

# The interplay between dengue morphological diversity and antibody recognition.

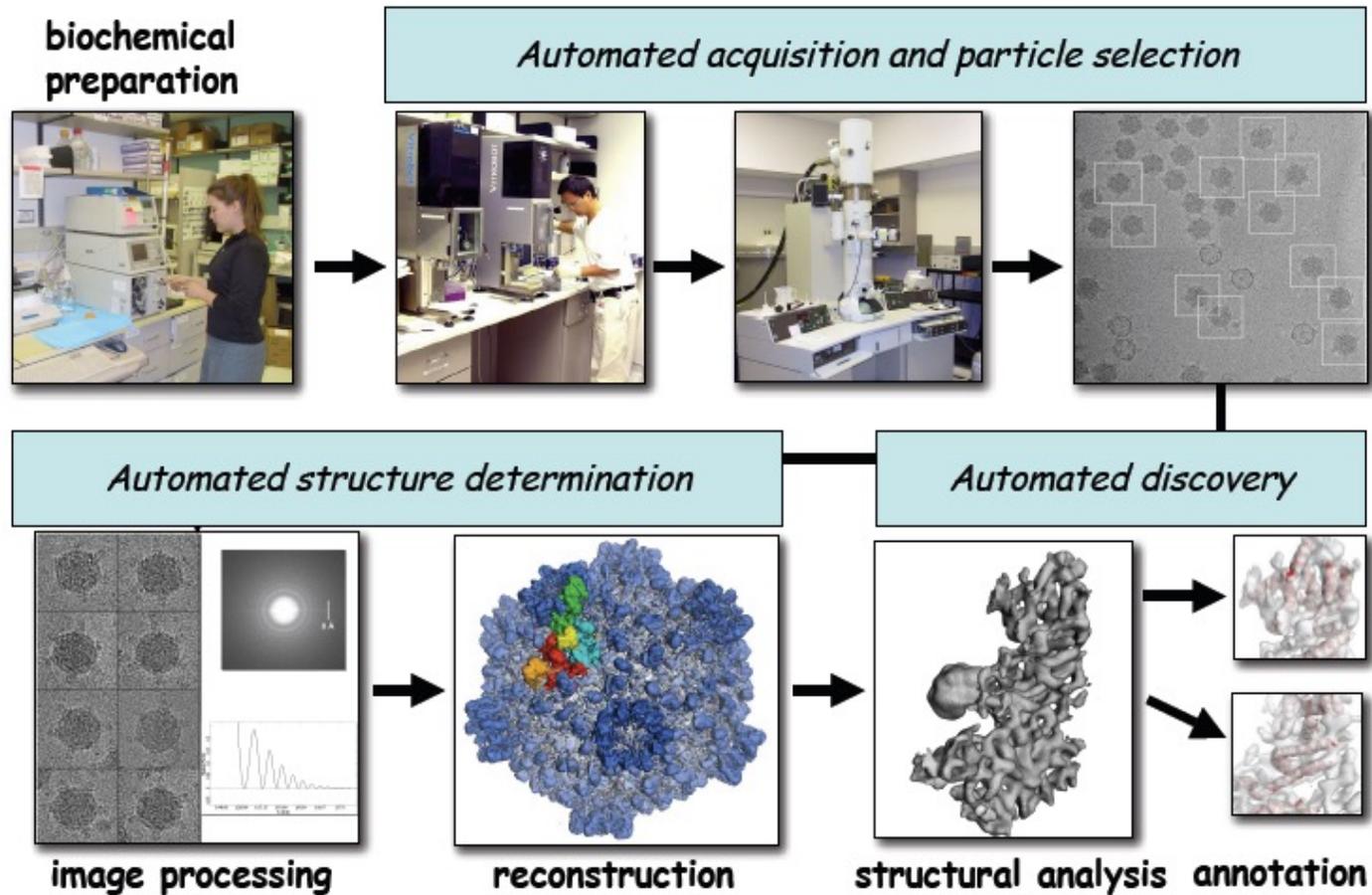
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Dept of Biological Sciences, CBIS, NUS



# Cryo-electron microscopy (CryoEM) single particle analysis



Courtesy by Wen Jiang

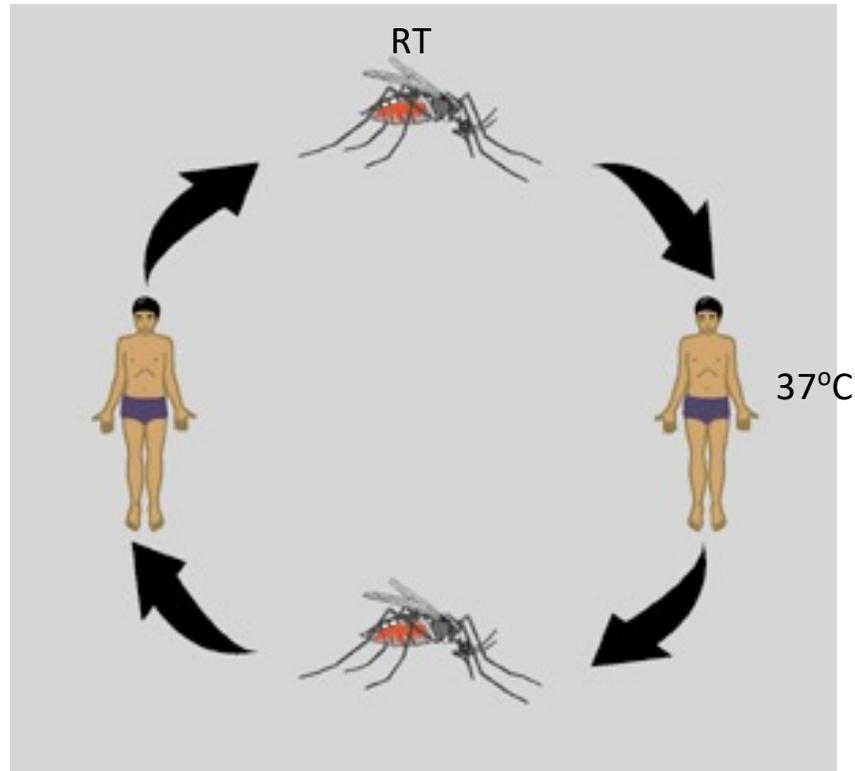
# Single Particle analysis

- There is a homogenous population of particles e.g. dengue virus
- The particles differ by orientation
- Able to average thousands to millions together
- Able to obtain high resolutions structure: latest 1.2Å apoferritin structure.

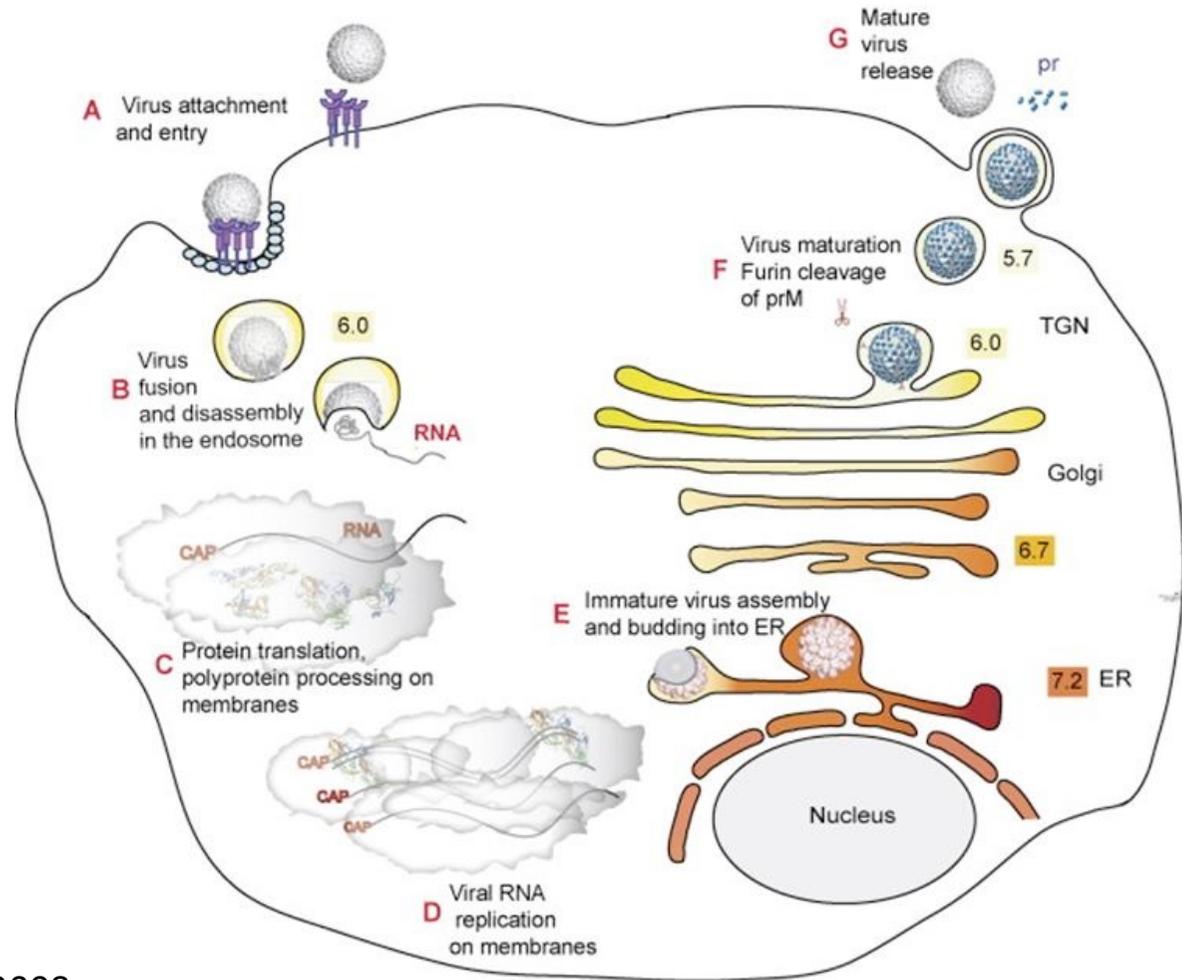
# Dengue virus

- Positive-sense RNA virus
- Dengue serotypes: dengue 1, 2, 3 and 4
- Disease
  - mild Dengue Fever: biphasic fever, rash, muscle and joint pain
  - more severe Dengue Hemorrhagic Fever (DHF) - all above + hemorrhagic manifestation + plasma leakage
- No highly effective vaccine
- No therapeutics

# Host-vector transmission



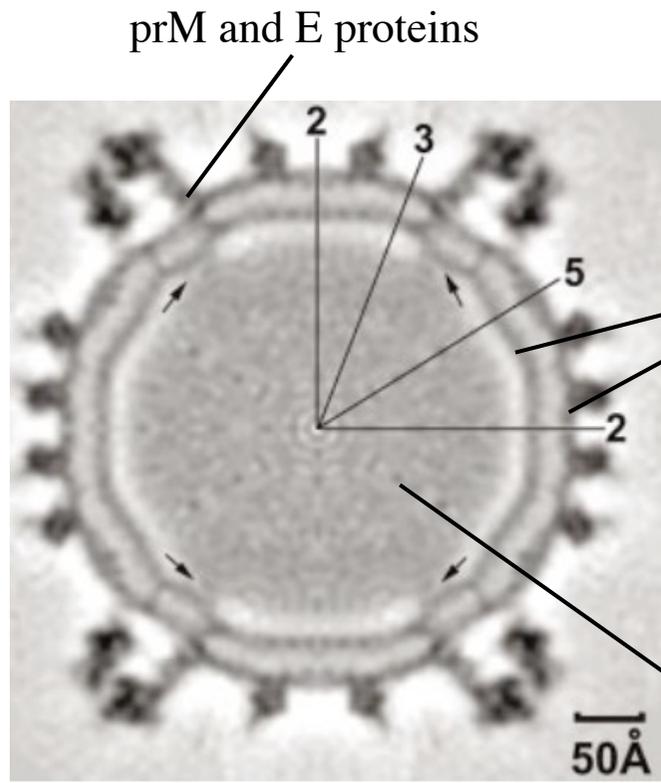
# Dengue virus infection cycle



Yu et al., 2008

[doi:10.1016/j.antiviral.2008.05.004](https://doi.org/10.1016/j.antiviral.2008.05.004)

