Structural studies of viral envelope proteins and insight for vaccine design

GIVRF, March 15, 2016

Félix Rey

Institut Pasteur, Paris

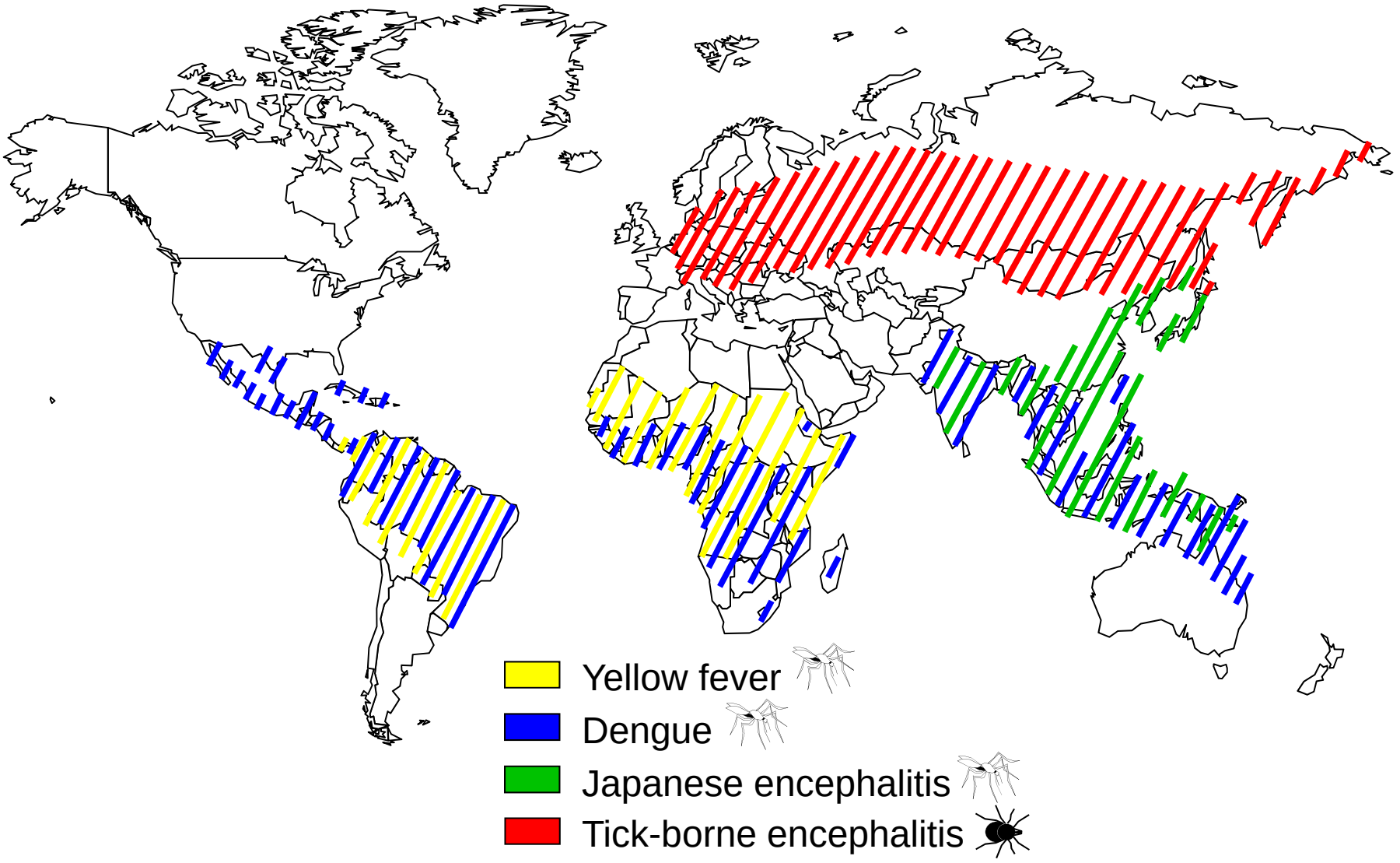
Dengue virus

- There are four different dengue viruses: serotypes 1-4
- The envelope protein E is the sole target of neutralizing antibodies. It shares 65% aa sequence identity between the most distant serotypes
- Infection by one serotype leads to life-long immunity against that serotype, but infection by other serotypes can be more serious
- Macrophages can become infected via Fcγ receptors through non-neutralizing antibodies bound to viral particles (ADE).

Flaviviruses

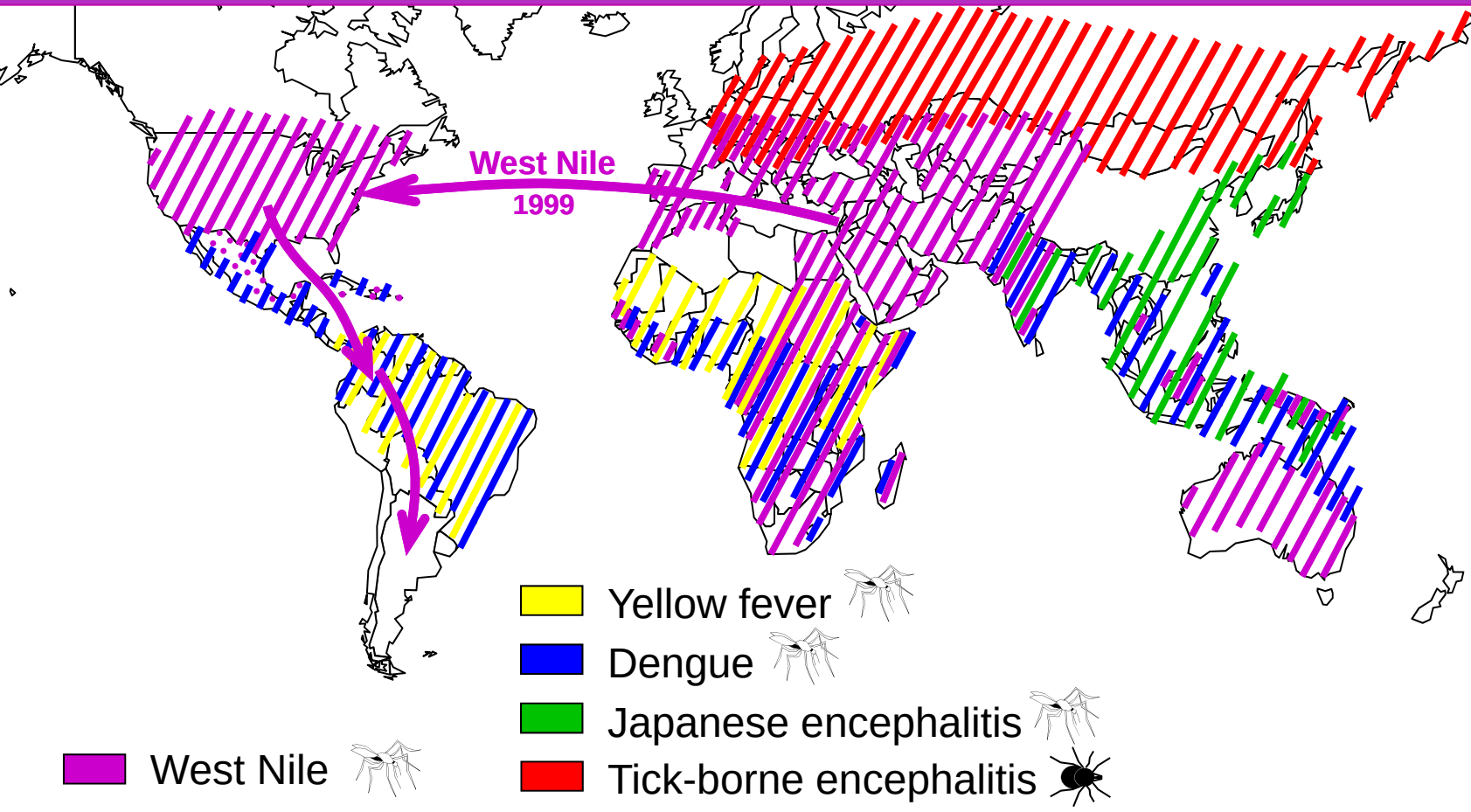
- Group of small enveloped viruses with a positive-sense, single stranded RNA genome, encoding a single polyprotein precursor to all viral proteins
- They cause serious disease and are of global concern. These include encephalitis, fevers, hemorrhagic fevers.
- The geographic distribution generally follows the distribution of the arthropod vector (insects or ticks)

Flaviviruses

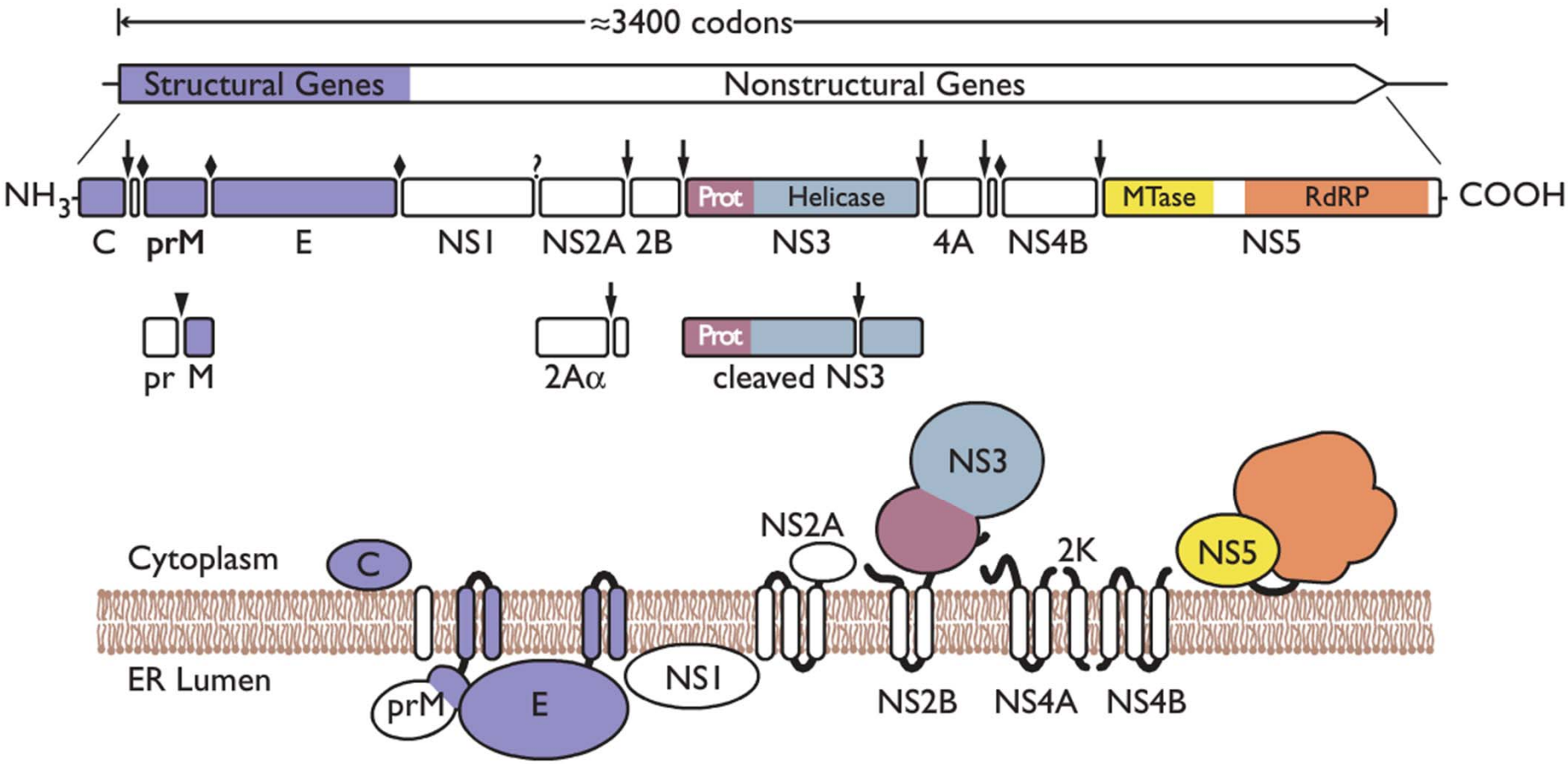


Flaviviruses

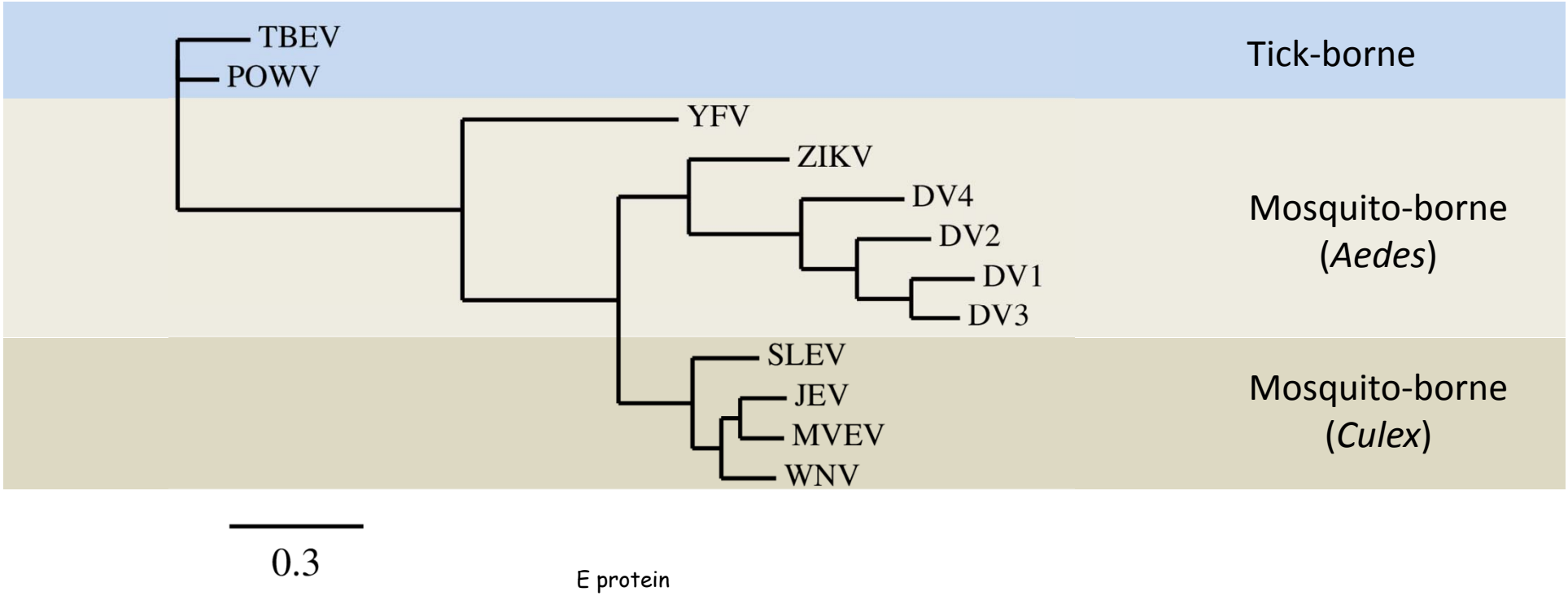
Similarly, now zika virus appeared in South America and is expanding toward the Caribbean islands, after having initially spread from Africa to South East Asia and then to Polynesia



