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Atomic model of vesicular stomatitis virus and mechanism of assembly

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Like other negative-strand RNA viruses (NSVs) such as influenza and rabies, vesicular stomatitis virus (VSV) has a three-layered organization: a layer of

Background

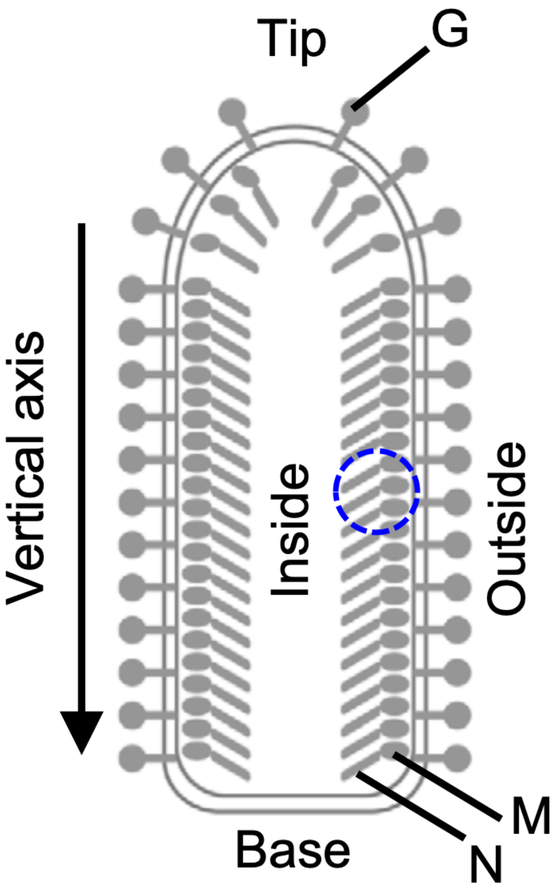
The importance of studying VSV assembly

- Vesicular stomatitis virus（水疱性口炎病毒）is a kind of Negative-strand RNA Virus(NSVs).
- Enveloped, bullet-shaped; is the prototypical NSV, which has long been used as a model for NSVs.
- Widely used for engineering pseudotypes as vaccines and anti-cancer agents.
  - promising potential in curing COVID-19, HIV and so on.
- Having a good understanding of the atomic details of VSV assembly is very important, especially how these molecular interactions take place and govern virus assembly.

Background

A brief introduction about structural proteins of VSV

- VSV contains three structural proteins: nucleocapsid protein(N), matrix protein(M) and glycoprotein(G).
- M is located between membrane envelope(containing G) and nucleocapsid(containing N and ssRNA), like a sandwich.



Scientific questions:

- Previous studies did not clearly show the near-atomic structures of M and N, the precise arragement of M, N, G and how they interact with each other to trigger VSV assembly.

Main idea of this study

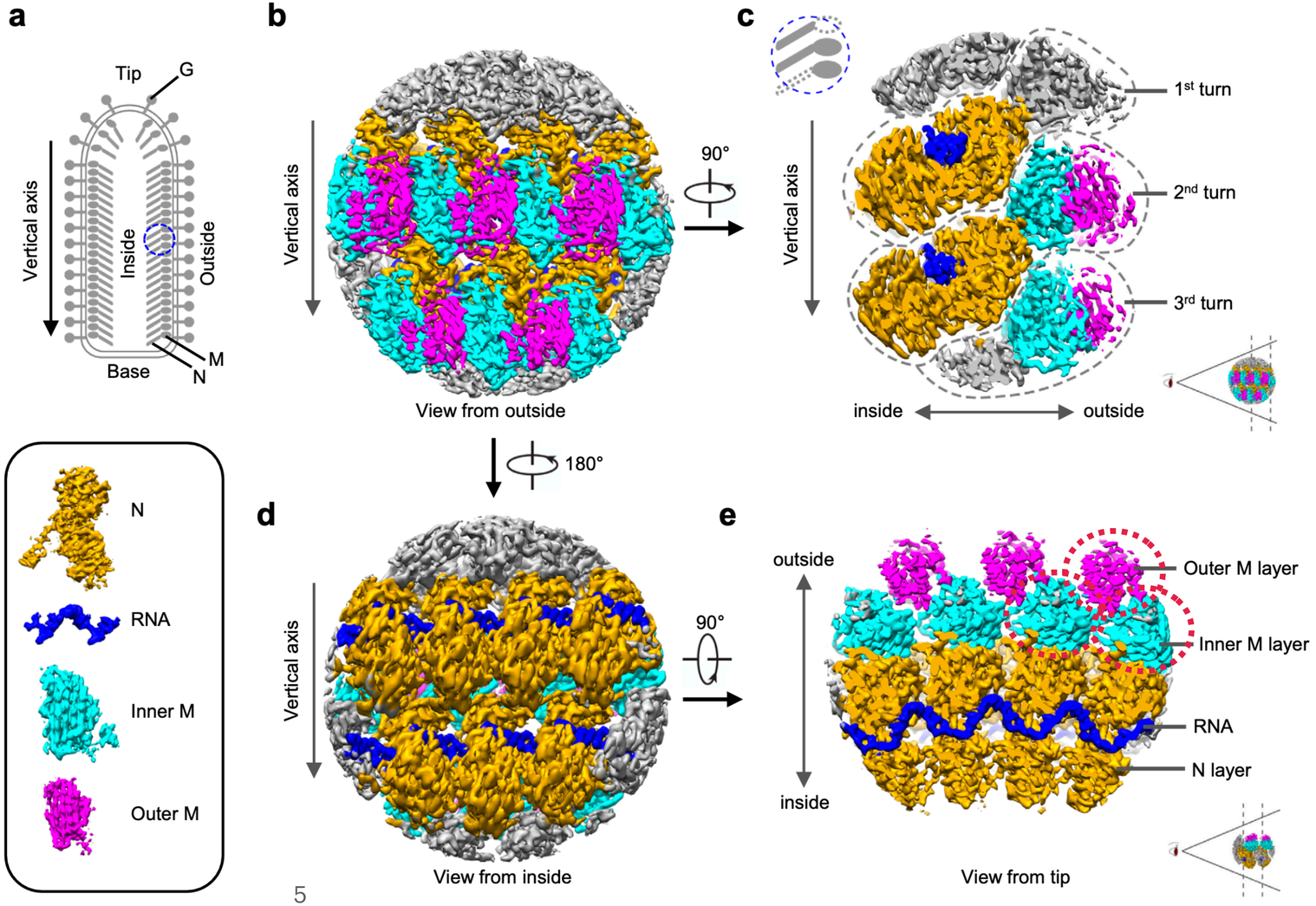
- Helical cryoEM sub-particle reconstruction & cryoET STA:
  - Determine the near-atomic resolution in situ structures of M and N;
  - Distribution of G trimers.
- Establish the mode of molecular interactions among RNA/N/M/G;
- Get pseudo-atomic model of an entire VSV virion
- Two transformational discoveries:
  - N:M sites=1:2; A double layer of M surrounds a layer of N;
  - The pattern of N-IM-OM-G and G-endodomain-OM association.

