

Journal Club

Zhen

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Multi-modal cryo-EM reveals trimers of protein A10 to form the palisade layer in poxvirus cores

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Check for updates

Julia Datler ^{1,2}, Jesse M. Hansen^{1,2}, Andreas Thader¹, Alois Schlögl ¹,
Lukas W. Bauer¹, Victor-Valentin Hodirnau ¹ & Florian K. M. Schur ¹ 

Poxviruses are among the largest double-stranded DNA viruses, with members such as variola virus, monkeypox virus and the vaccination



The palisade layer of the poxvirus core is composed of flexible A10 trimers

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Jiasui Liu ^{1,12}, Simon Corroyer-Dulmont^{2,3,12}, Vojtěch Pražák ^{2,4},
Iskander Khusainov ¹, Karola Bahrami^{5,10}, Sonja Welsch ⁶,
Daven Vasishtan^{2,4,7}, Agnieszka Obarska-Kosińska ¹,
Sigurdur R. Thorkelsson^{2,11}, Kay Grünewald ^{2,3,7,13} ,
Emmanuelle R. J. Quemina ^{2,8,13} , Beata Turoňová ^{1,13}  &
Jacomina Krijnse Locker ^{5,9,13} 

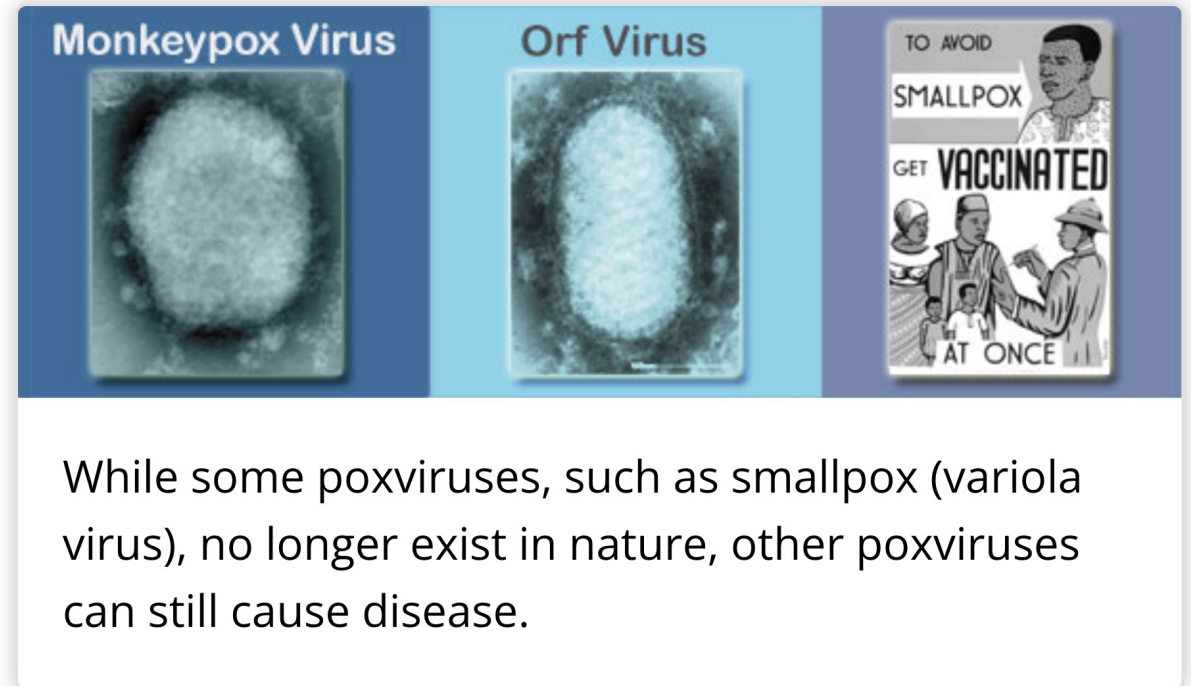
Background

Poxviruses: the largest dsDNA viruses

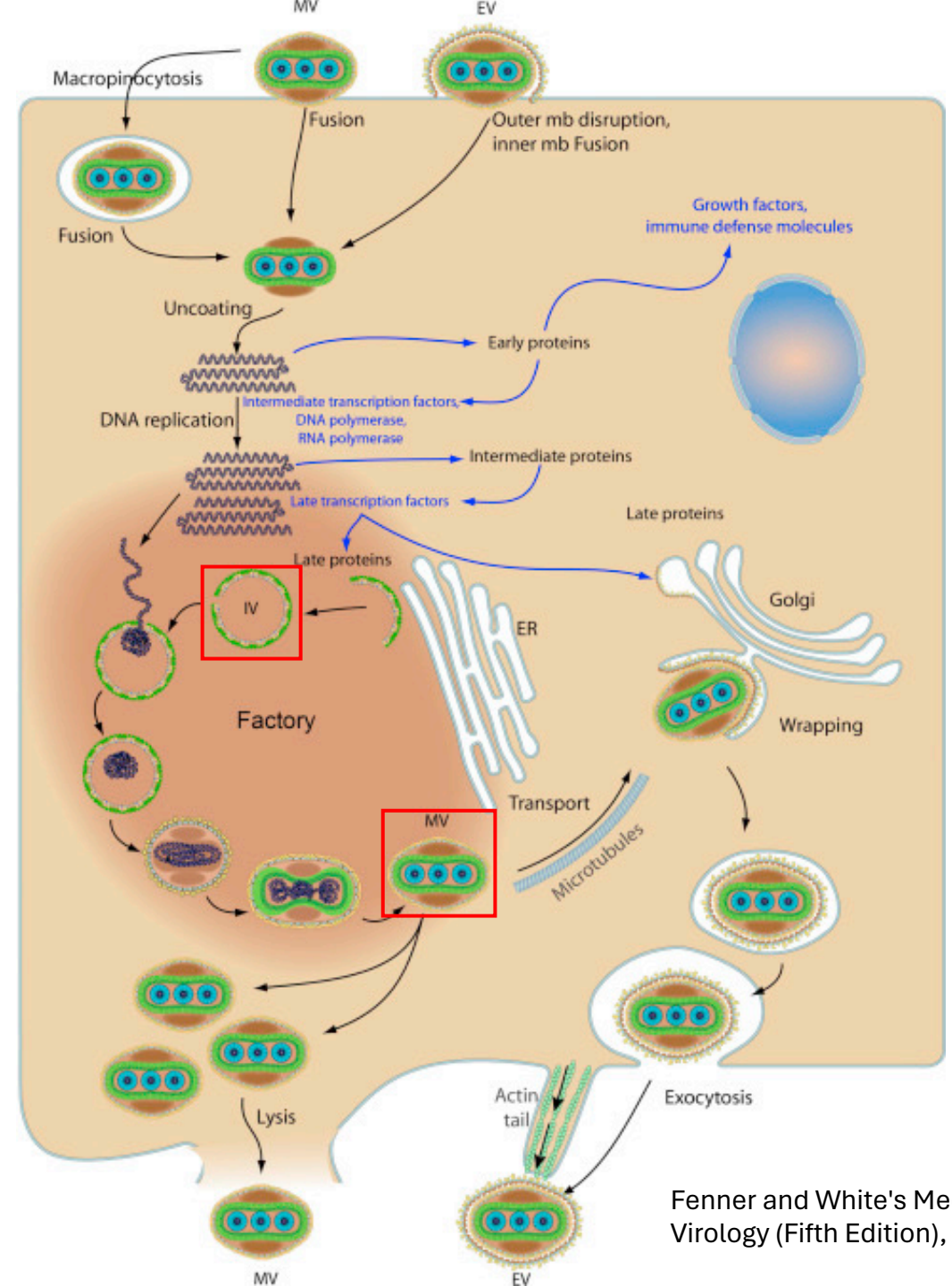
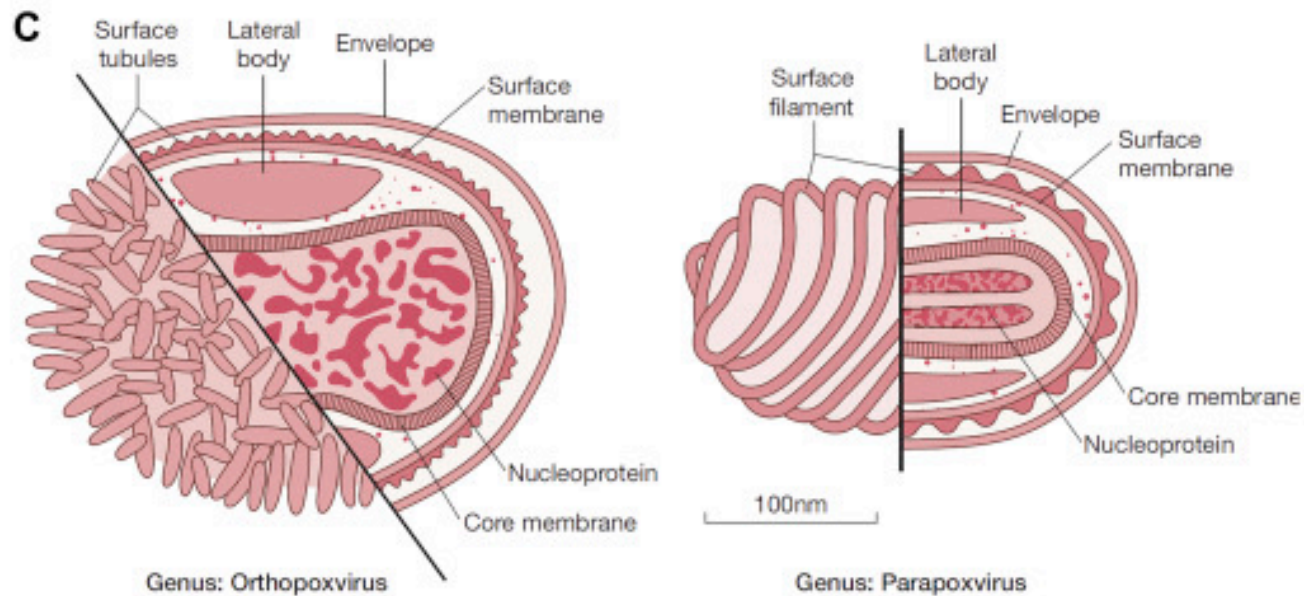
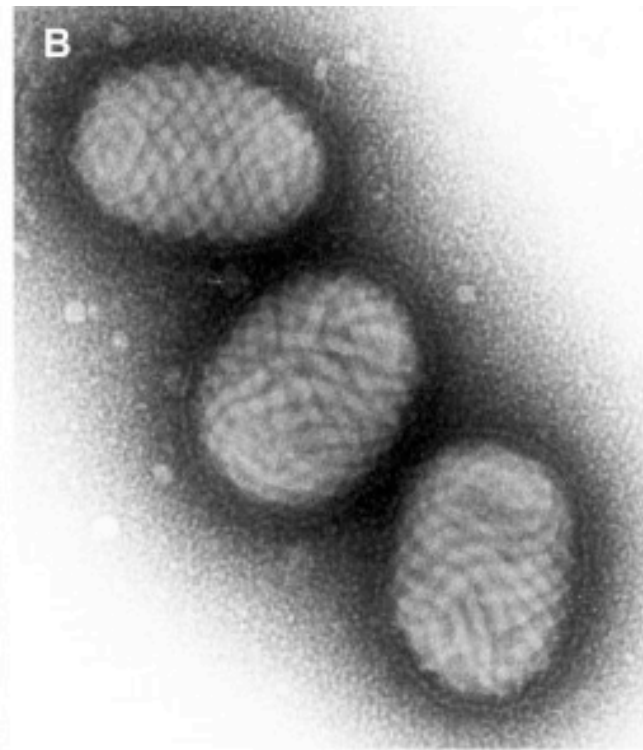
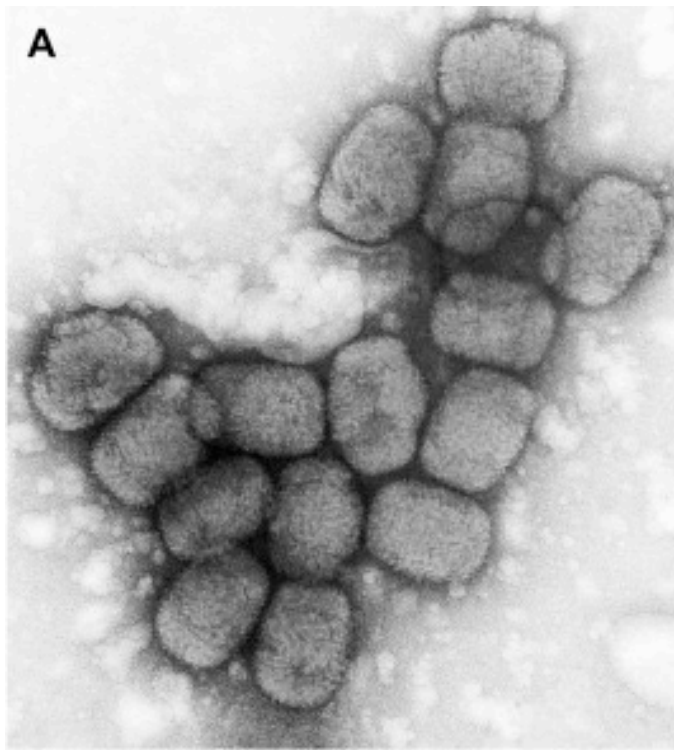
- brick or oval-shaped
- large double-stranded DNA genomes
- replicate and assemble entirely in the cytoplasm

Members like:

- Variola virus (smallpox)
- Monkeypox
- Vaccination strain vaccinia virus (VACV)

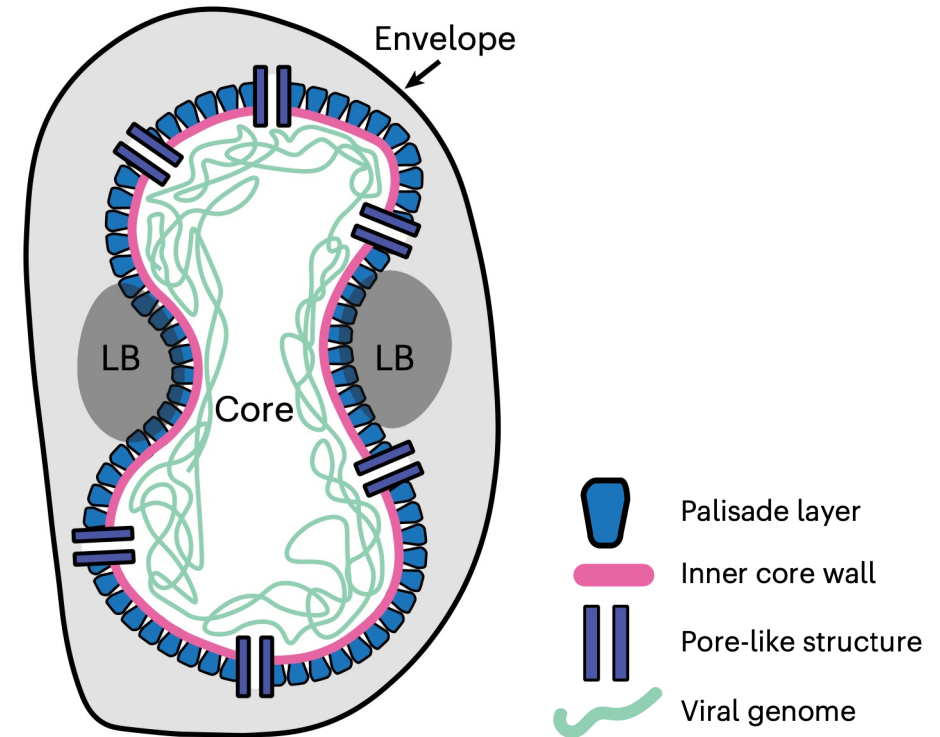


<https://www.cdc.gov/poxvirus/index.html>



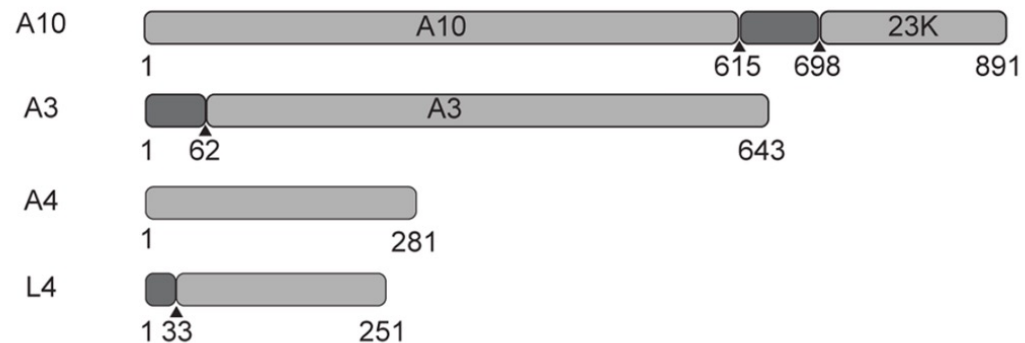
What have we known so far

- discontinuous palisade layer (formed of spike-like assemblies)
- pseudo-hexameric lattice
- pore-like structures with unknown function
- structural proteins:
 - core wall and palisade layer: **A10, A3, A4, L4**
 - LB: F17



