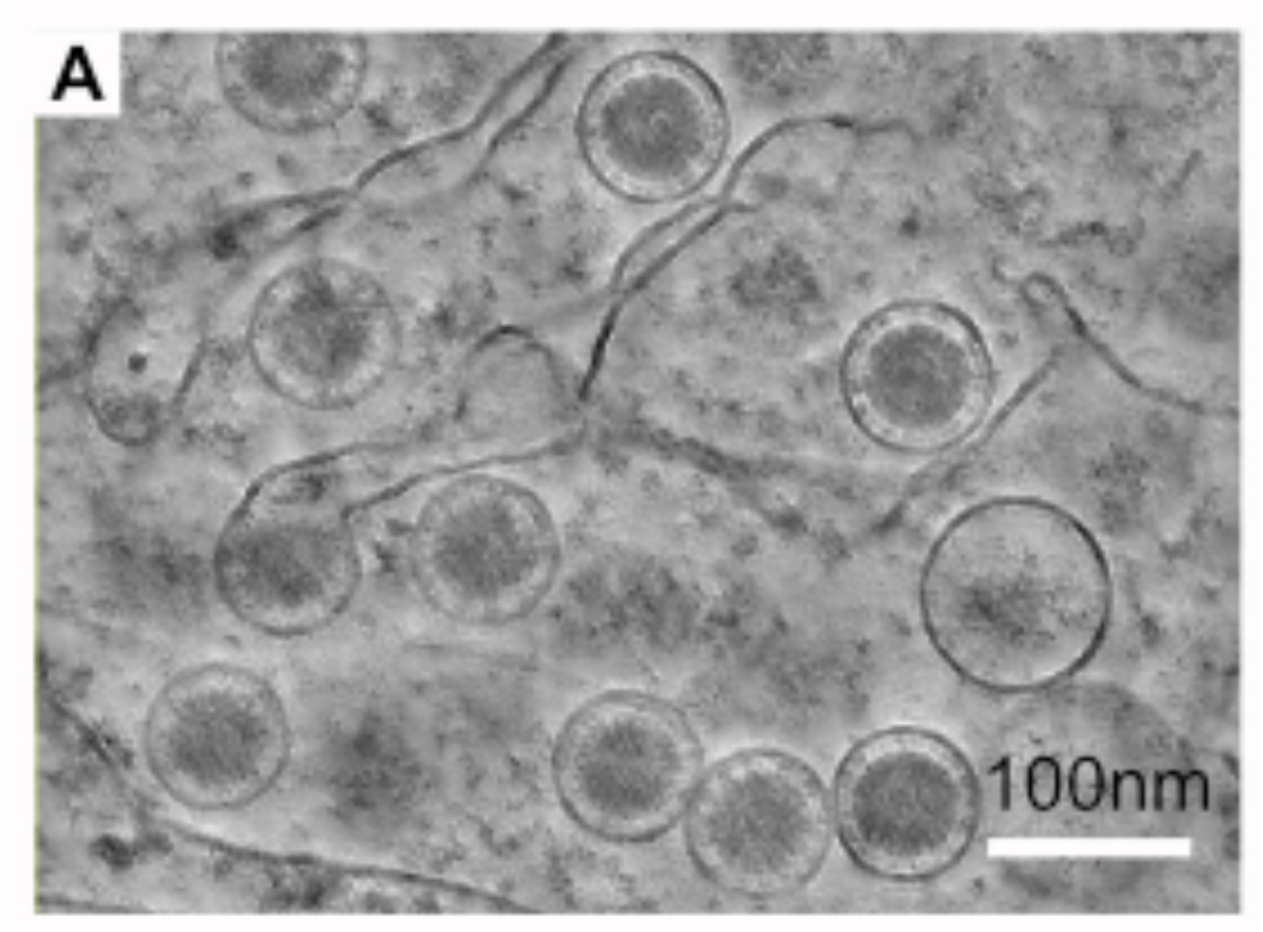
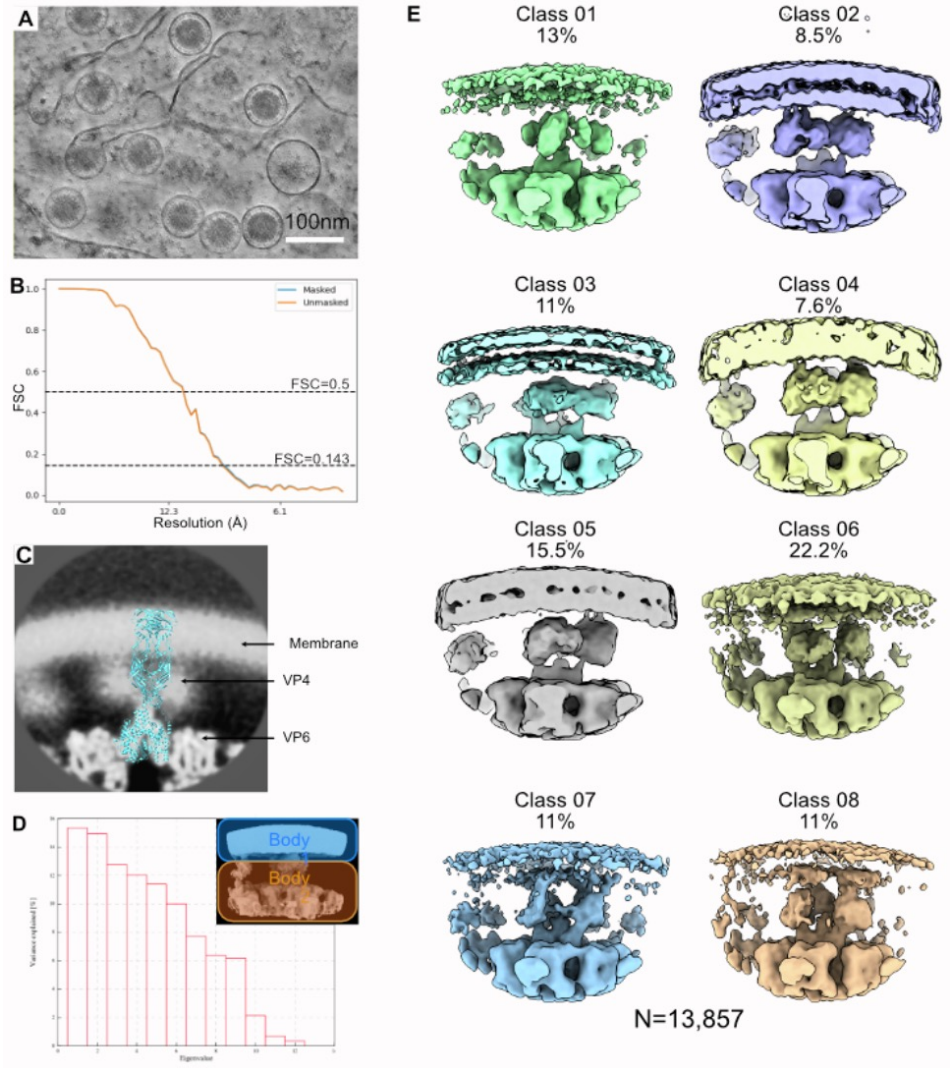