# Immature DENV



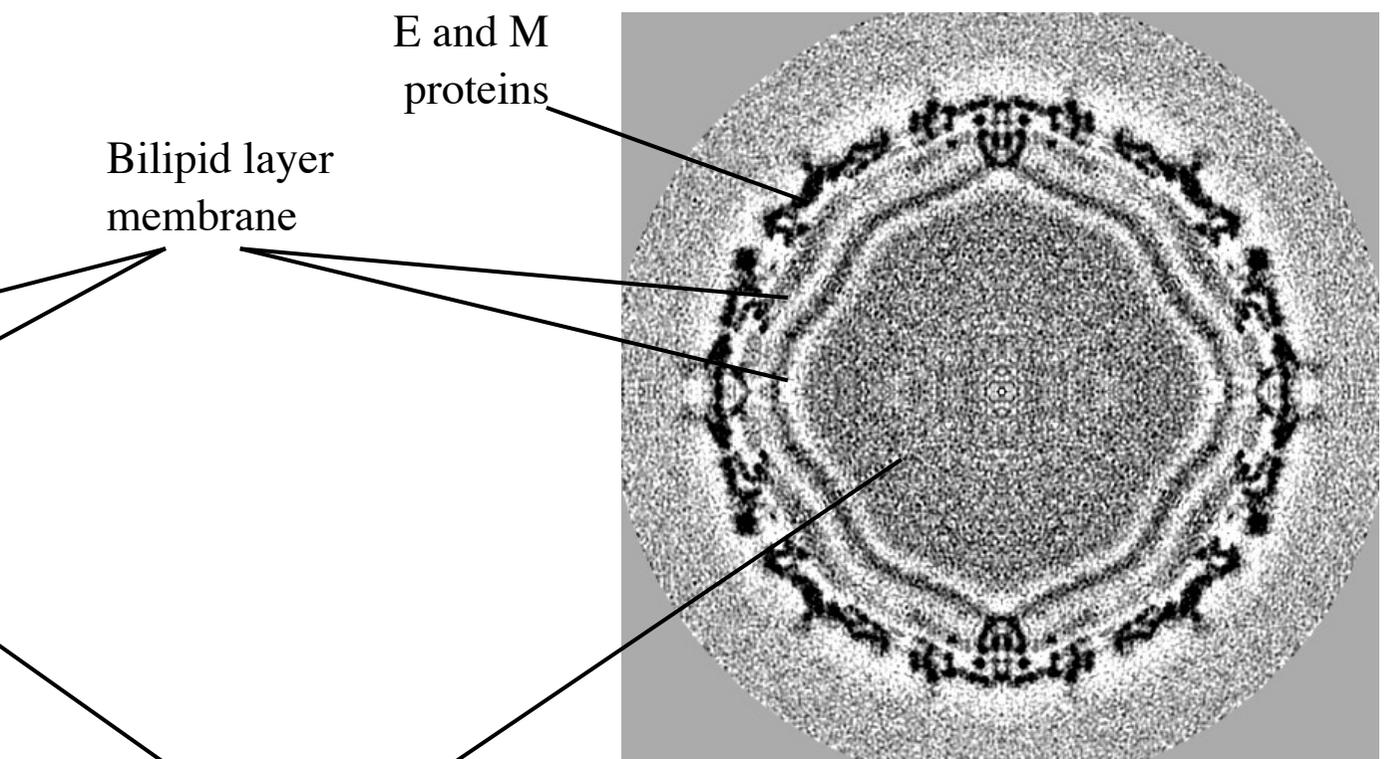
prM and E proteins

2 3 5 2

50Å

Zhang *et al.*, 2003

# Mature DENV



E and M proteins

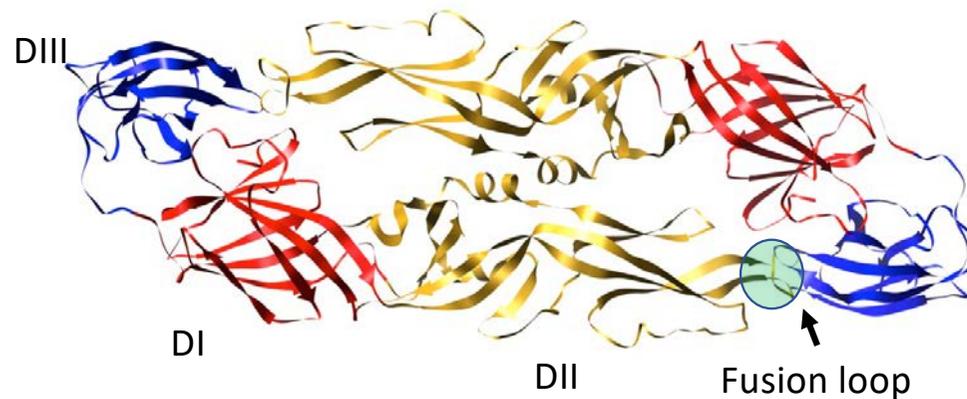
Bilipid layer membrane

Positive-sense RNA with capsid proteins

Kostyuchenko *et al.*, 2013

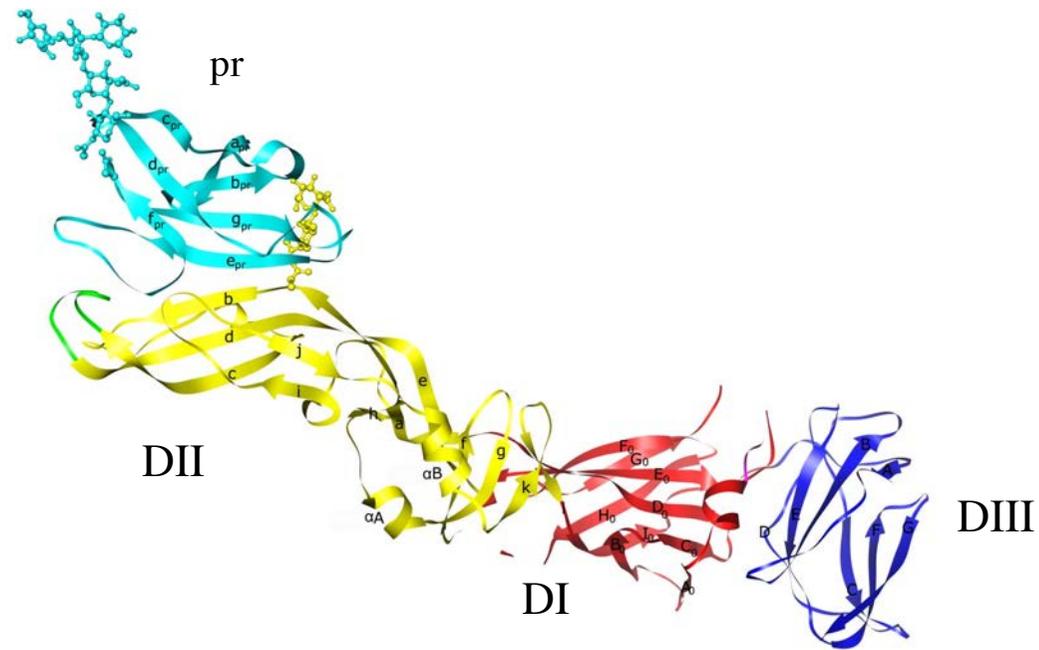
# Crystal structure of E protein dimer similar to that on the mature virus

- Major surface protein targeted by antibodies
- Important for entry: receptor binding and fusion to the endosomal membrane



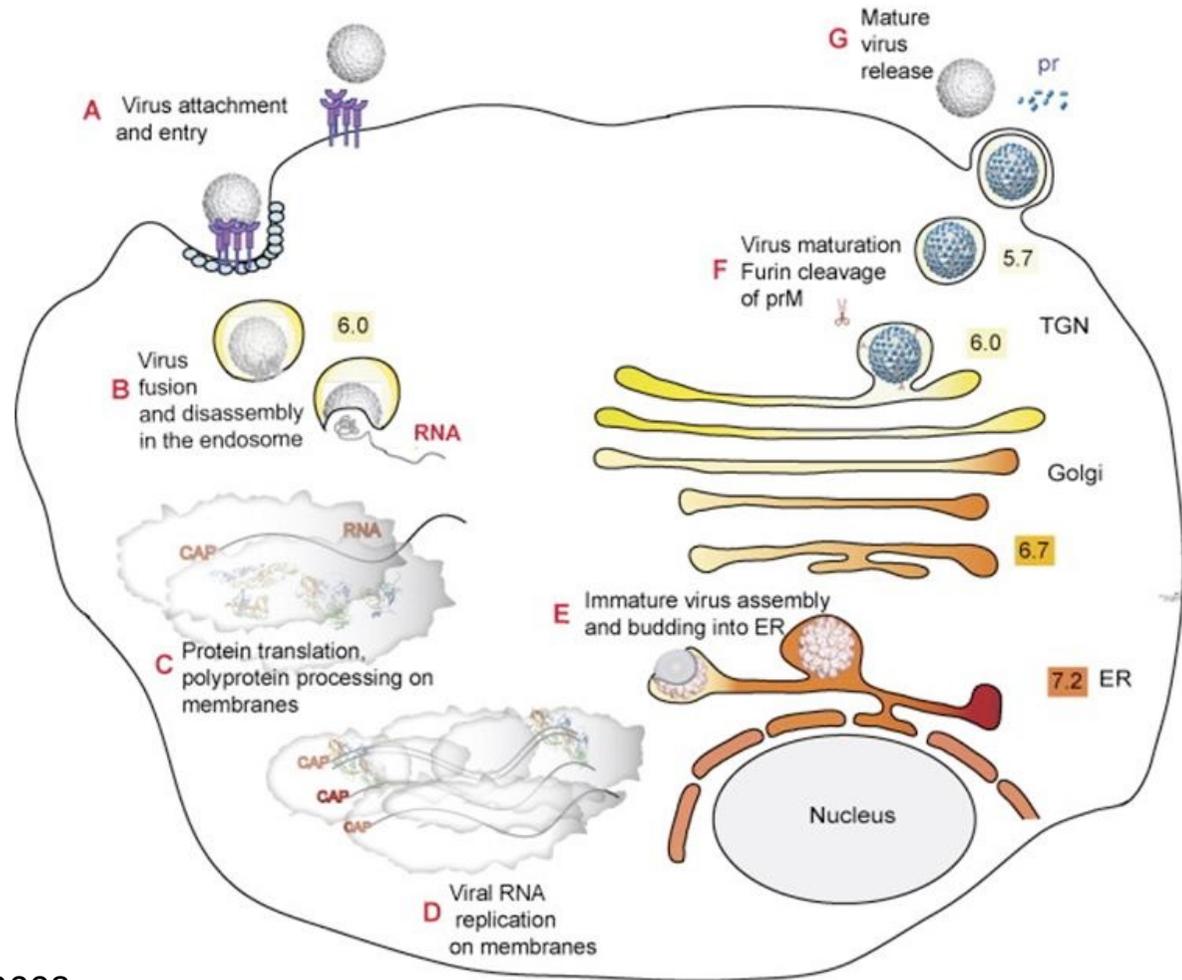
Modis *et al.*, 2003, Zhang *et al.*, 2004