Putative major structural core proteins & their precursor proteins

a



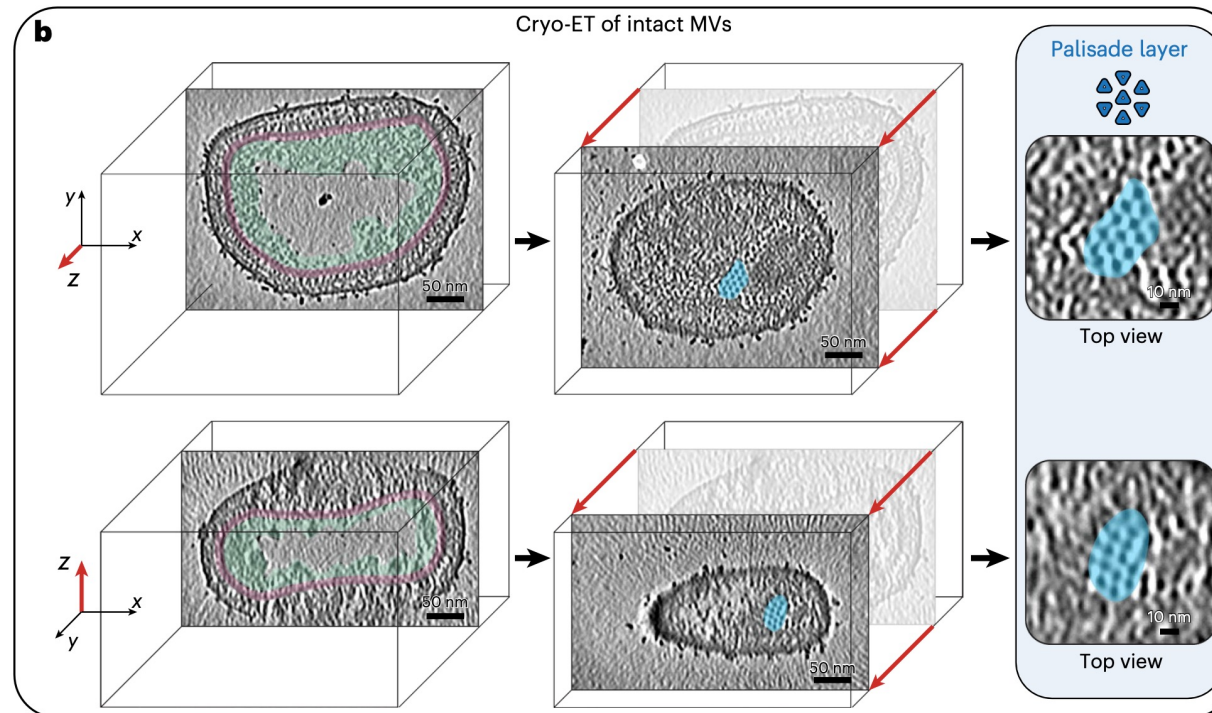
b

Protein (UniProt name)	Residues	MW (kDa)	UniProt ID
A10 (4a)	1 - 614	62	P16715
A3 (4b)	62 - 643	60	P06440
23K (cleavage product of A10 precursor)	698 - 891	23	P16715
A4 (p39)	1-281	31	P29191
L4 (VP8)	33 -251	25	P03295

Questions

Biological questions

- the structures of these protein candidates?
- no direct structural proof to assign any of these to specific structural core features
 - **Precise structure and molecular composition of MVs and its core?**
- the functions of these proteins?



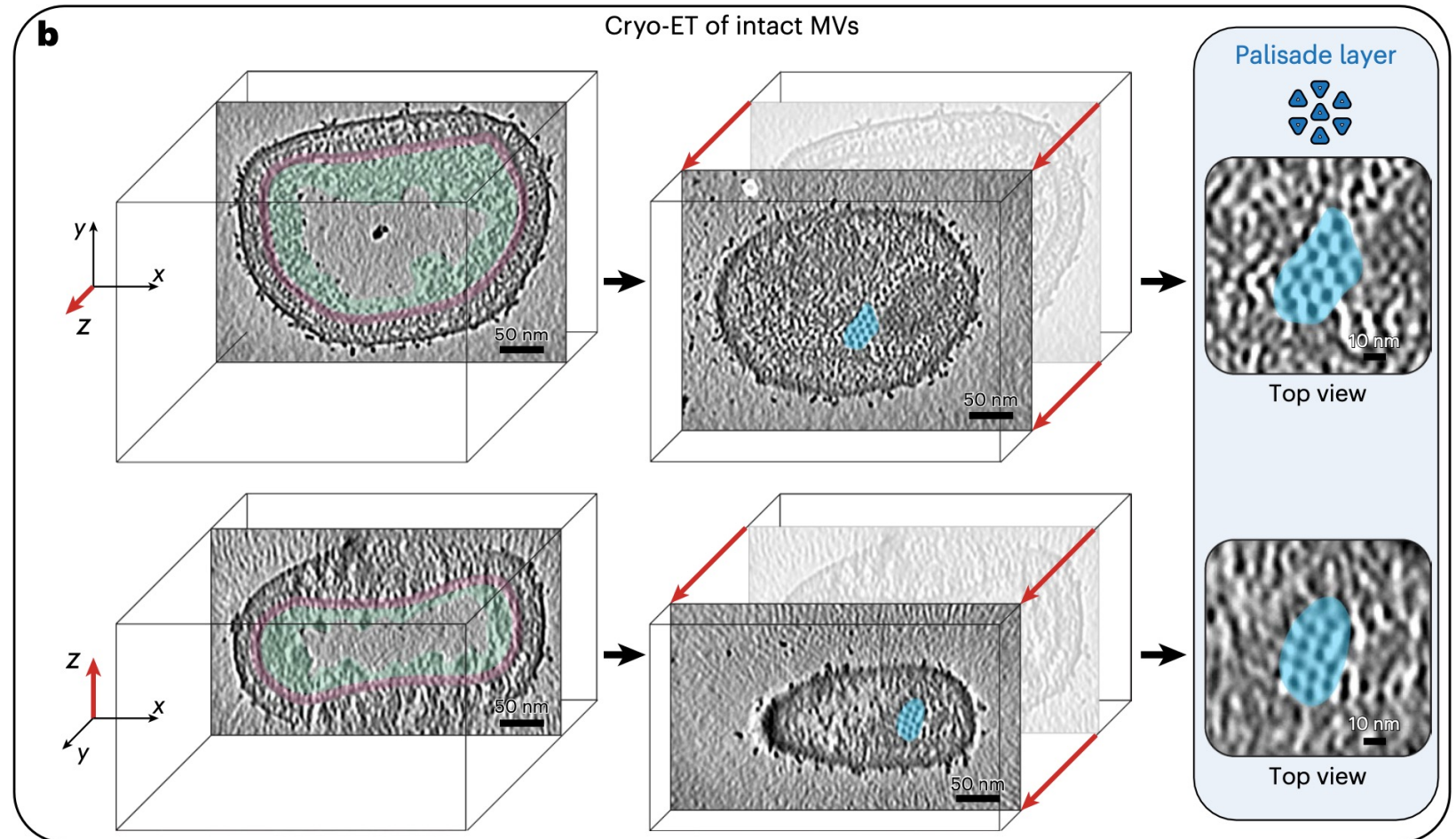
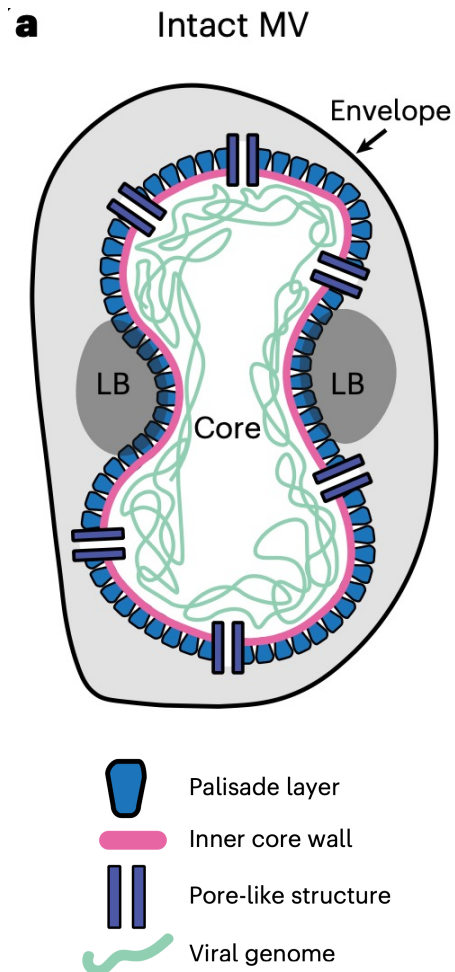
Methods and Materials

Methods: multi-modal cryo-EM

- cryo-ET and Subtomogram Average (STA)
- Single Particle Analysis
- AlphaFold2 (AF2)
- Software like: Dynamo, cryoCAT, Isonet, novaSTA, napari

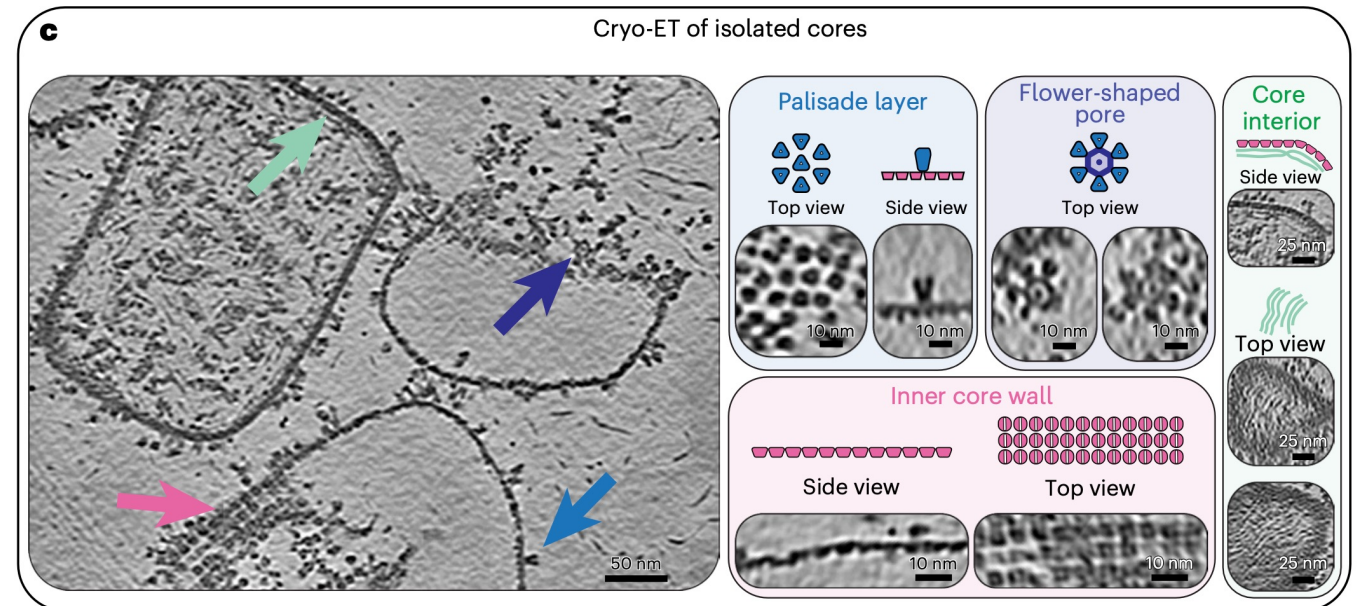
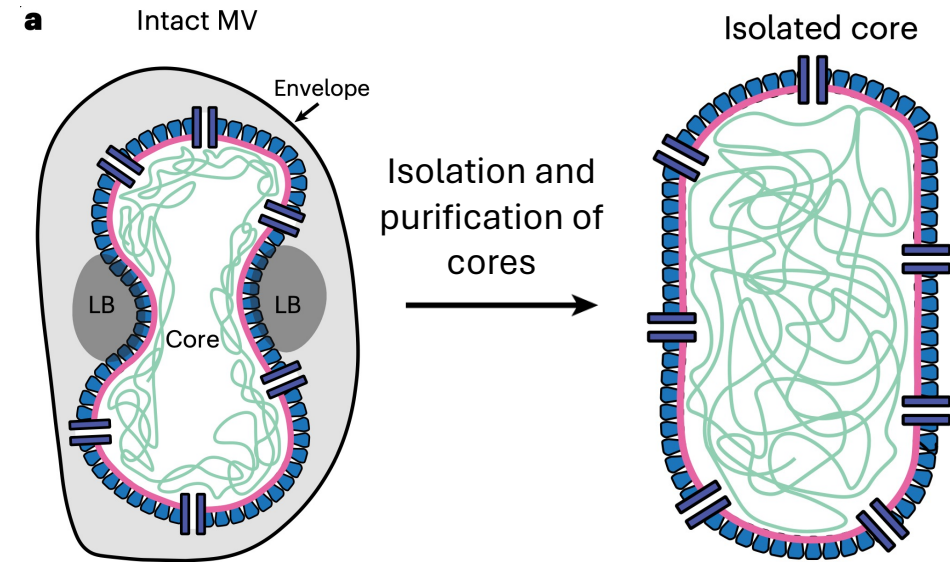
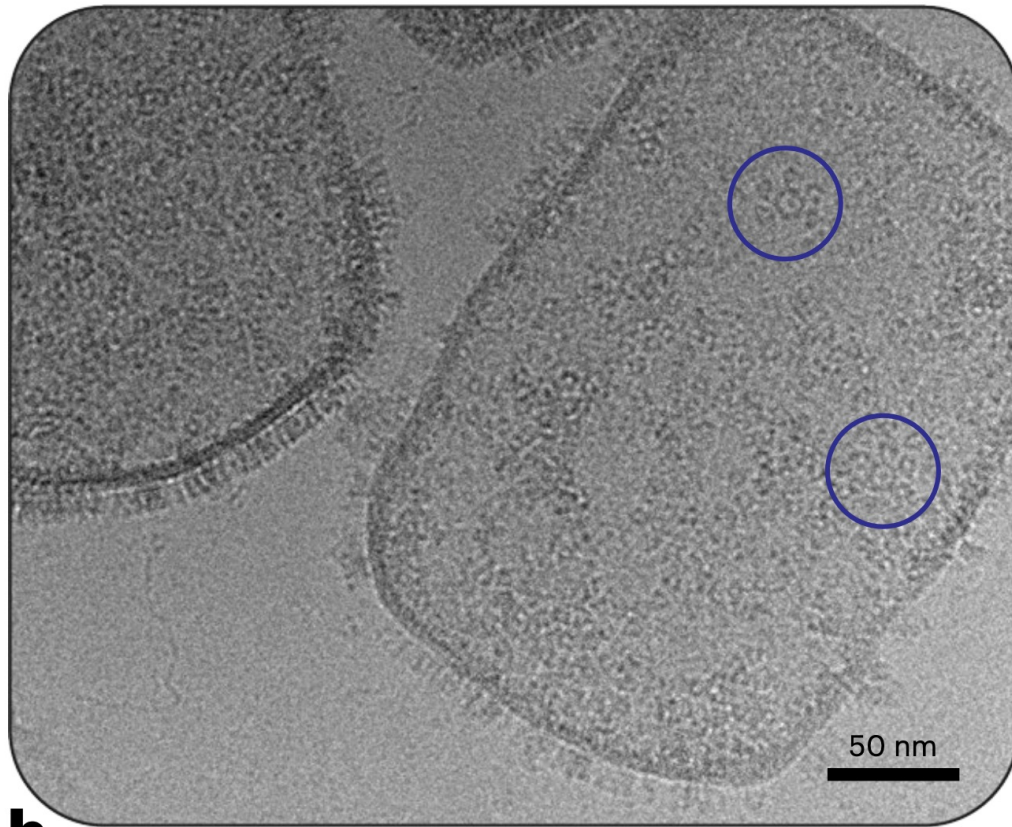
Materials