Thapsigargin毒胡萝卜素



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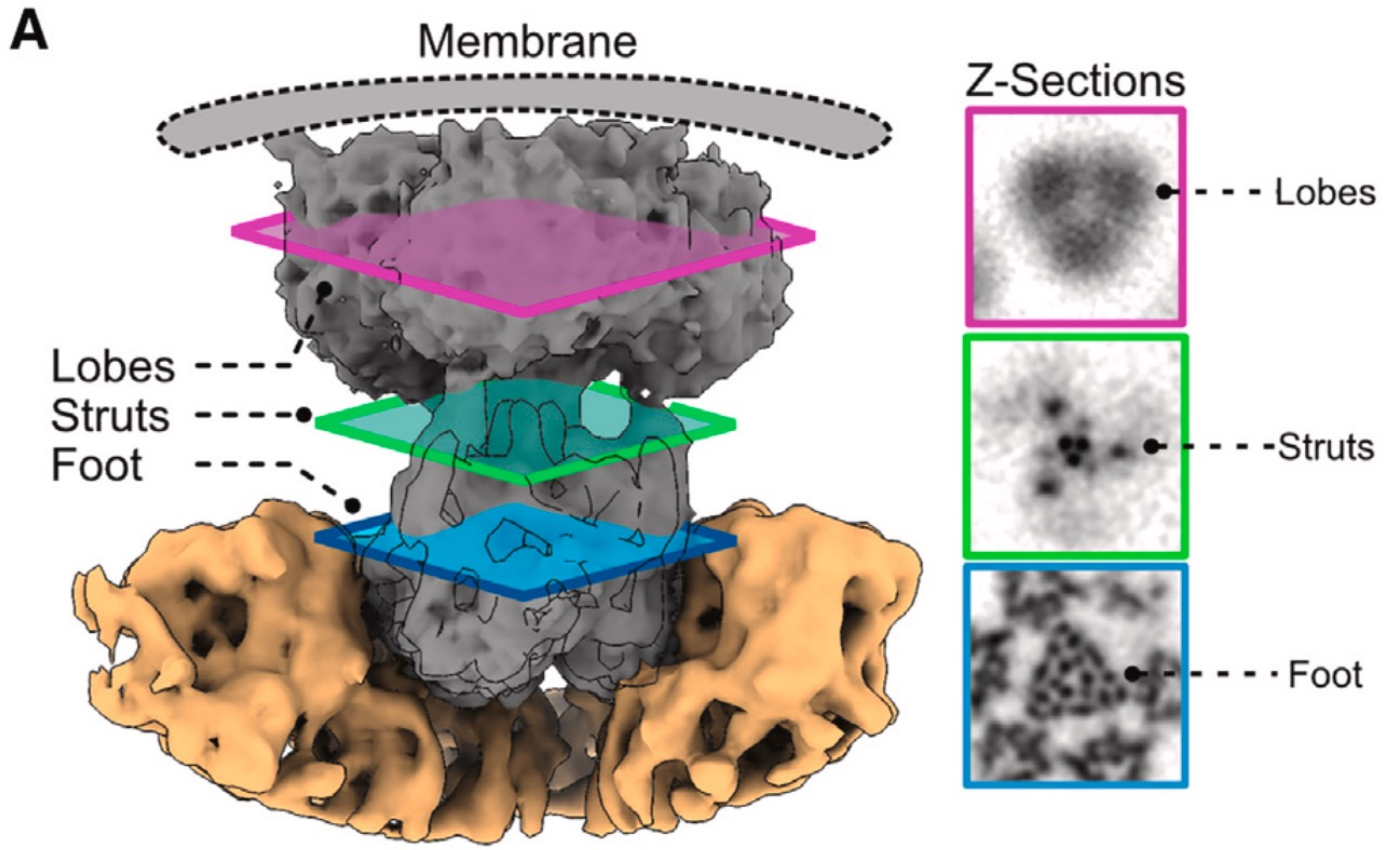

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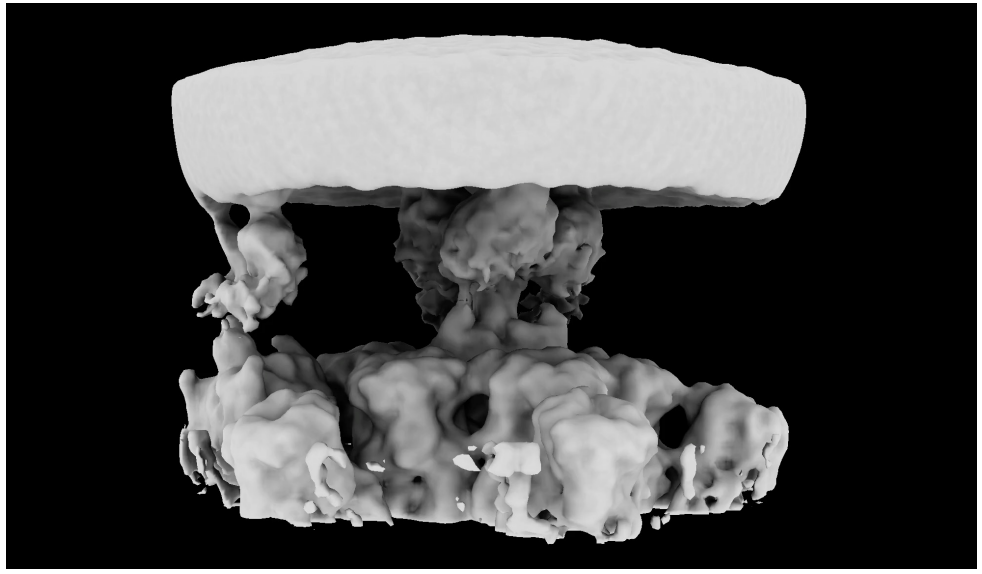