# Crystal structure of prM-E complex of immature virus



Li et al., 2008, Science

# Dengue virus infection cycle



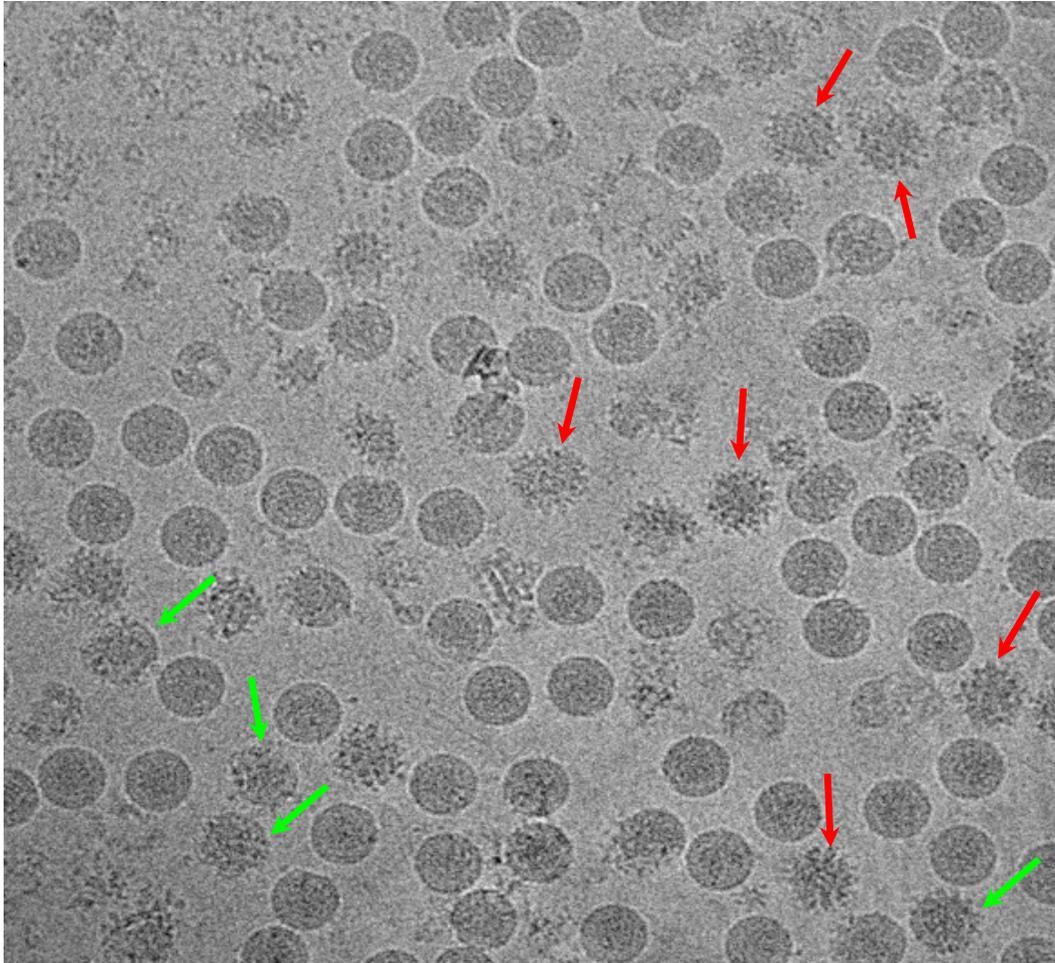
Yu et al., 2008

doi:10.1016/j.antiviral.2008.05.004

# Heterogeneity of DENV virus particle morphology

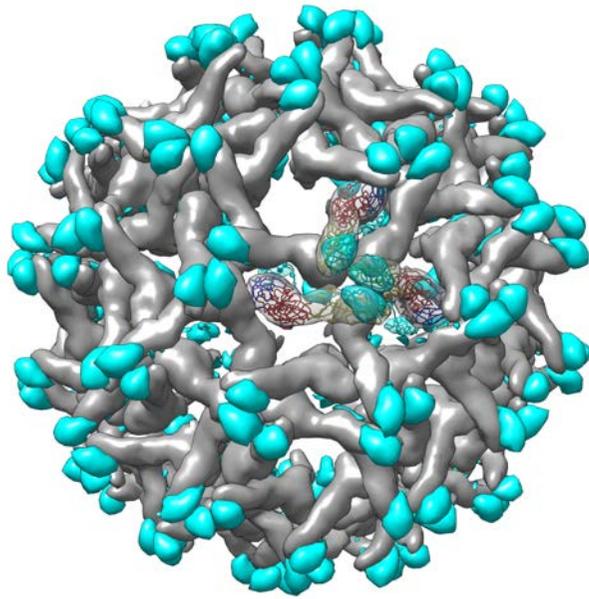
## Part 1: different maturation states

## Dengue virus when infected in mosquito cell line (C6/36) at 29°C

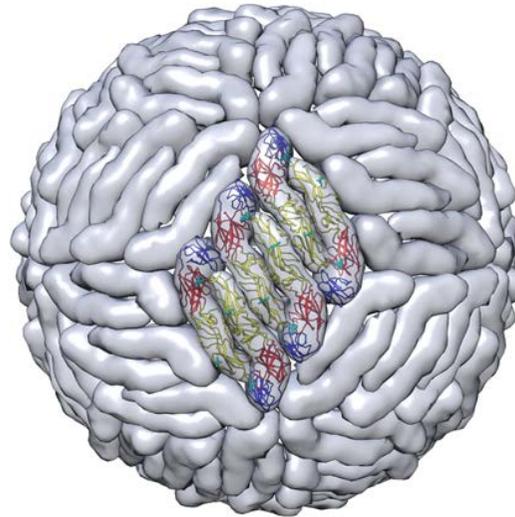


- Mature virus
  - Smooth round spherical particles
- Highly immature virus
  - Spikey particles
- Partially immature/mature virus
  - part smooth and part spikey particles
- The maturation process of virus inside cell is not efficient

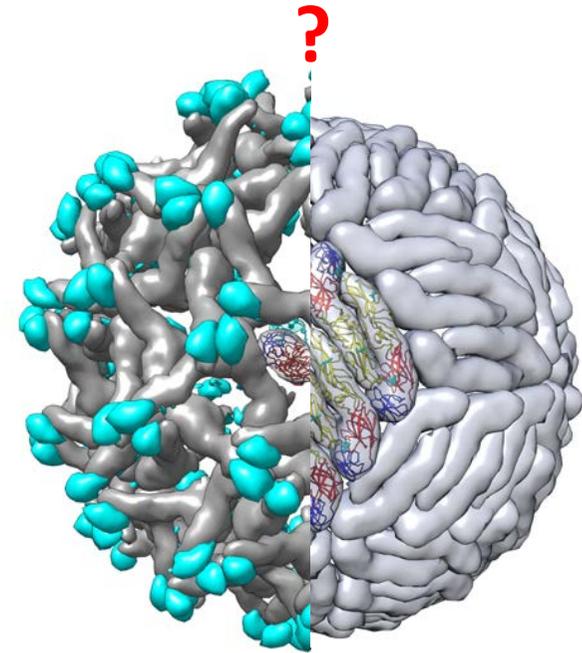
# Virus released from the cell, can have different maturation states



Fully immature virus  
7Å resolution



Compact smooth surface  
mature virus  
3.7Å resolution



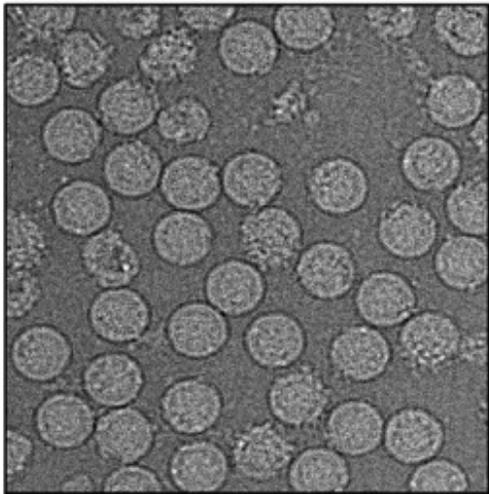
Partially immature/mature

Part 2: Mature virus can have an  
alterative structure

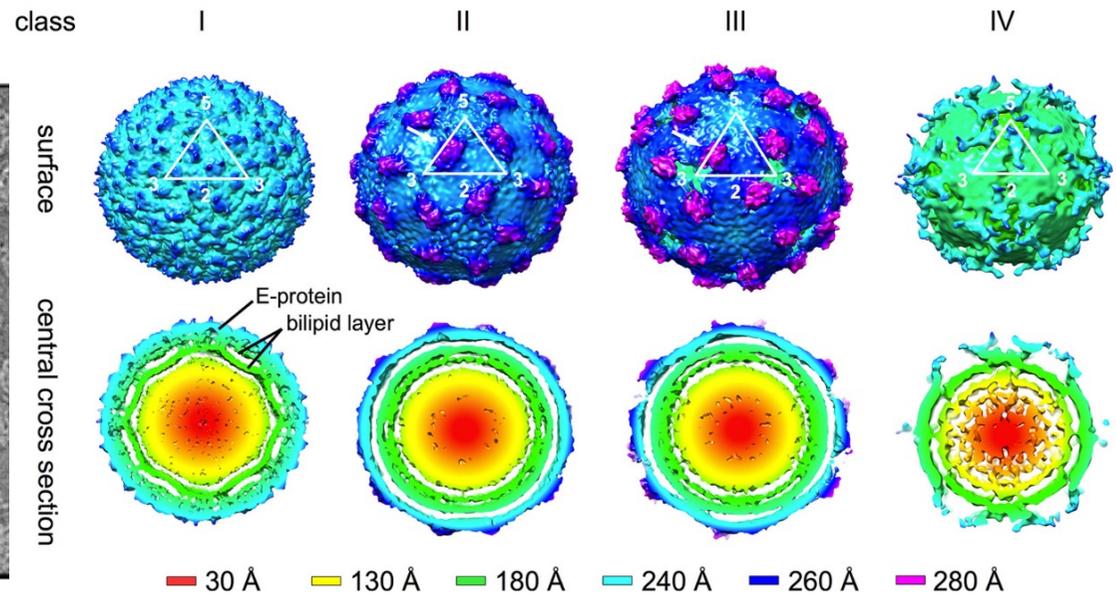
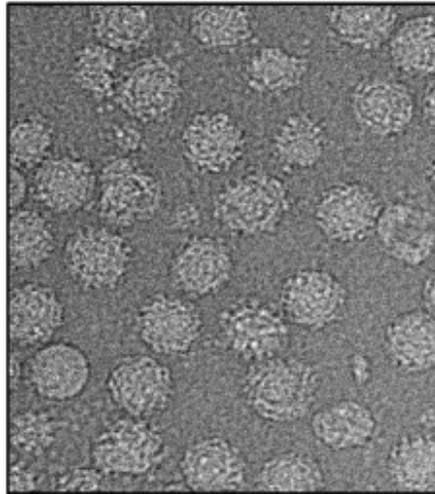
The warm bumpy surface structure