(1) intact VACV MV virions purified from infected HeLa cells



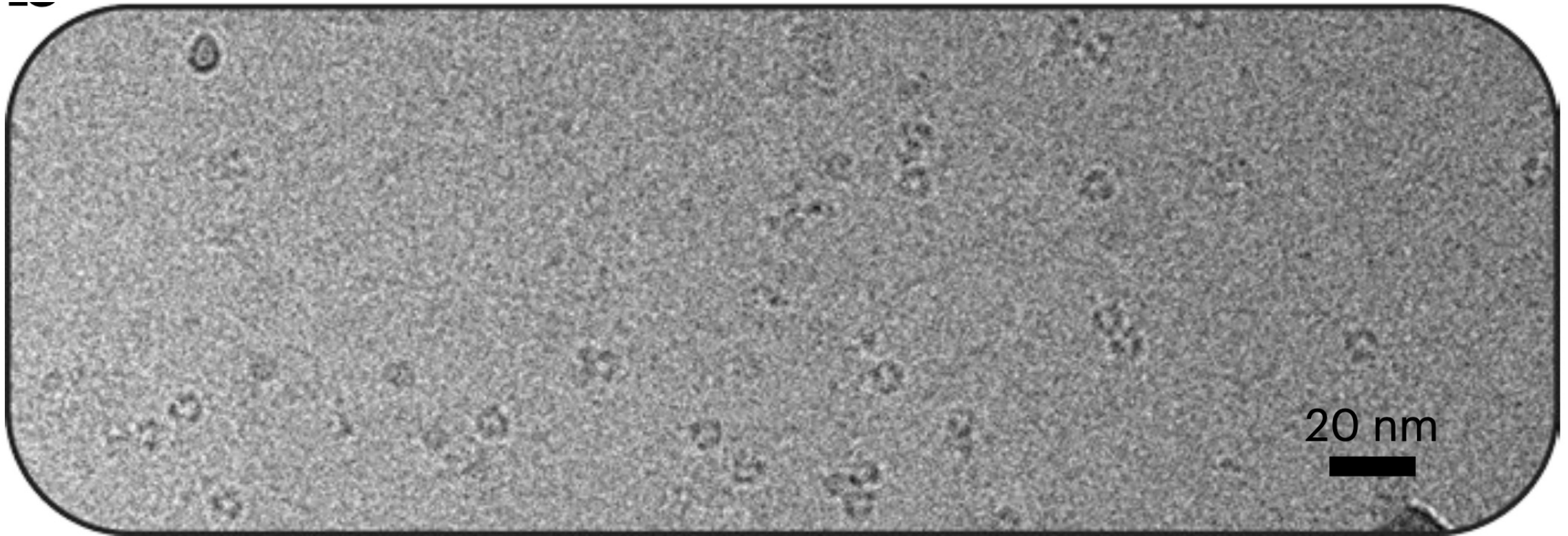
Materials

(2) isolated *in vitro* MV core



Materials

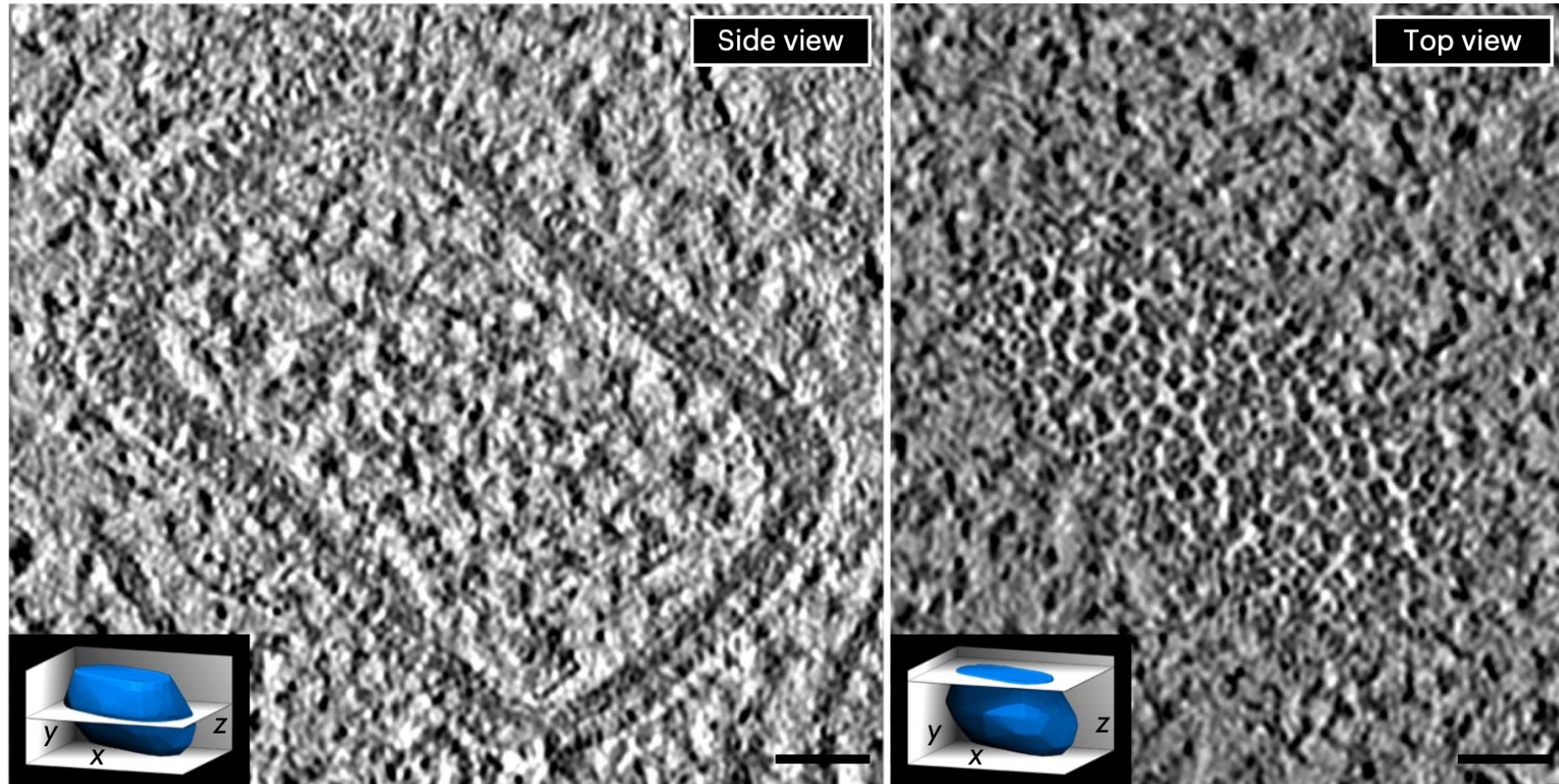
(3) individual components released from cores: isolated MV core + additional centrifugation purification step



Materials

(4) *in situ* VACV cores in the cytoplasm shortly after entry in HeLa cells

a



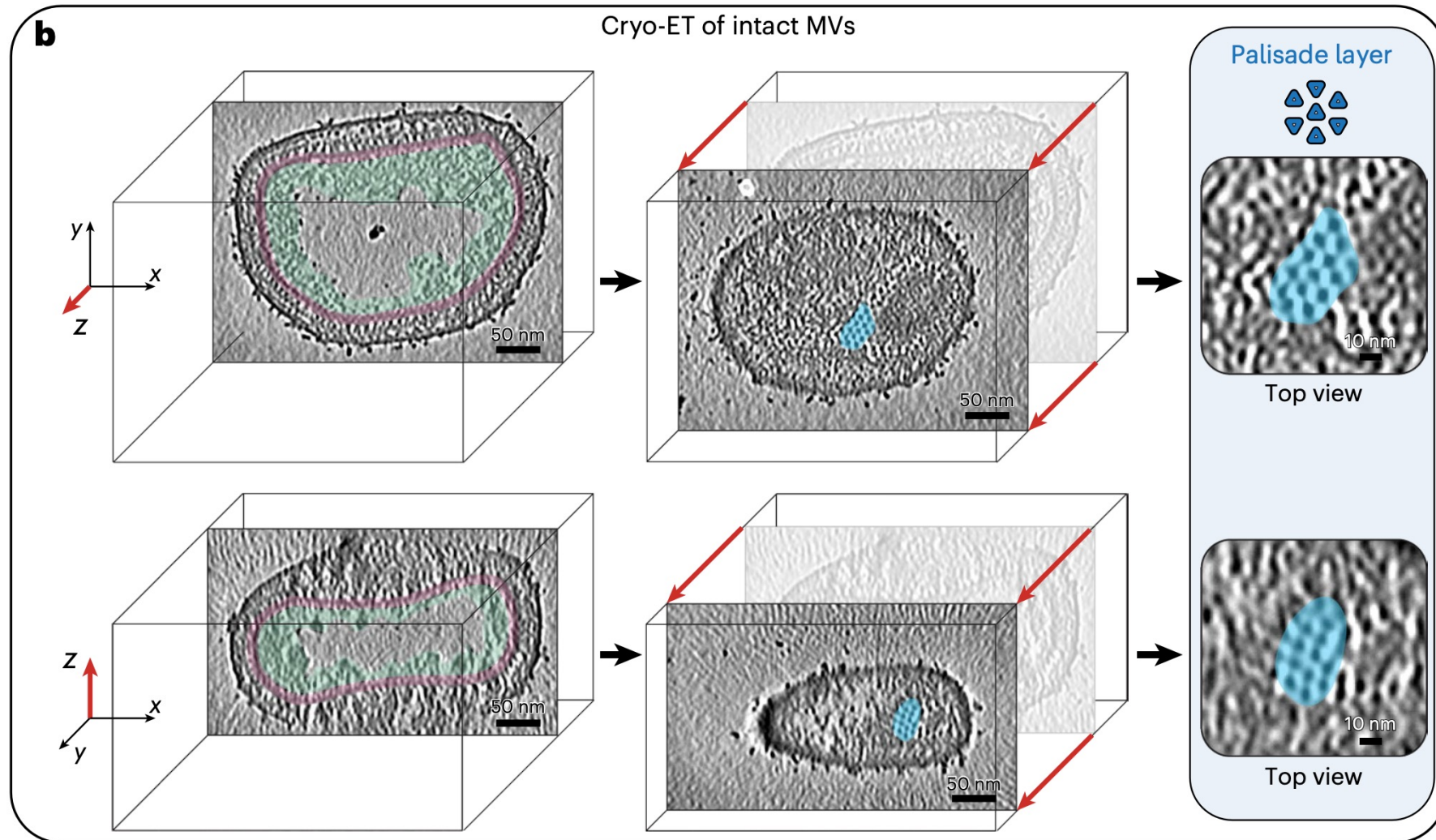
Main results

Main results

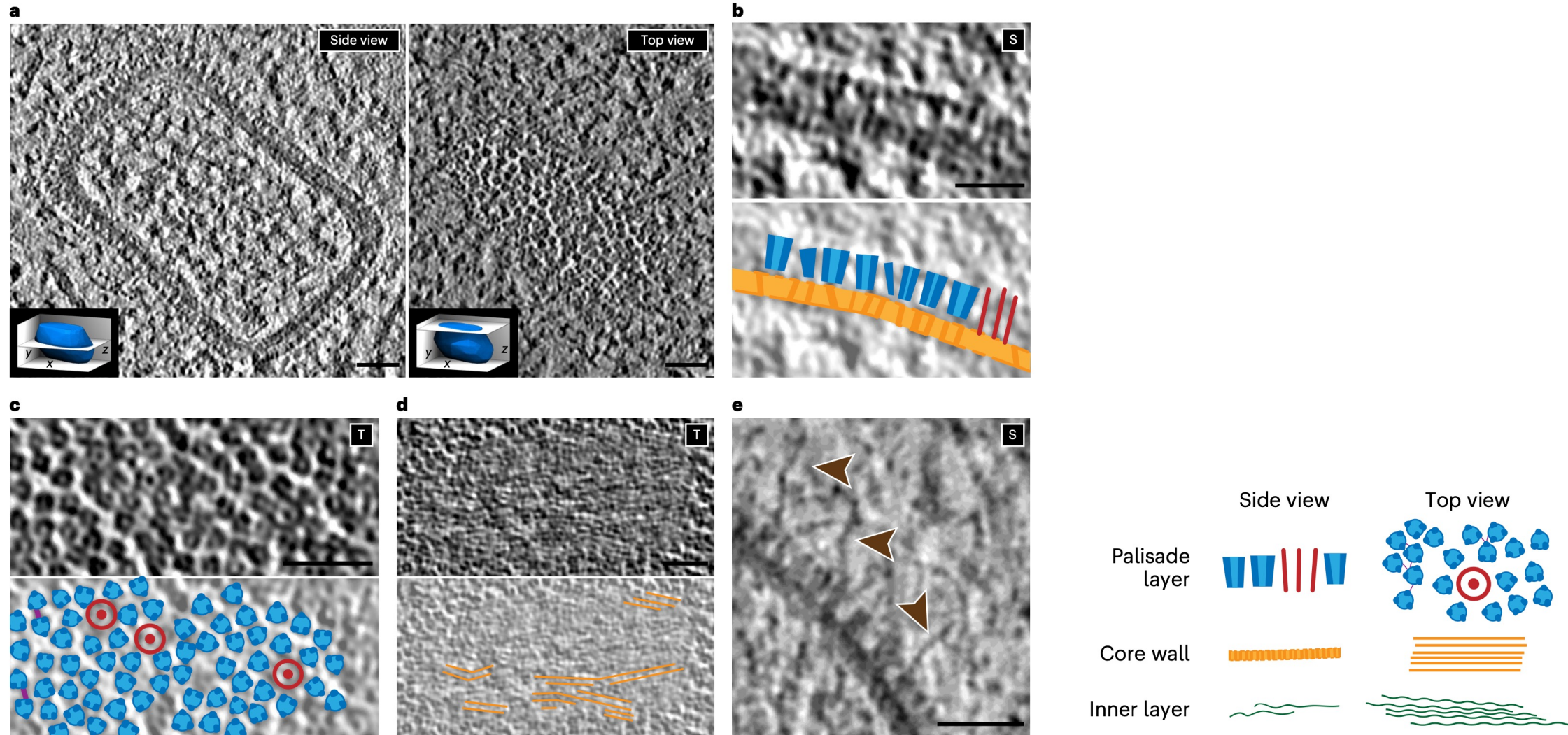
- the structures of key **core proteins** in MVs
- **trimers of A10** form the palisade layer
- a refined model of the molecular architecture of the **viral core**

Results

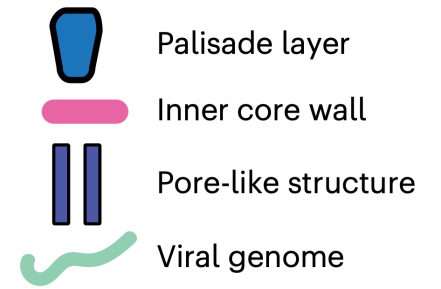
Cryo-ET of *in situ* VACV mature virus



Cryo-ET of *in situ* VACV cores in the cytoplasm shortly after entry in HeLa cells