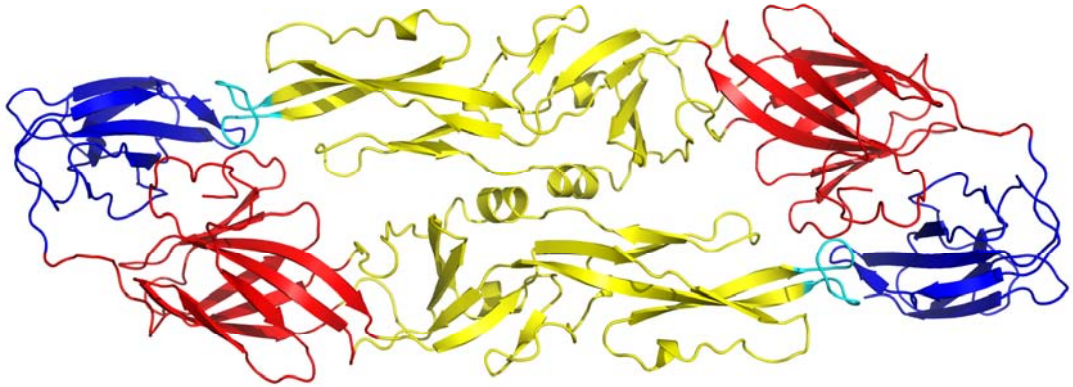
Flavivirus Genomic Organization:



Flaviviruses: phylogenetic tree based on protein E



The main antigen of dengue virus: protein E

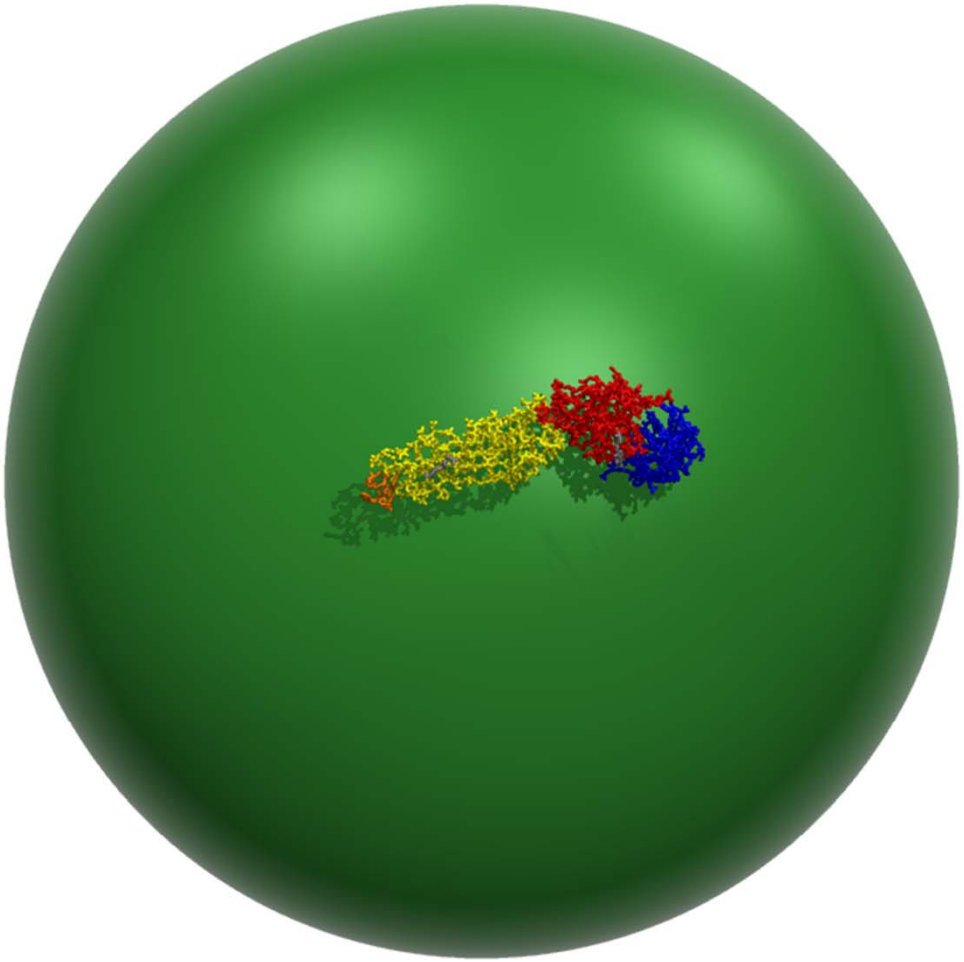


Pre-fusion form (dimer)

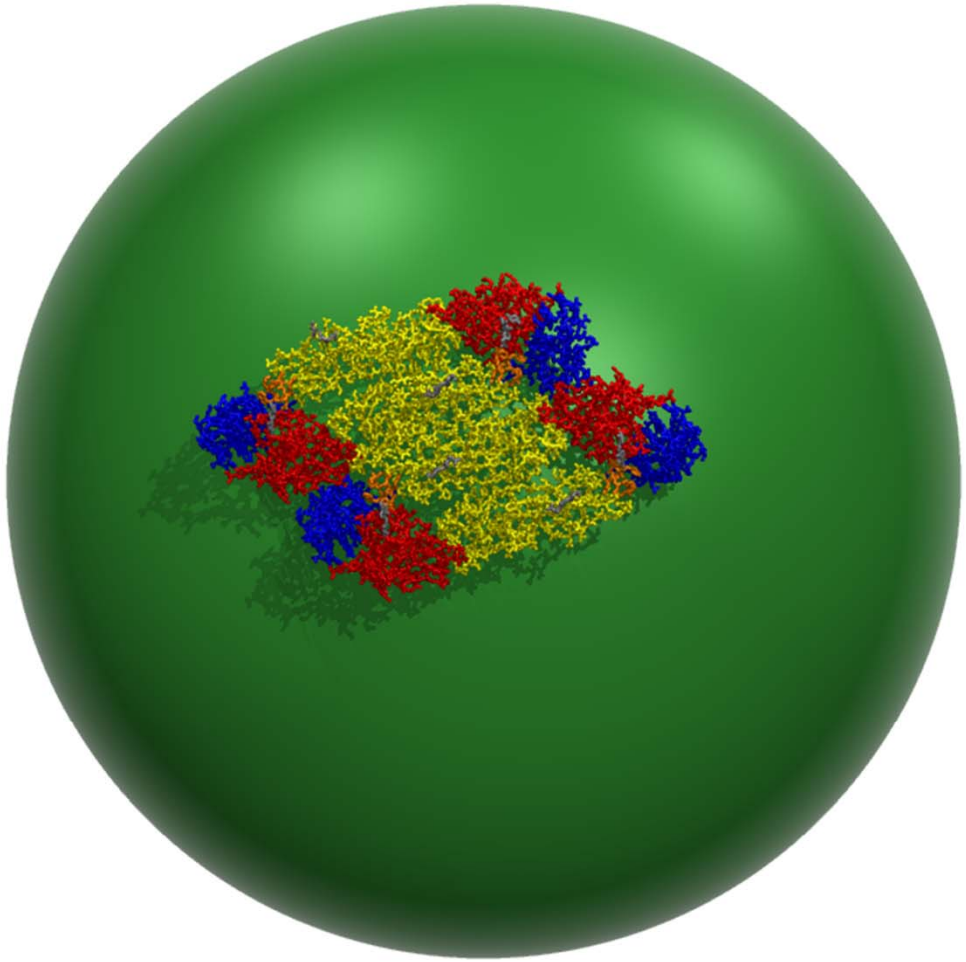
Modis et al, 2003

Rey et al, 1995

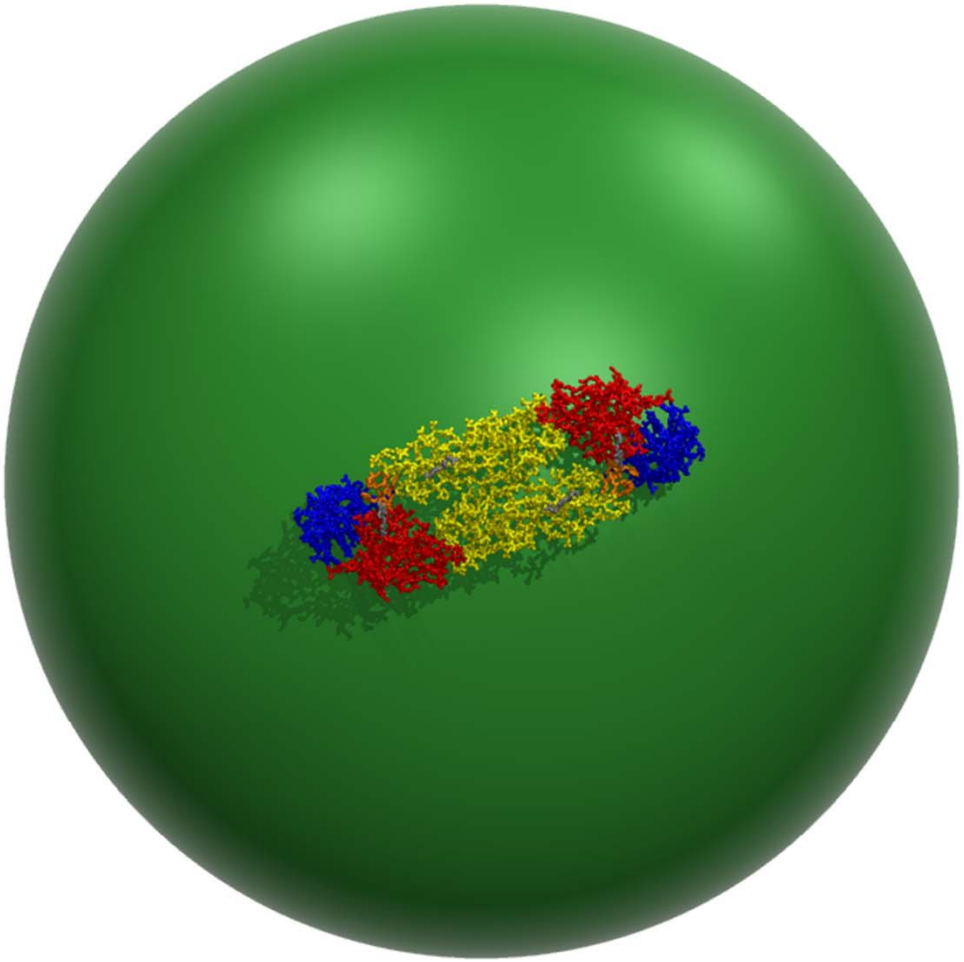
Mature flavivirus particle:



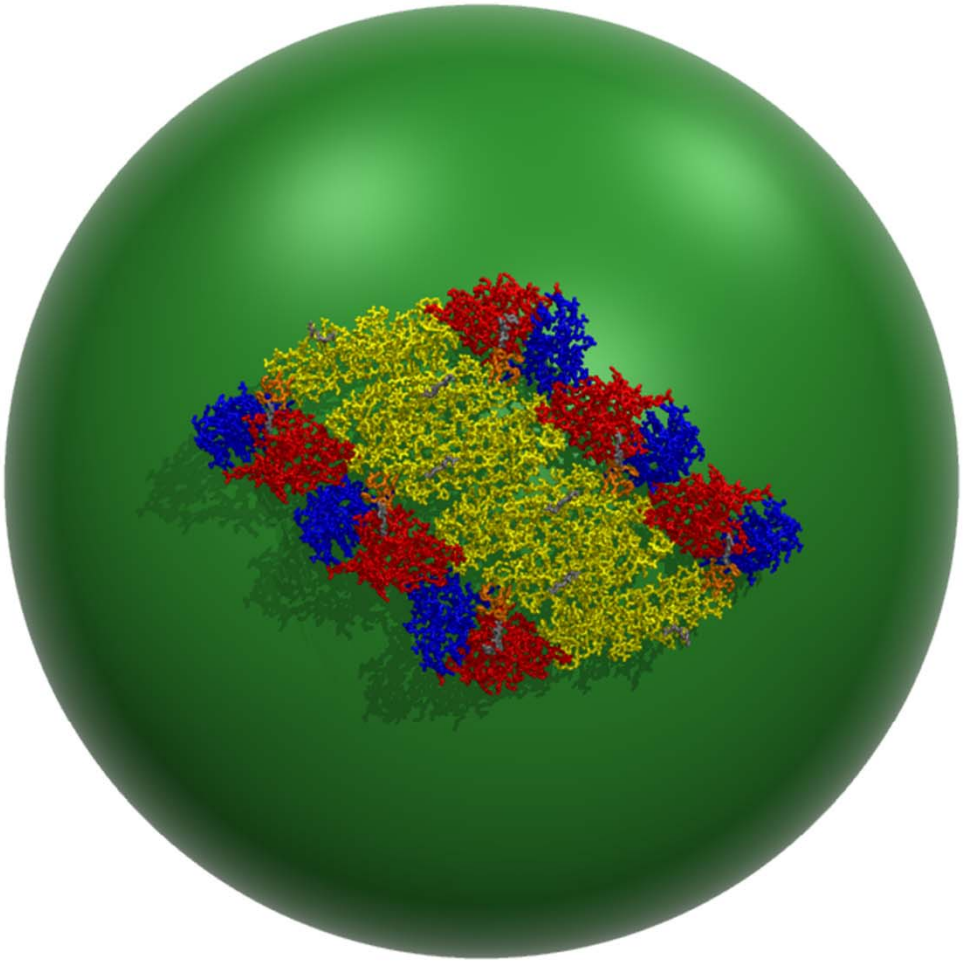
Mature flavivirus particle:



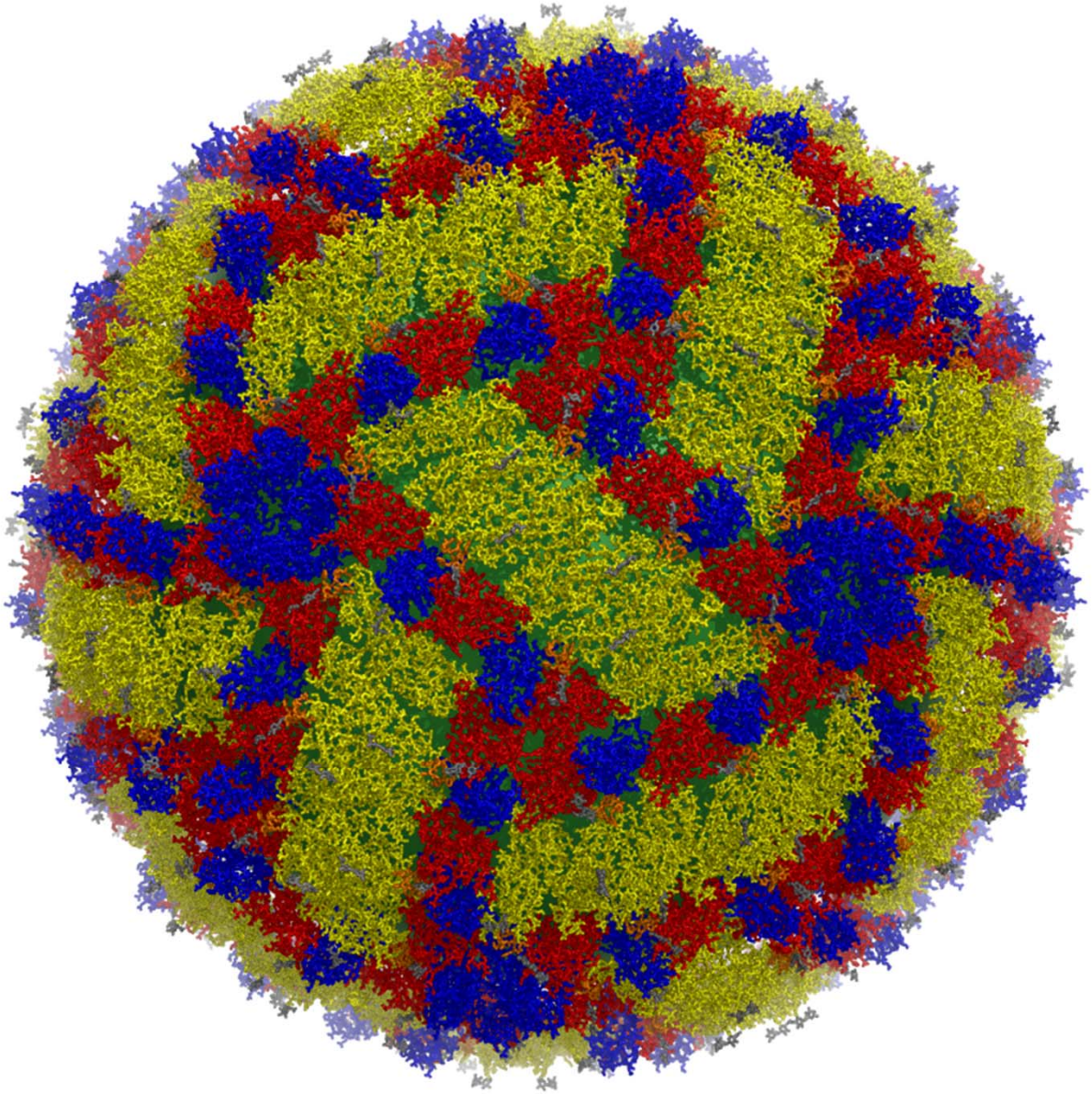
Mature flavivirus particle:



Mature flavivirus particle:



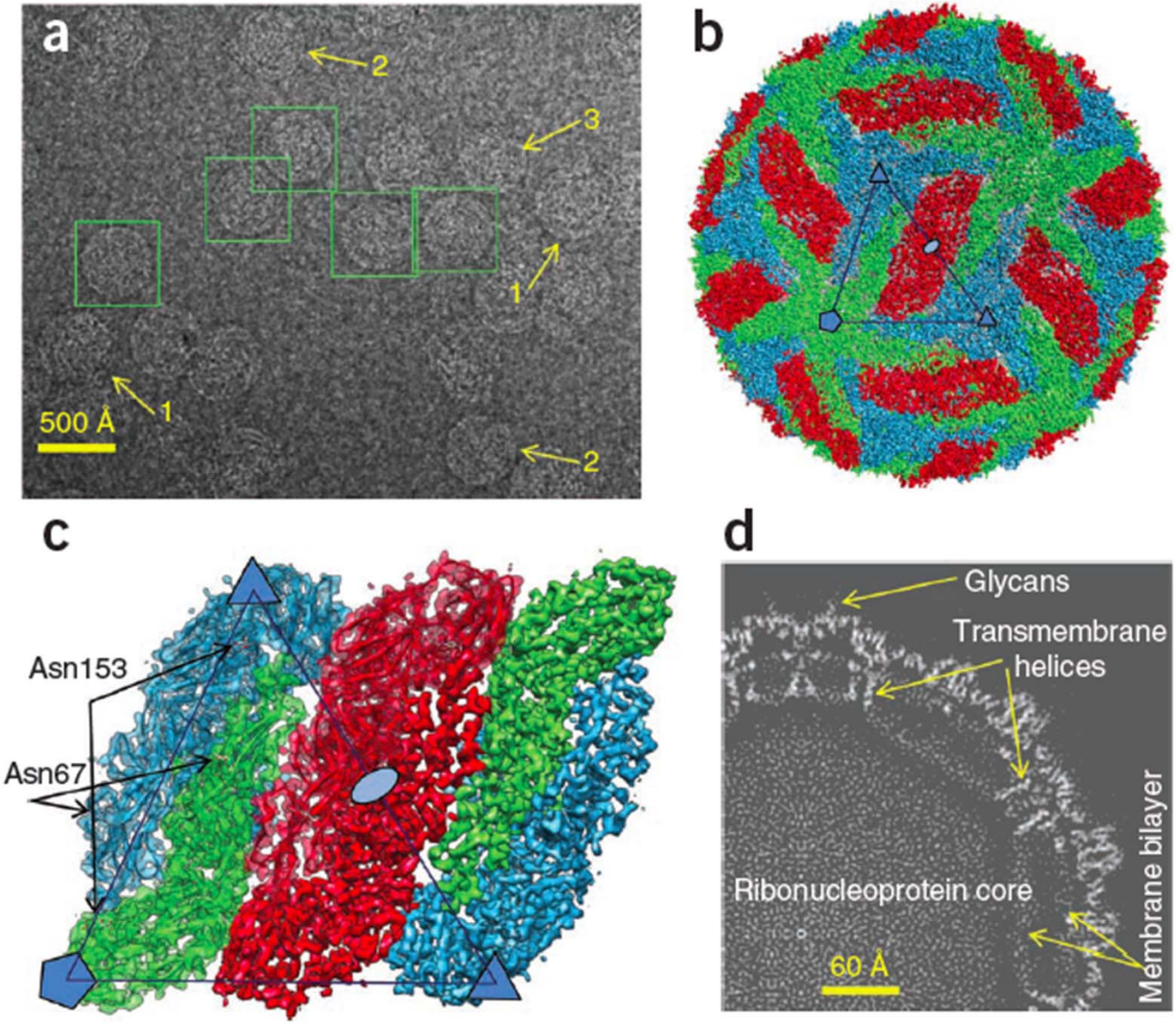
Mature flavivirus particle:



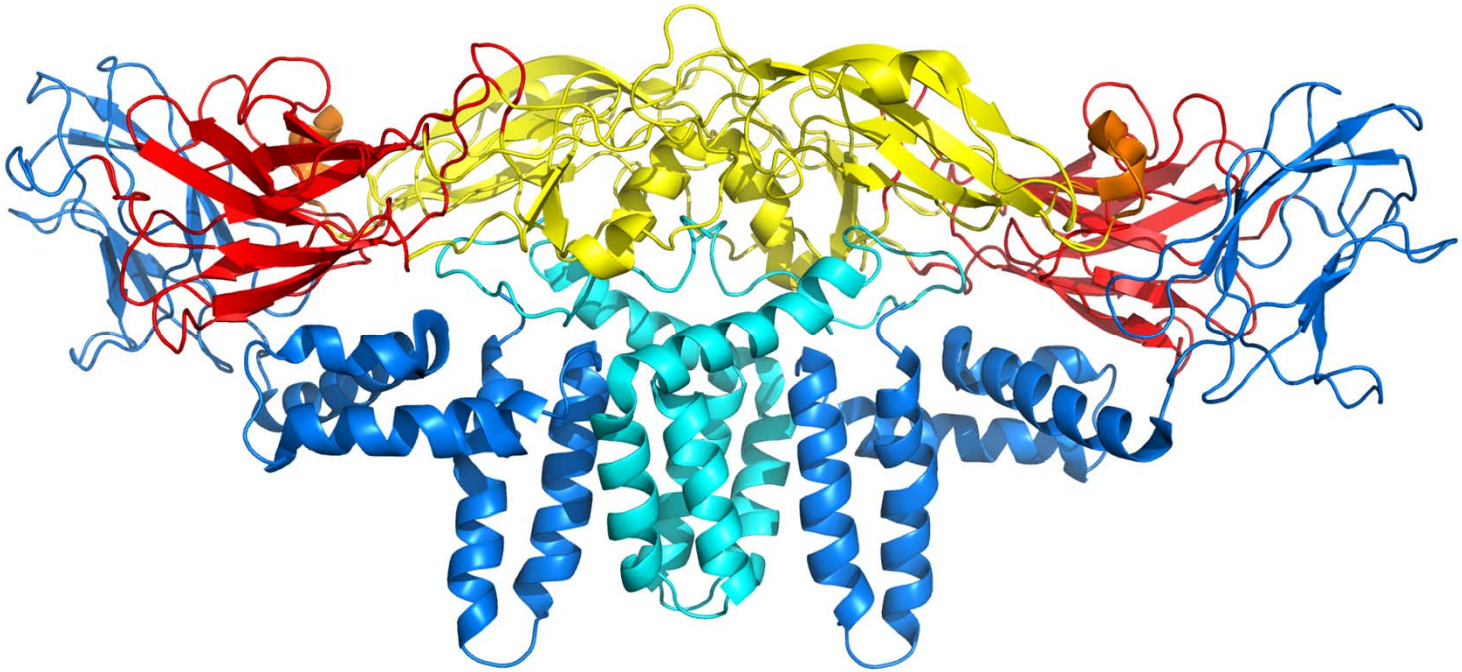
Cryo-EM structure of the mature dengue virus at 3.5-A resolution

Xiaokang Zhang[1–5,9], Peng Ge[1–3,9], Xuekui Yu[1–3], Jennifer M Brannan[3,8], Guoqiang Bi[4,5], Qinfen Zhang[6], Stan Schein[2,7] & Z Hong Zhou[1–5]