# DENV2 (lab passaged strains) at 37°C

29°C



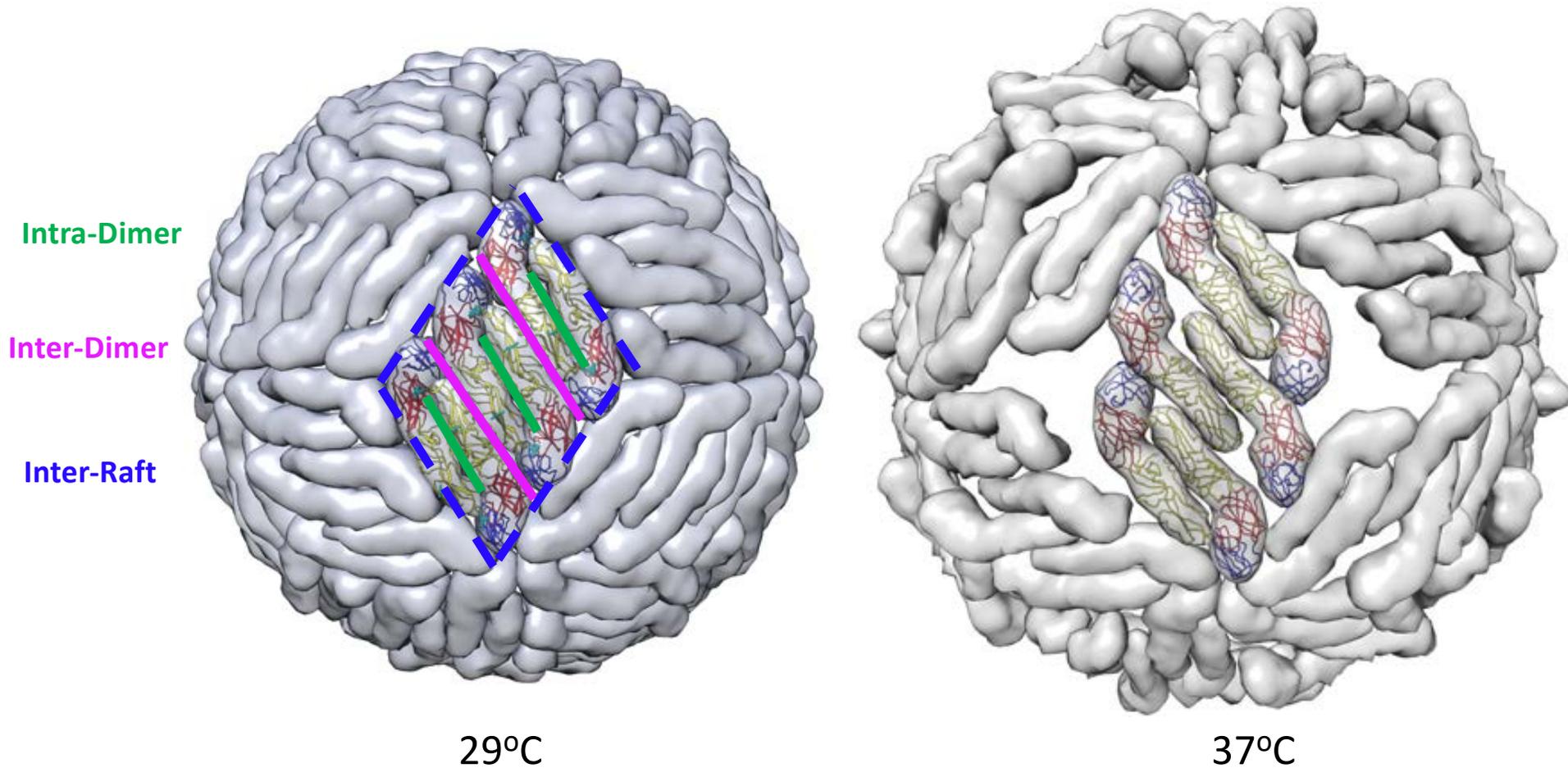
37 °C



- The increase temperature from 29°C to 37°C result in the structural change of DENV2 NGC from smooth compact to bumpy expanded morphology
- Different structures in mosquitoes and human hosts

Fibriansah *et al.* (2013) Journal of Virology

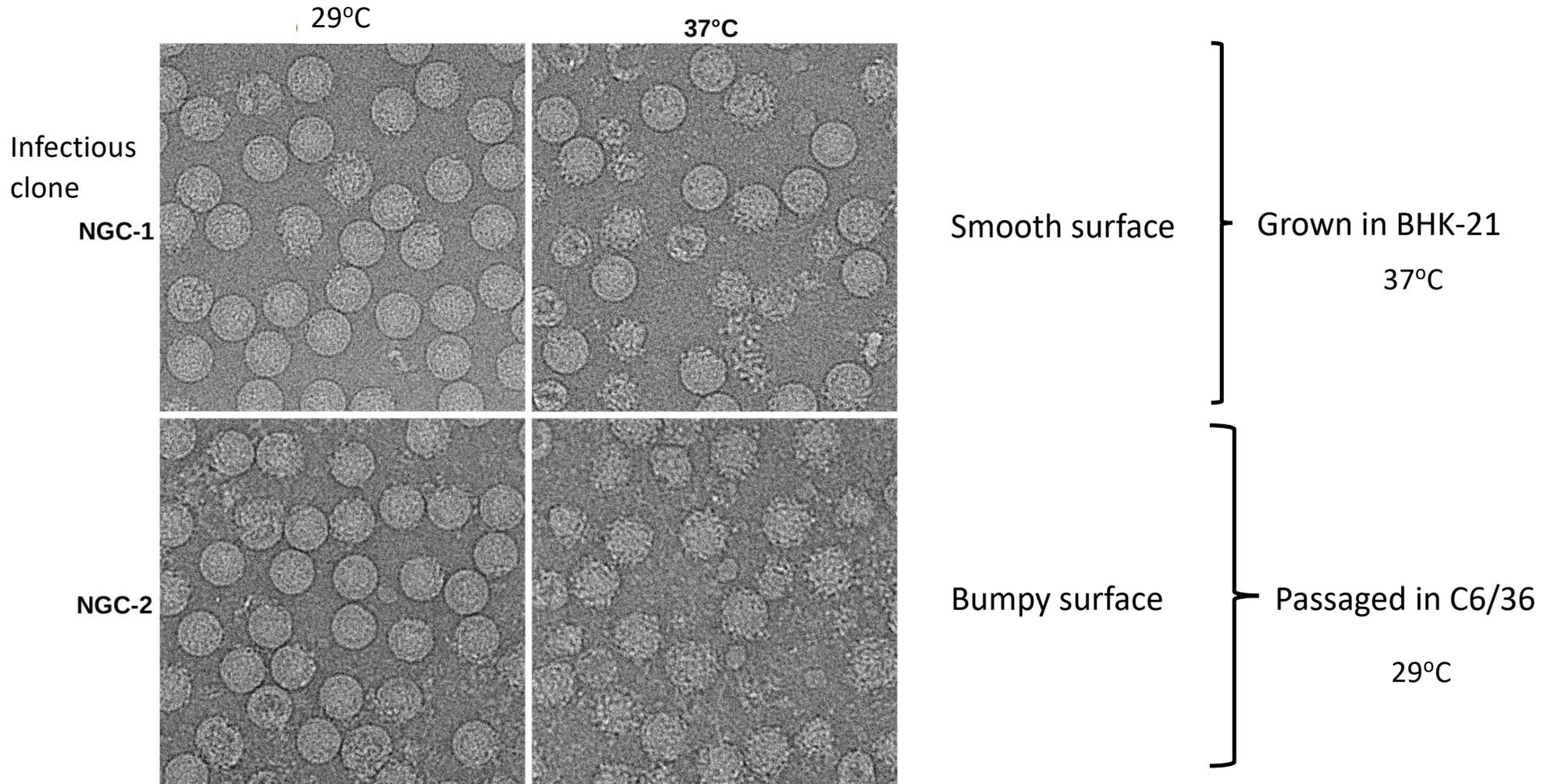
## Bumpy mature DENV2 structure at 37°C



Virus quaternary structure can change when induced by elevated temperature

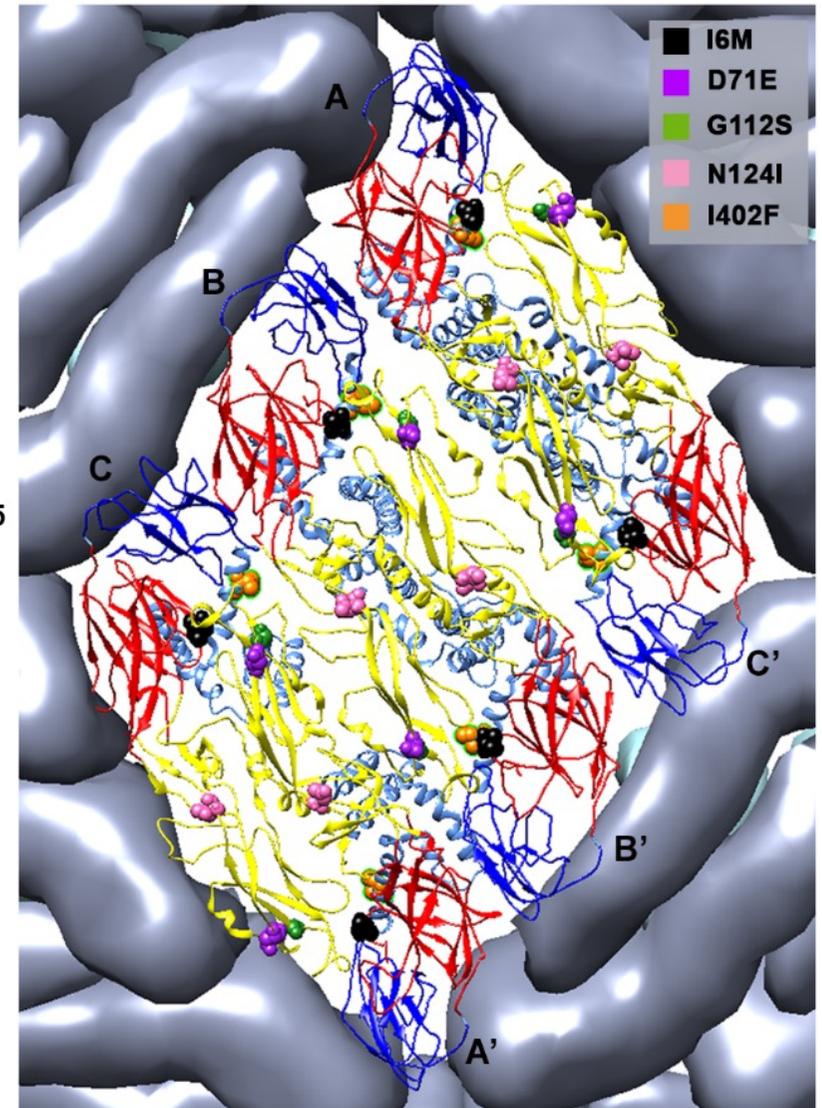
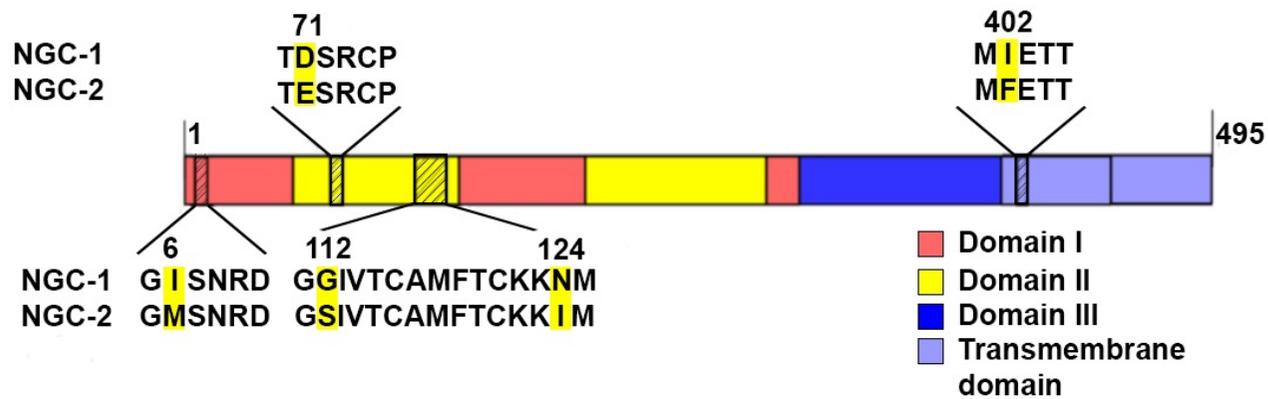
What are the E protein molecular determinants that cause the change from smooth to bumpy surface particles?