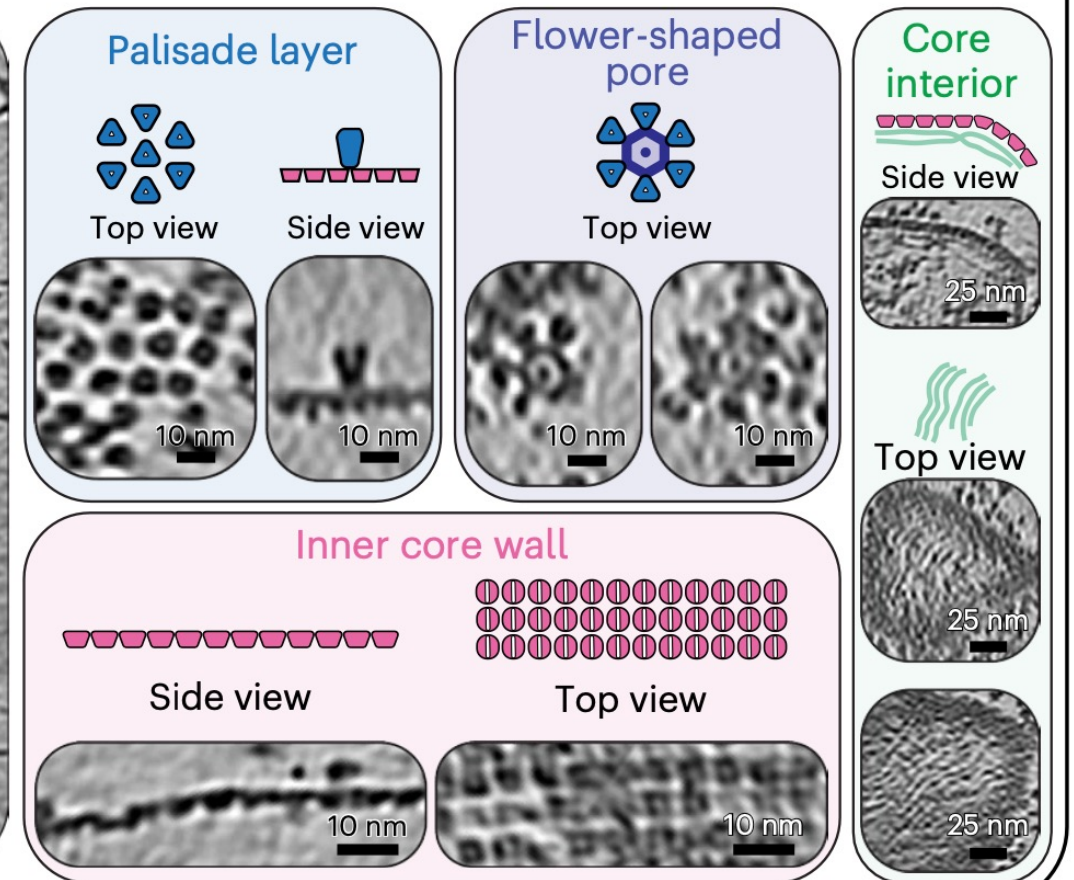
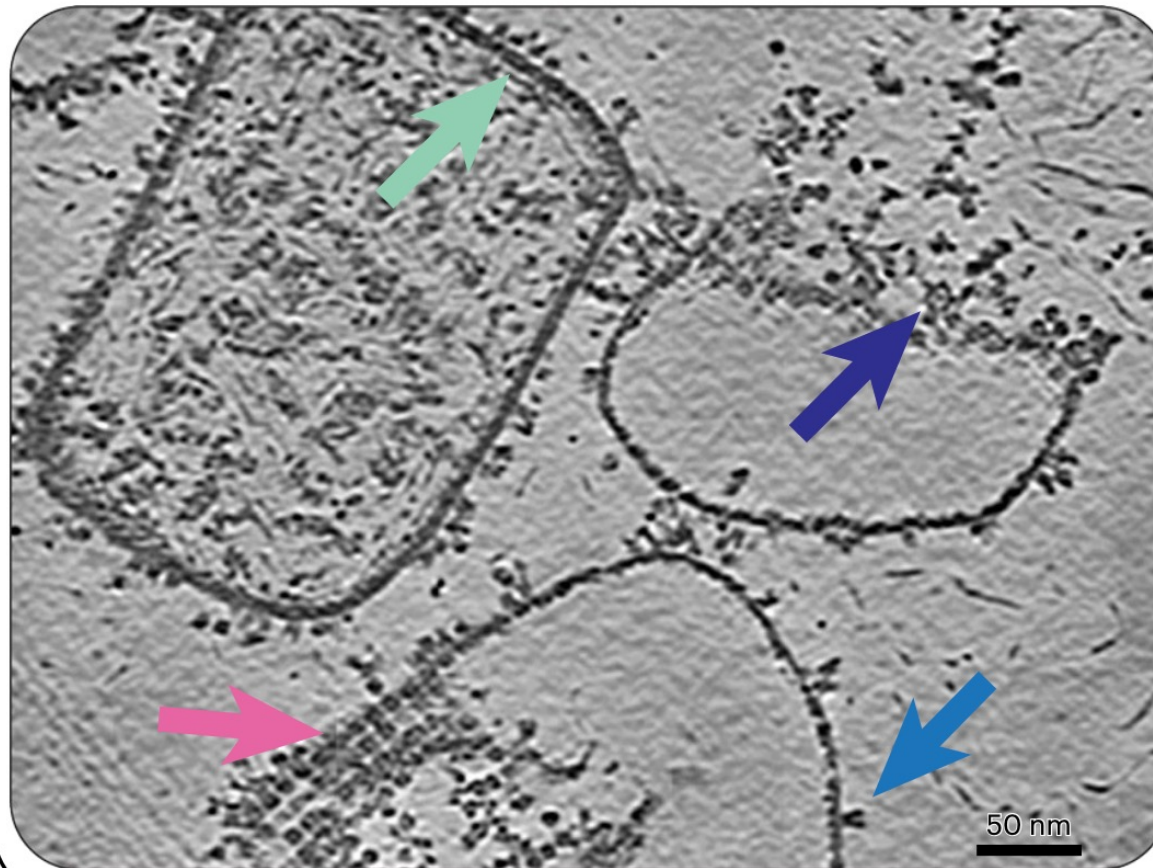


Cryo-ET of *in vitro* isolated VACV cores

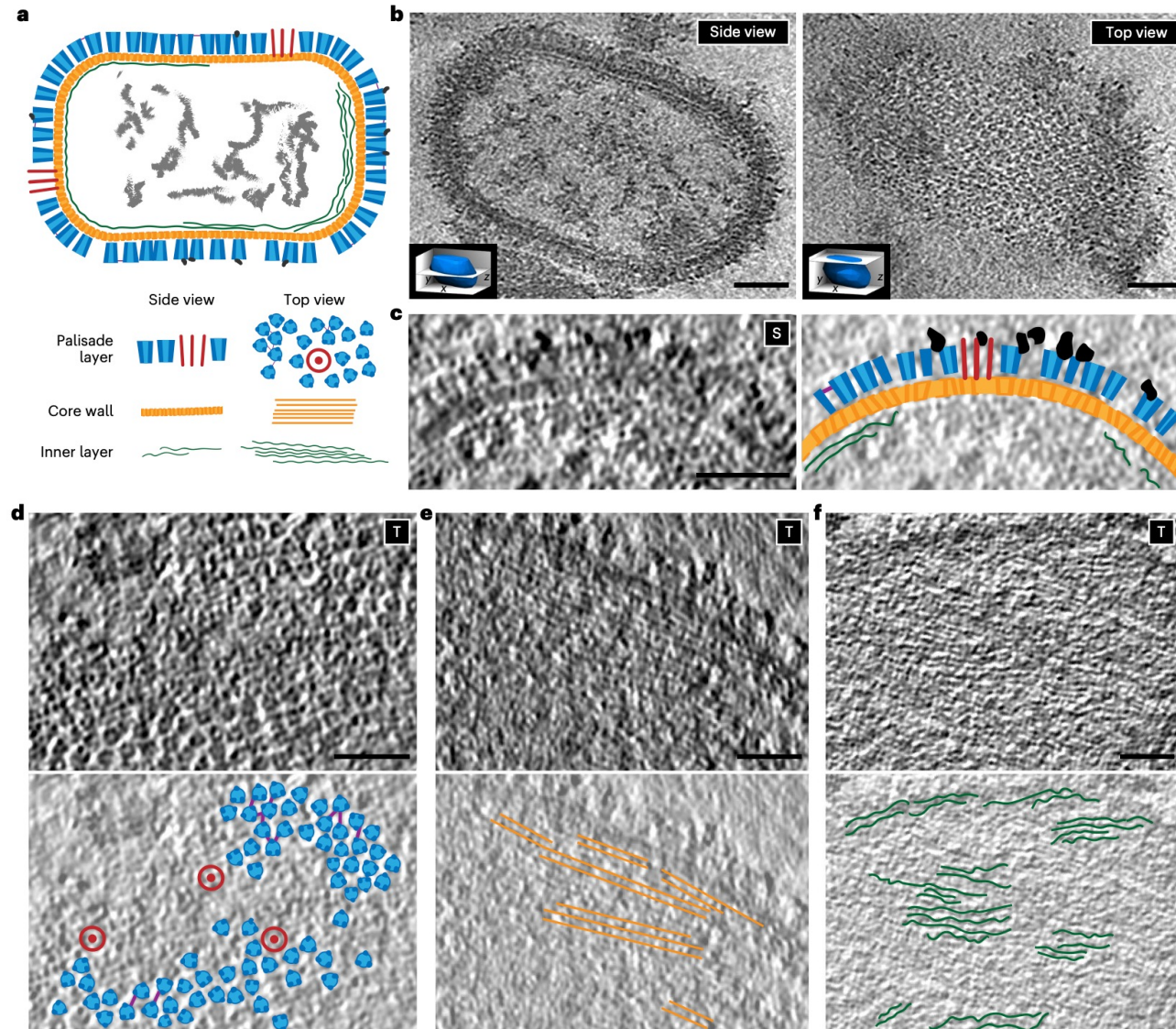


c

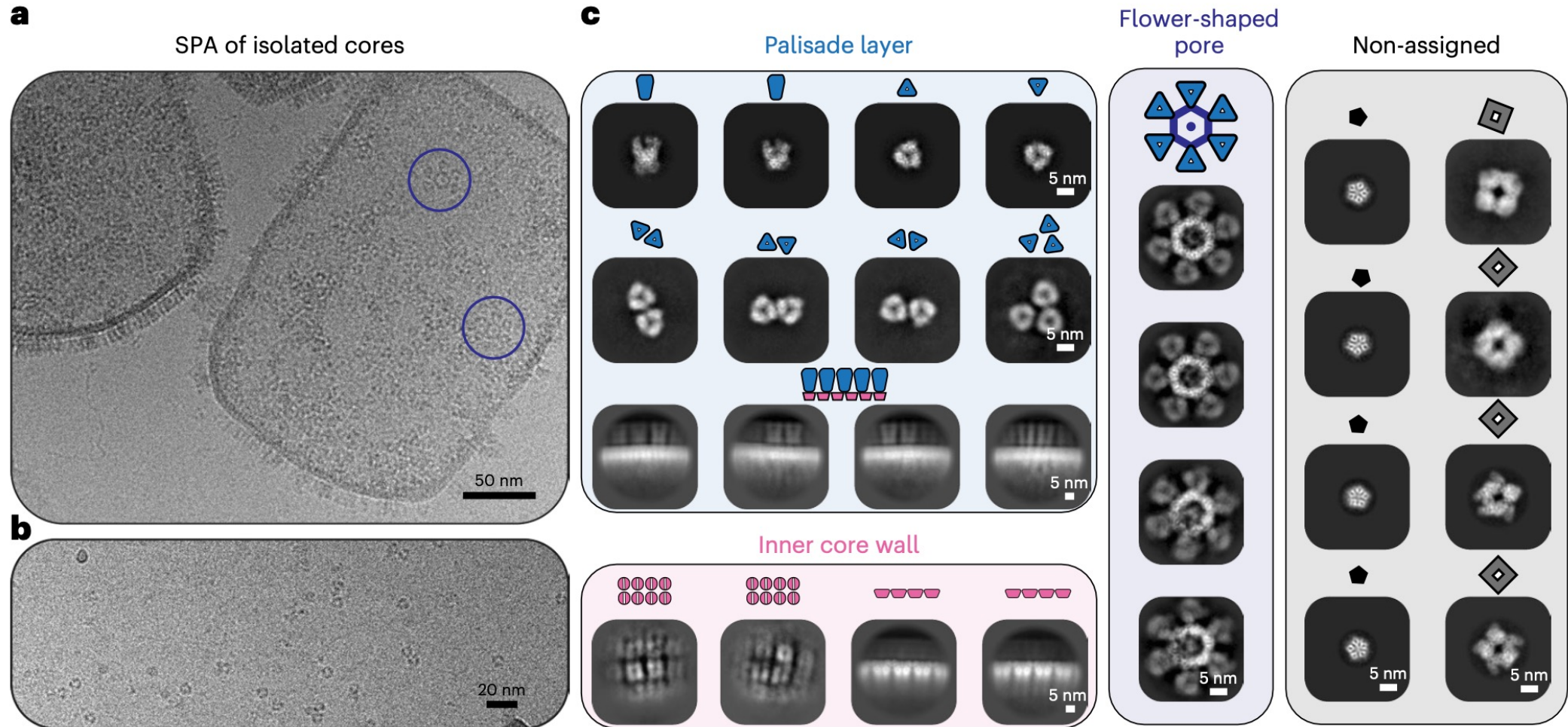
Cryo-ET of isolated cores



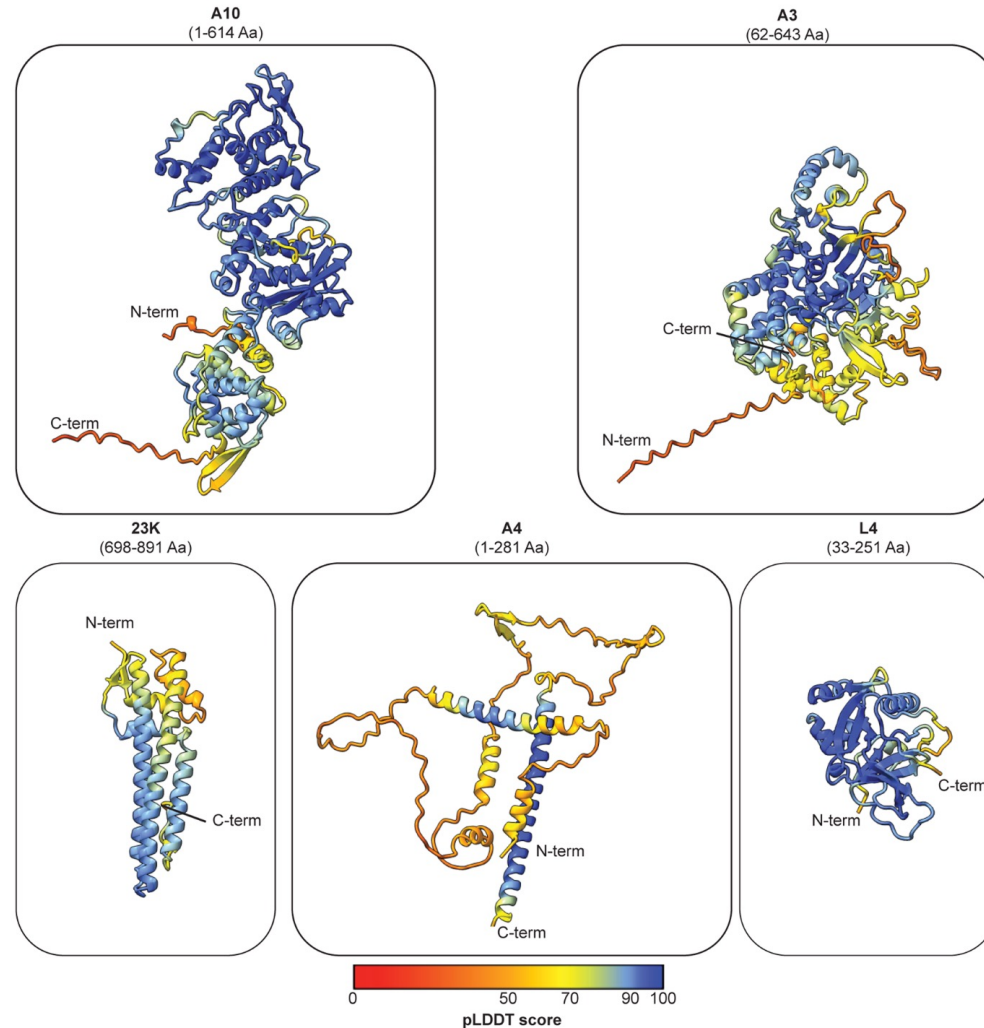
Cryo-ET of *in vitro* isolated VACV cores