Results

Atomic in situ structures of M and N & encapsidated RNA

Key results:

- Define an asymmetric unit: 1 N subunit and 2 M subunits.
- Within the same turn, each IM interacts with 2 N, one from the same unit and another from a neighboring unit.
- Each IM is also inlaid between 2 OM.



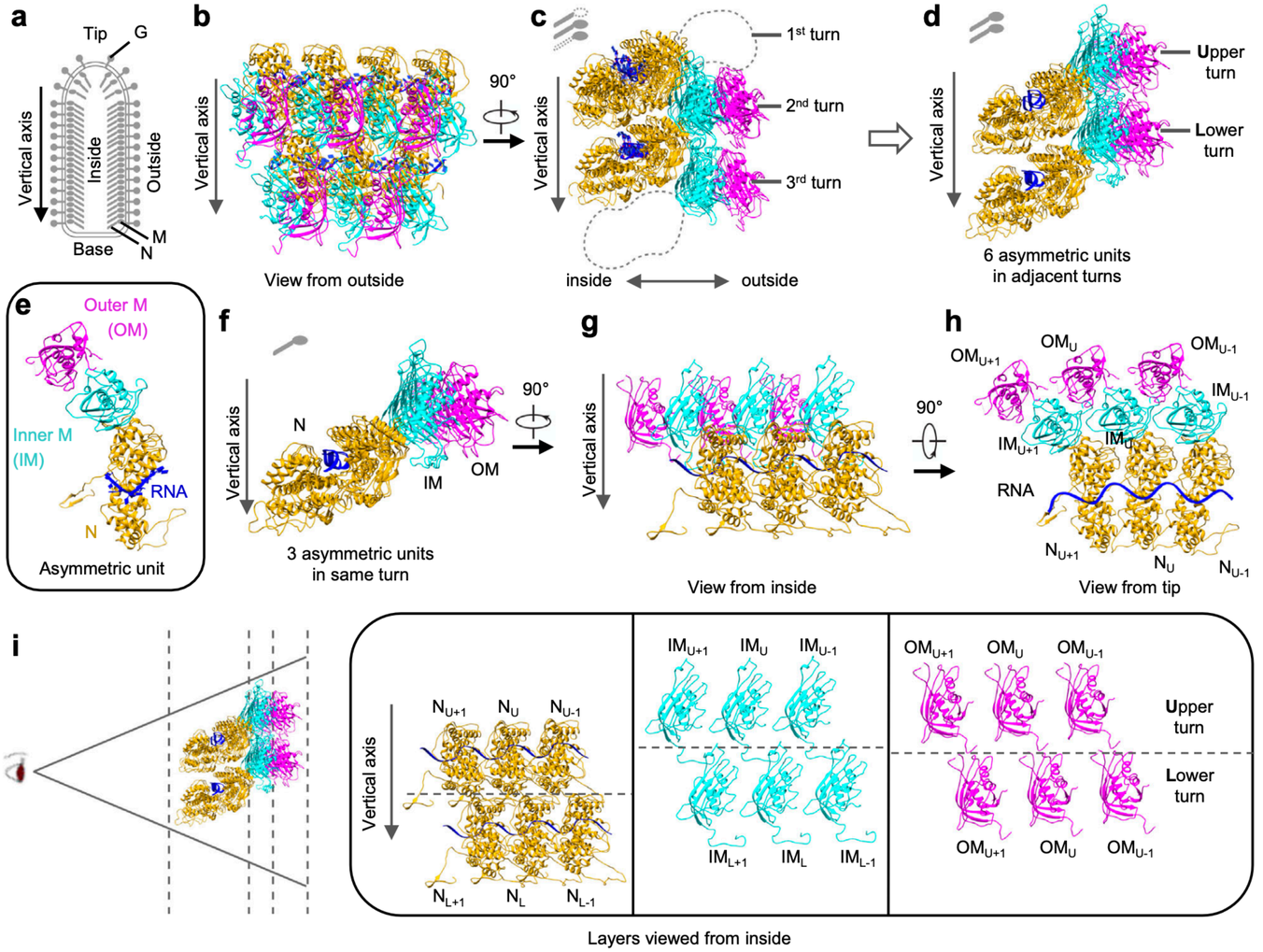
Results

Atomic in situ structures of M and N & encapsidated RNA

Key results:

- VSV trunk to 3.47Å resolution in situ
- N:M = 1:2
- Each N accommodates 9 nucleotides of RNA

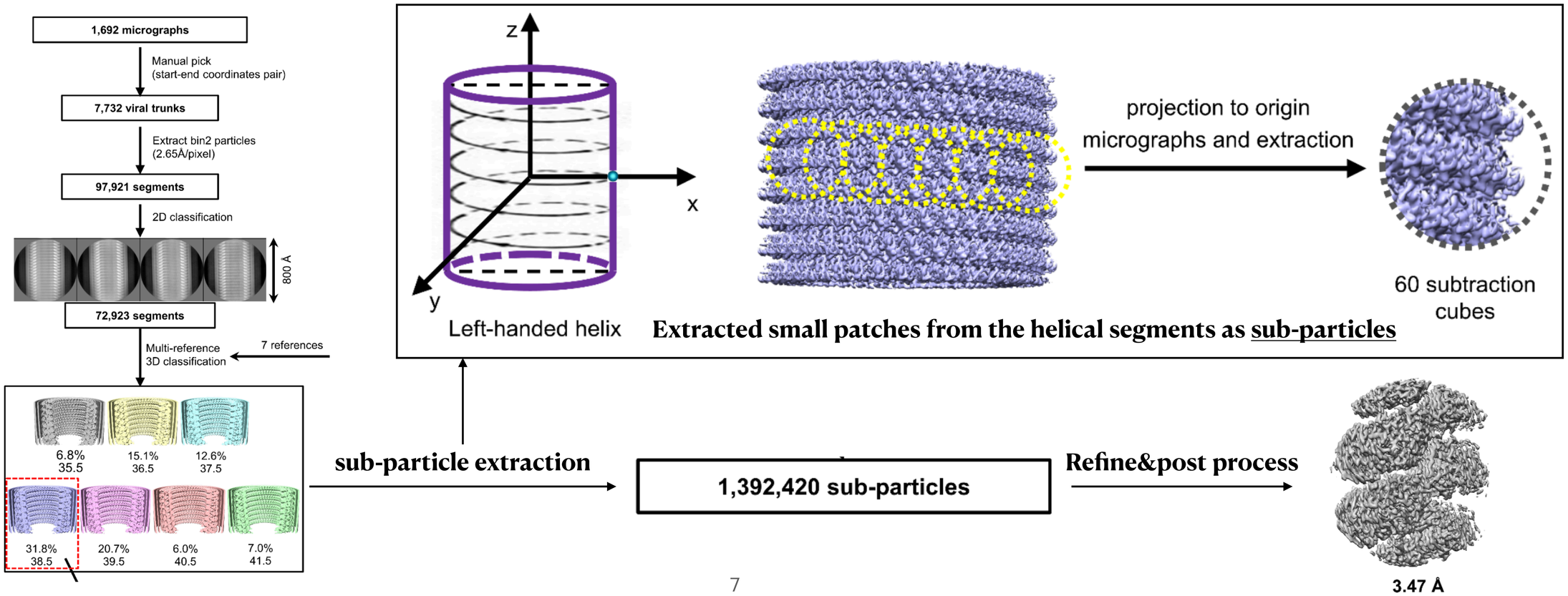
How?