# DENV2 NGC strains with different passage history

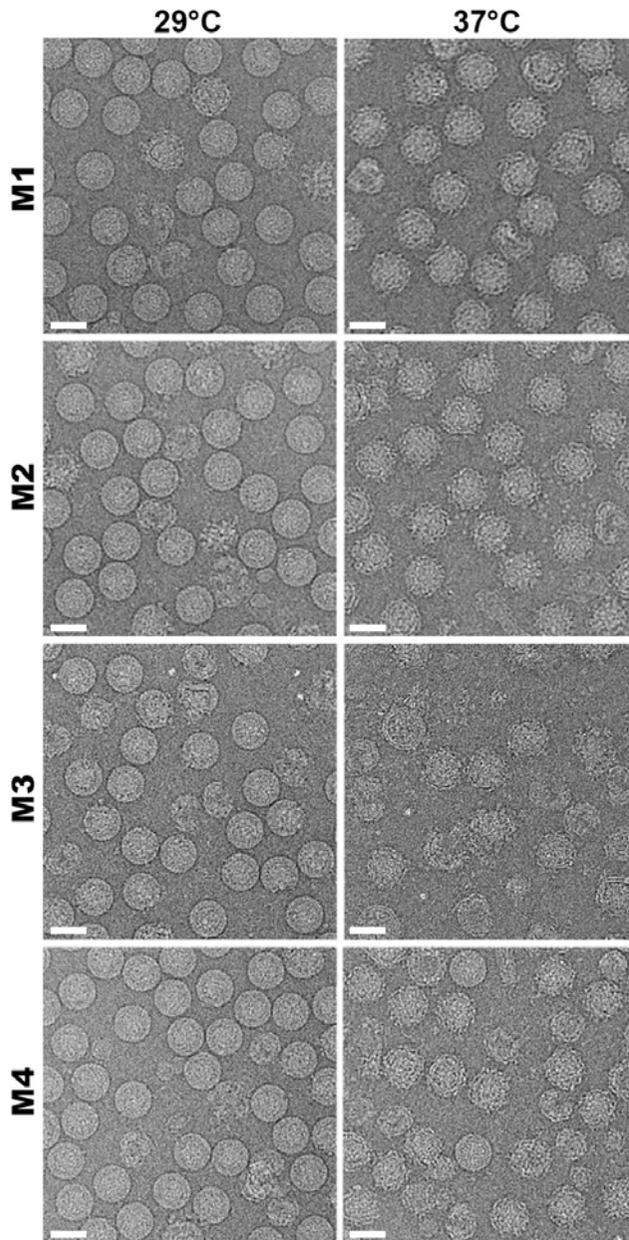


# Between NGC-1 and NGC-2

- Only 5 residues differences



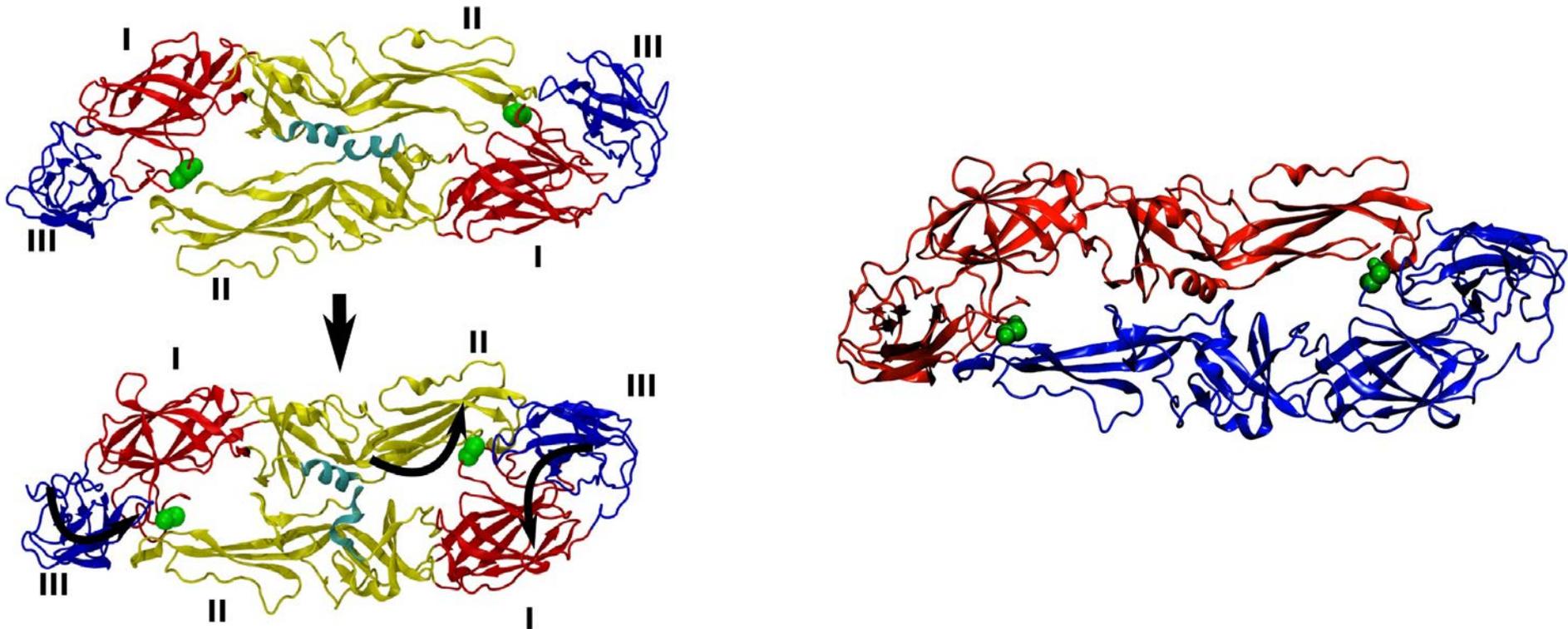
Mutations done on the smooth surfaced NGC-1 infectious clone to that of NGC-2



Mutant		M1	M2	M3	M4
Mutations done on NGC-1 backbone	I6M	✓	✓		✓
	D71E	✓	✓	✓	
	G112S	✓	✓	✓	
	N124I	✓			
	I402F	✓	✓	✓	

Different mutations can cause virus to turn bumpy

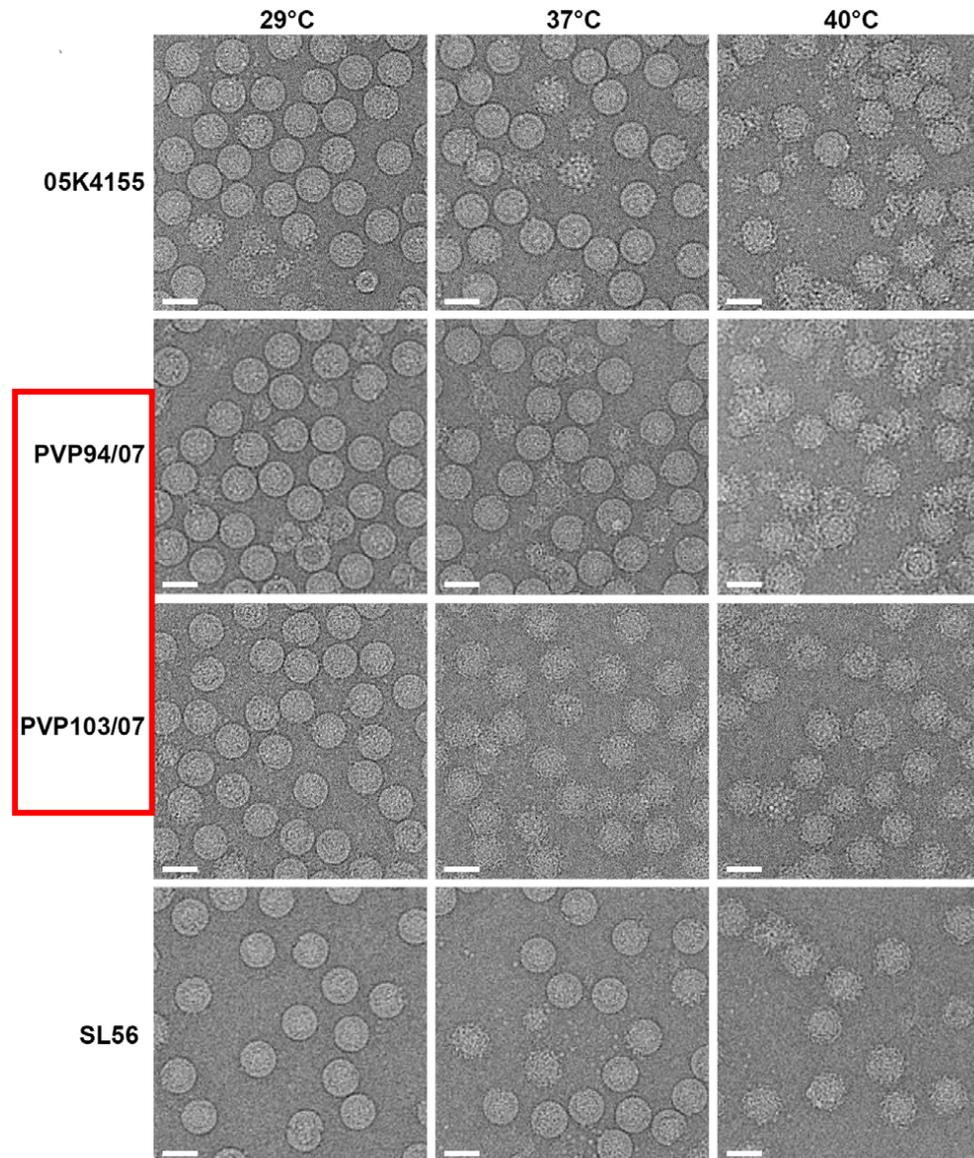
# How I6M mutation on NGC-1 affects the E protein dimer conformation?



- Domain I shifts “outwards” with respect to domain III of each chain
- Domain II then reoriented leading to a change in the relative position of the two antiparallel helical segments at the centre of the dimer

# DENV2 clinical isolates

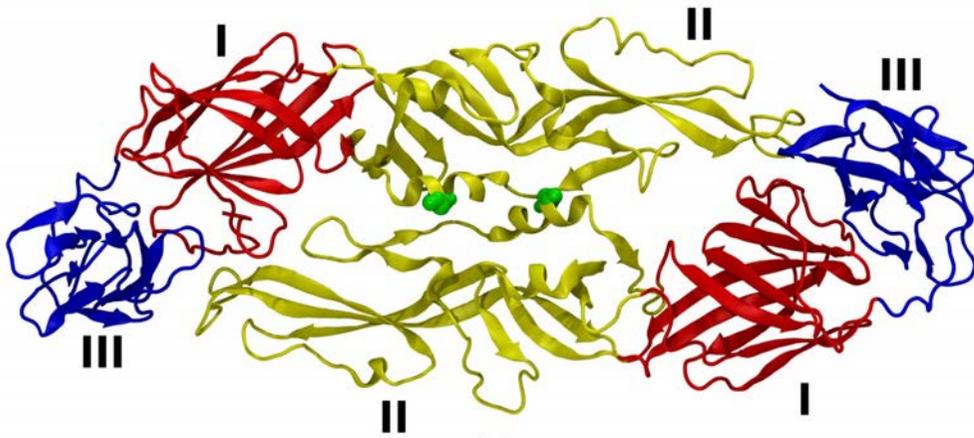
- Implications for
- (1) Vaccine
  - (2) Prophylactic antibody treatment
  - (3) Therapeutic antibody treatment