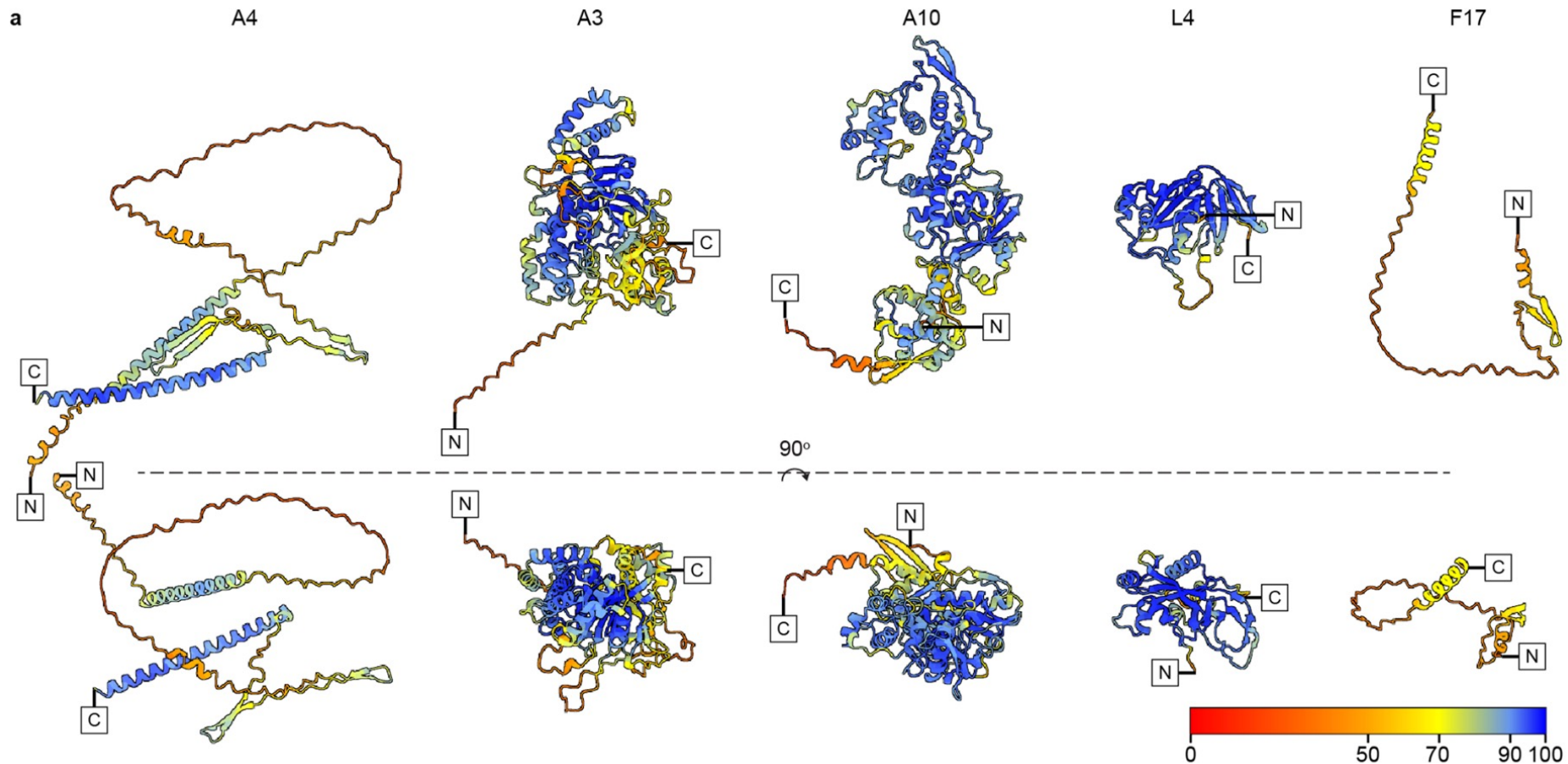
SPA of *in vitro* cores & soluble core proteins



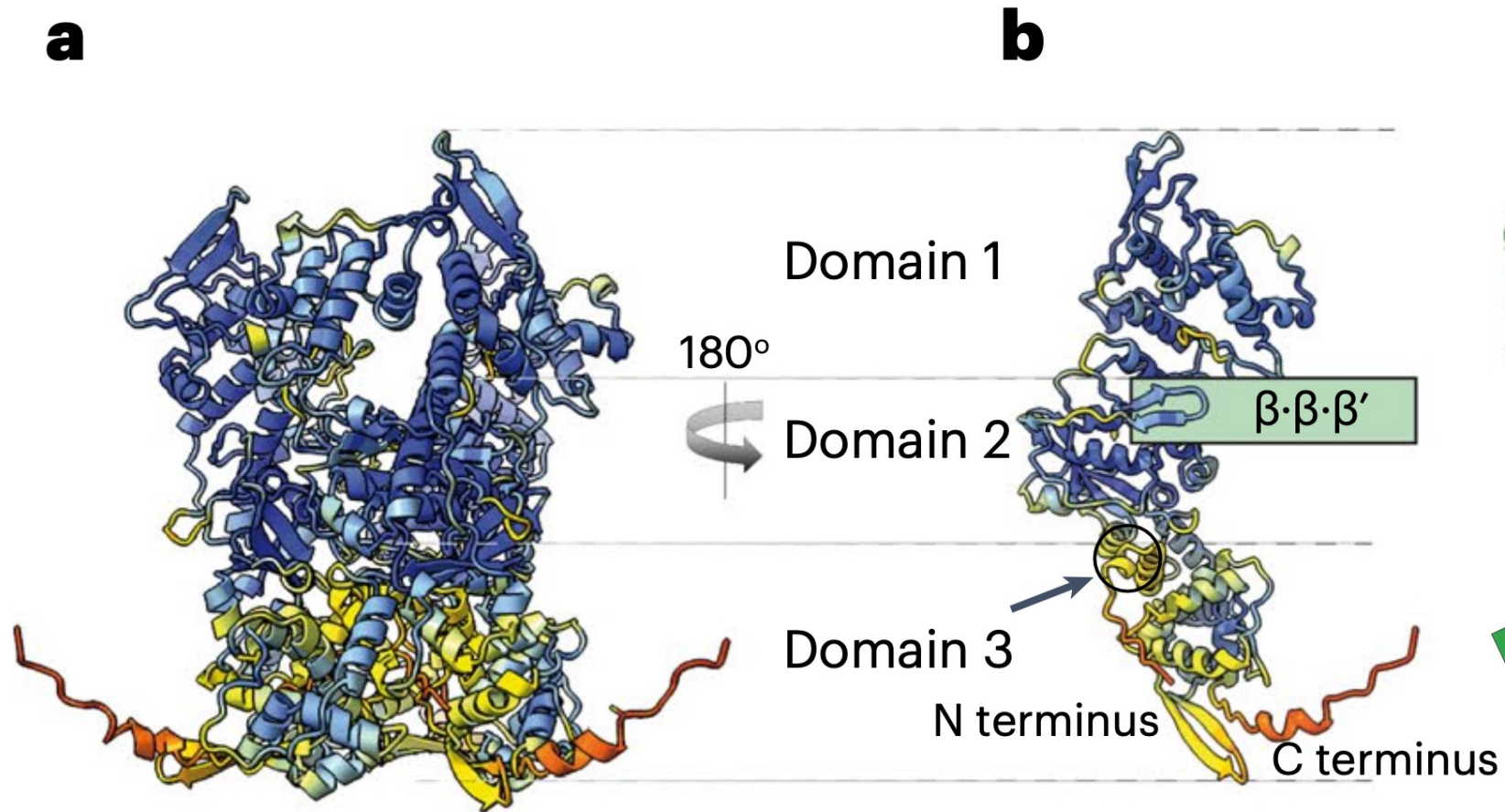
AlphaFold-predicted models of major structural core proteins