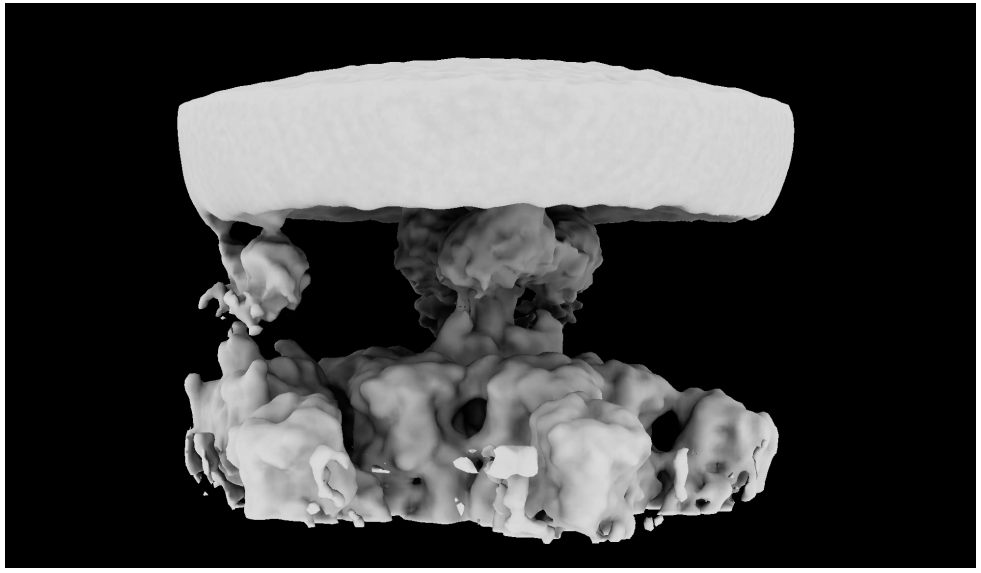

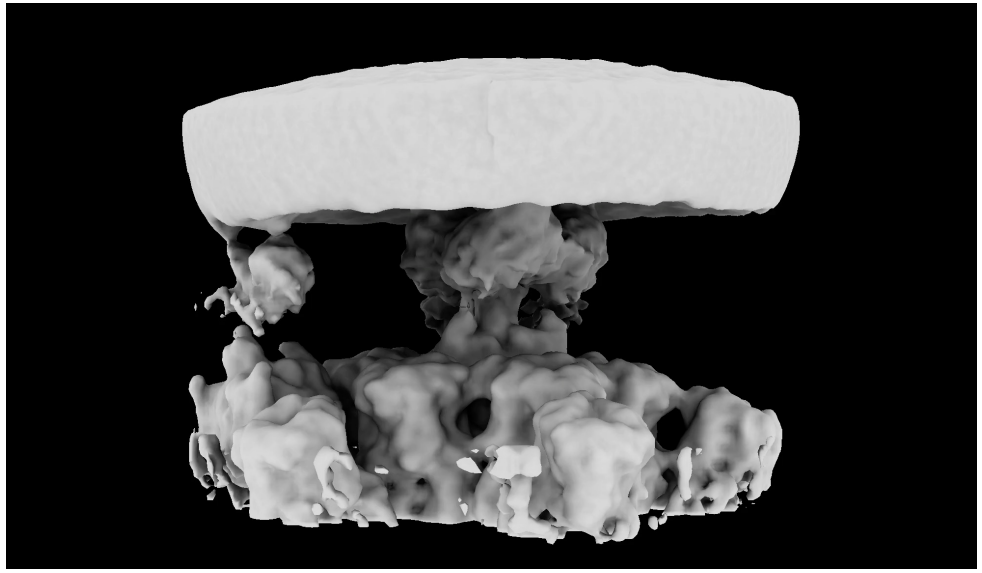

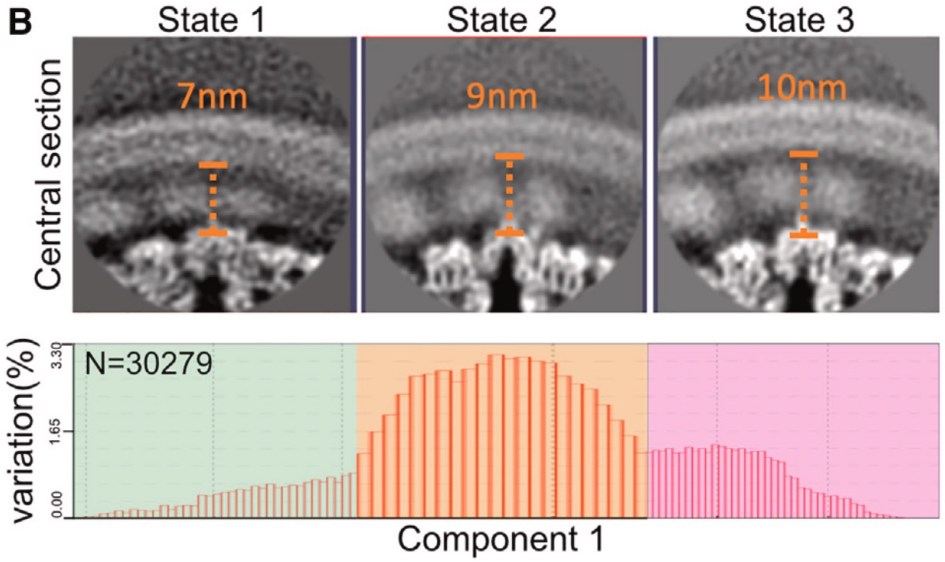

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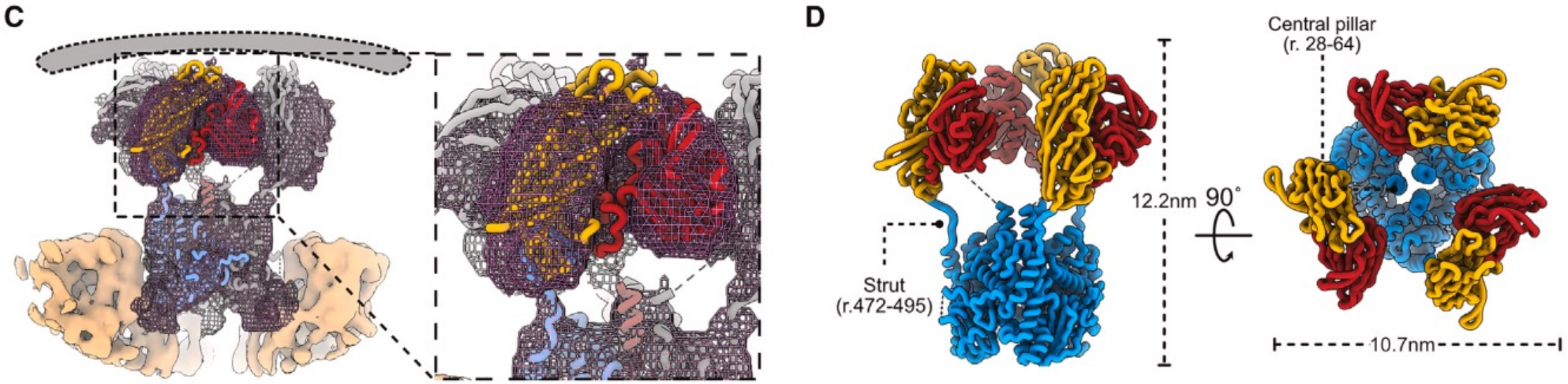