Results

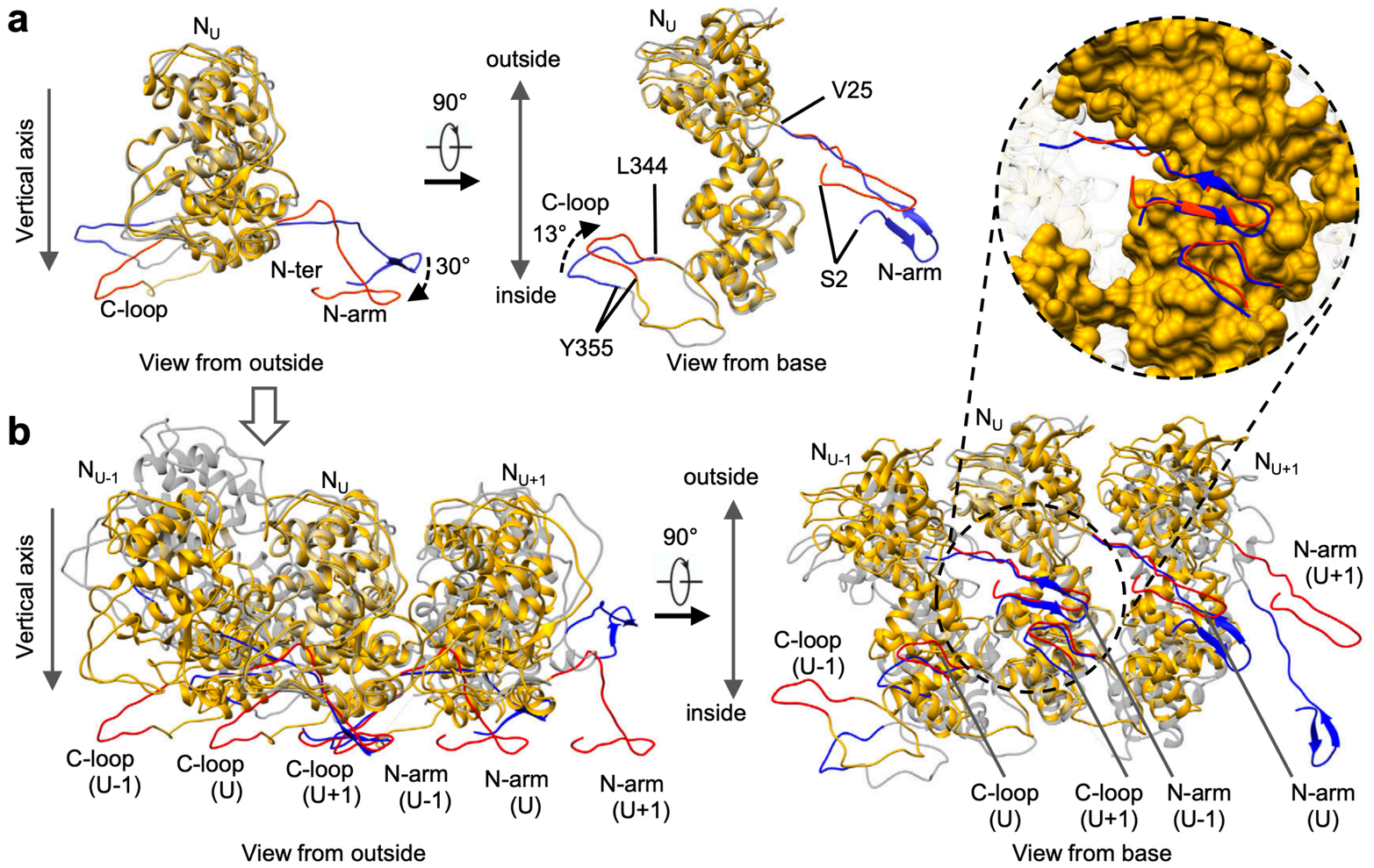
Helical sub-particle reconstruction

- Conventional helical reconstruction method is not strong enough in atomic VSV trunk reconstruction due to the large variable range of numbers of subunits per turn(the helix is flexible).



Results

Comparison between in situ and crystal structures of N



Results

Comparison between in situ and crystal structures of N

(1) Structure of single N:

- Main body and RNA-binding pattern remain the same;
- Both have interlocking anchor: the interactions between 3 N subunits

(2) N subunits number is different:

- Crystal structure contains 10 subunits in one turn;
- In situ structure contains 35.5-41.5 subunits in one turn.

(3) N-terminal arm and an extended loop are very different:

- Compared to crystal structure, N-arm rotates downwards by 30 degrees, and C-loop rotates outwards by 13 degrees.

Results

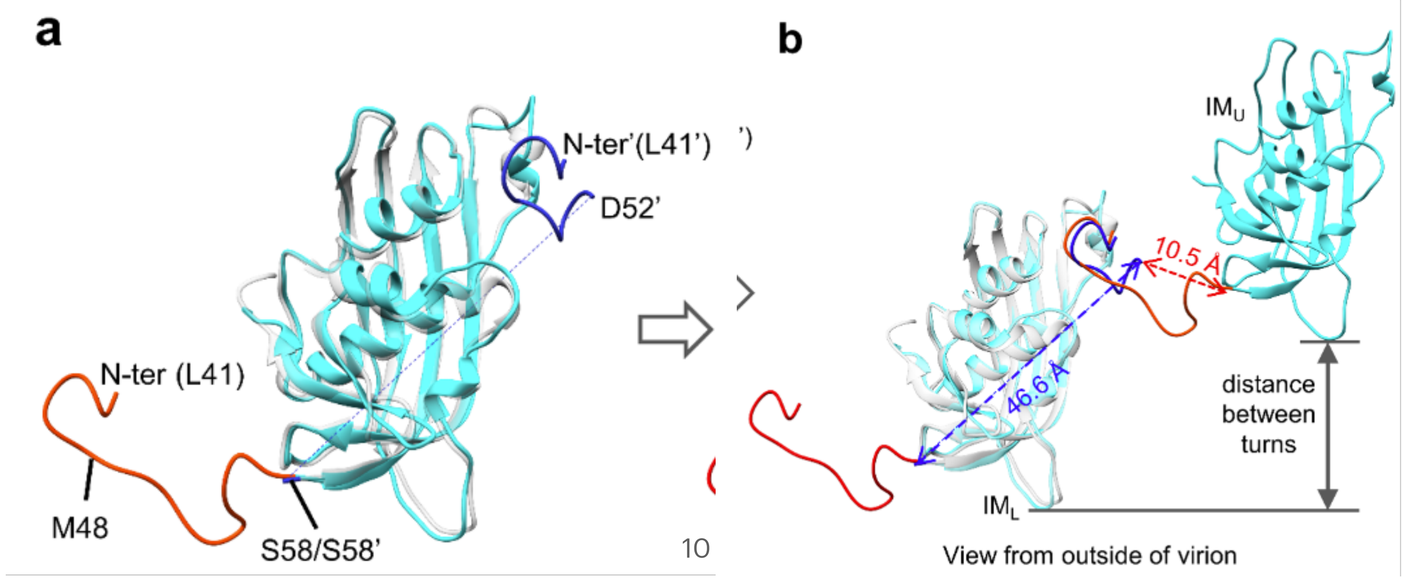
Comparison between in situ and crystal structures of M