AlphaFold-predicted models of major structural core proteins

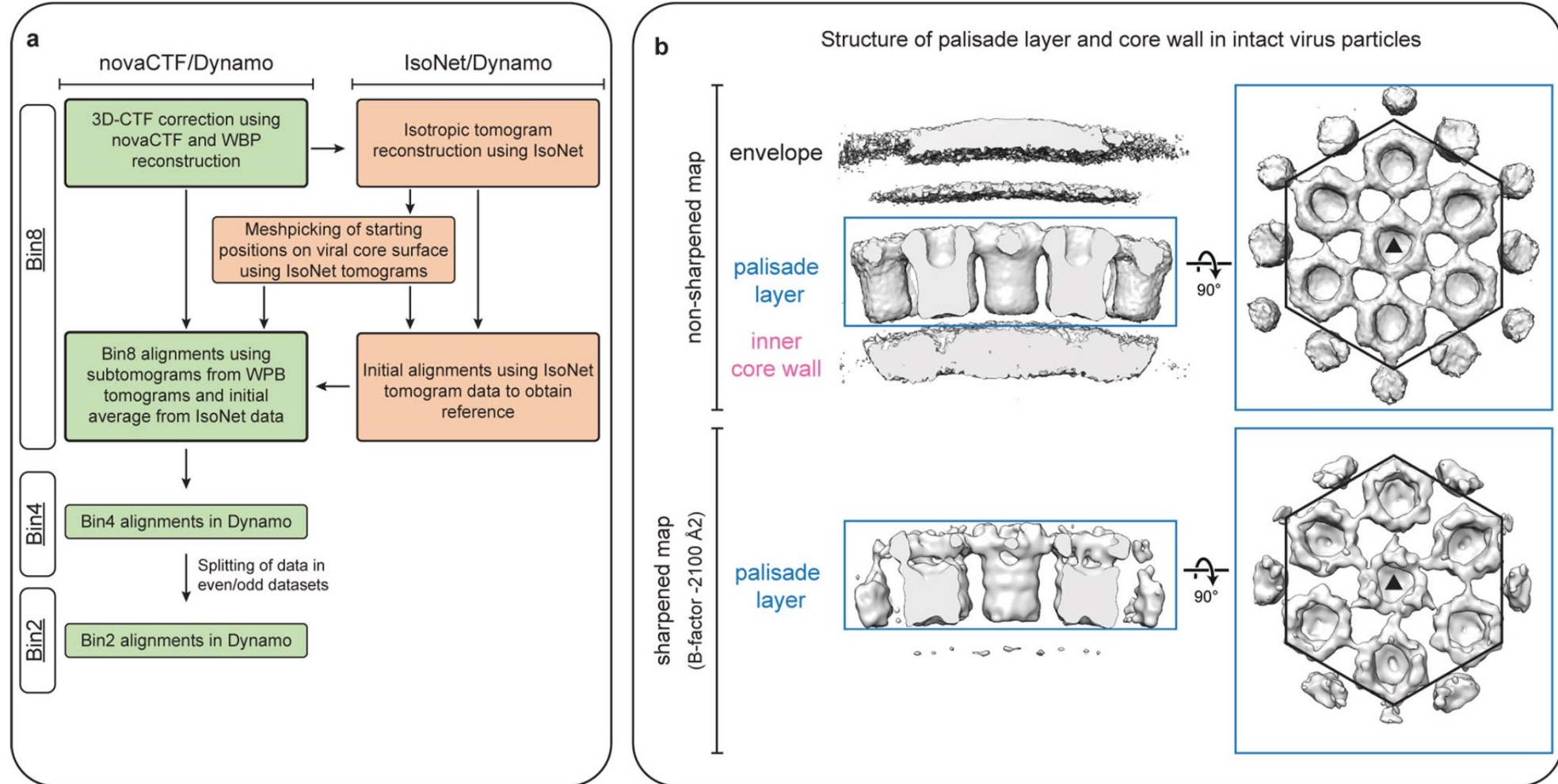


Multimer predictions

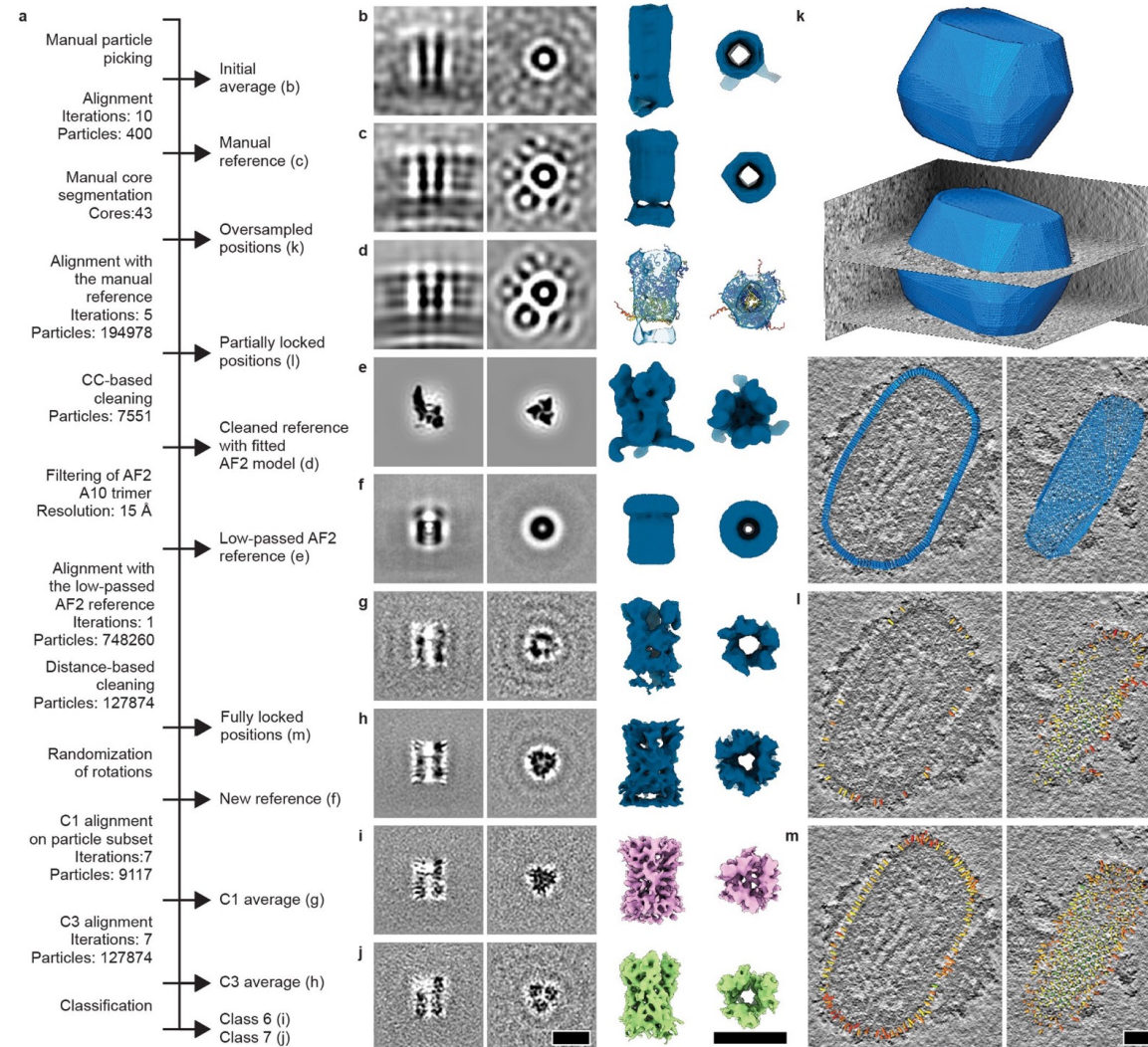


A3 and L4 could form dimers, A10 most likely forms a trimer

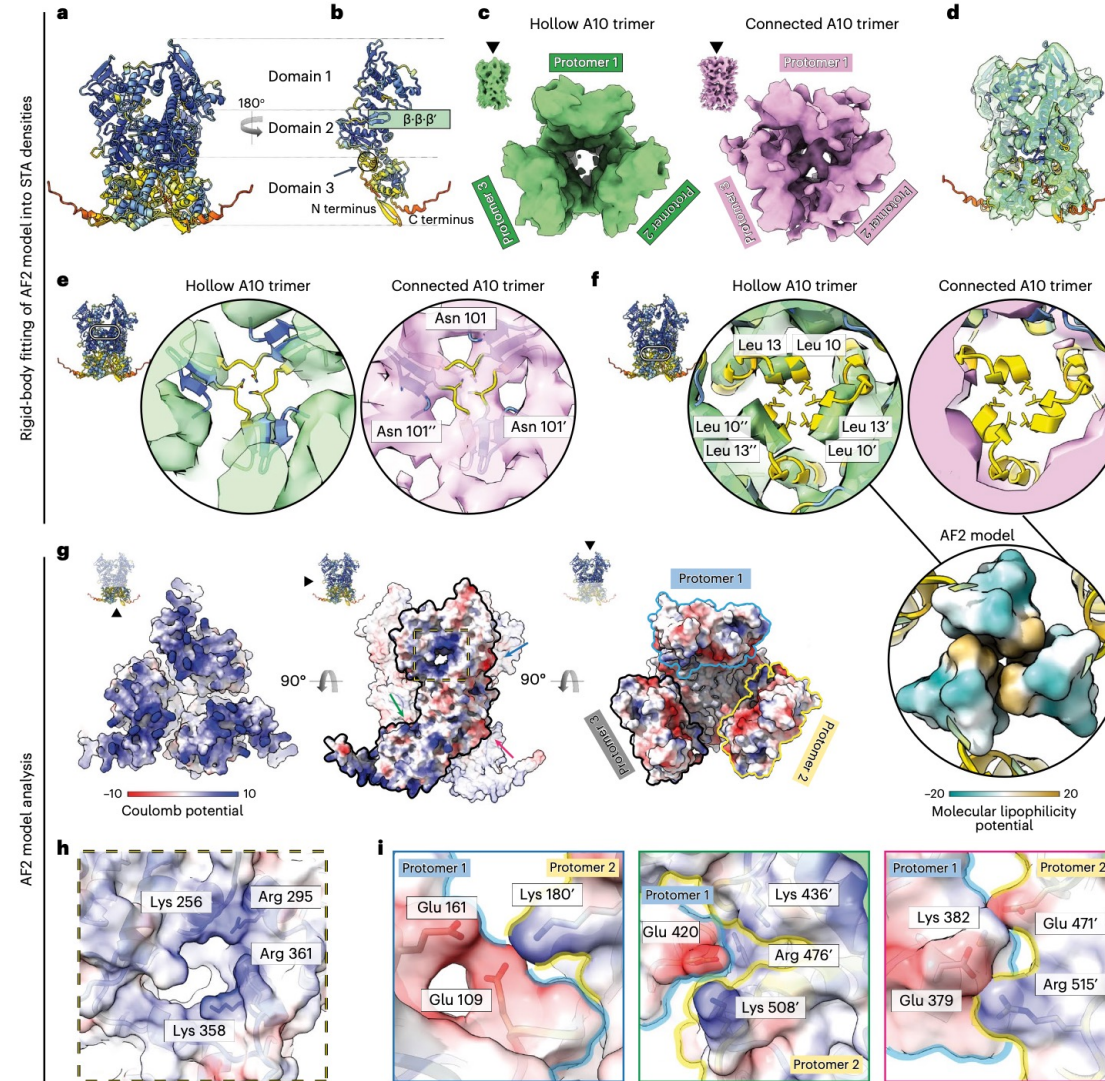
STA of the *in situ* VACV core wall in intact MV



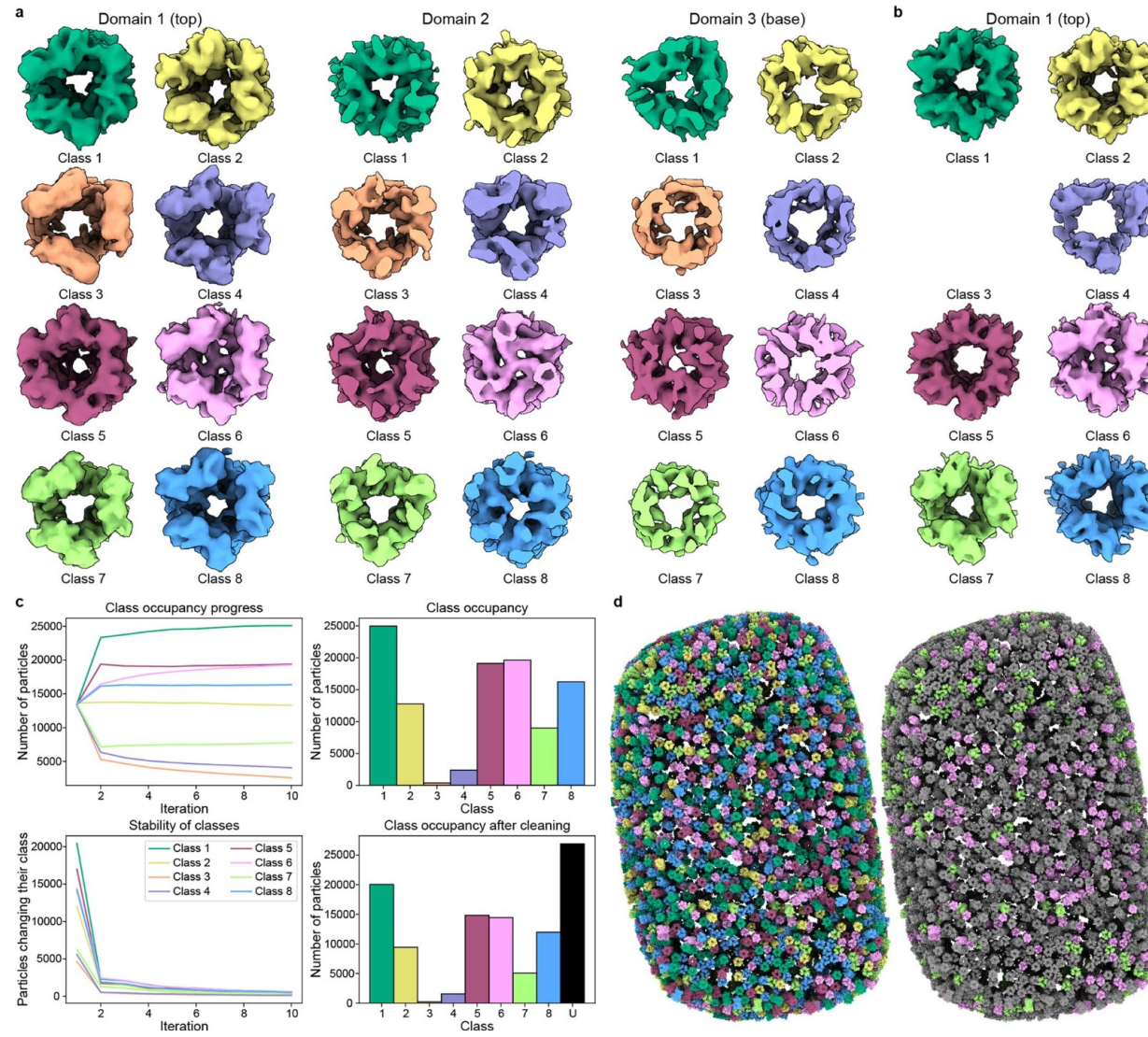
The palisade consists of trimers of major core protein A10



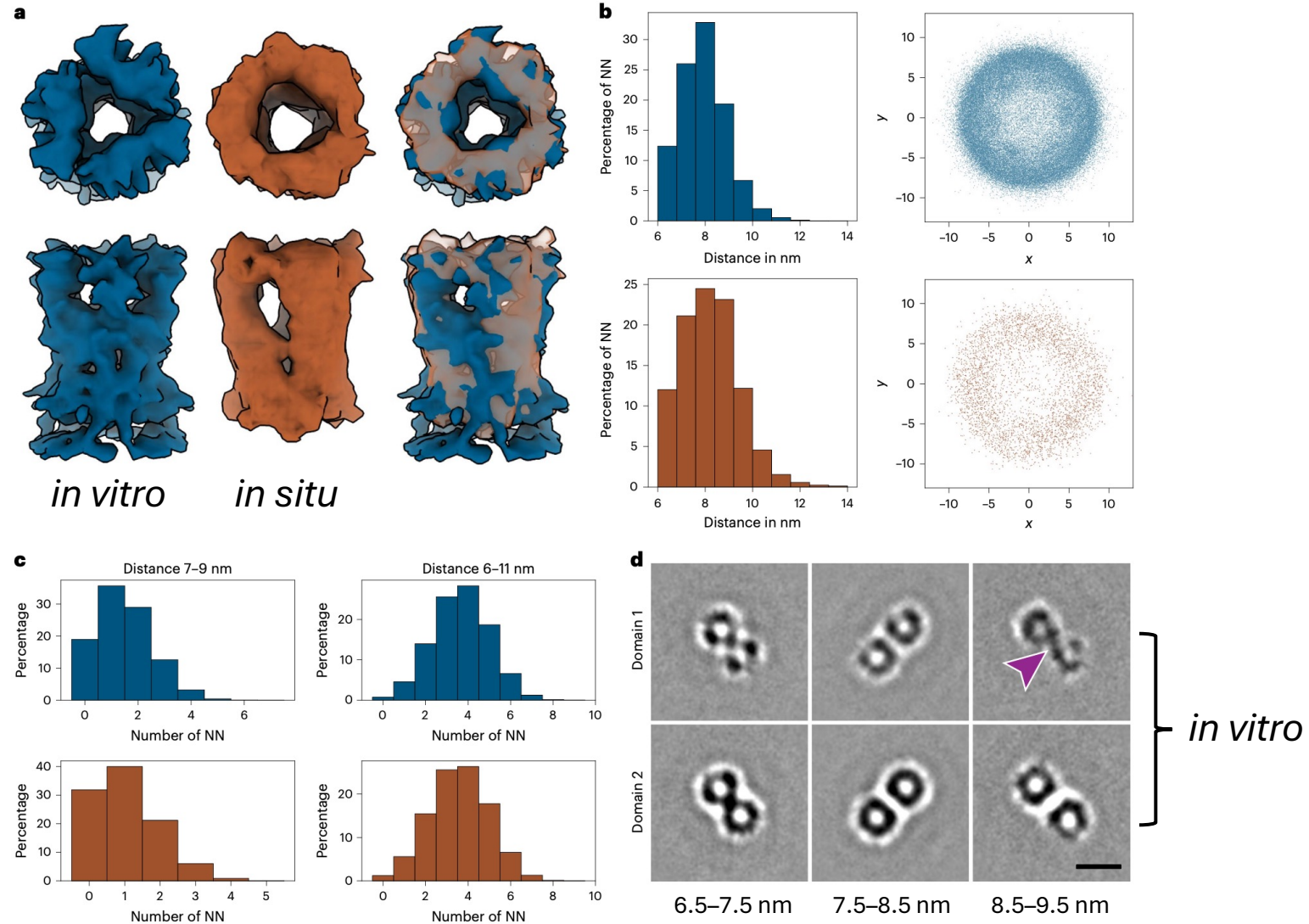
The palisade consists of trimers of major core protein A10



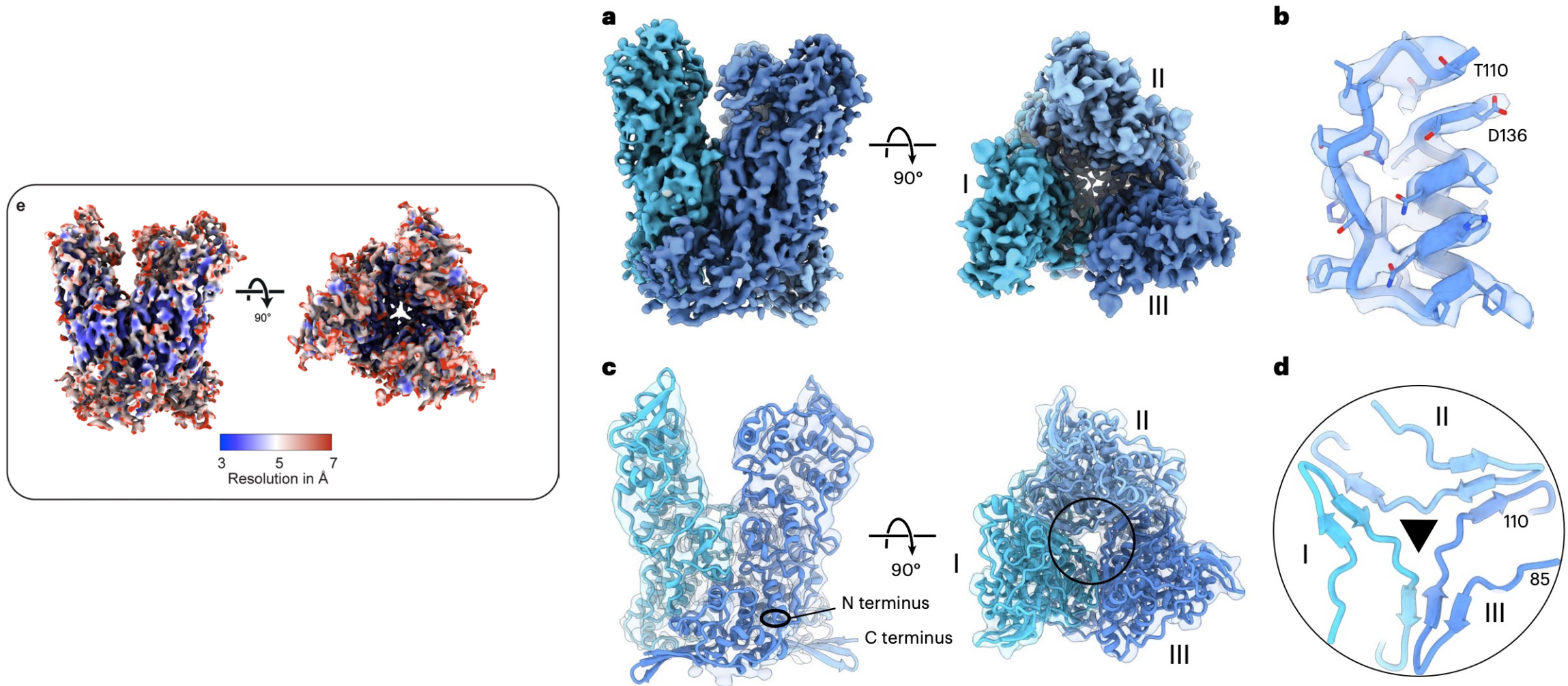
Classification of A10 trimers from VACV *in vitro* core palisade