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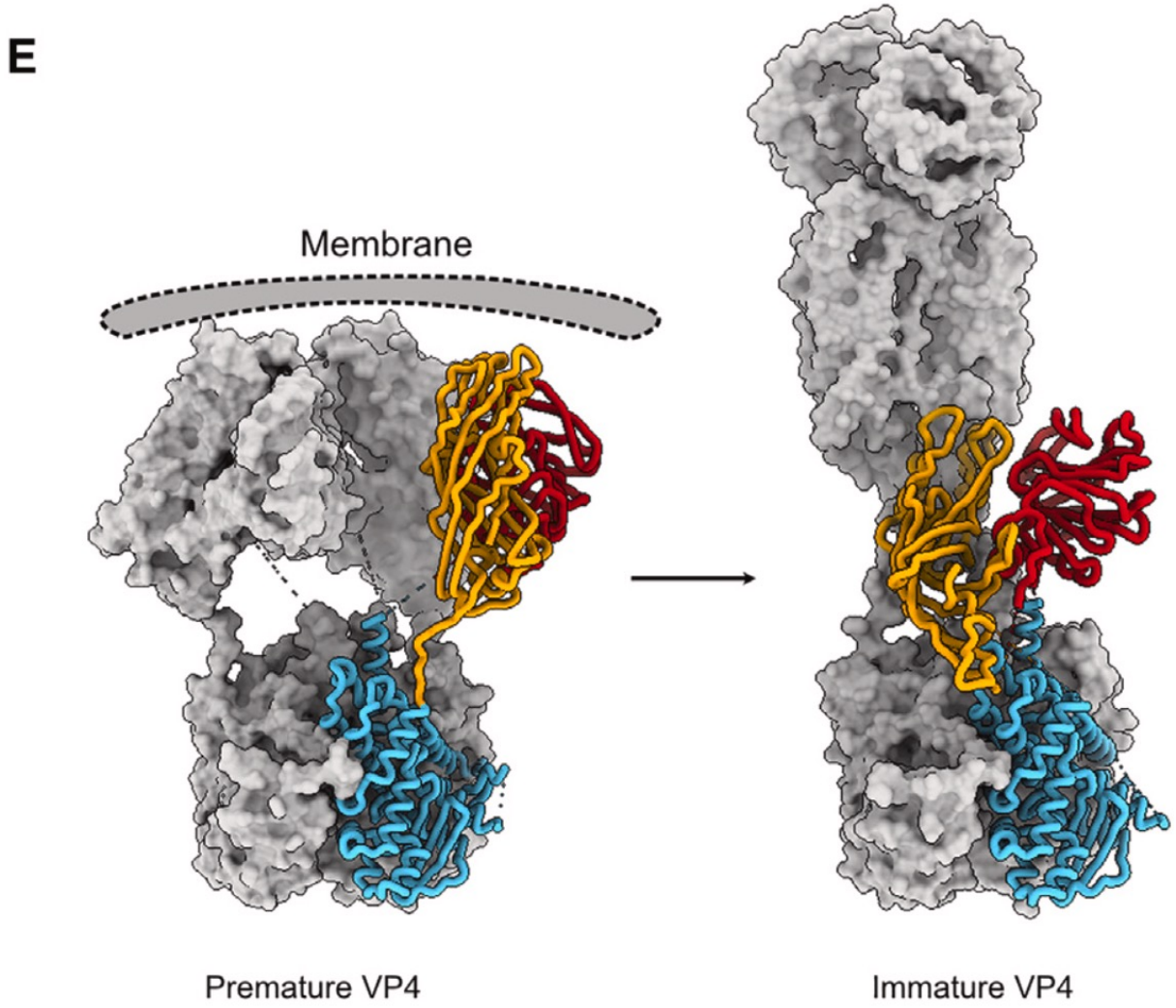
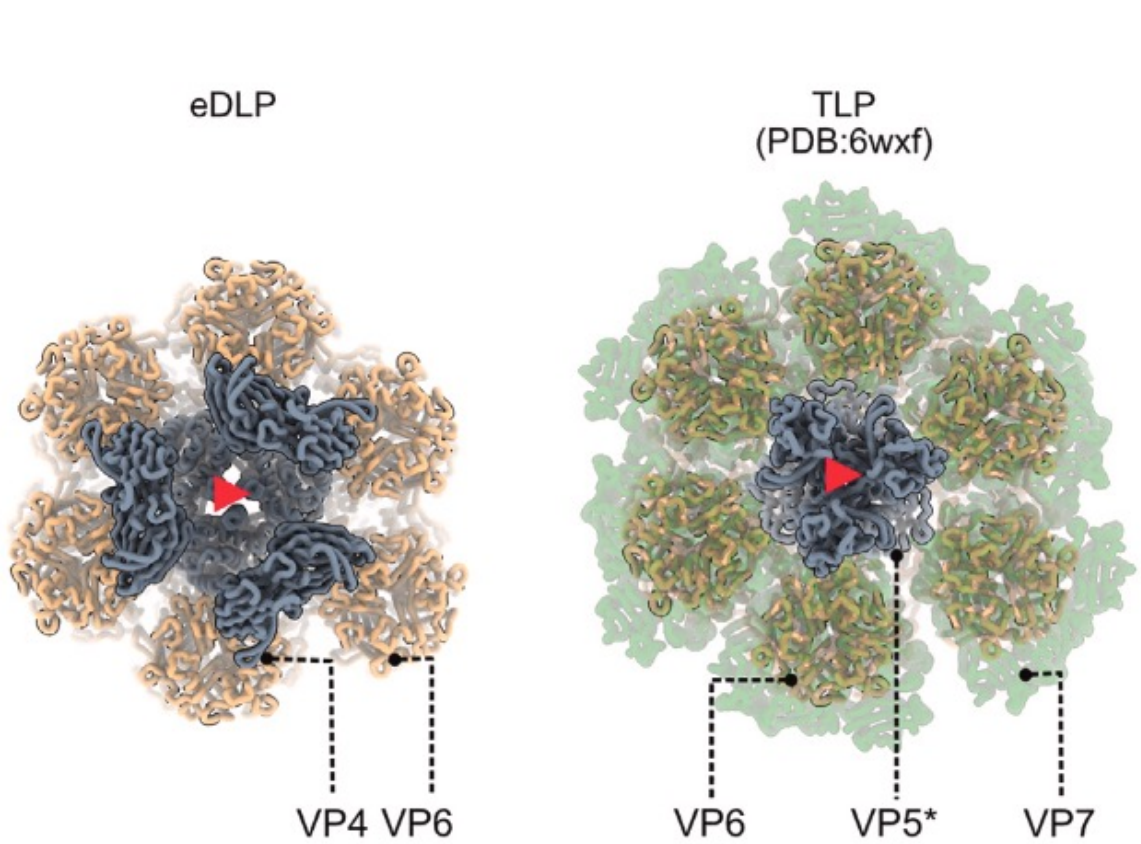
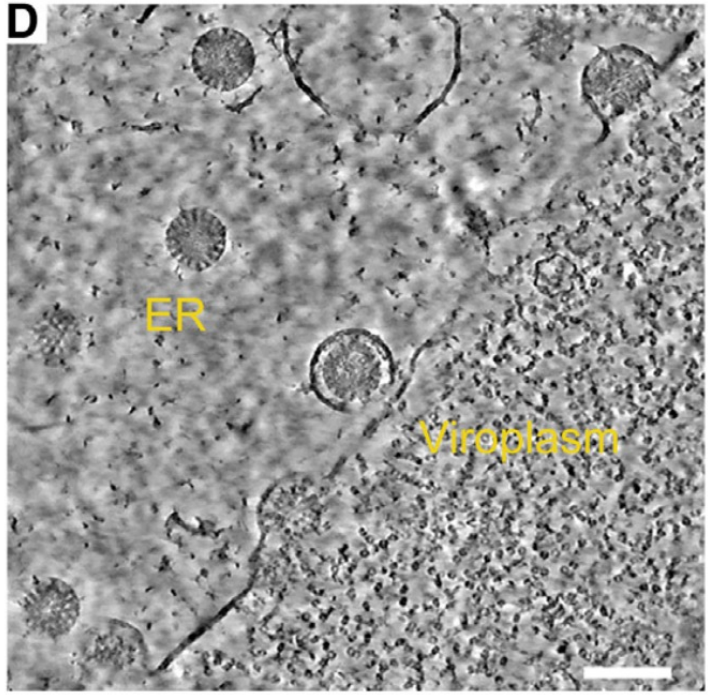
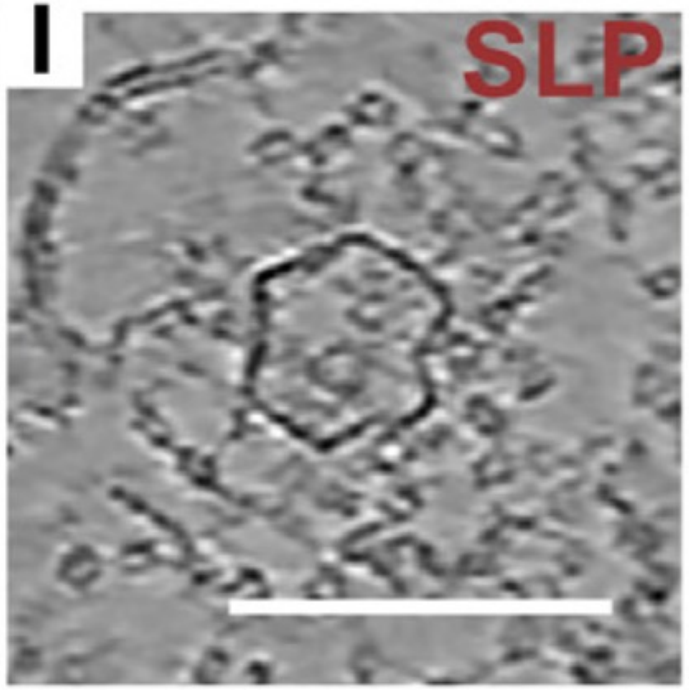