(1) double layer of M:

- IM between N and OM;
- no direct contact between N and OM; IM and IM, OM and OM;

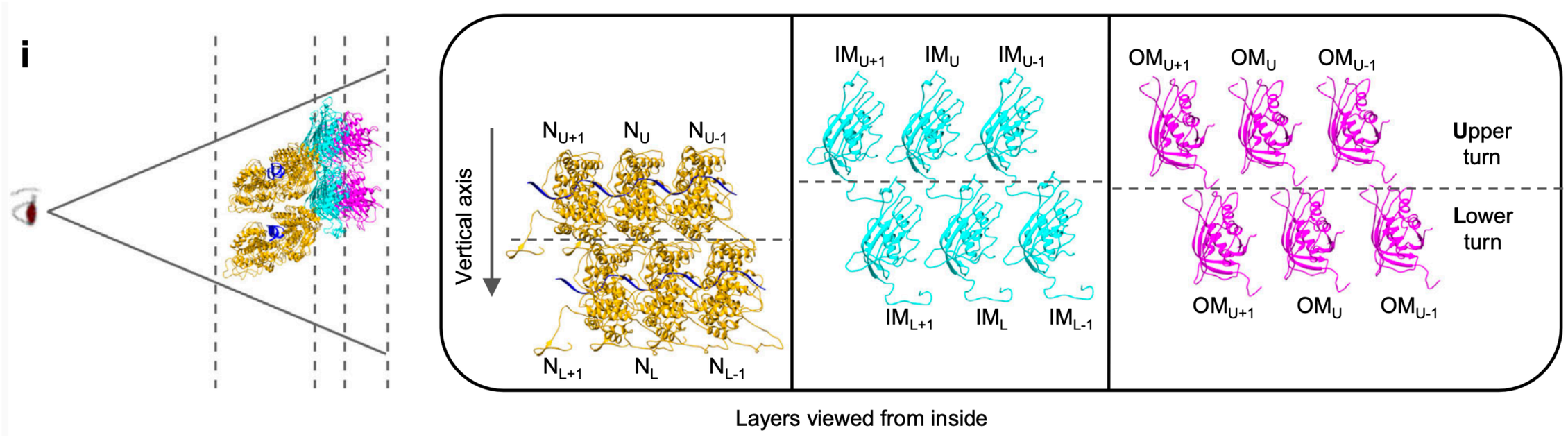
(2) in situ structure of IM:

- The blue part belonging to IM_U is more reasonable.



Results

Comparison between in situ and crystal structures of M



Results

Comparison between IM and OM

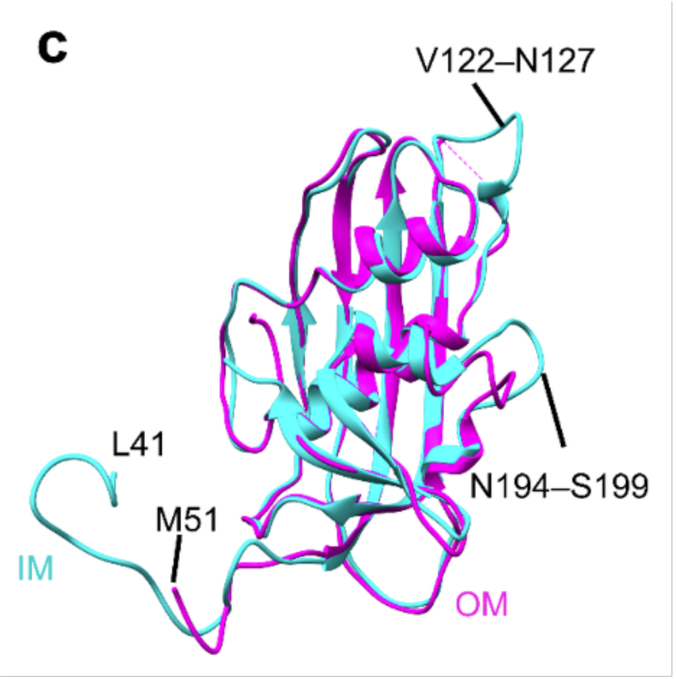
(1) Leu41-Asp50 remains unresolved in OM, maybe due to flexibility.

(2) Val122-Asn127 in OM are also unresolved.

(3) Asn194-Ser199 in OM, the conformation is different.

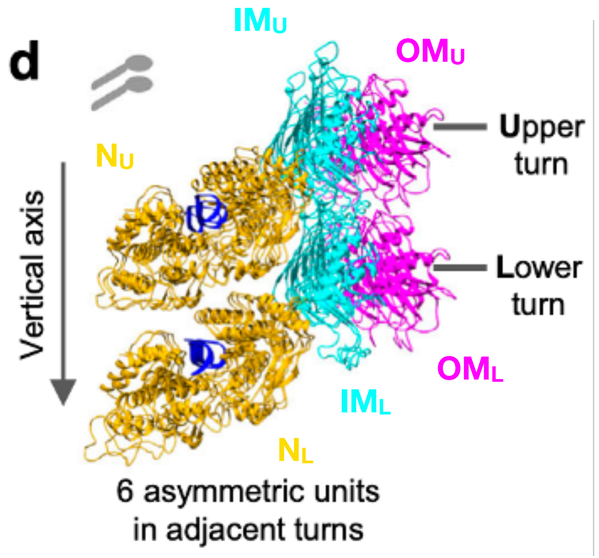
The conformational differences above are located at **outward-facing regions**, next to the viral envelope.

(4) The orientation of IM and OM is completely different.