Lim et al., 2019  
PLOS Pathogen

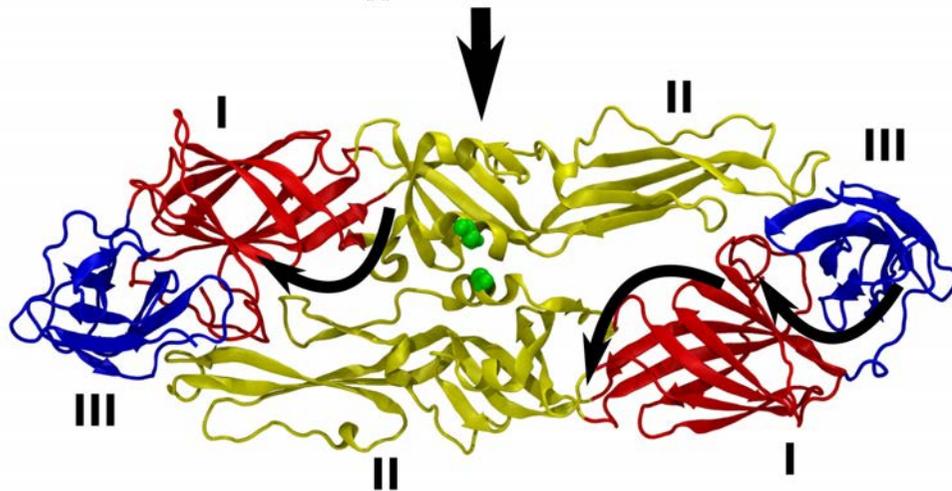


## T to M mutation at position 262



Round compact virus structure

T262



Bumpy surface virus structure

M262

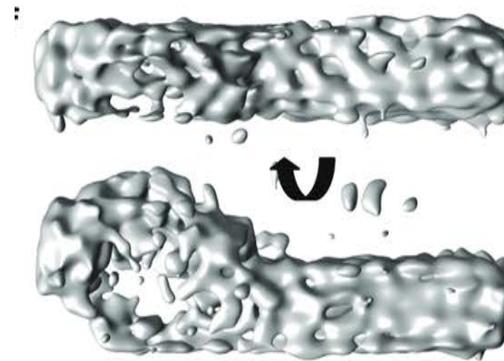
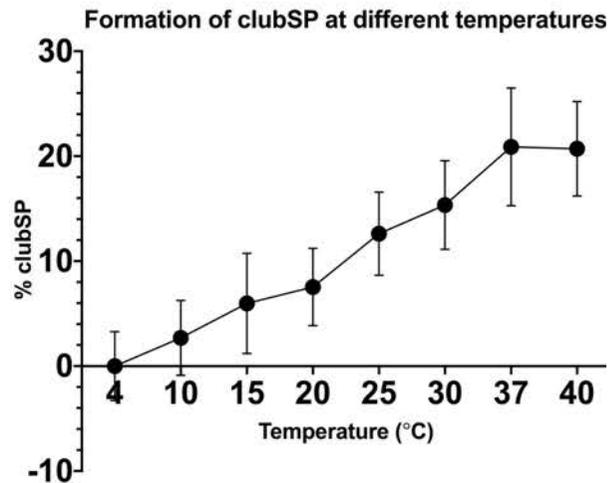
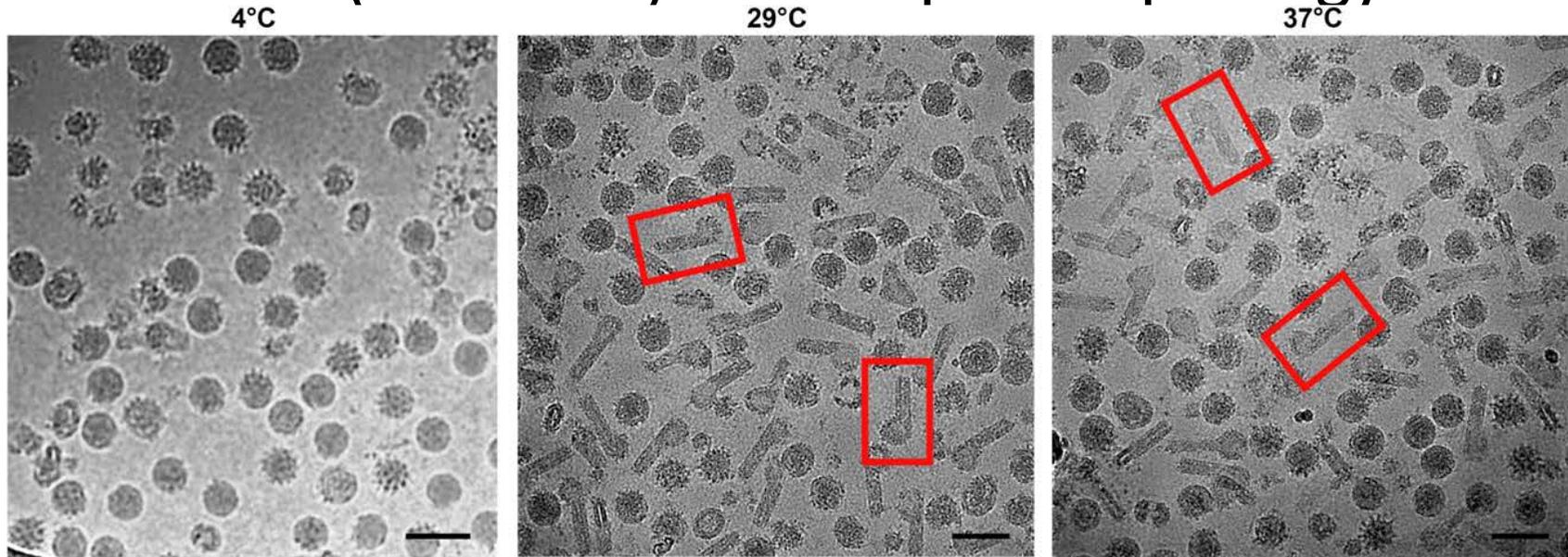
# Summary

- Subtle mutation at different residues can have effect on DENV2 morphology
  - Unlikely to predict with confidence the morphology from the primary sequence
- Mutations (e.g. I6M and T262M) that affect the intra-dimer interface are likely to result in quaternary rearrangement of E protein at 37°C leading to bumpy virus morphology
- Amongst the clinical strains, most have smooth surface at 37°C, whereas the laboratory strains are mostly bumpy surfaced.

## Part 3: DENV3, a more structurally complicated virus

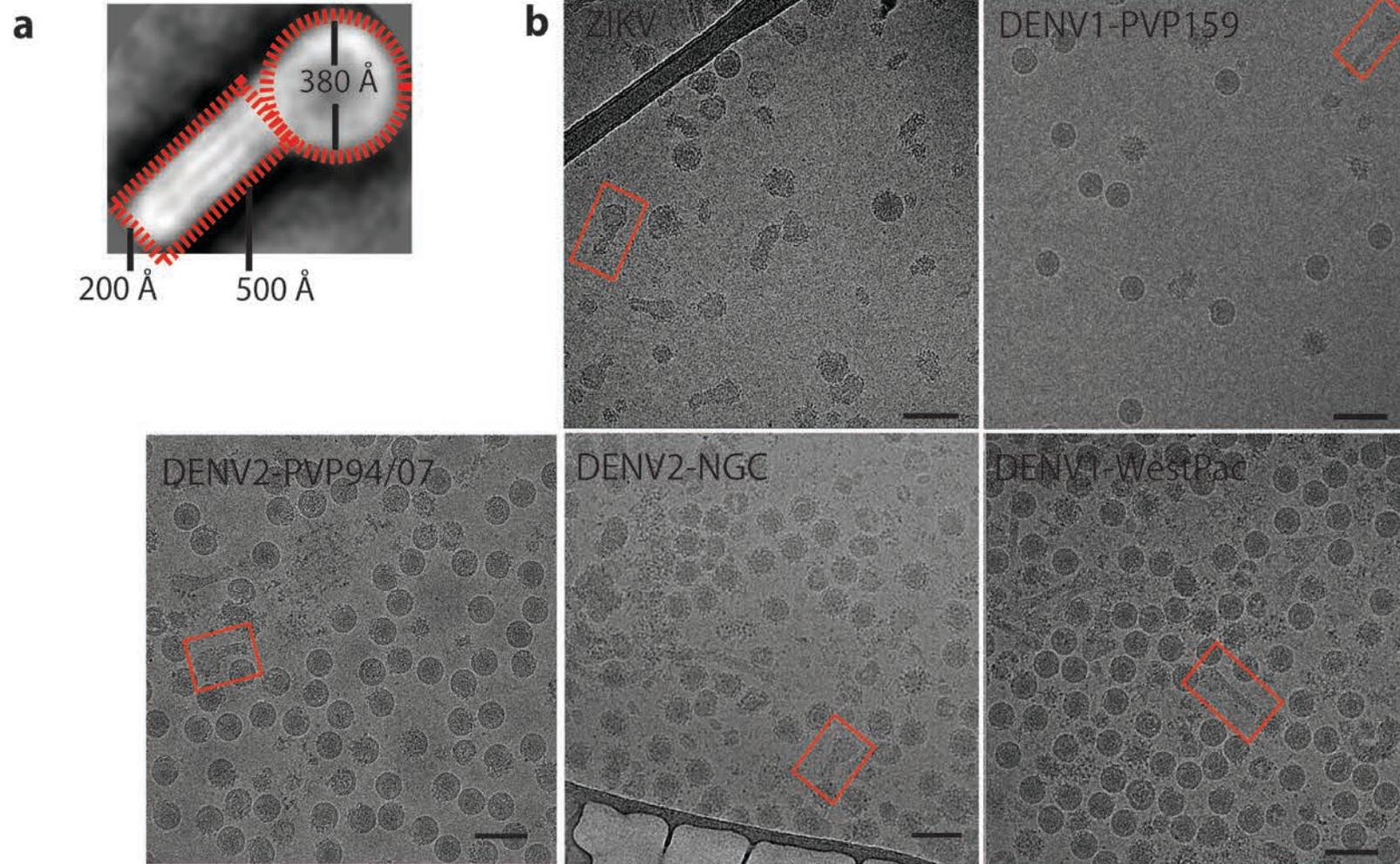
Different maturation states, breathing states and even a new morphology:  
clubshape structures

# DENV3 (CH53489) clubshape morphology



Morrone et al., 2020, Nature Communications

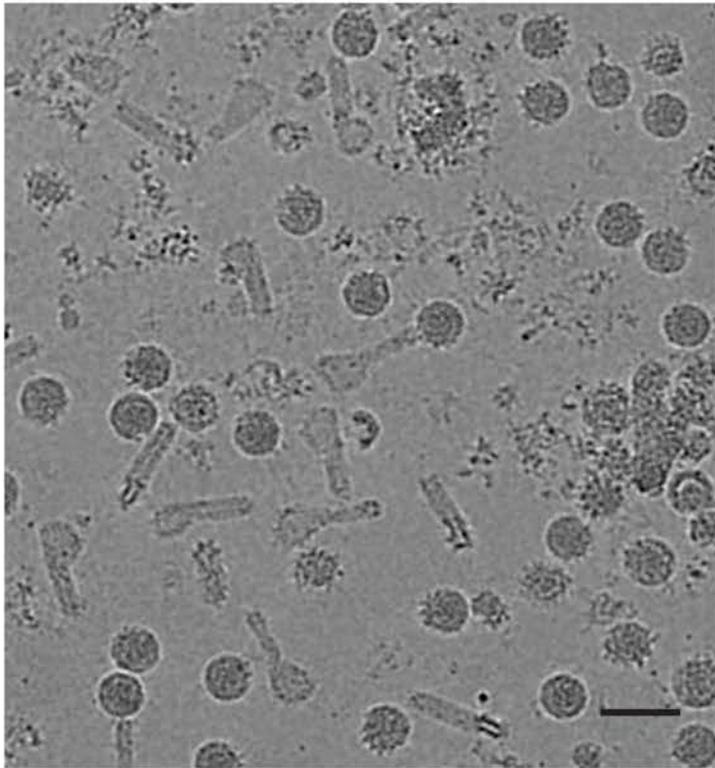
## Other DENV serotypes and zika



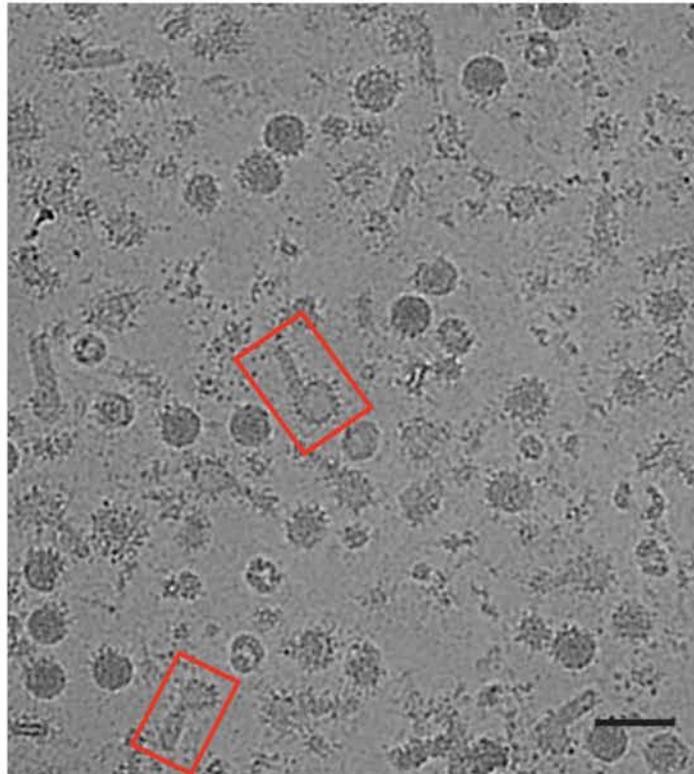
Morrone et al., 2020, Nature Communications