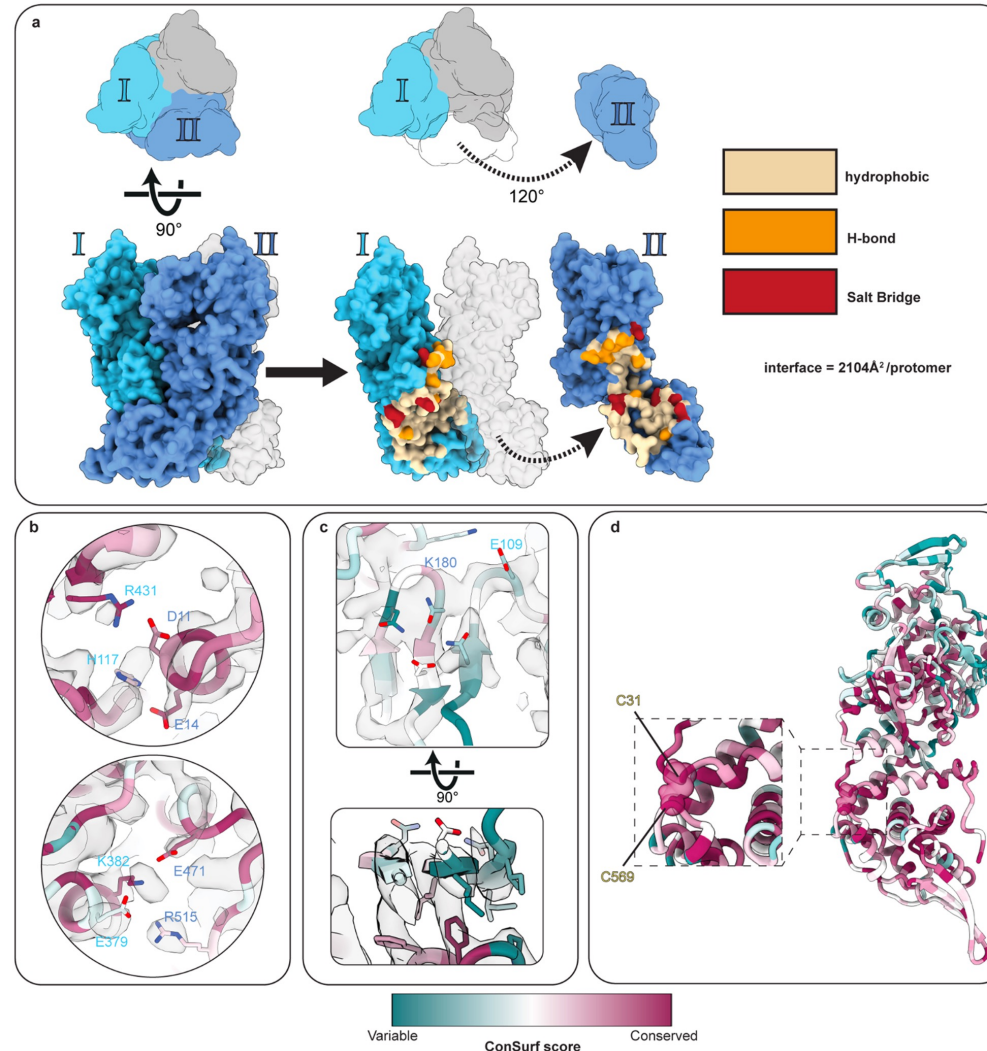
Cryo-ET structures of *in vitro* and *in situ* A10



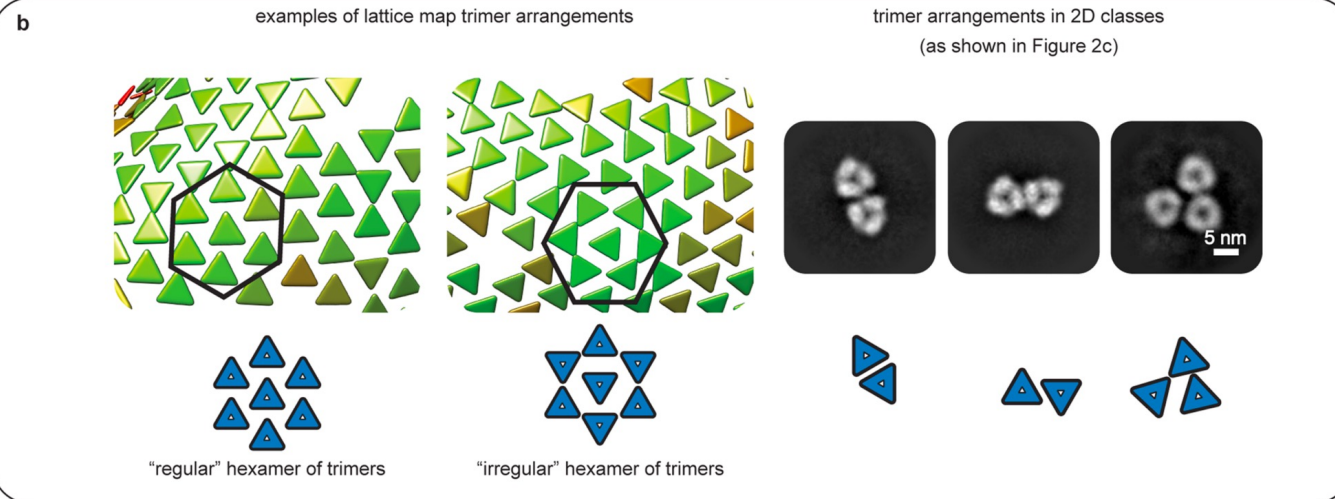
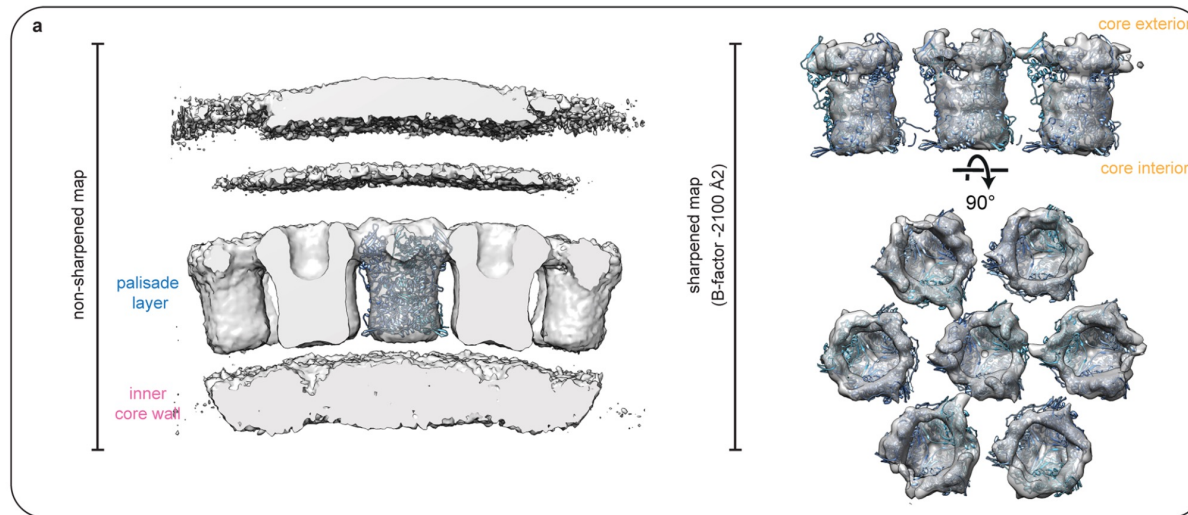
Single-particle cryo-EM structure of the A10 trimer



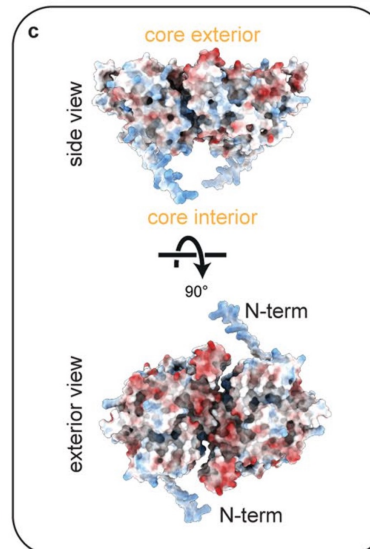
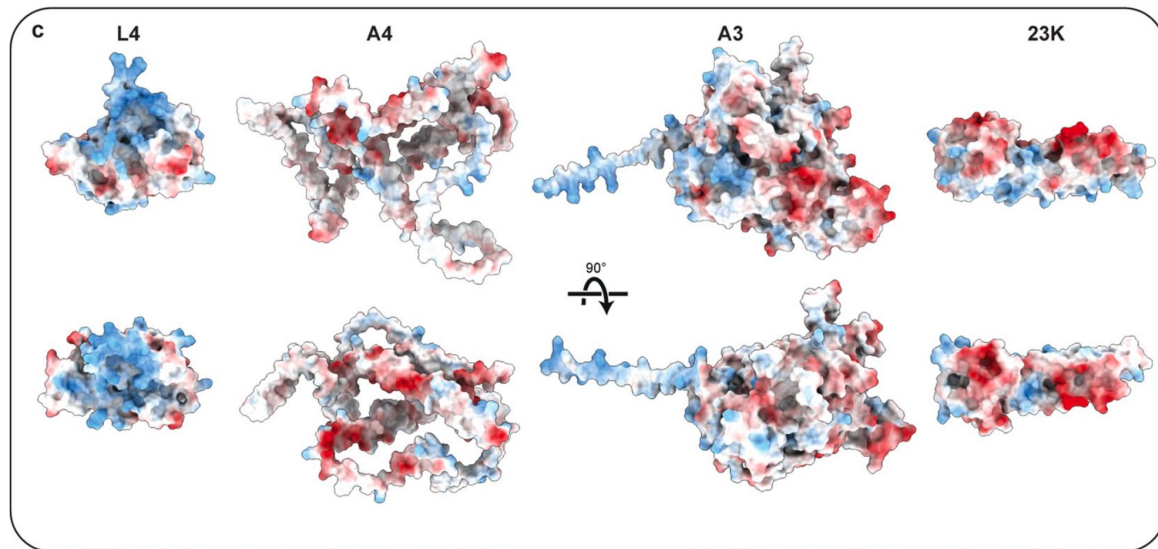
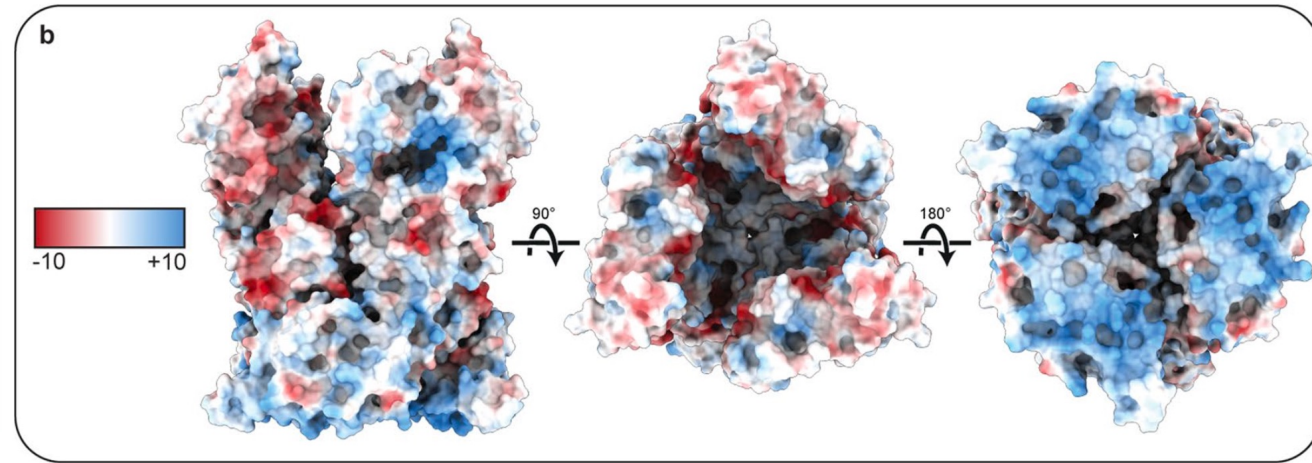
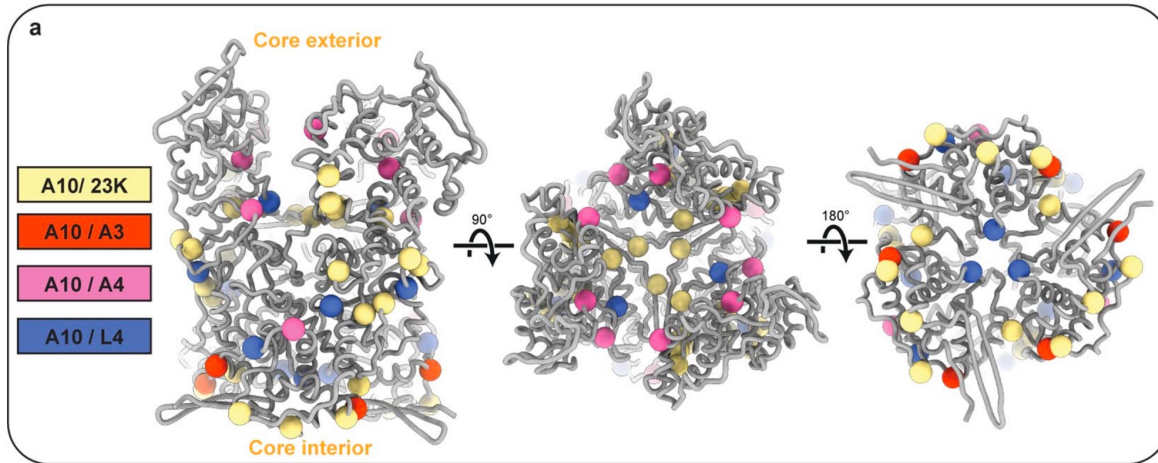
The A10 trimer is stabilized through extensive interactions



The palisade layer forms weak interactions between trimers

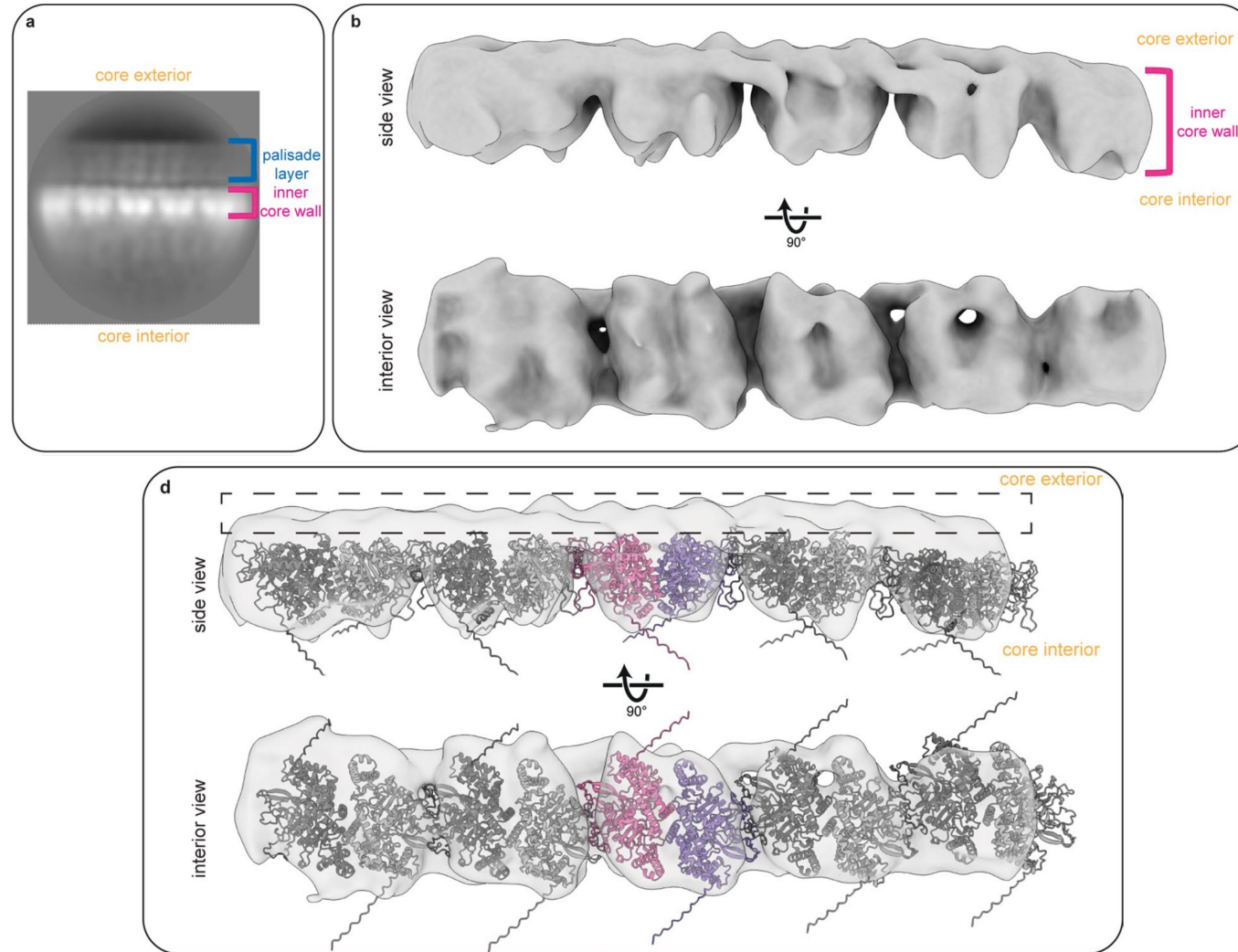


Possible interactions with other structural proteins



- **A3: maybe part of the inner core wall**
- A4: preferentially interacts with the exterior side of the A10