Result5: SLP is assembled in the viroplasm





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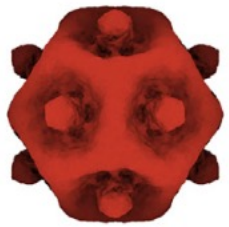

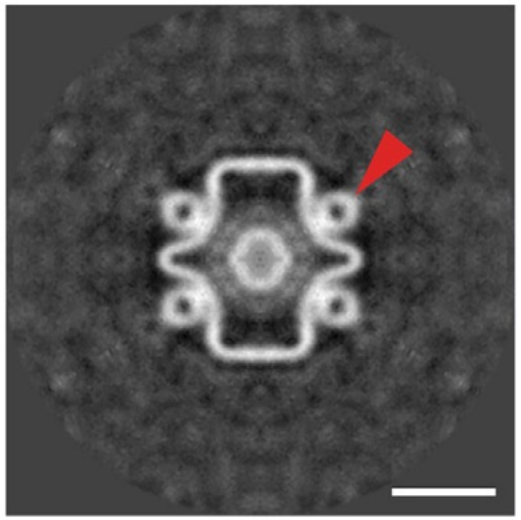

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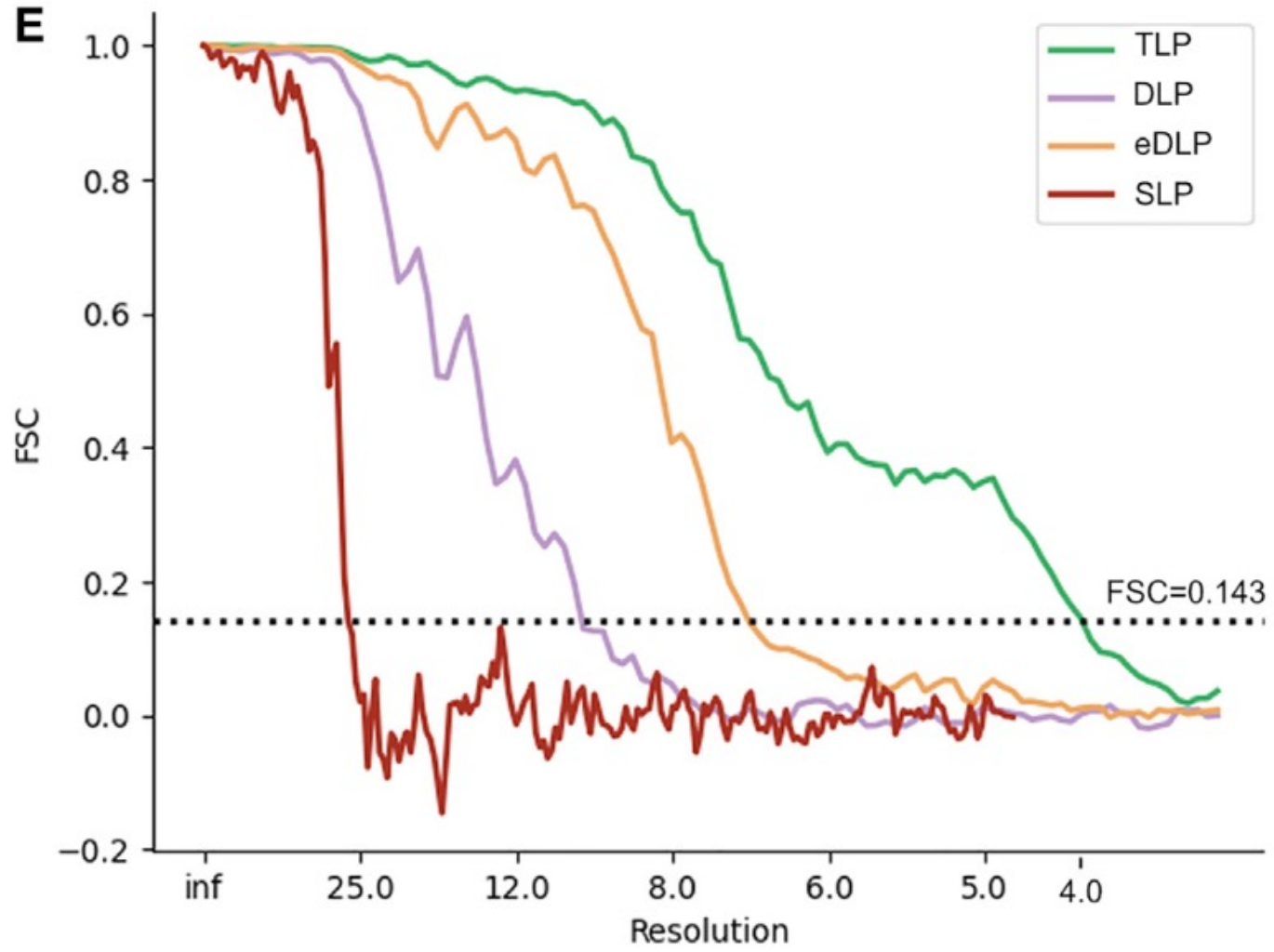