NSMB 2013

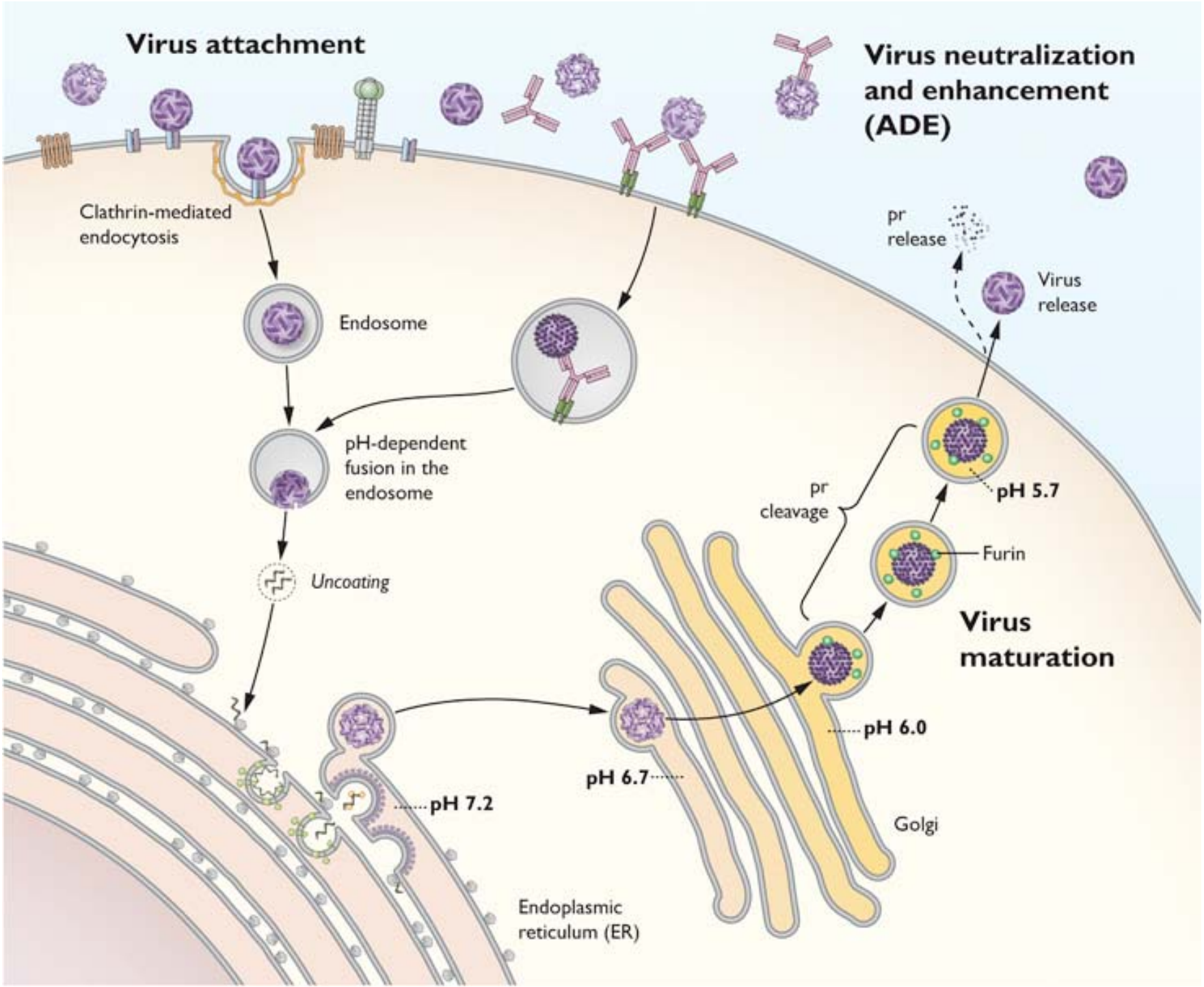


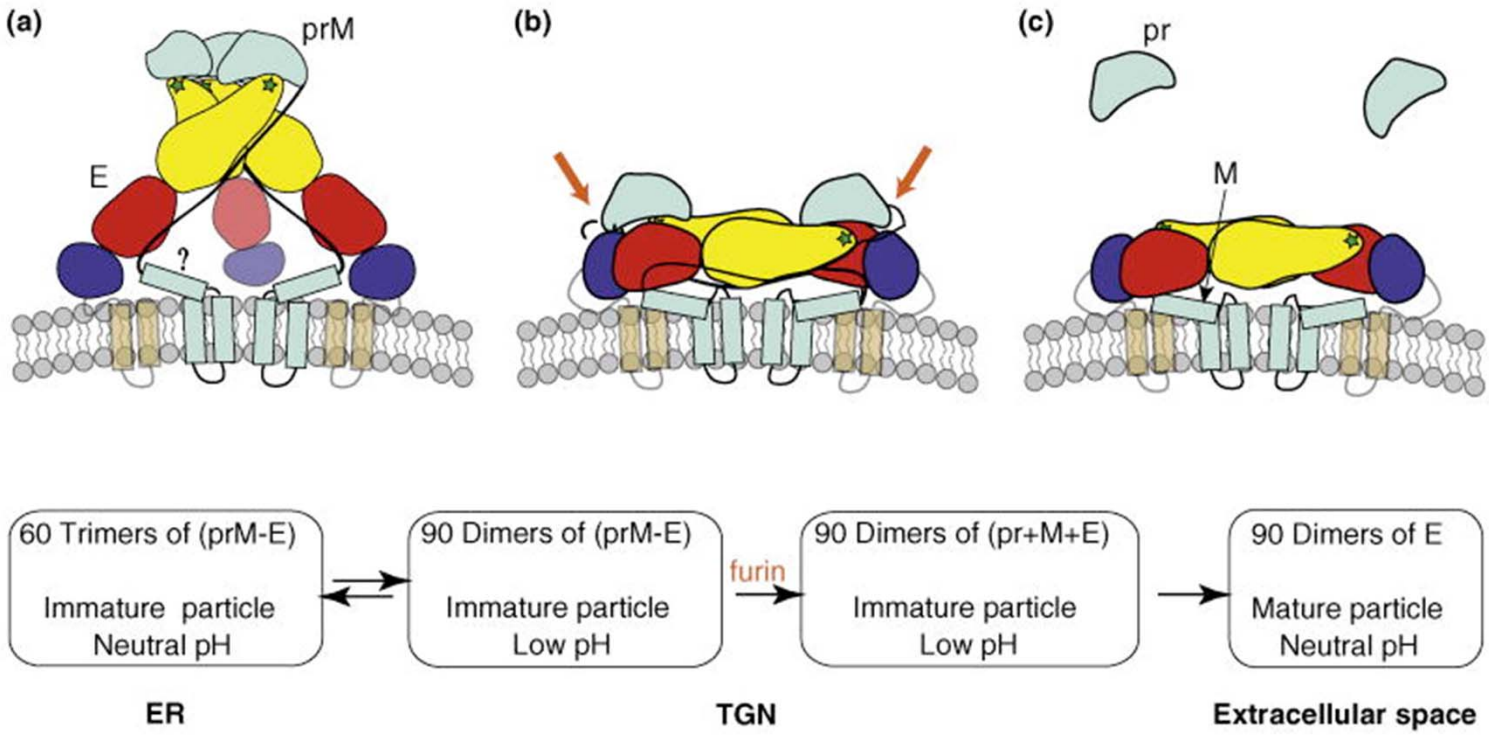
mature DENV-2 M-E dimer
(3J2P)

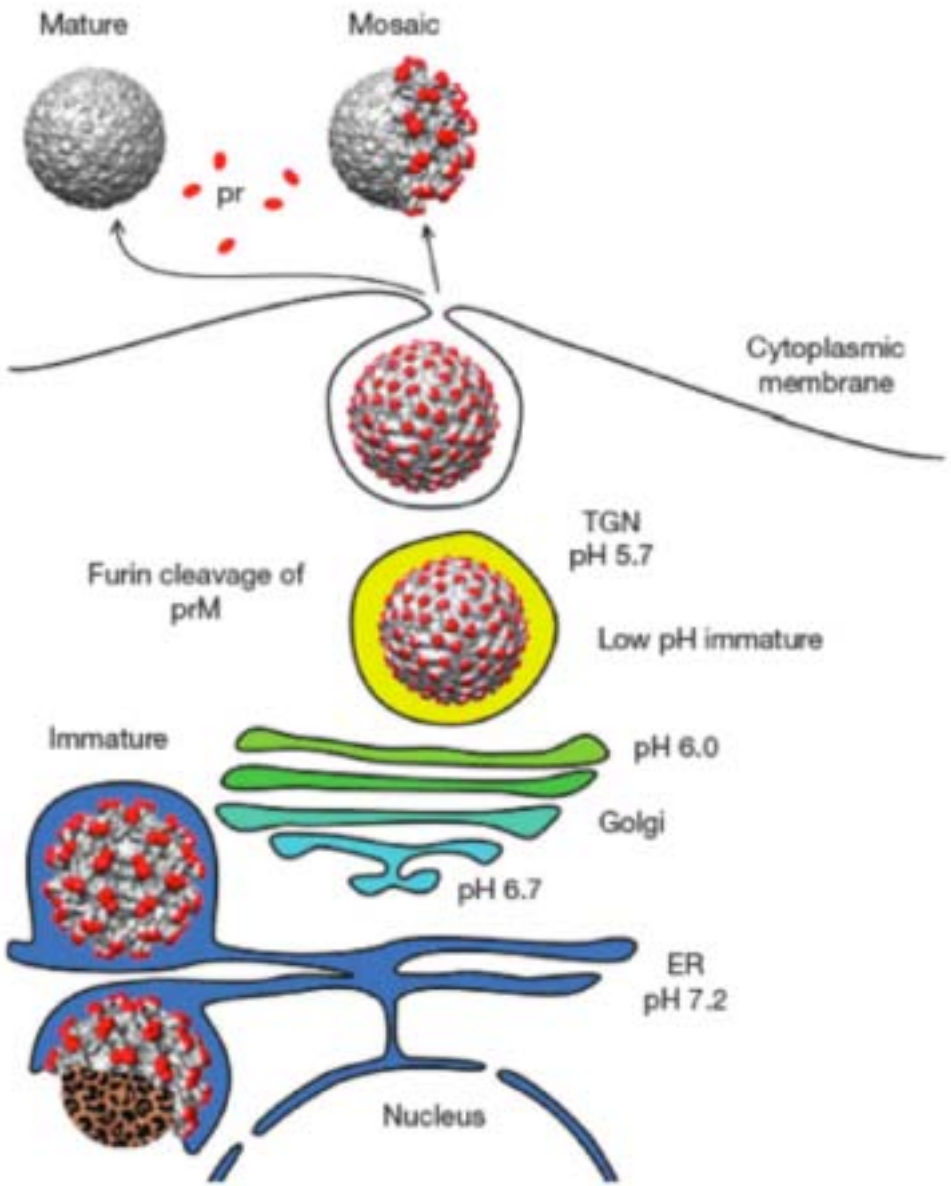


cryo-EM structure at 3.6 Å resolution
(Zhang et al, NSMB, 2012)

- **DENV buds as immature particles in the ER of the infected cell**
- **Immature DENV particles contain a heterodimer between the envelope protein E and the precursor membrane glycoprotein (prM)**
- **These particles have 60 trimeric spikes with prM/E subunits**


Virus attachment
Virus neutralization and enhancement (ADE)
Clathrin-mediated endocytosis
Endosome
pH-dependent fusion in the endosome
Uncoating
pH 7.2
pH 6.7
pH 6.0
pH 5.7
pr release
Virus release
pr cleavage
Furin
Virus maturation
Golgi
Endoplasmic reticulum (ER)


(a)
prM
E
?
(b)
(c)
pr
M
60 Trimers of (prM-E)
Immature particle
Neutral pH
90 Dimers of (prM-E)
Immature particle
Low pH
furin
90 Dimers of (pr+M+E)
Immature particle
Low pH
90 Dimers of E
Mature particle
Neutral pH
ER
TGN
Extracellular space


Mature
Mosaic
pr
Cytoplasmic membrane
TGN
pH 5.7
Furin cleavage of prM
Low pH immature
Immature
pH 6.0
Golgi
pH 6.7
ER
pH 7.2
Nucleus

- The extent of maturation depends on cell type.
- In some cells, the amount of unprocessed prM is more than 60%.
- This means that dengue virus is presented to the immune system in a very heterogeneous way.
- Antibodies against prM are non-neutralizing, and can be strong enhancers of the infection (ADE)
- The epitopes of the neutralizing antibodies produced in humans have been a matter of debate.

- **The structure of the mature virion, displaying dimers tightly packed, is only a medium (or time-average) structure**

- **Mature dengue particles show a highly dynamic behavior**

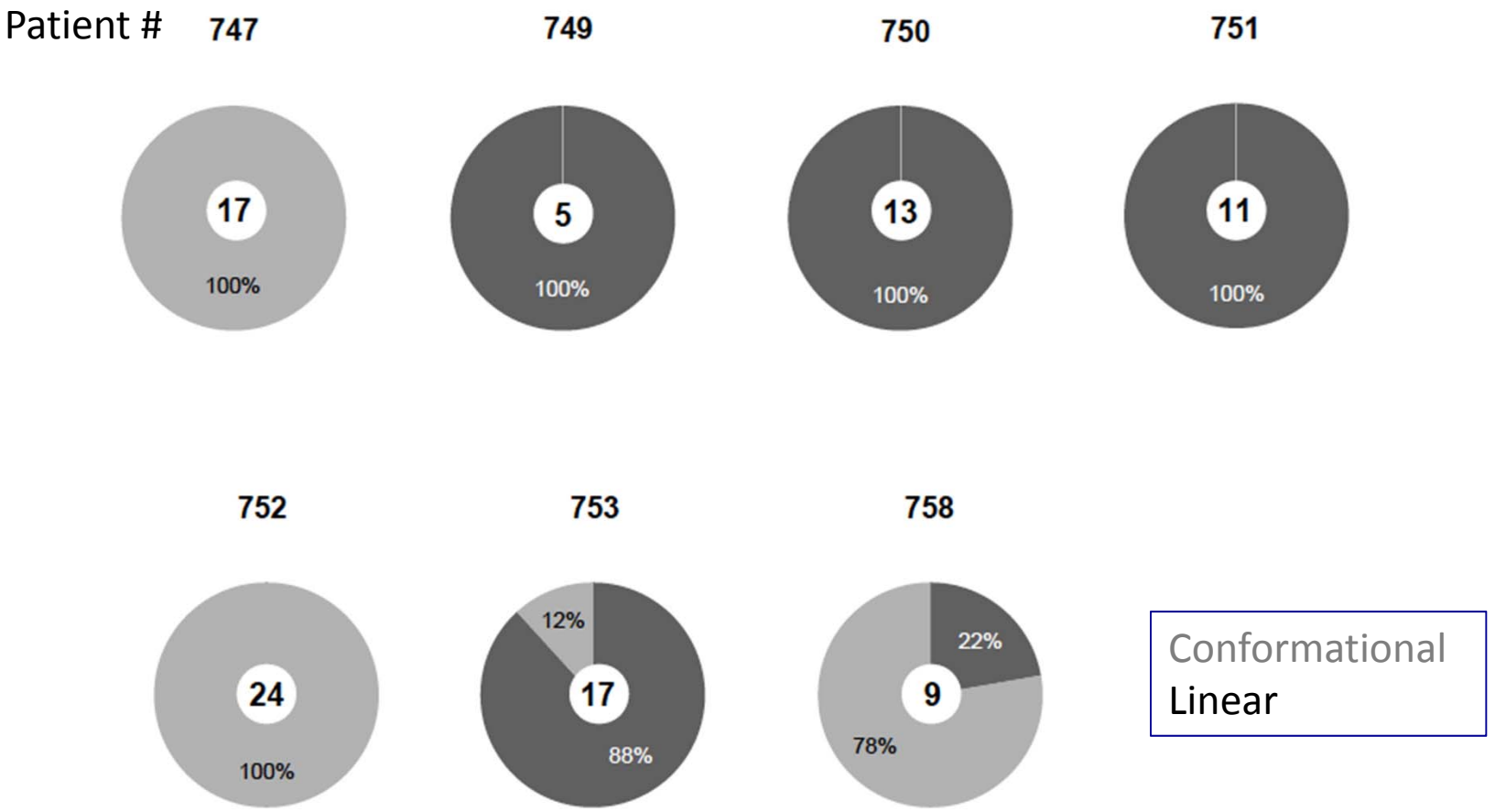
- **This behavior is a consequence of the metastability of the protein, which needs to undergo a major conformational change to induce fusion.**