# Antibodies can have different interact with different parts of the clubshape particle

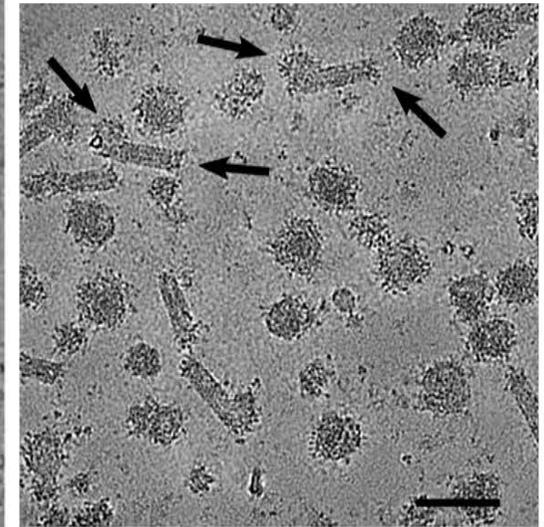
**DENV3-CH53489 control**



**Fusion loop antibody  
DENV3-CH53489 + Fab4G2**

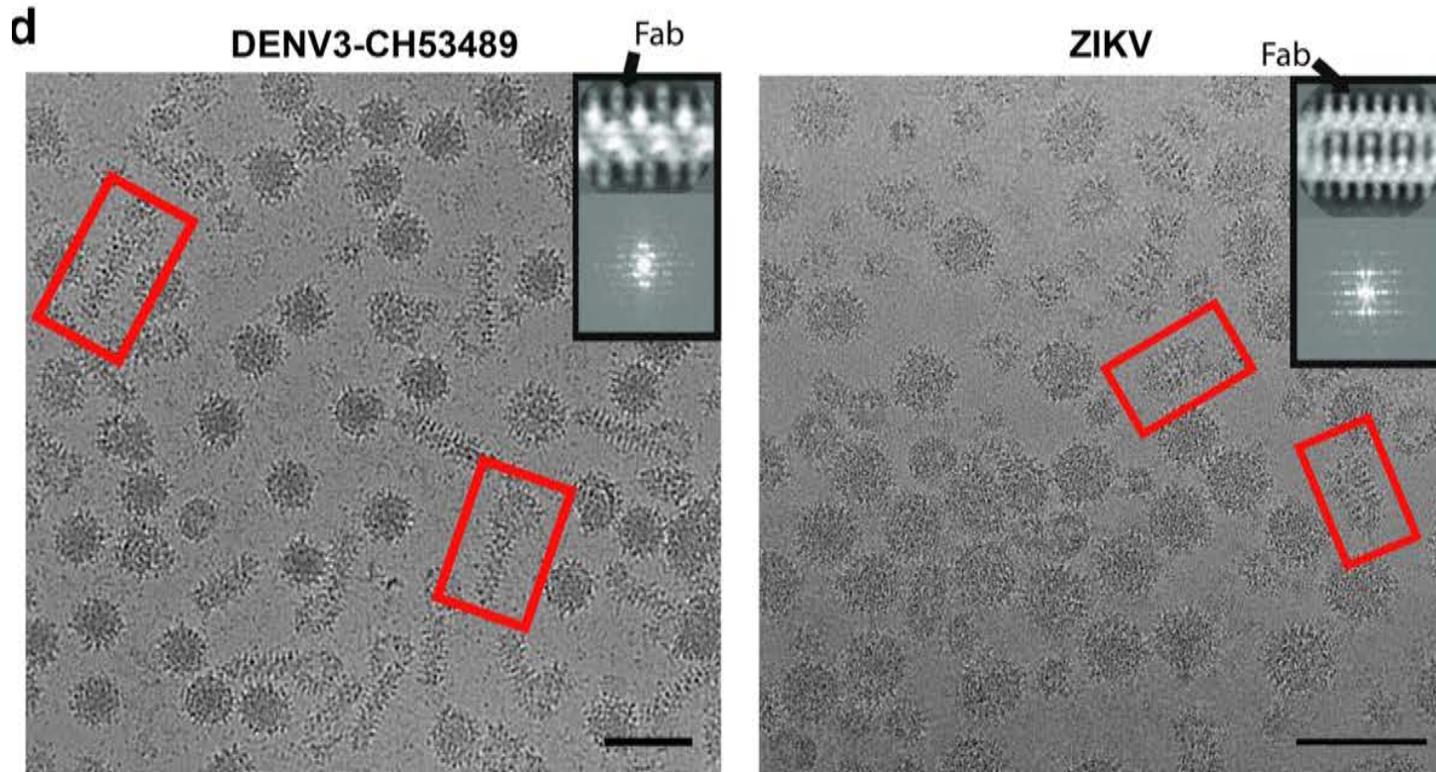


**DIII antibody  
Fab 8A1**



## E protein dimer binding antibody

Complexed with Fab C10



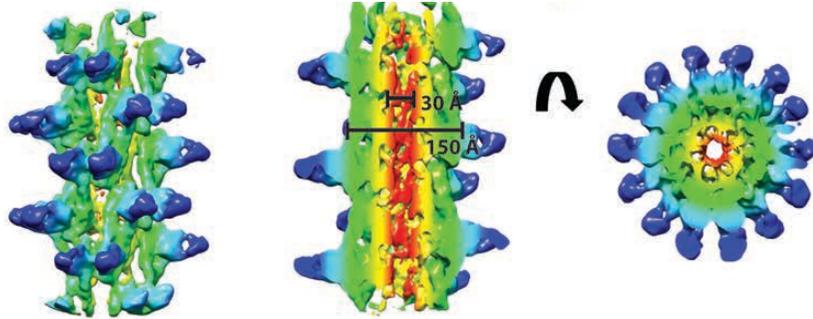
DENV3-Fab clubshape particles

Zika-Fab caterpillar structure

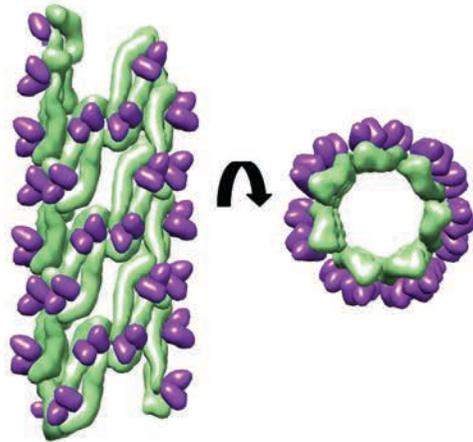
Morrone et al., 2020, Nature Communications

## Tail of DENV3-C10 clubshape particles

CryoEM maps

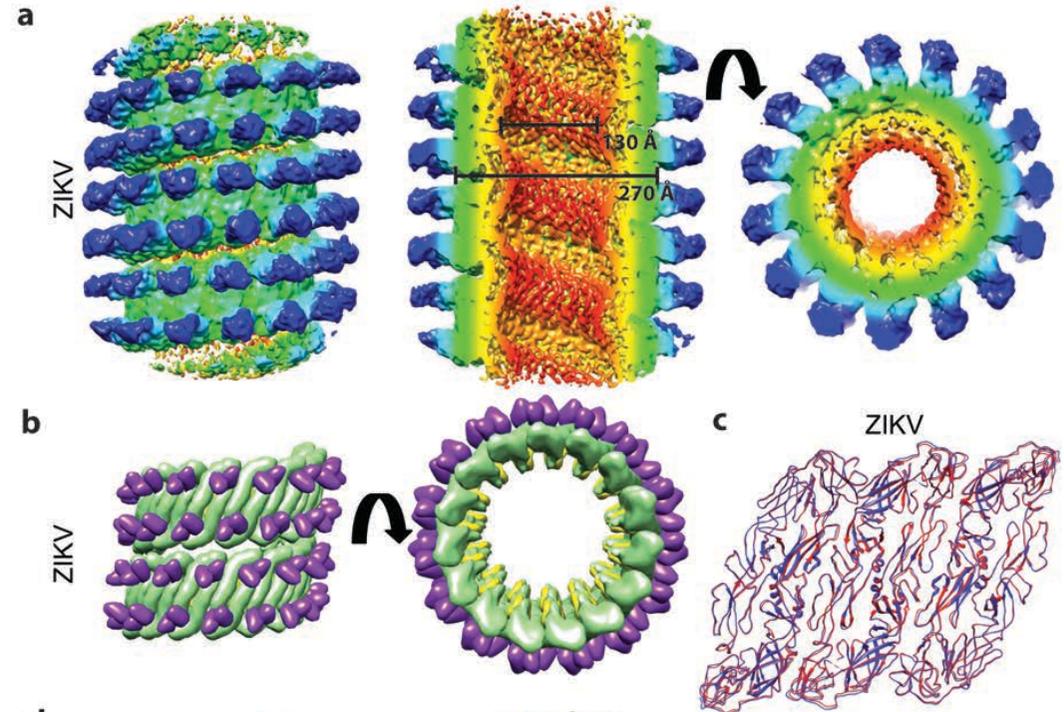


Fitted E and Fab C10 molecules



Fab C10 locks an E protein dimer by binding across two E proteins within a dimer

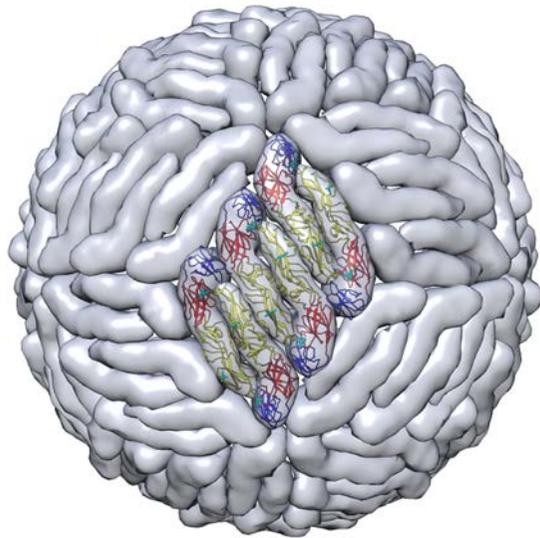
## ZIKV-C10 caterpillar structure



Fab 10, in addition to locking the E protein dimers, it also binds across dimers (inter-dimer interface), thus locking the three E protein dimers within a raft together.

Morrone et al., 2020, Nature Communications

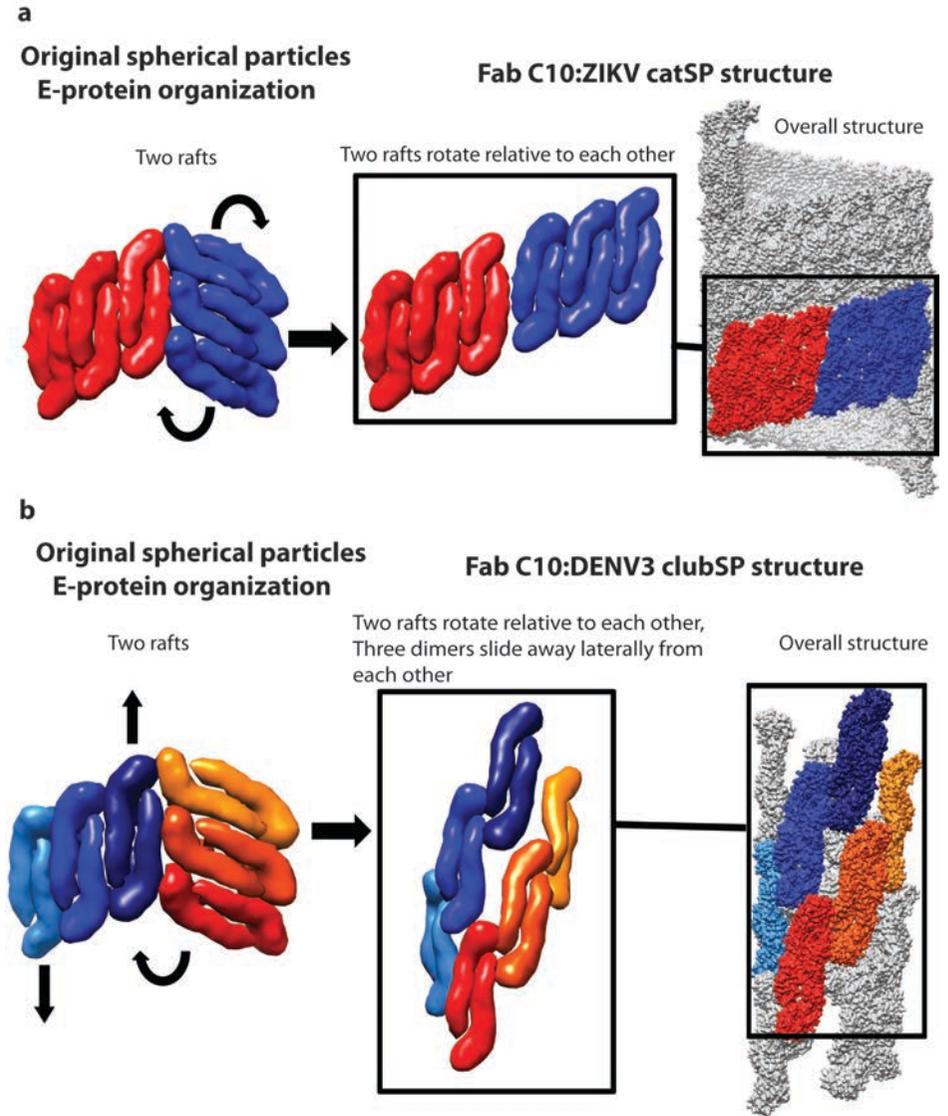
# E protein reorganization to form ZIKV-Fab caterpillar and DENV3-Fab clubshape structures