Results

Gibbs free energy to qualify inter-subunit interactions



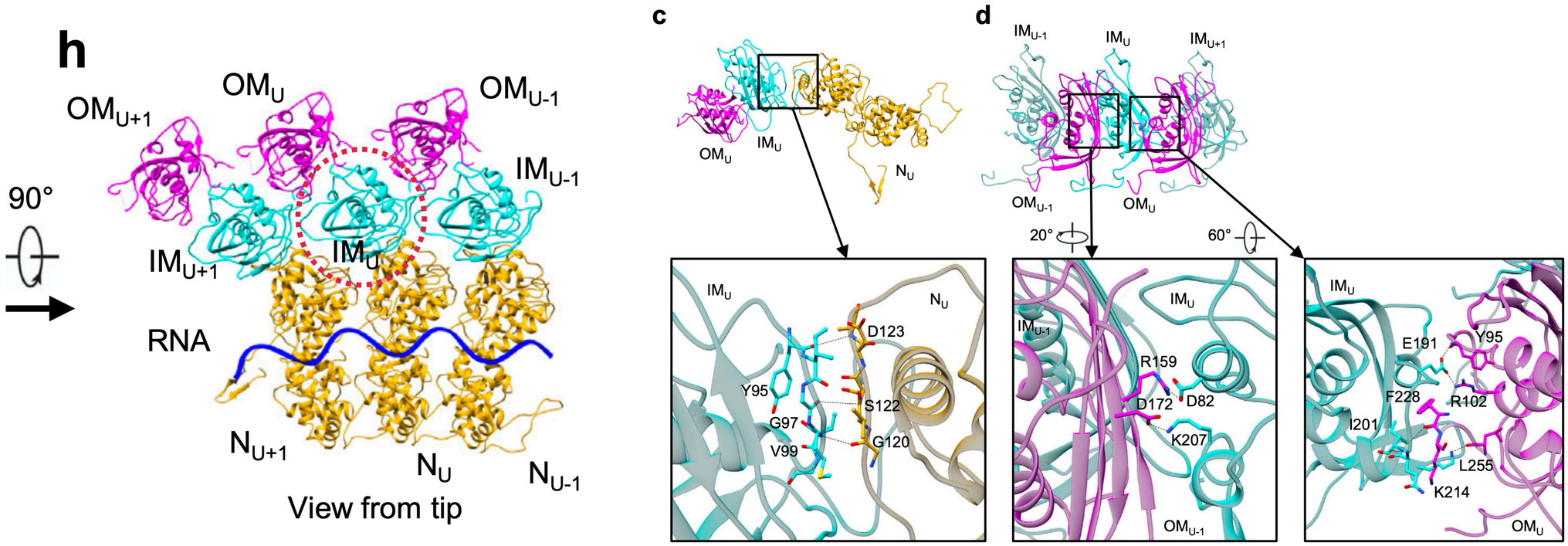
Subunit 1	Subunit 2	Interface area (Å²)	ΔⁱG (kcal/mol)
N_U	N_{U+1}	2551.6	-20.8
N_{U-1}	N_{U+1}	321.4	-5.4
N_U	N_L	29.5	0.1
N_U	N_{L+1}	180.8	0.5
IM_L	N_U	451.8	0
IM_U	**N_U**	398.9	-4.4
IM_U	N_{U+1}	229.1	-2.3
IM_U	IM_L	413.2	-4.3
IM_U	**OM_U**	884.2	-8.5
IM_U	OM_{U-1}	689.5	-2.9

Supplementary Table 1 | Interface area and ΔⁱG between subunits analyzed by PISA software.

- Lateral interactions of adjacent N are the strongest, even N_{U-1}-N_{U+1} is significant.
- But there are no significant vertical interactions between N, despite there is a buried interface between IM_L and N_U.

Results

IM interactions



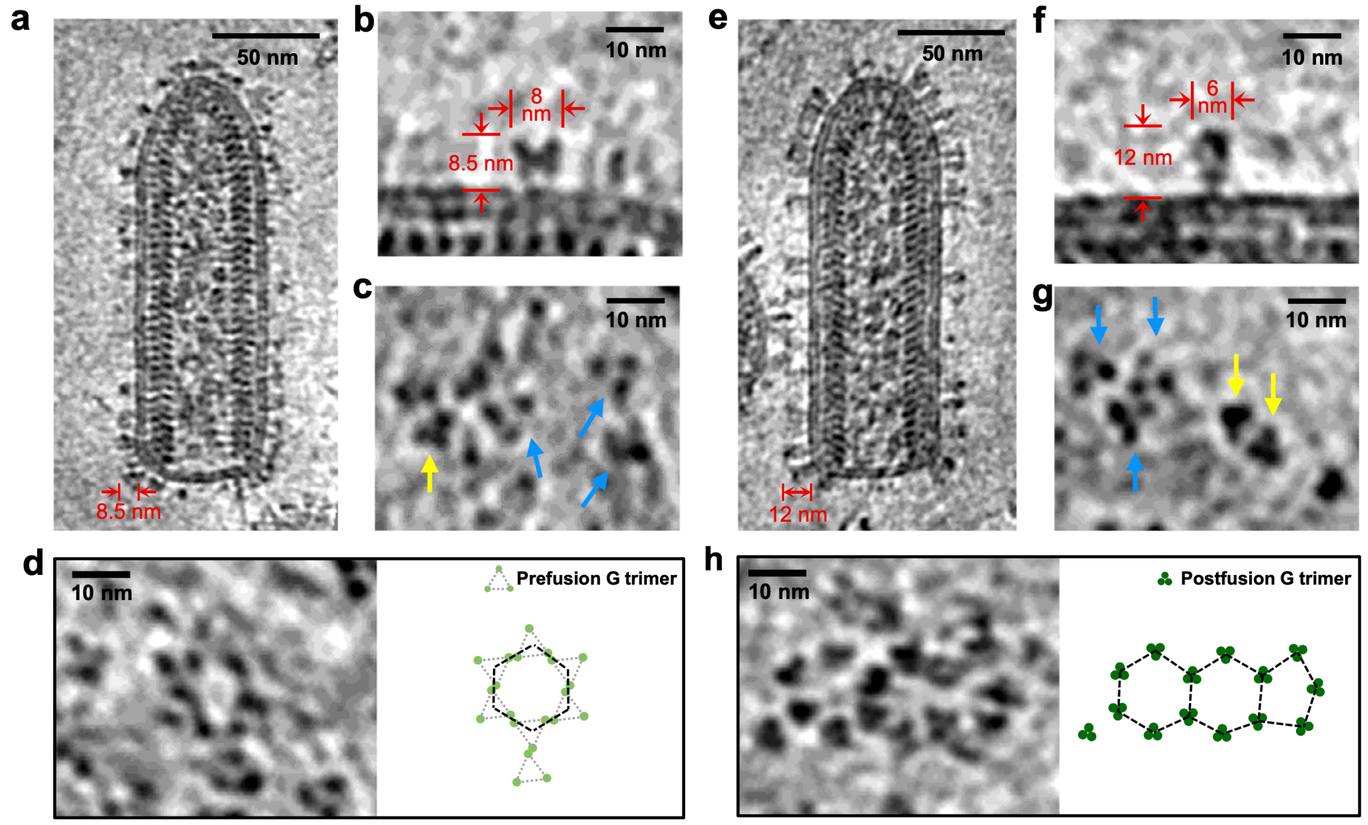
- Although IM is between 2 N subunits in different turns(Lower and Upper), IM does not interact with N_U and N_L, but N_U and N_{U+1}. These interactions occur at inner side of the IM.
- IM interacts with 2 independent OM(OM_U and OM_{U-1}), and the binding between IM_U and OM_U is stronger than IM_U and OM_{U-1}.