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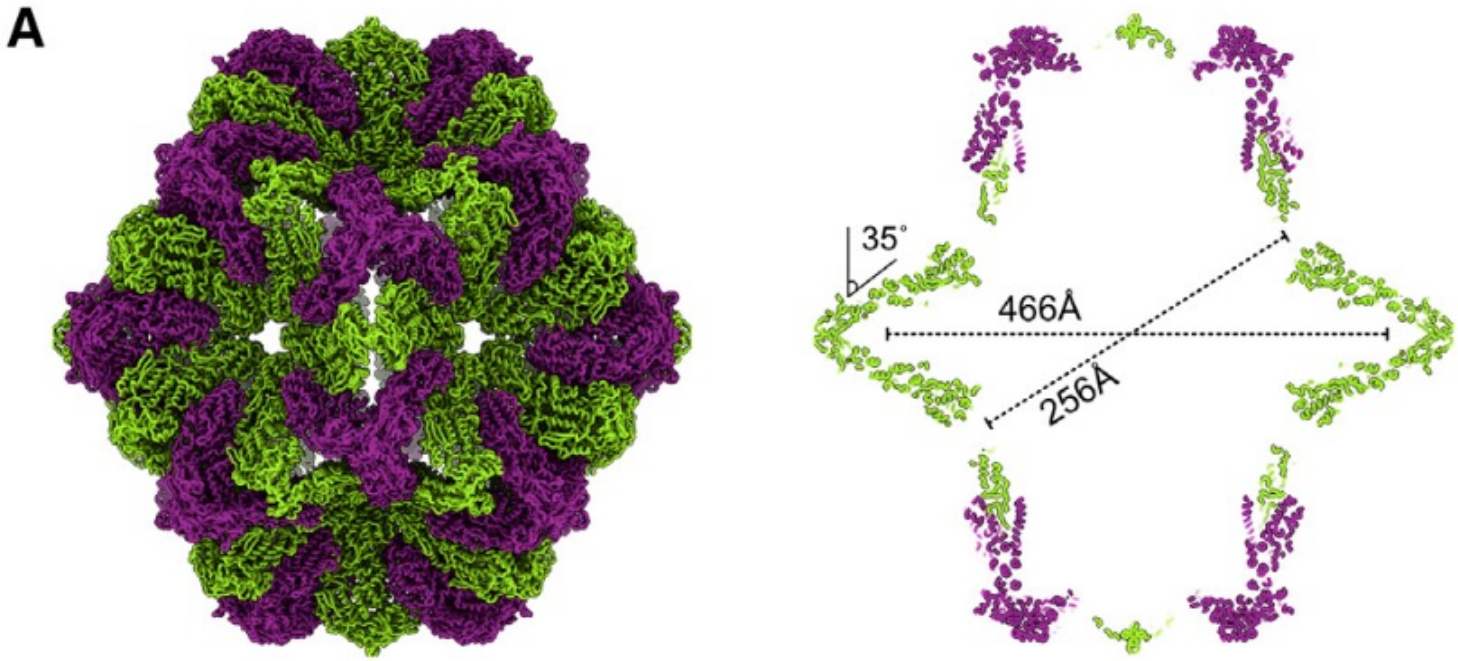

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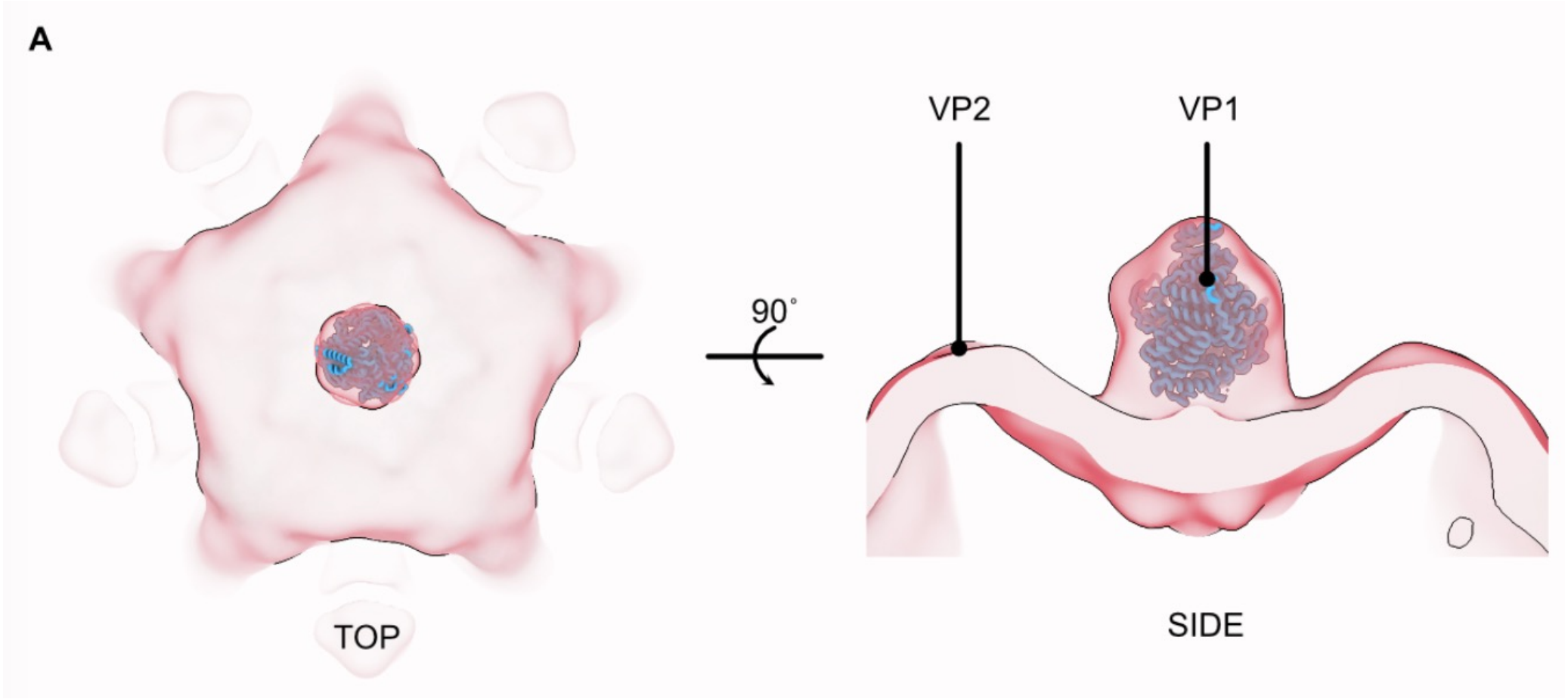