Dengue/zika  
round particle organization

ZIKV-Fab caterpillar  
structure

DENV3-Fab clubshape  
tail structure

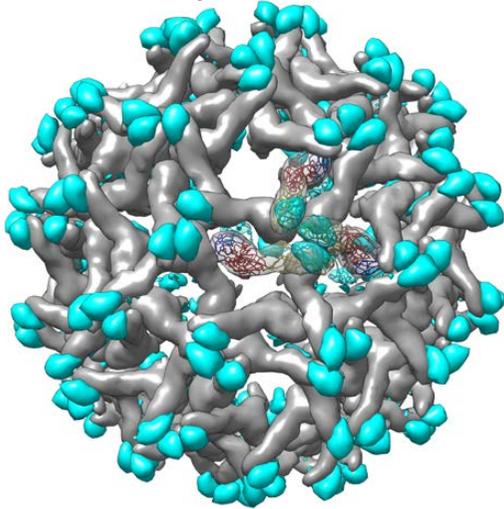


# Summary

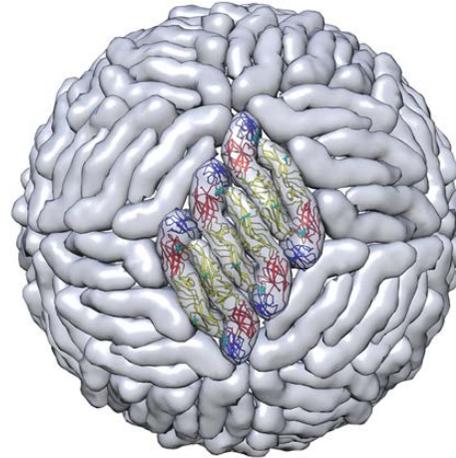
- We showed a new clubshape morphology that can be displayed by most flaviviruses.
- Different parts of the virus can have different accessibility to binding by different antibodies.

# Part 4: Does the presentation of different morphologies confer advantages to the survival of virus?

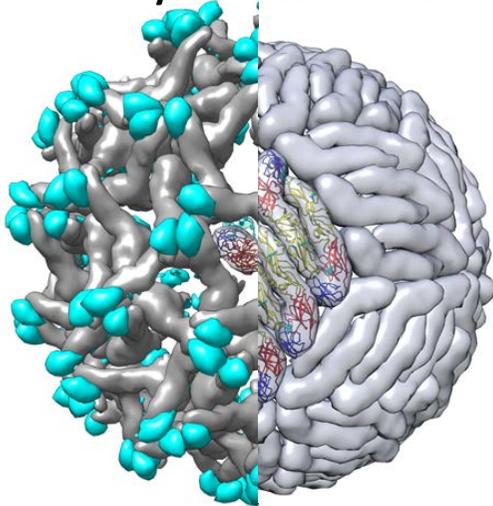
Fully immature virus



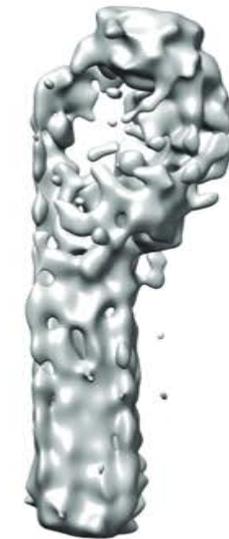
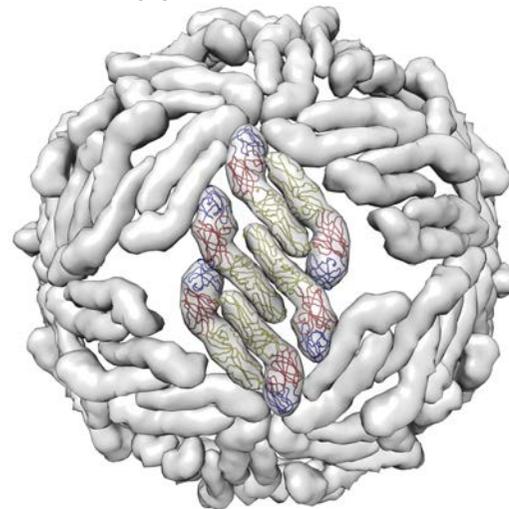
Compact smooth surface mature virus



Partially immature virus



Bumpy surface mature virus

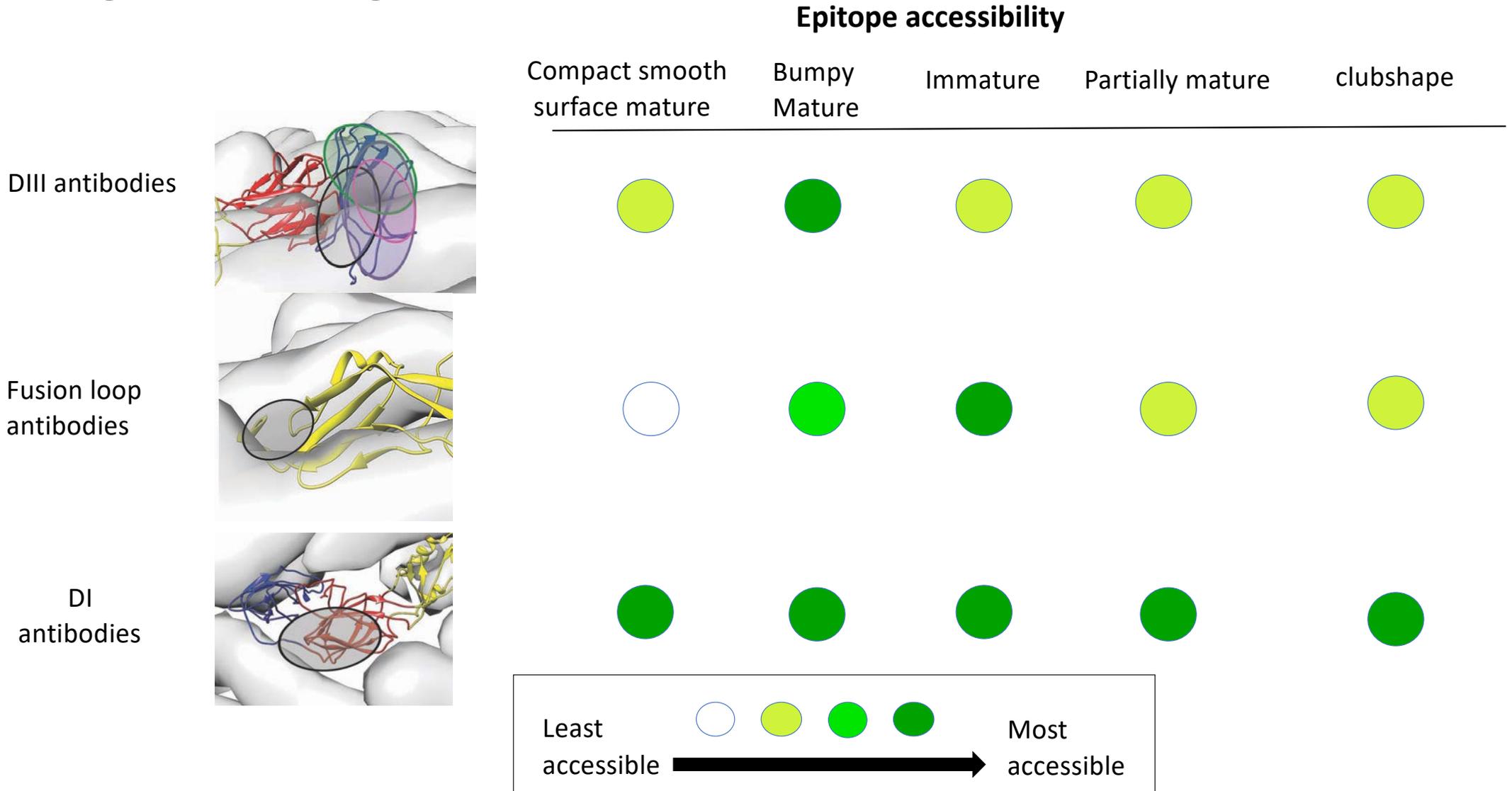


Clubshape particles

## They are all infectious and we can't ignore them

- **Mature smooth compact surface virus particles**
  - very infectious. Infect cells by binding to attachment factors such as heparin sulfate, TIM, and DC-SIGN. May or may not bind to a specific receptor.
- **Mature bumpy surface virus particles**
  - As infectious as the compact structure.
- **Immature virus** – non-infectious by itself. But can infect Fc receptor positive cells when virus is complexed with anti-prM, certain anti-E antibodies and also DC-SIGN
- **Partially mature virus** can enter cells by pathways utilized by both the fully mature and immature virus.
- **Clubshape particles** contains RNA genome, no detectable reduction in the amount of virus attached to cells after clubshape formation.

# Dengue neutralizing antibodies



### Epitope accessibility

Mature compact

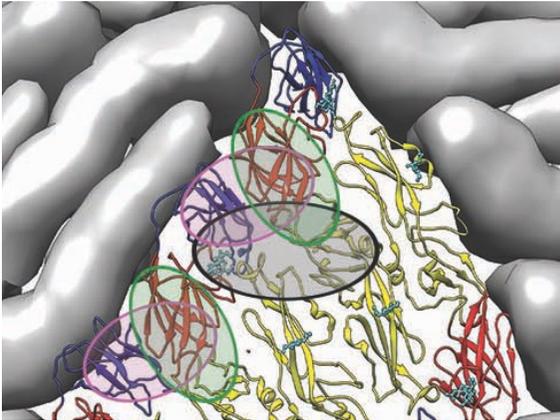
Mature expanded

Immature

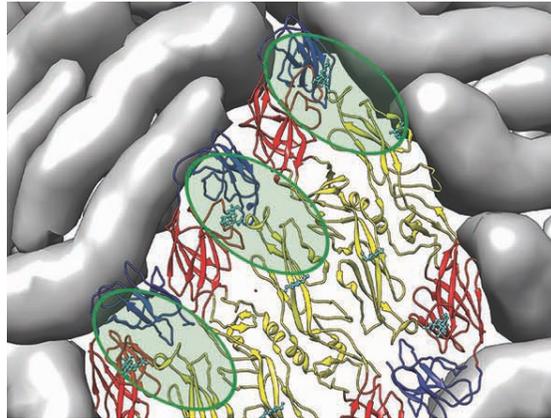
Partially mature

clubshape

Virus quaternary dependent epitope antibodies



E protein dimer binding antibodies



# Antibody therapeutics and vaccine development

- Is there an antibody that could neutralize all serotypes and also all morphologies?? We may need an antibody mixture for prophylactic and therapeutics development...
- Vaccine – should all these morphologies be represented?

# Acknowledgement

## Collaborators

### My lab members

- Guntur Fibriansah
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- Ralph Baric (UNC)
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