Results

Local clustering of G trimers on the virion surface

G protein: primarily as <u>trimers</u> on membrane envelope, in different conformations

- without density gradient step: <u>prefusion</u> mainly
- with density gradient step: <u>postfusion</u> mainly



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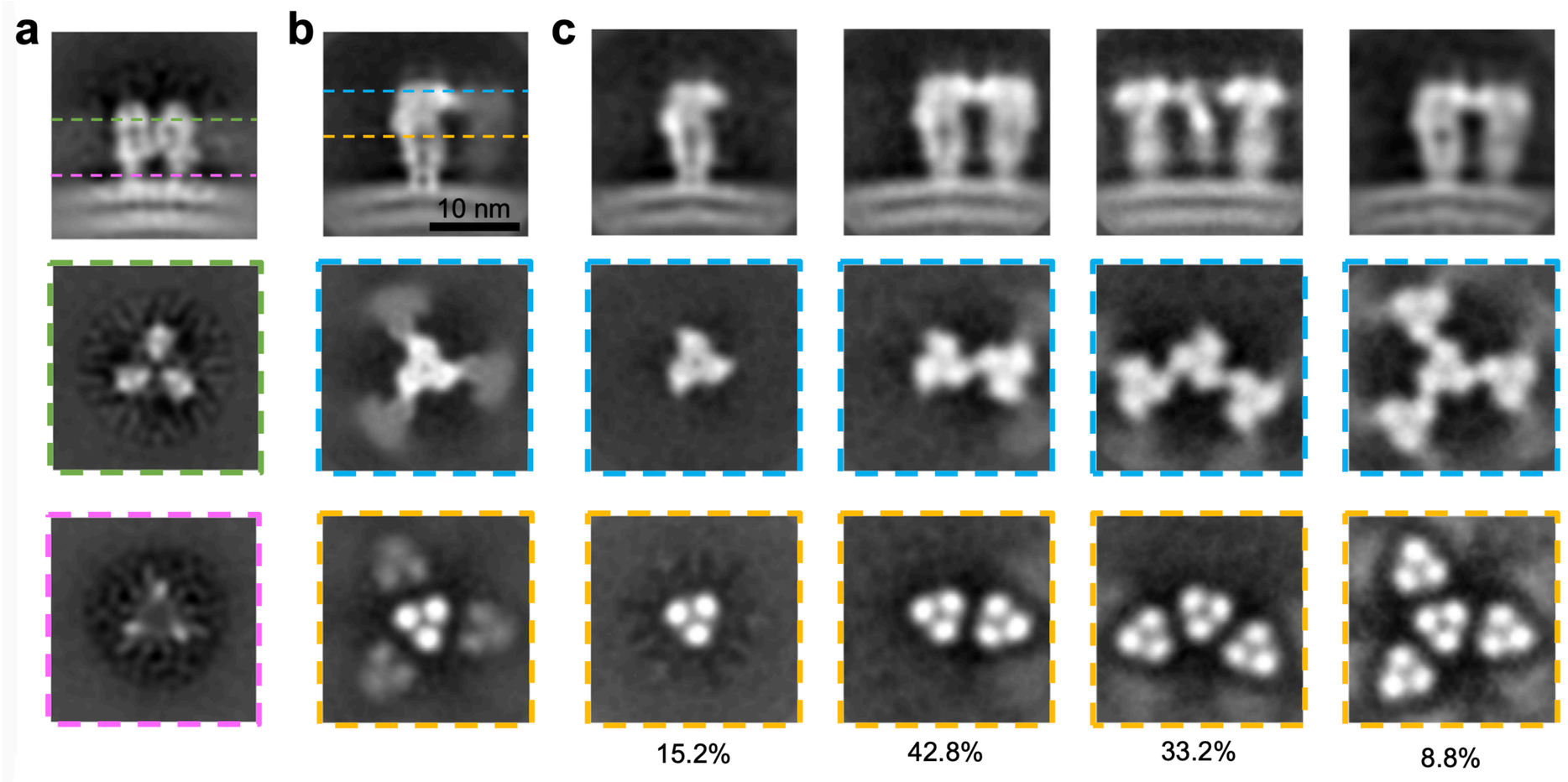
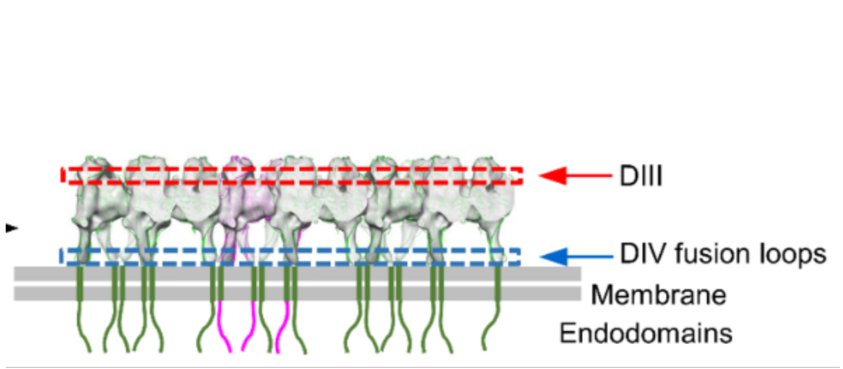
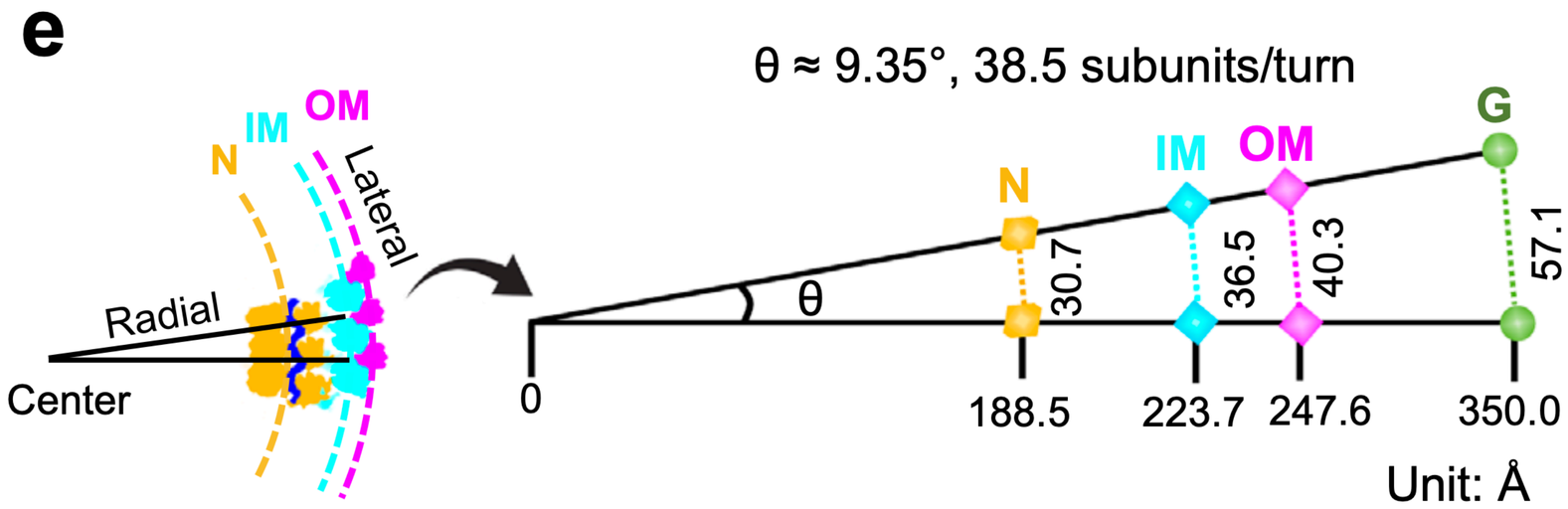