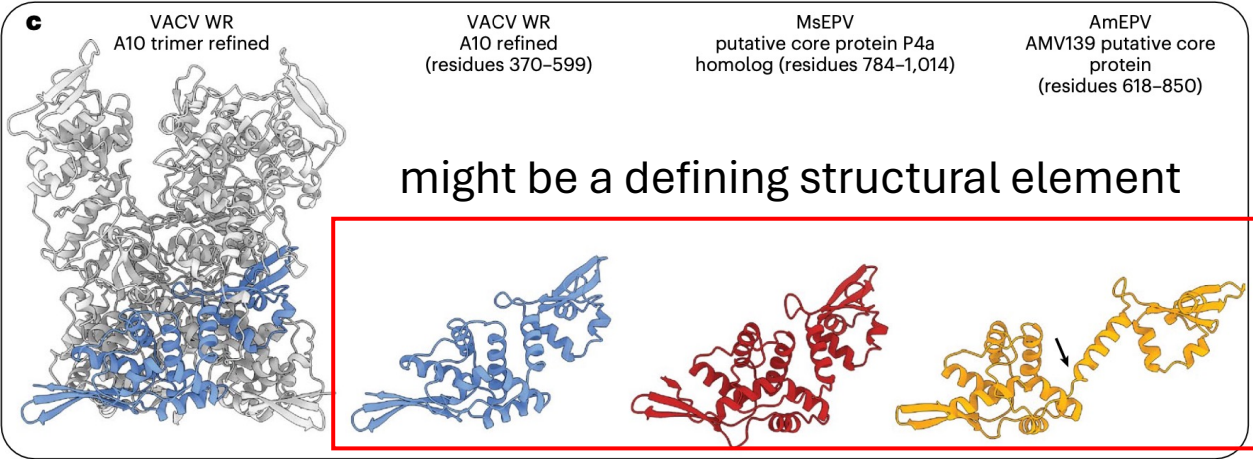
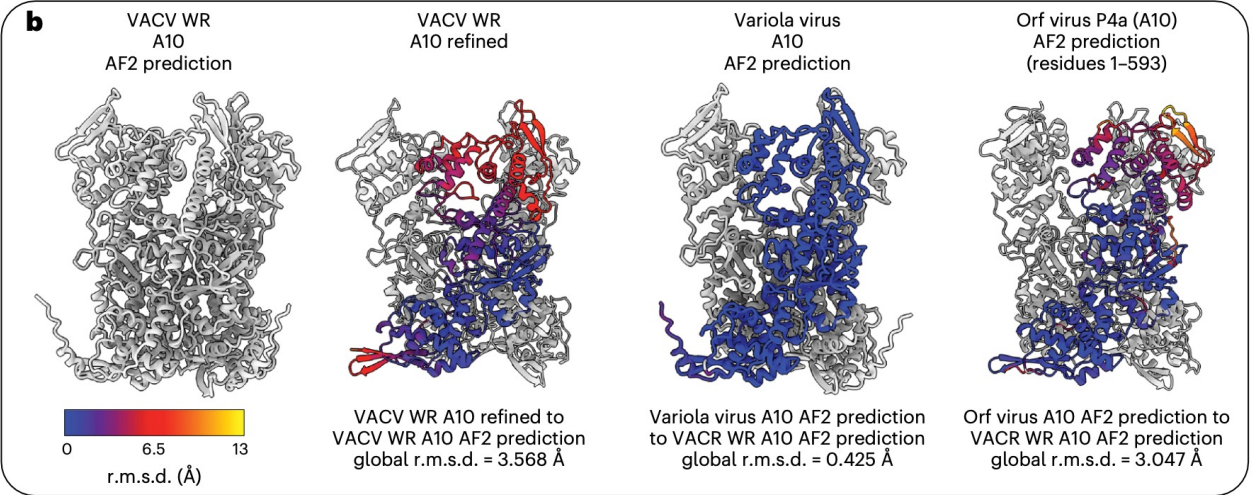
An A3 dimer fits to the density of the inner core wall units



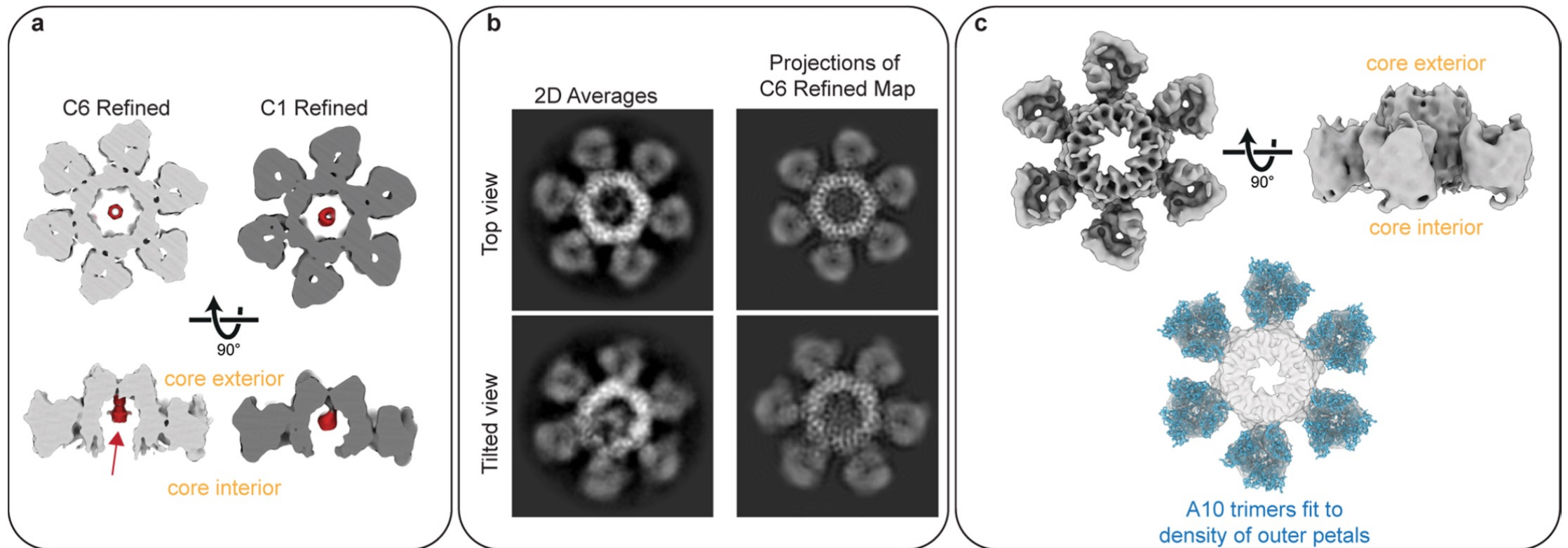
Comparison of VACV Western Reserve A10 trimers to other members of the poxvirus family

a

Taxonomy	Genus	Protein	GenBank Protein ID	Alignment length (nt)	Sequence identity (%)
Variola virus	Orthopoxvirus	A10	ABF23487.1	615	96.6
Monkeypox virus	Orthopoxvirus	A10	YP_010377118.1	614	96.6
Rabbitpox virus	Orthopoxvirus	A10	AAS49831.1	615	99.2
Cowpox virus	Orthopoxvirus	A10	ADZ29251.1	614	97.6
Ectromelia virus	Orthopoxvirus	A10	NP_671631.1	614	96.3
Orf virus	Parapoxvirus	P4a (A10)	AY386264.1	511	39.5
<i>Amsacta moorei</i> entomopoxvirus	Entomopoxvirus	AMV139 (putative core protein)	NP_064921.1	145/114	22.1
<i>Melanoplus sanguinipes</i> entomopoxvirus	Entomopoxvirus	Putative core protein P4a homolog	AF063866.1	293	21.2



Single particle cryo-EM structure of the flower-shaped pore



A10 as a shape-defining structural protein in the core wall

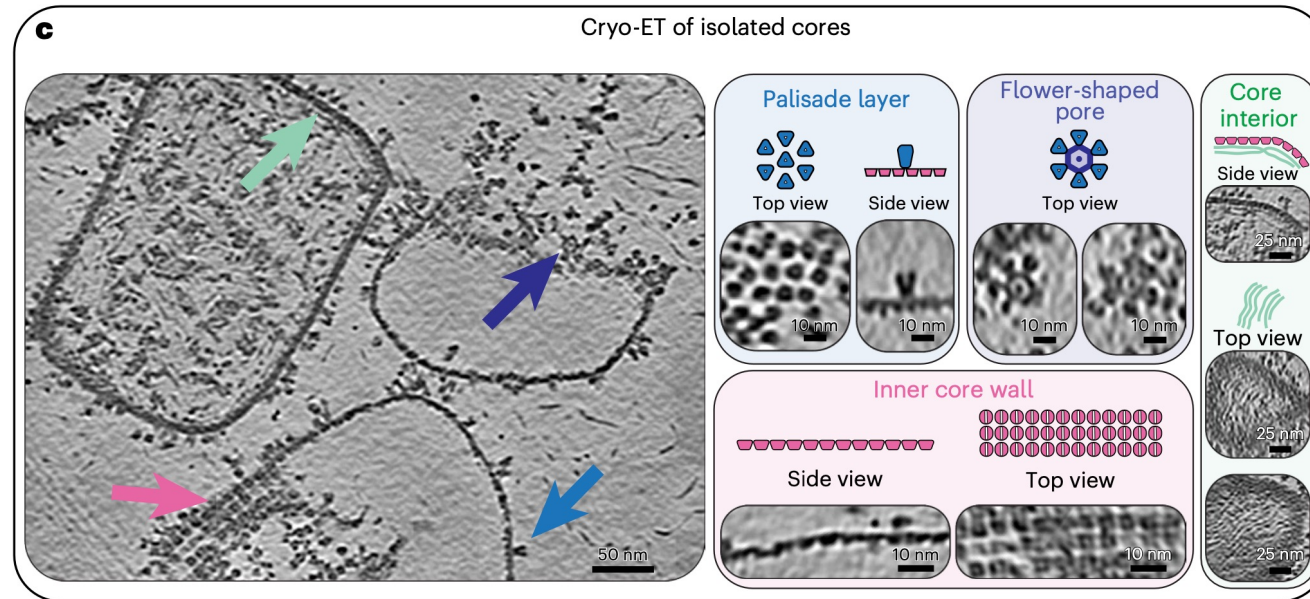
- A4在病毒核心外侧
- A10形成palisade layer
- A3形成inner core wall
- 23K有可能像之前说的在三聚体下方，也有可能pore内
- L4有可能也在pore内，或者在core腔内。

A10三聚体之间相互作用很弱，所以它不应该只起到稳定core的作用；

core的完整性应当由另一个layer实现，比如inner core wall，或者由A4在三聚体更外侧辅助

- 可以推测，**A10 trimers** 决定了**core**的形状，使其能够形成高度复杂的哑铃型。

A10 as a shape-defining structural protein in the core wall

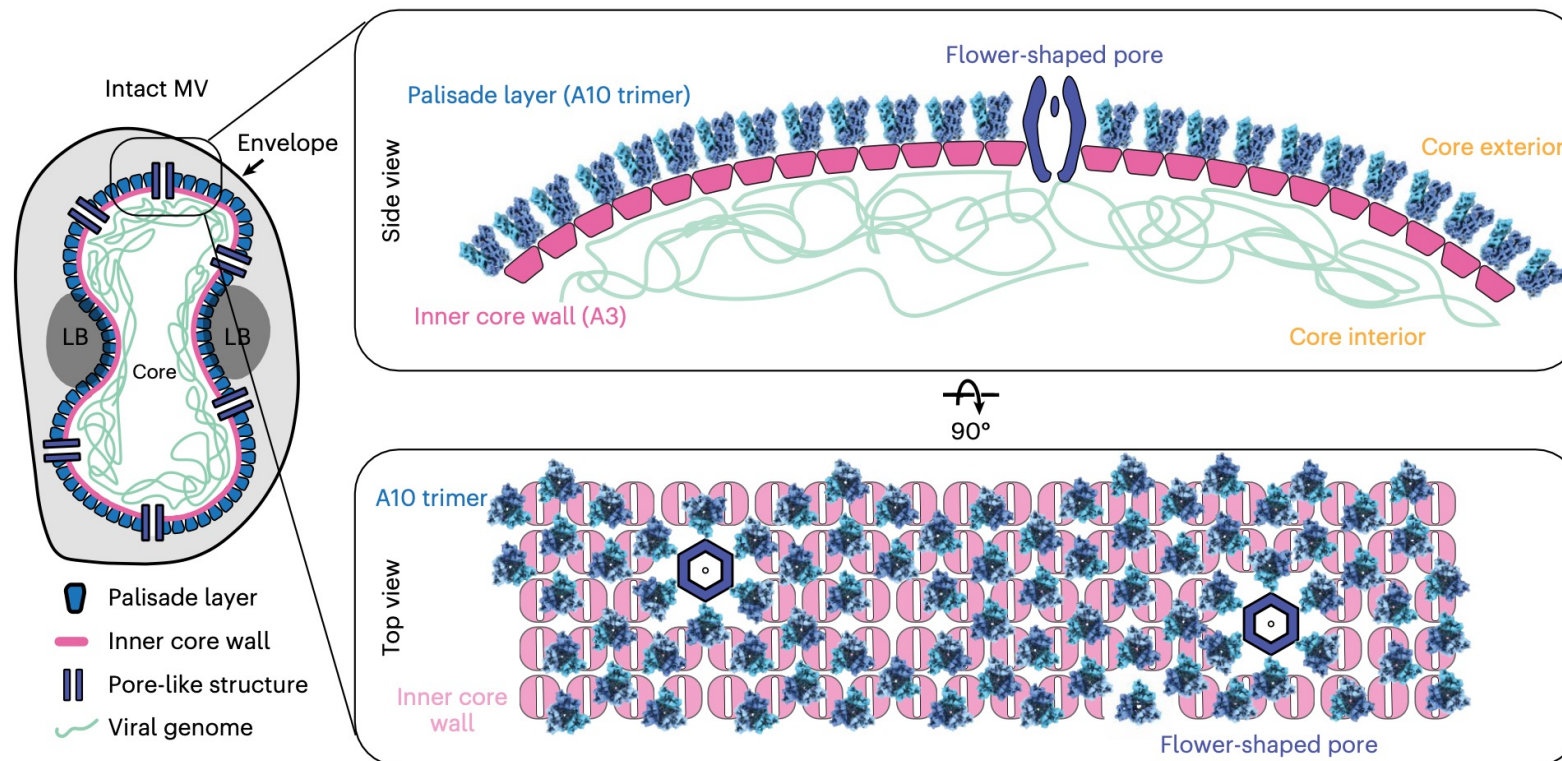


- A10的缺失会导致MVs无法正确聚集，而此时inner core wall会变成stacks或者sheet-like architecture。说明A10主要影响viral core的形状和曲率，而inner core wall则更倾向于通过更强的侧向相互作用起到稳定core的作用。
- 在isolated cores中也可以看出，虽然部分trimers已经从core表面脱落，但是core和inner core wall本身是完整的

Summary

Summary

- place previously obtained descriptions of protein interactions and locations within the core wall into perspective
- provide a more detailed model of poxvirus core architecture



Future work...

- change the purification protocols for cores and improve sample vitrification protocols to reduce the observed preferred orientation
- in vitro reconstitution of structural core proteins
- the experimental structures and functions of L4/23K/A4/F17
- flower-shaped pore