- **In addition, about 1000 to 10000 particles are needed in average for an infection event**

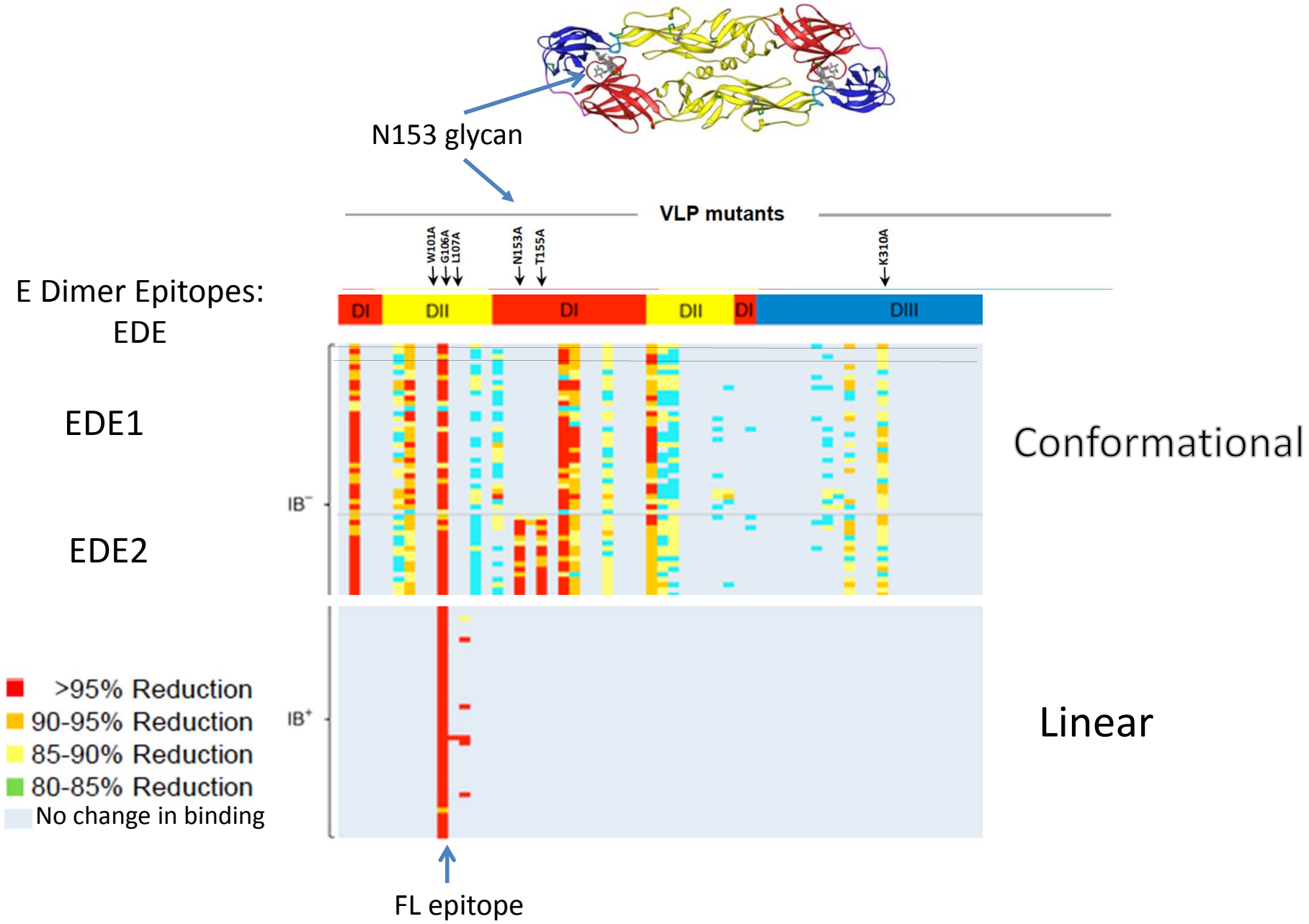
- **The vast majority of dengue particles displays regions that are buried in functional, well structured dimers required for entry into cells.**

- The challenge is to protect against all four dengue viruses simultaneously
- The Screaton and Mongkolsapaya laboratories at Imperial College recently isolated antibodies out of plasmablasts from dengue patients, following a cohort in Thailand

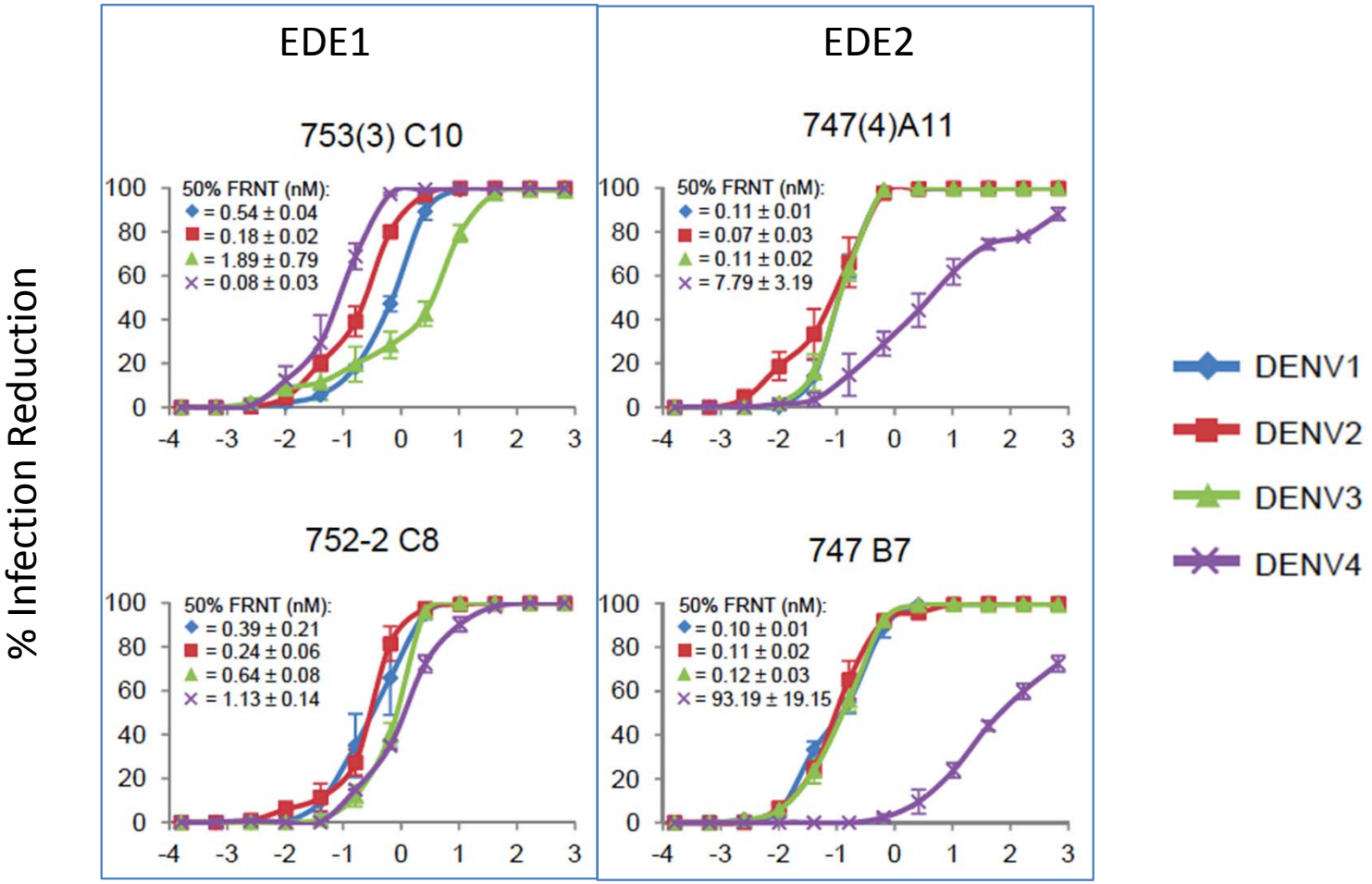
Abundance of conformational Abs in select patients



Mapping epitopes by Ala scanning mutagenesis on VLPs

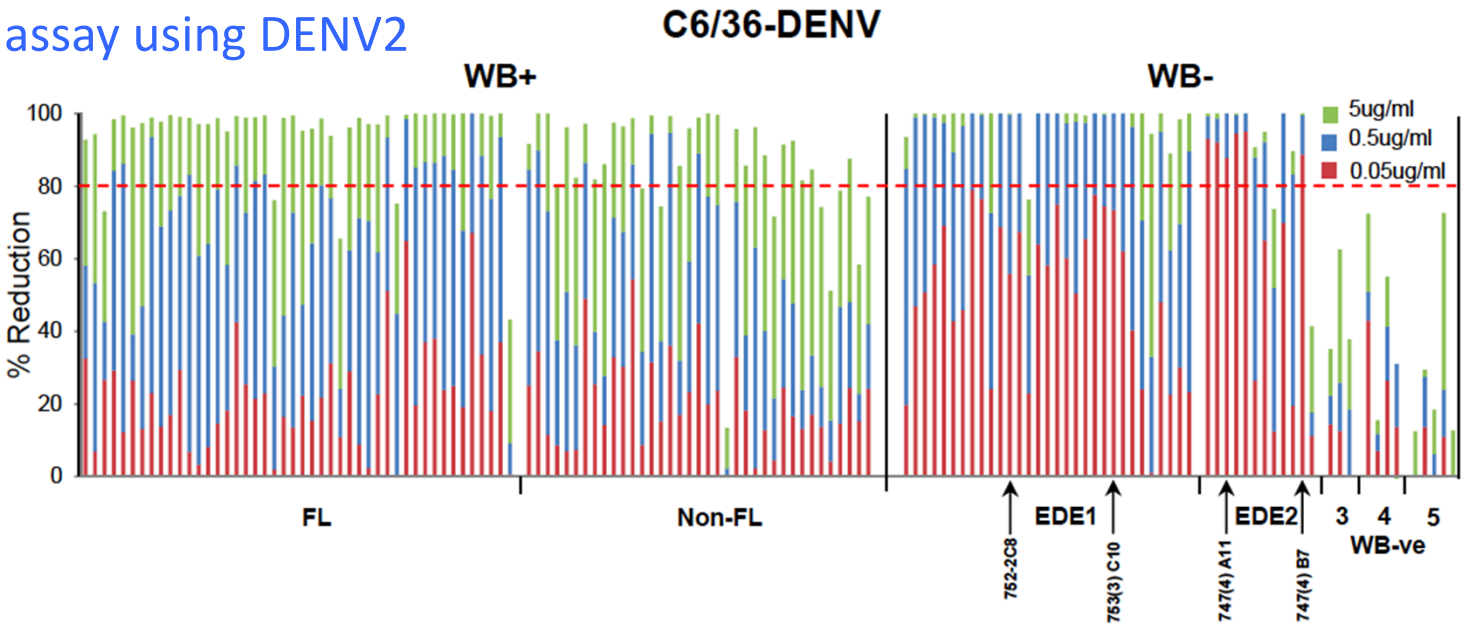


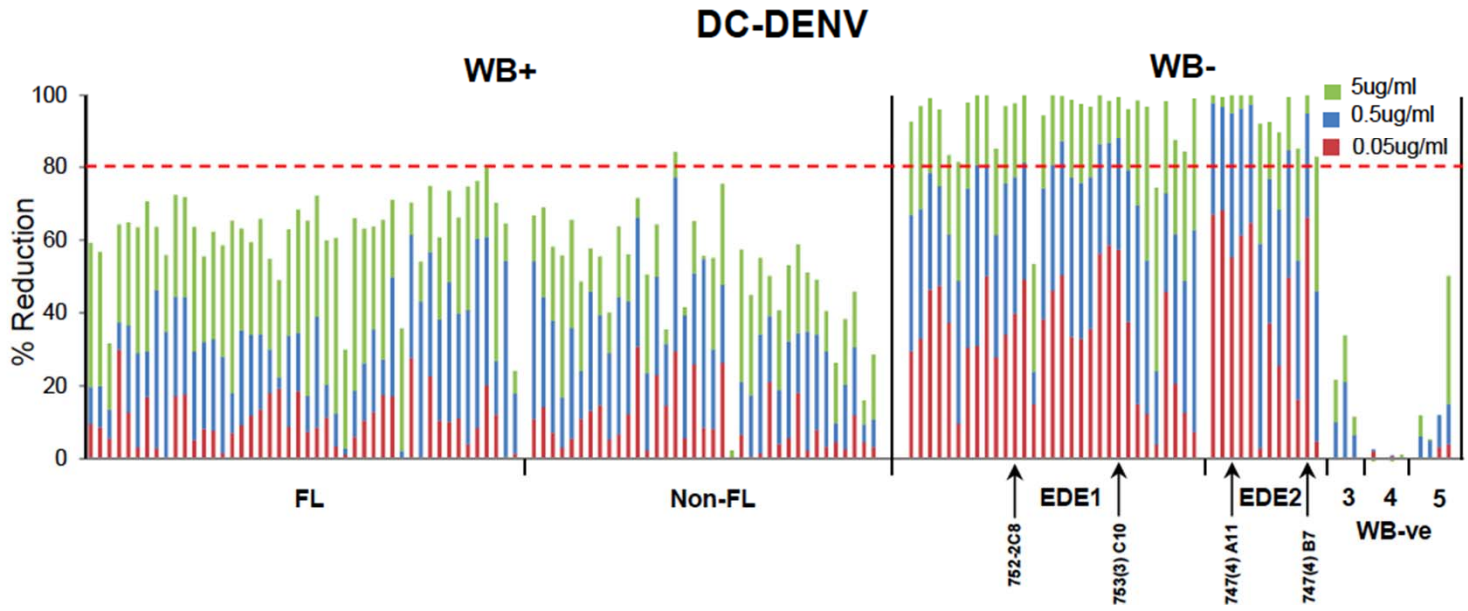
Infection reduction by select Abs assayed on all four serotypes