Partial occupancy: 4 different categories of G trimer supercomplexes

Results

The pattern of N-IM-OM-G and G-endodomain-OM association

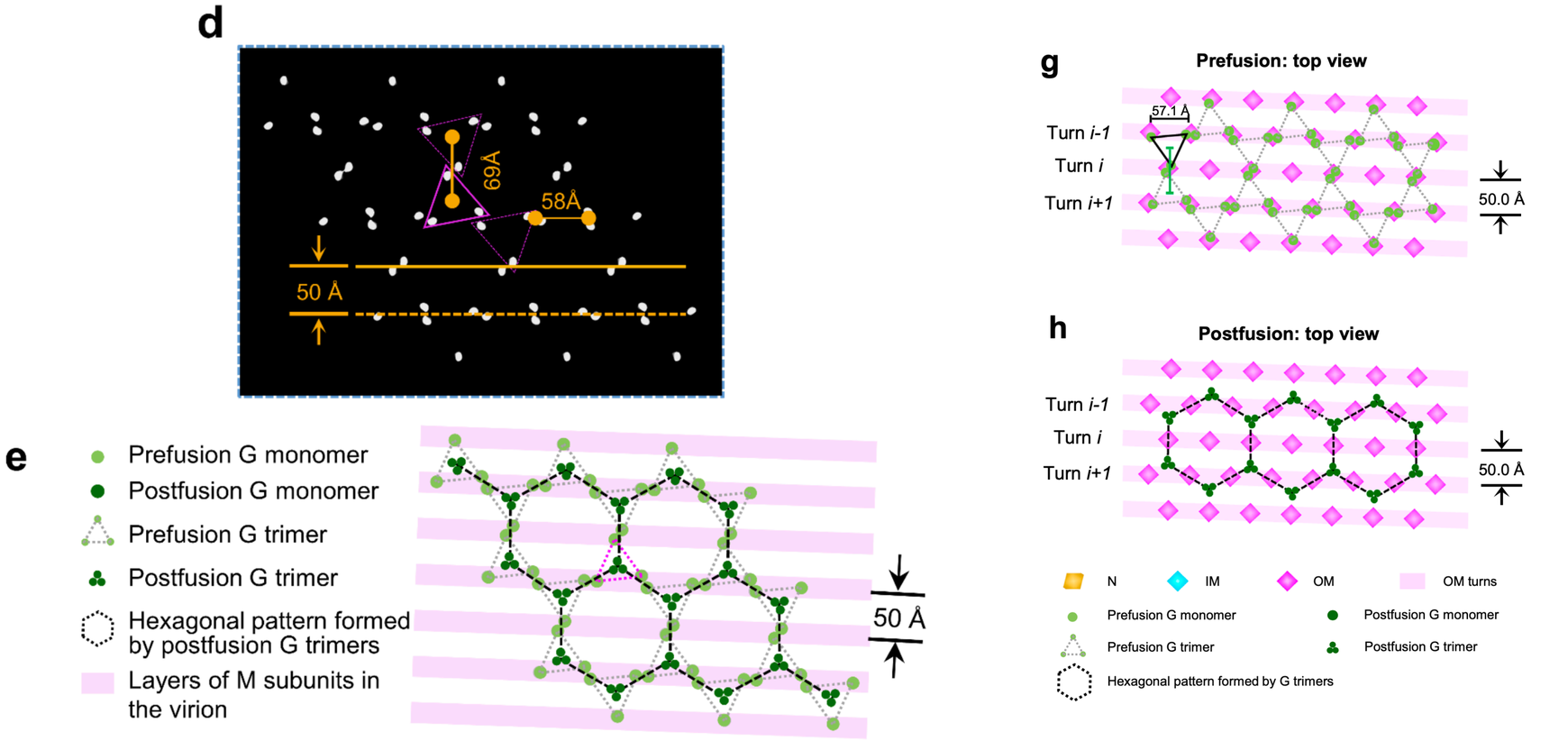




- N:IM:OM=1:1:1, and N/IM/OM are coaxial;
- Verified by crystal structure: the side length 57.1Å is about the same as the distance between the neighboring DIV pairs in the crystalline lattice of prefusion G trimers

Results

The pattern of N-IM-OM-G and G-endodomain-OM association



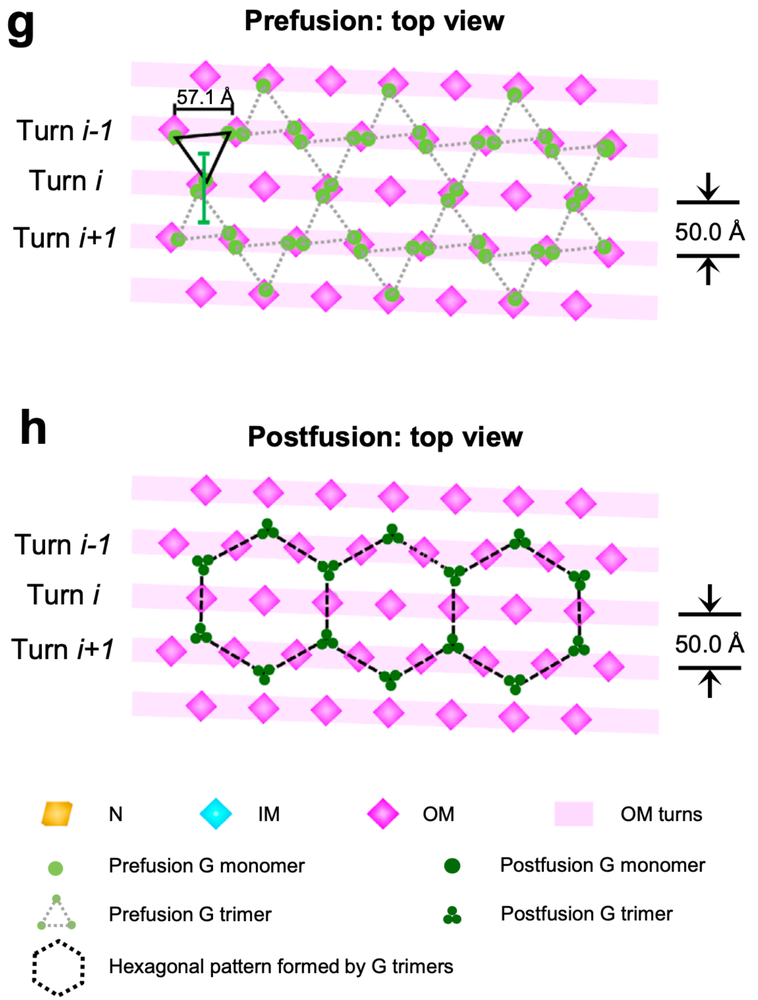
(1) 3 endodomains of a prefusion G trimer reach out to 3 OM sites spanning 2 turns

(2) Each OM site accommodates a DIV pair.