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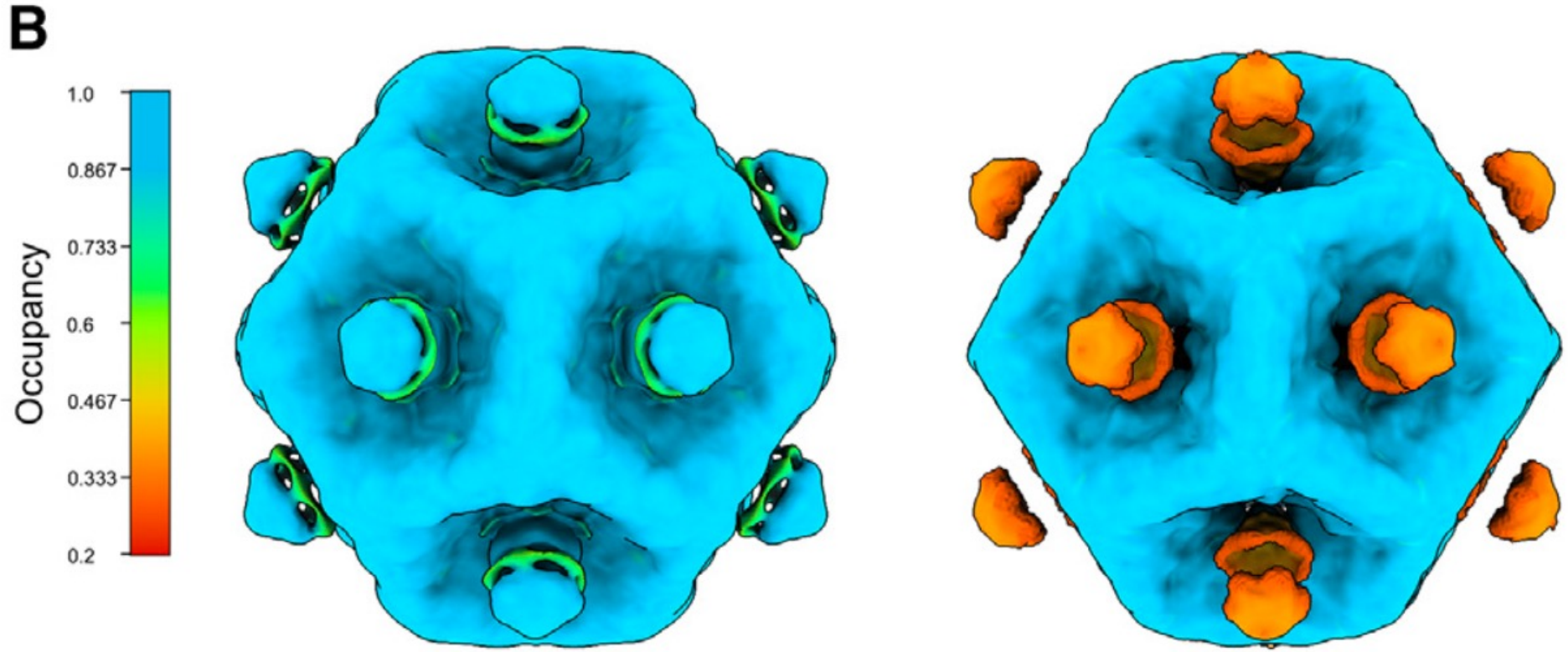

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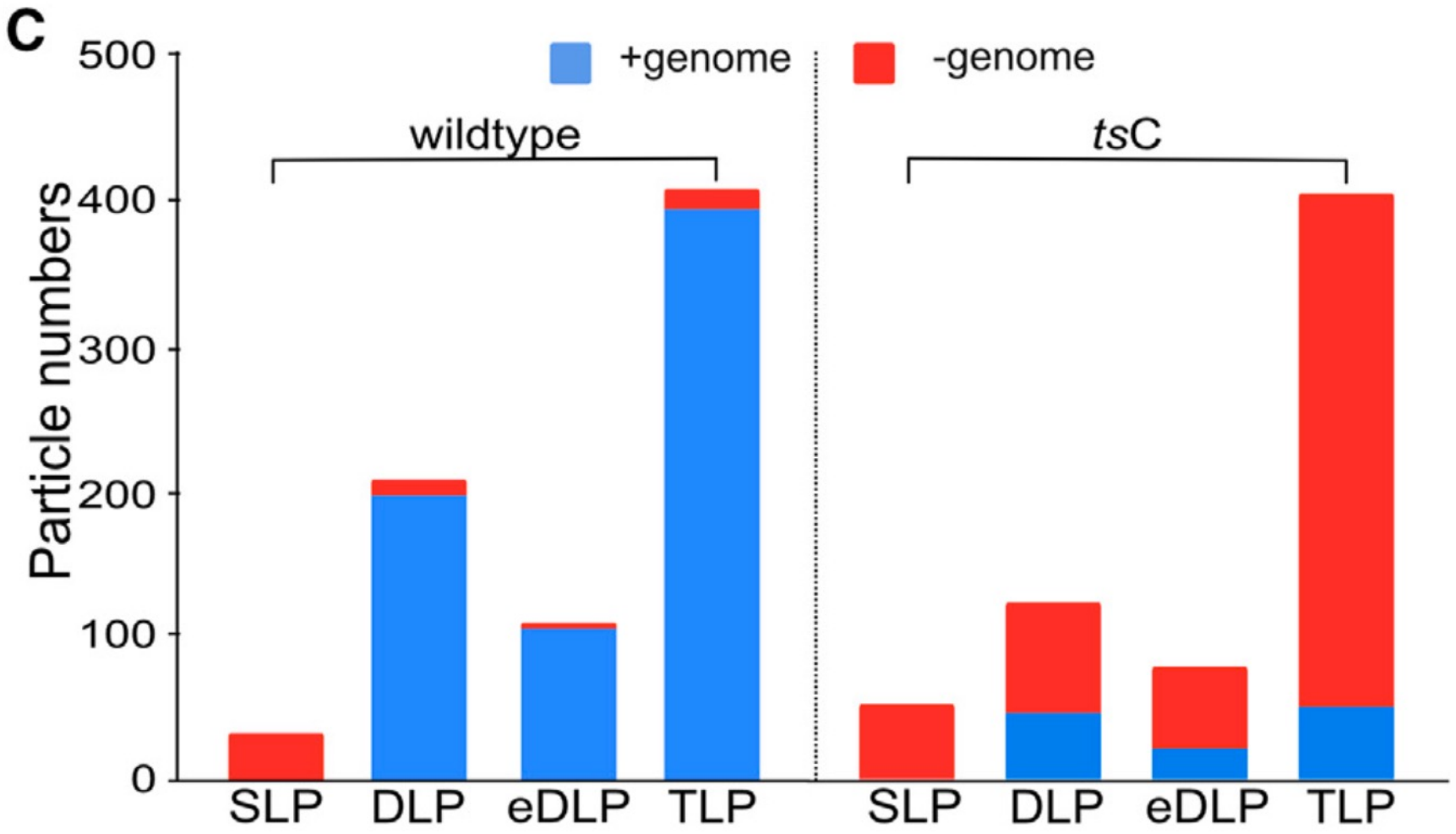
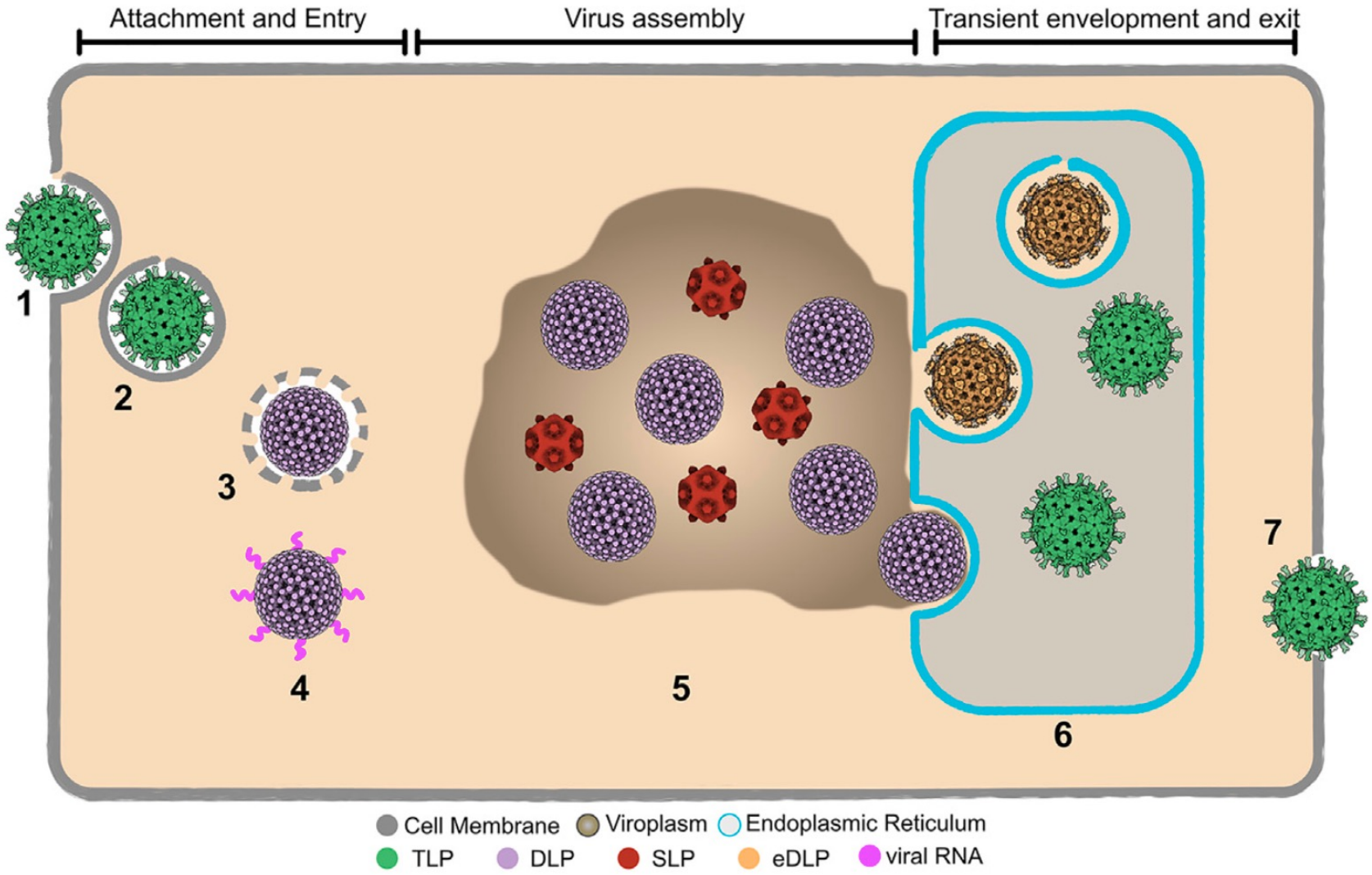