Results

The pattern of N-IM-OM-G and G-endodomain-OM association

- This association would like to break when G trimers convert from prefusion to postfusion conformation, and their transmembrane regions coalesce together.
- Probably facilitating the uncoating of membrane during viral entry.



Results

Alternative mechanism of VSV assembly

(1) Genome replication

(2) Nucleocapsid assembles assisted by IM

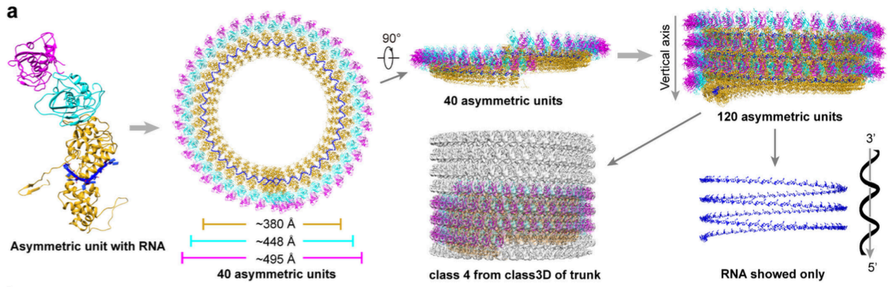
(3) Form a regular mesh on host cell membrane.

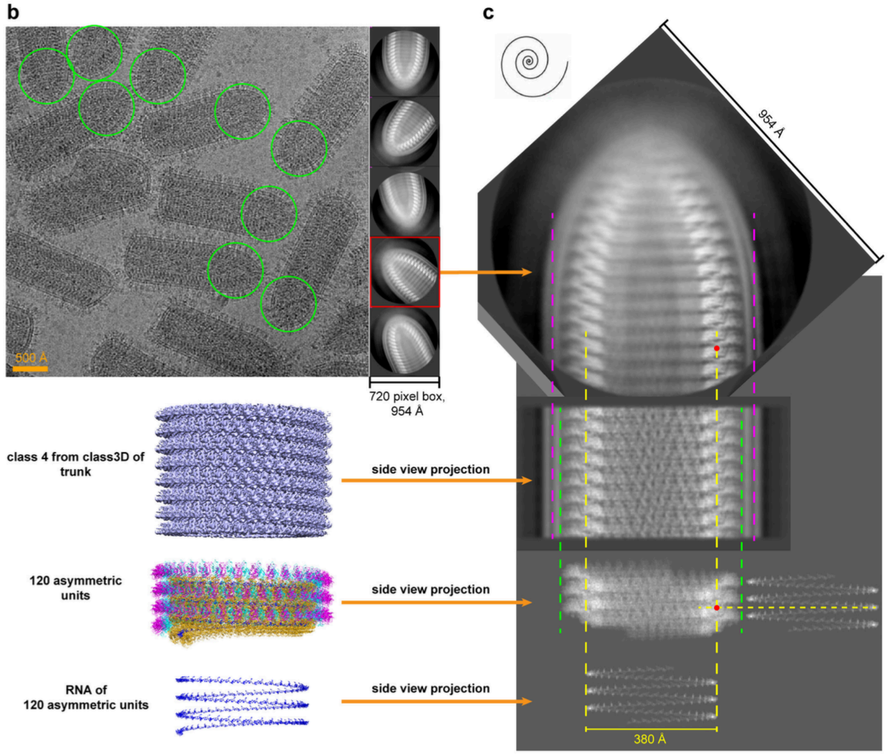
(4) Nucleocapsid binds underneath microdomains containing G clusters and introduces curvature

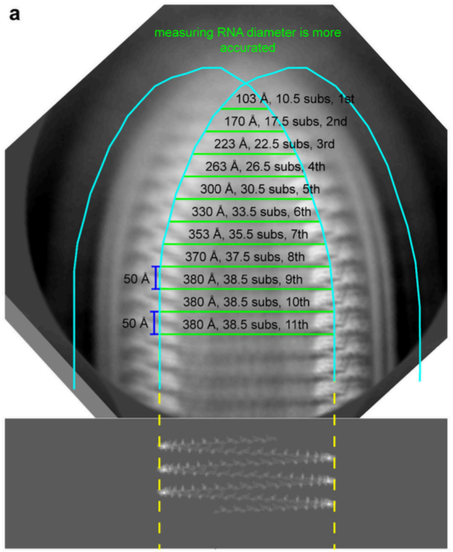
(5) Budding of an infectious virion.

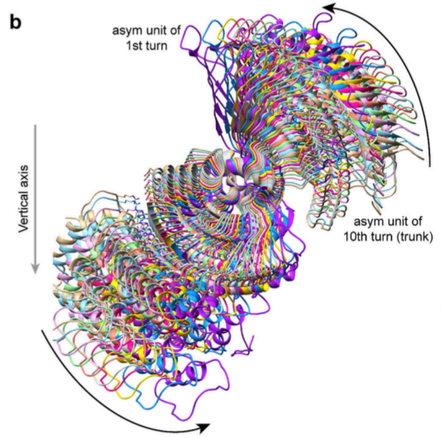
Results

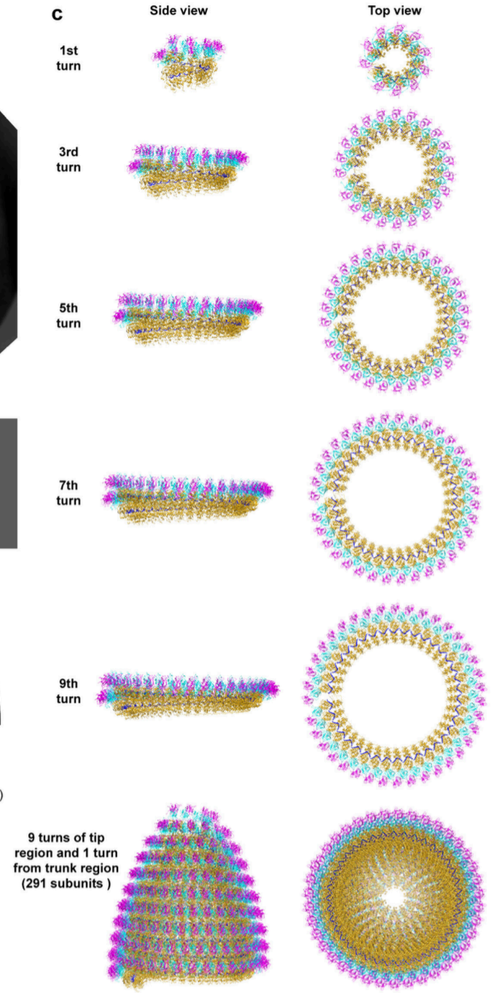
Pseudo-atomic model of an entire VSV virion











Summary

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