Discussion


Attachment and Entry
Virus assembly
Transient envelopment and exit
1
2
3
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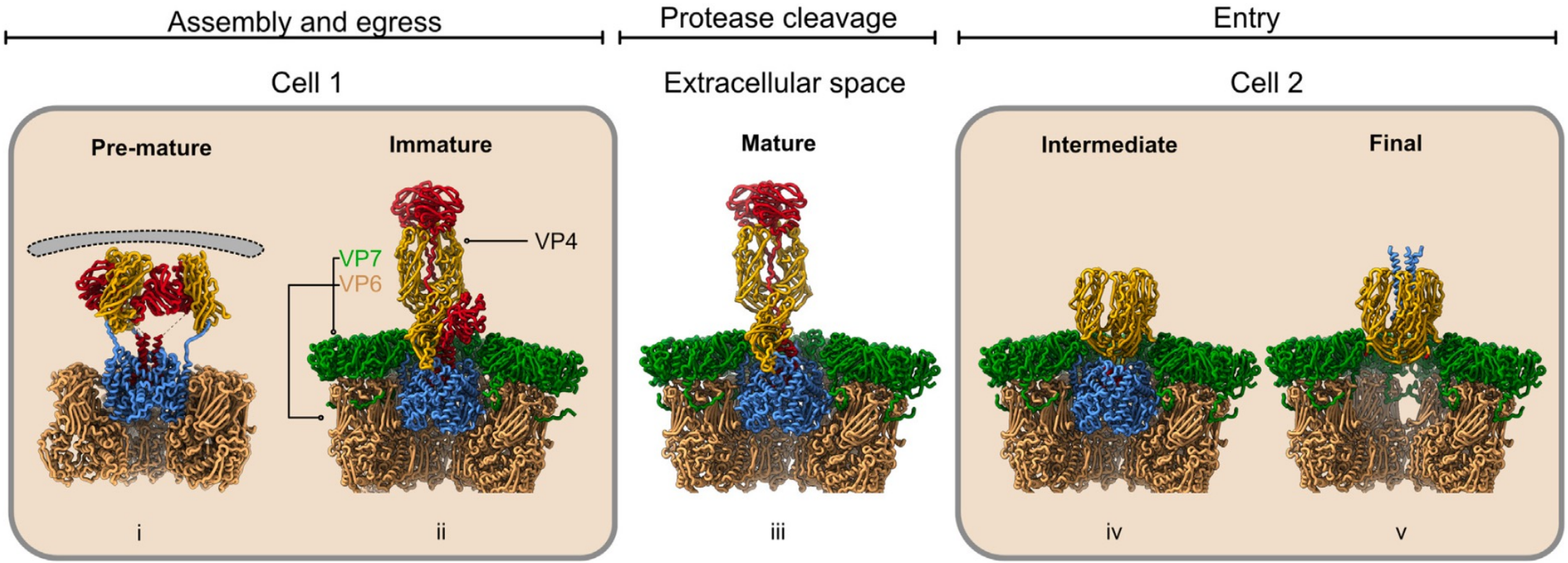
5
6
7
Cell Membrane
Viroplasm
Endoplasmic Reticulum
TLP
DLP
SLP
eDLP
viral RNA

Discussion



- have detected and characterized at the molecular level assembly stages not previously detectable;
- how the assembly events relate spatially to the cellular structures
- delicate, short-lived forms that cannot be purified outside of their native milieu