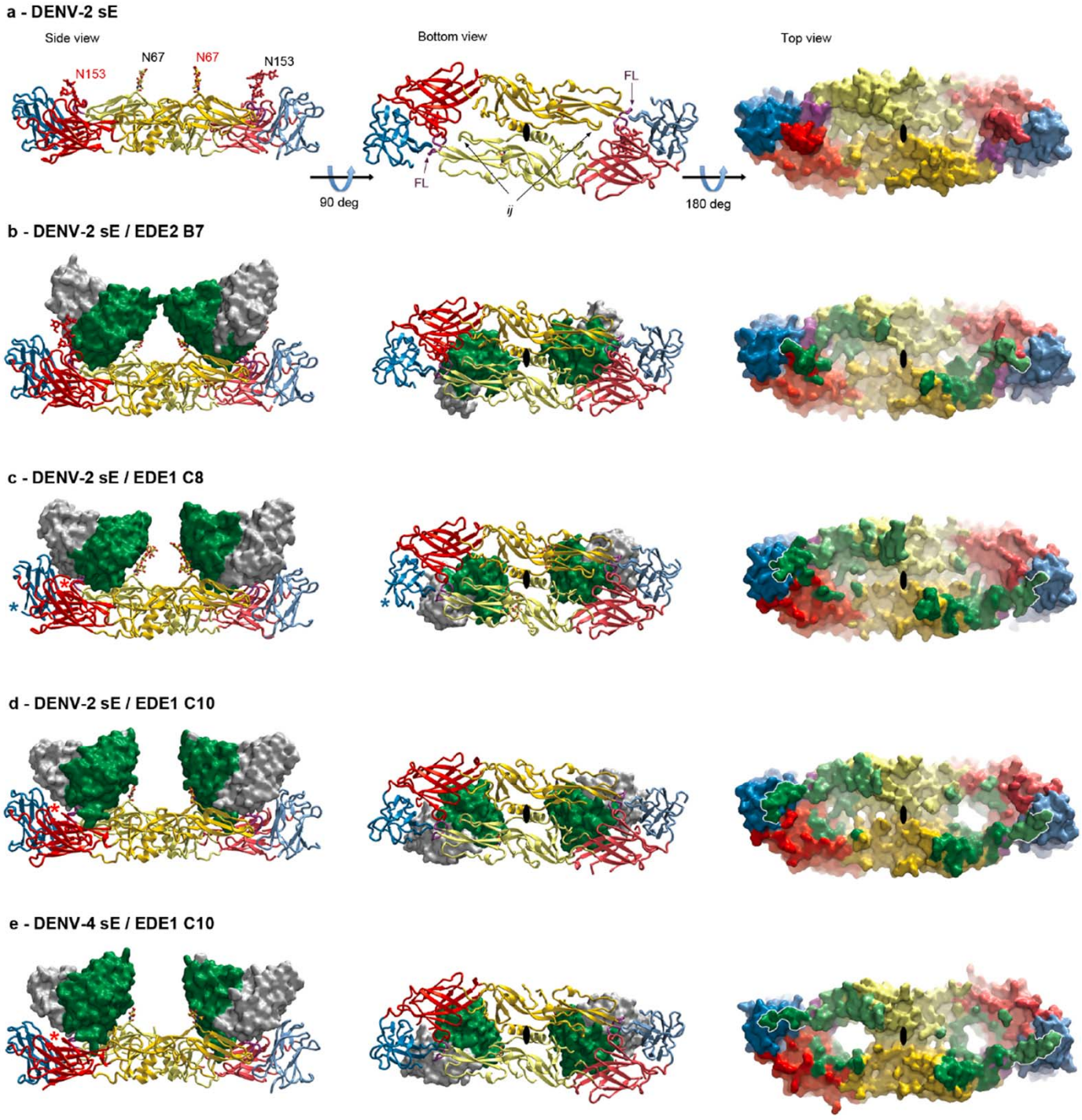


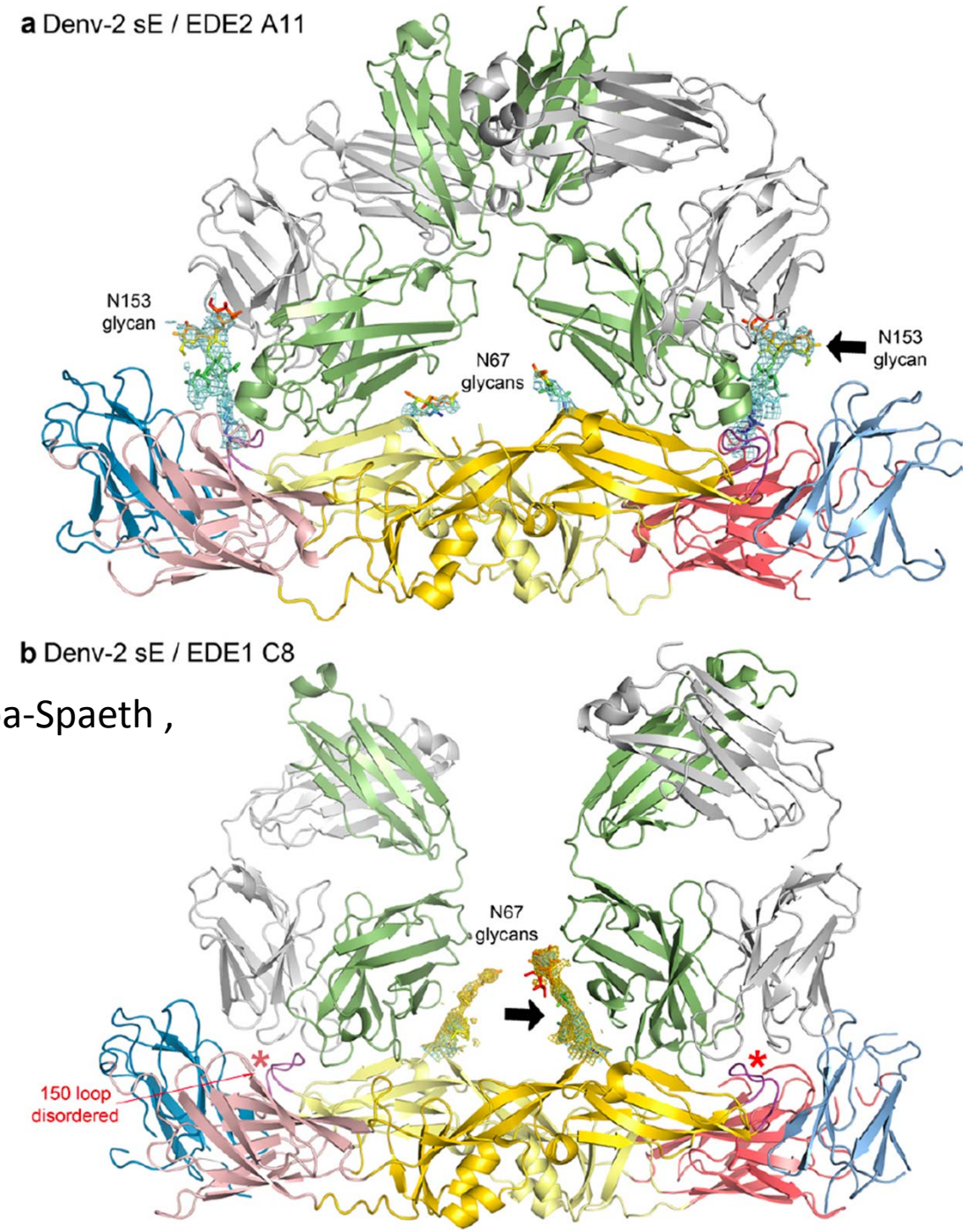
Dejnirattisai et al
Nature Immunology 2015

Infection reduction assayed by focus-forming assay using DENV2



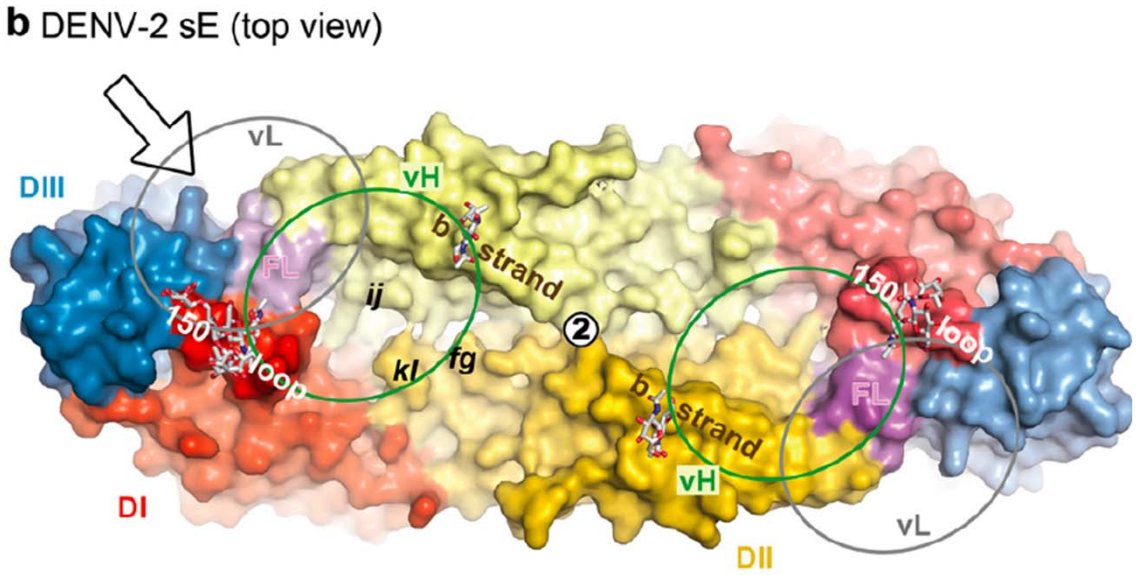



a - DENV-2 sE
Side view
N153
N67
N67
N153
Bottom view
FL
FL
ij
Top view
90 deg
180 deg
b - DENV-2 sE / EDE2 B7
c - DENV-2 sE / EDE1 C8
d - DENV-2 sE / EDE1 C10
e - DENV-4 sE / EDE1 C10



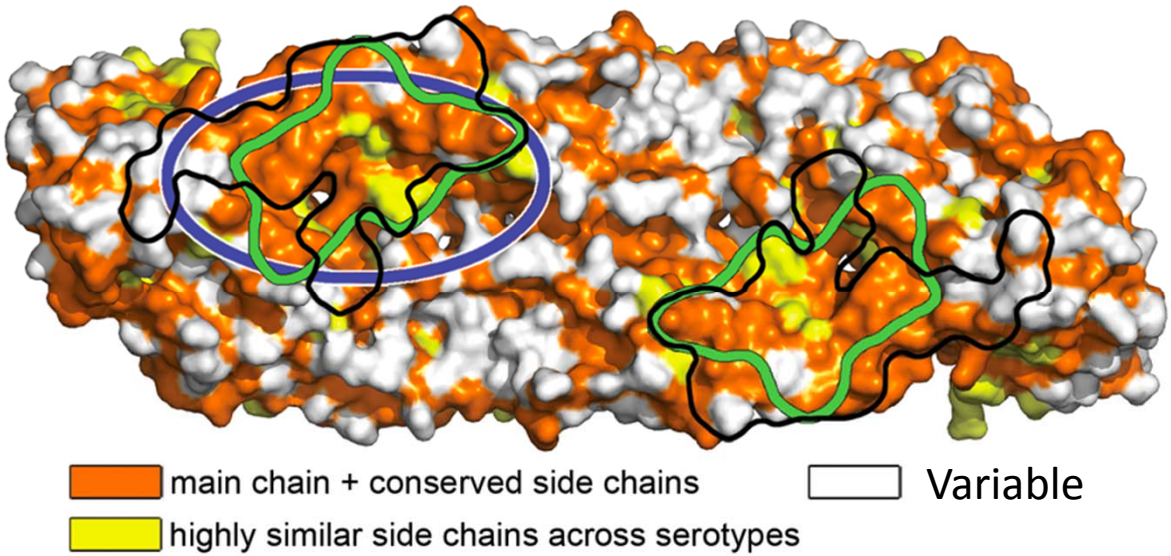
Rouvinski, Guardado-Calvo, Barba-Spaeth , et al.
Nature 2015

The heavy chain spans the distance between the two glycans across the dimer interface:

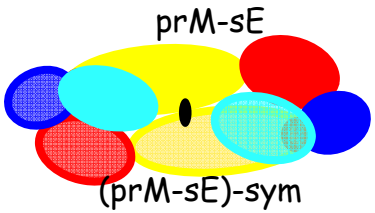


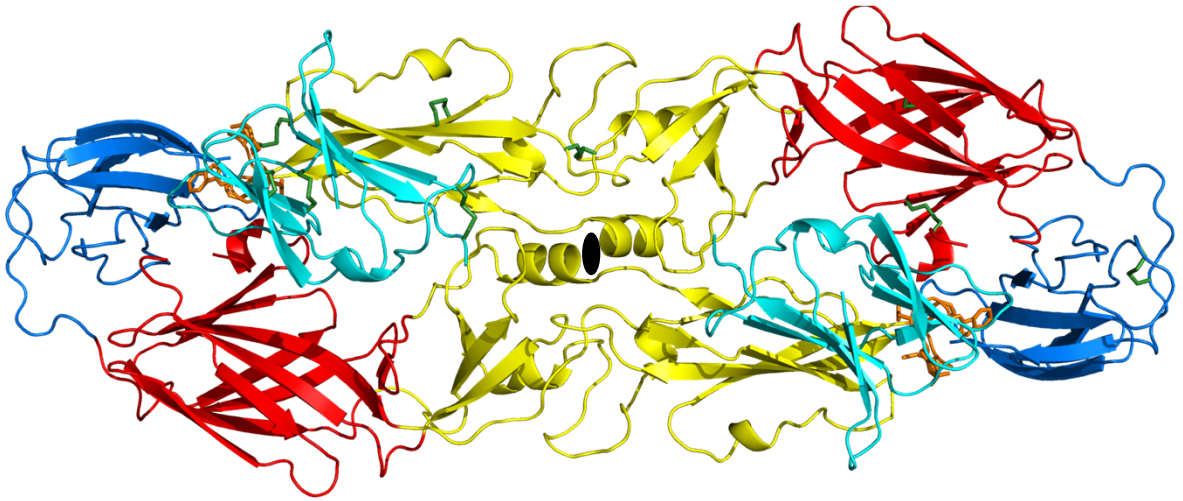
Rouvinski, Guardado-Calvo, Barba-Spaeth ,
et al.
Nature 2015

The sE surface patch corresponding to the epitope is highly conserved across serotypes:

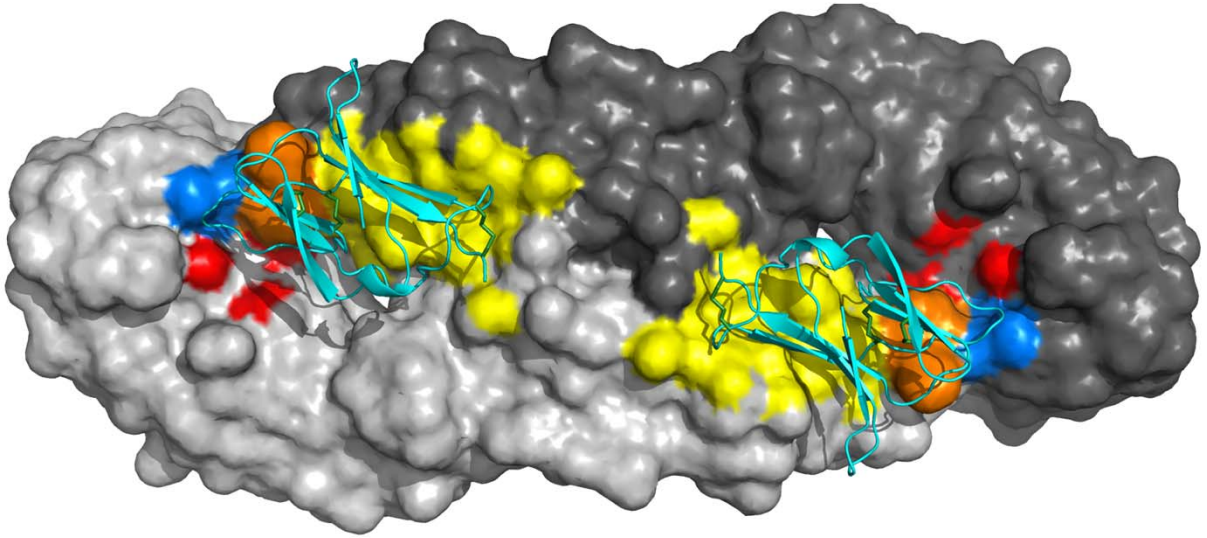


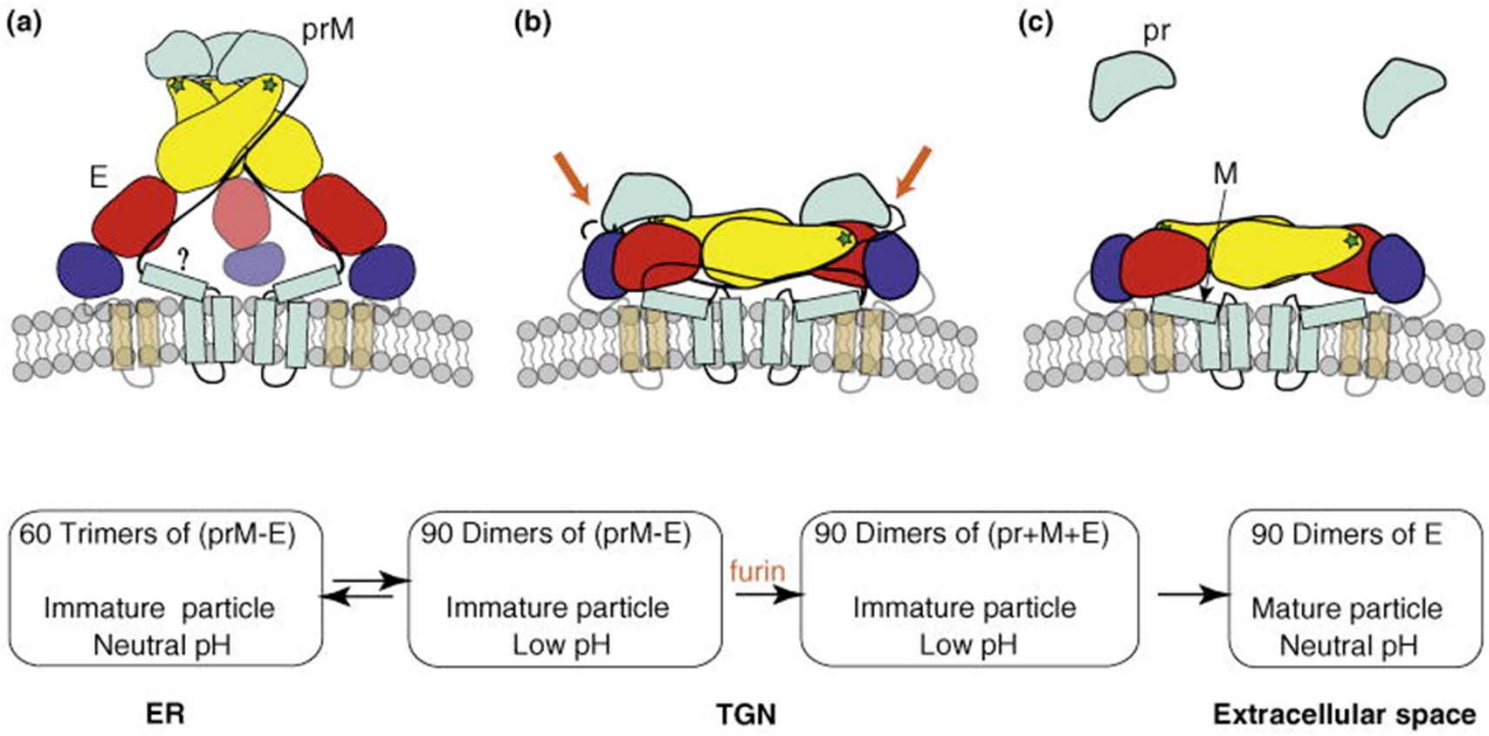
Crystal structure of the TBEV E dimer in complex with pr:





Marie-Christine Vaney




(a)
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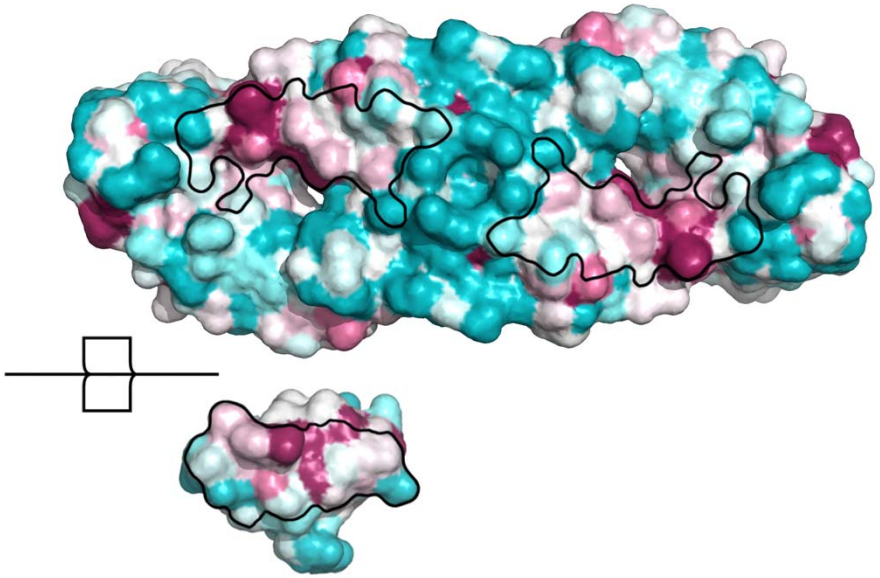
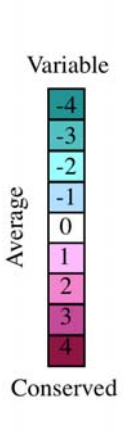
90 Dimers of (pr+M+E)
Immature particle
Low pH
90 Dimers of E
Mature particle
Neutral pH
ER
TGN
Extracellular space

Amino acid conservation among the flaviviruses

Database with 1732 prME full-length and non redundant sequences
Sequence information (identity, deviation, ...) obtained was applied on surfaces of pr and E

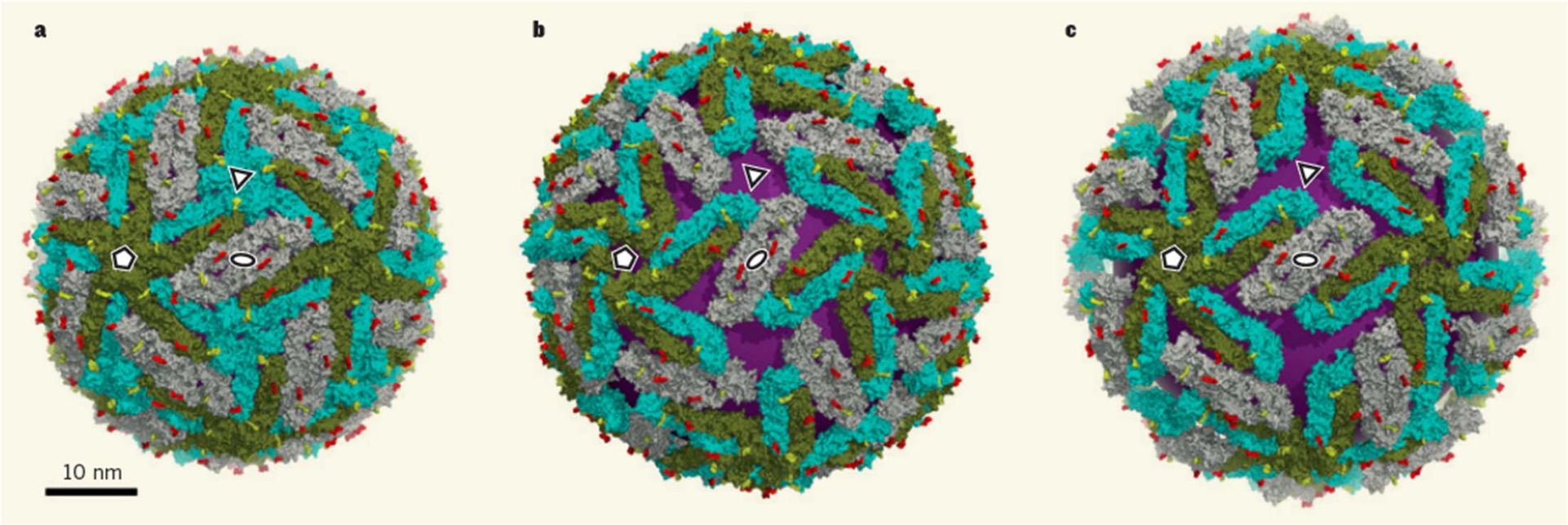
Mariano Delarolle





The dengue virus particle expands at 37°C:

F Rey, Nature N&V 2013

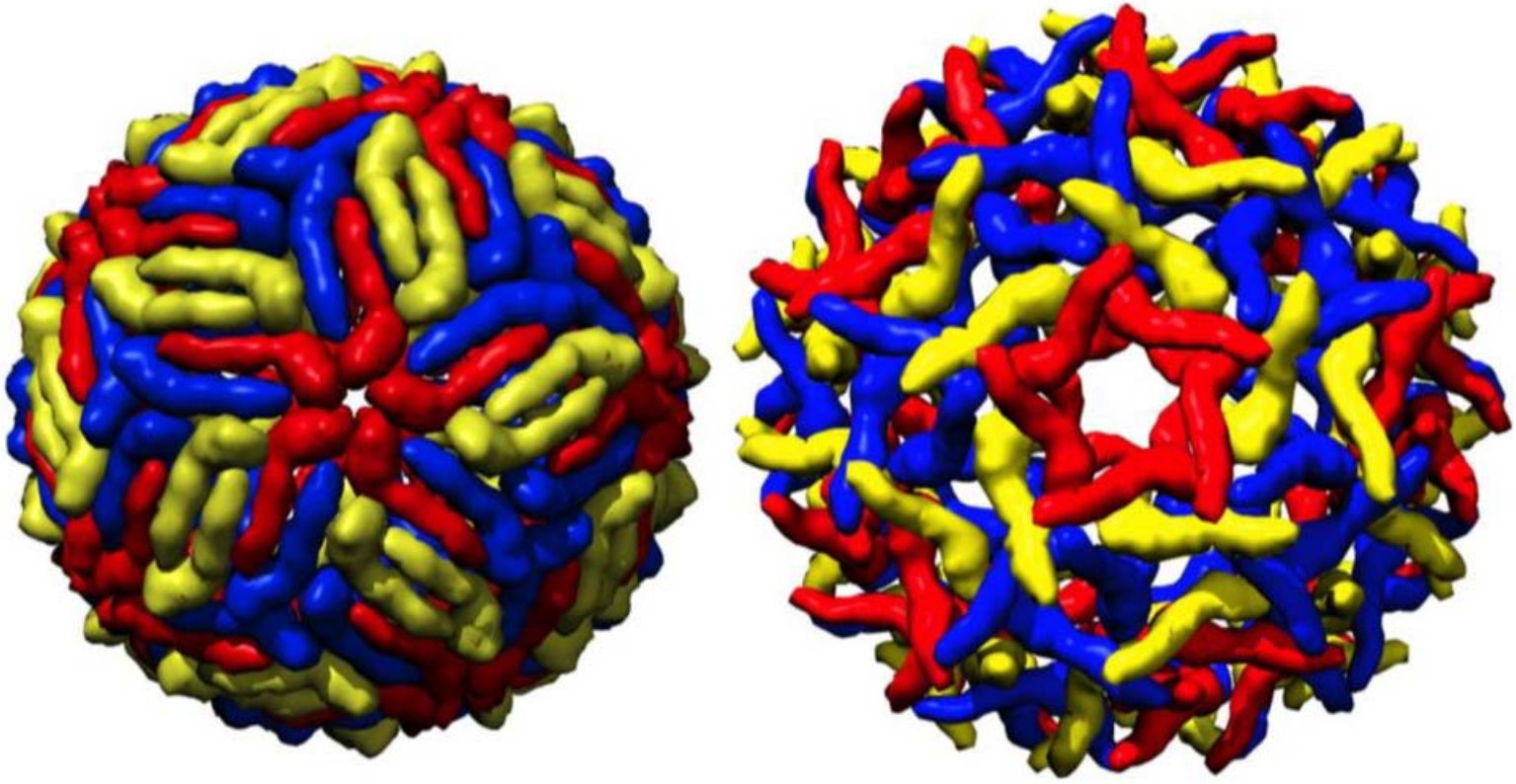


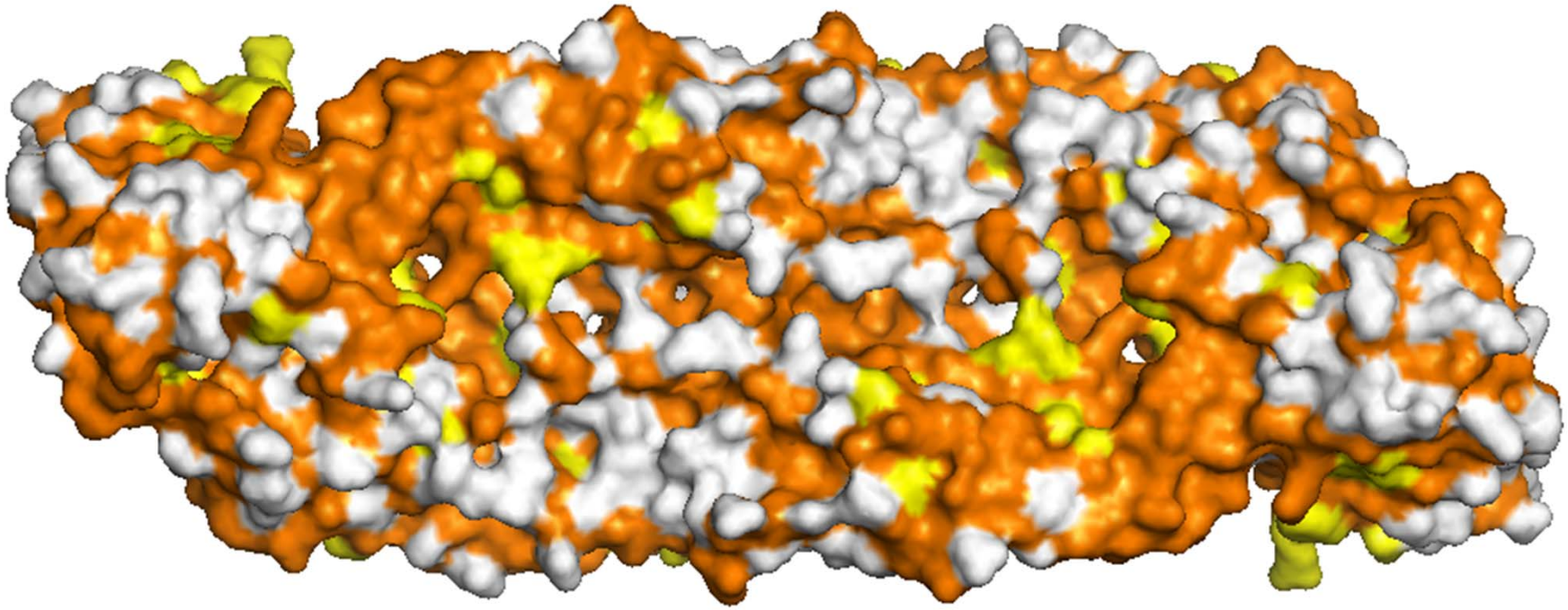
(N153 glycan in red, and N67 in yellow)

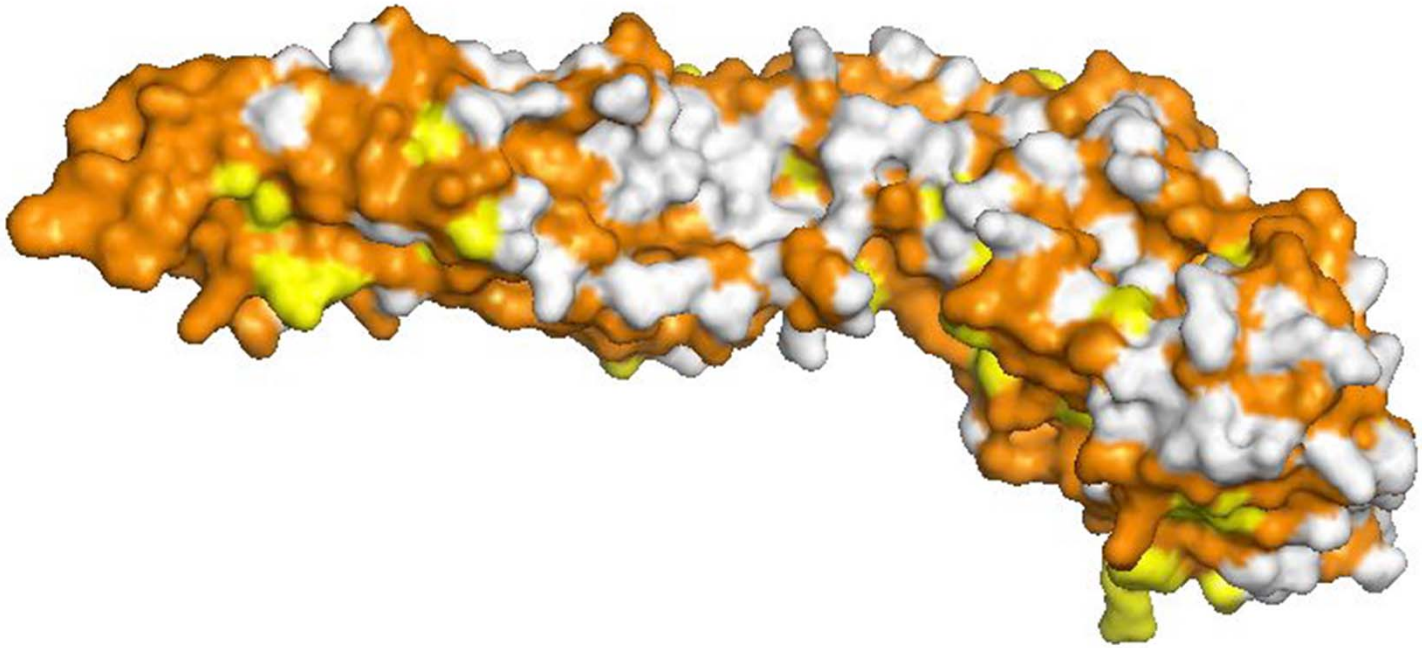
Zhang, X. *et al.* and Rossmann, M.
Dengue structure differs at the temperatures of its human and mosquito hosts. *Proc Natl Acad Sci U S A* **110**, 6795-6799 (2013)

Fibriansah, G. *et al. and Lok, S.M.*
Structural changes of dengue virus when exposed to 37°C.
J Virol, doi:10.1128/JVI.00757-13 (2013).

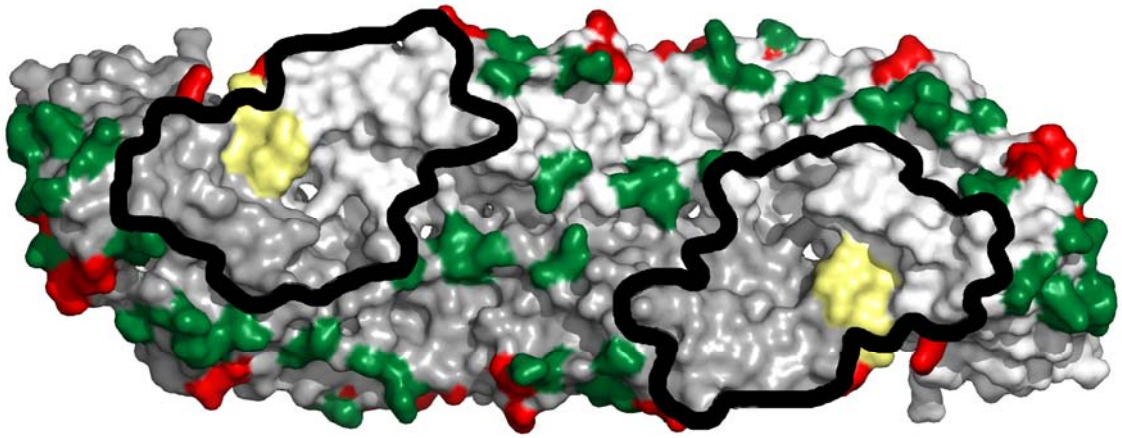
Dengue virus particle “breathing”:





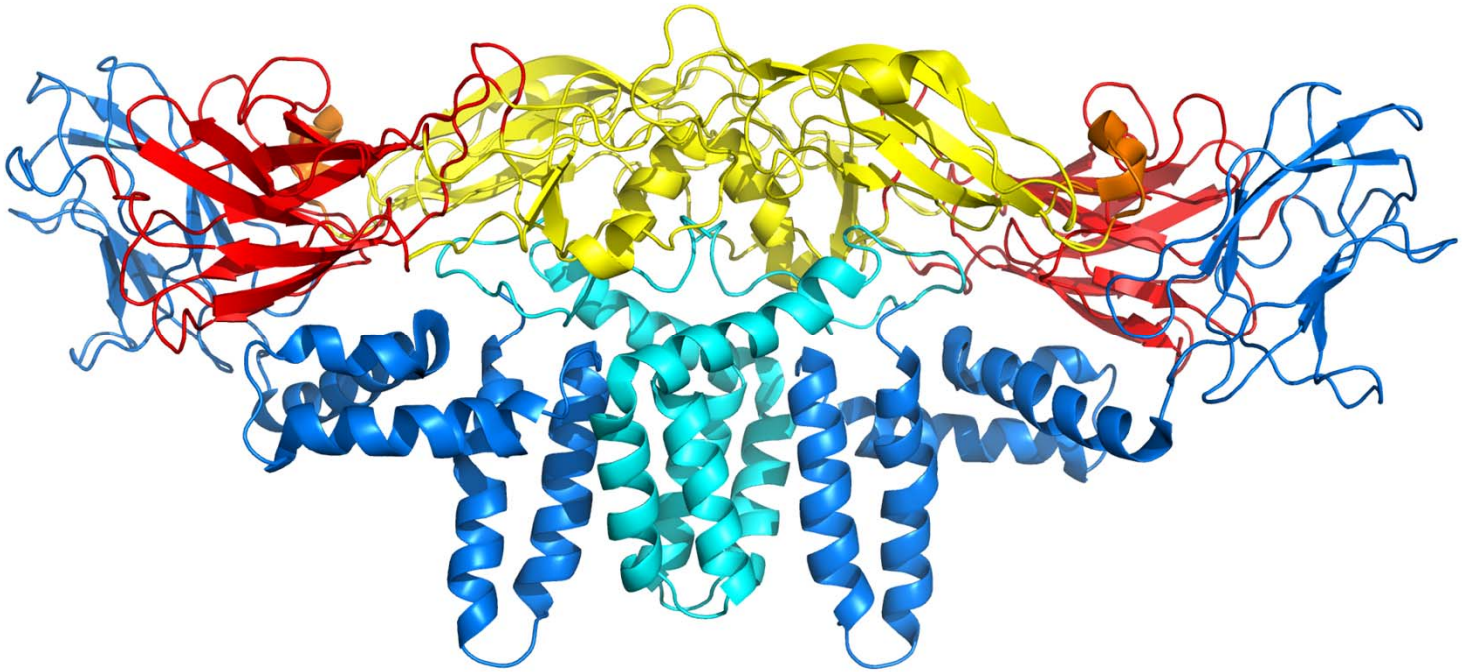


Dimer stabilization and resurfacing



The E dimer is shown in surface representation one subunit in white and the other in grey, with the fusion loop region in yellow. The outline of the EDE is in black. Candidate residues for resurfacing are displayed in bright colors: green and red for serotype variable and conserved residues, respectively.

mature DENV-2 M-E dimer
(3J2P)



cryo-EM structure at 3.6 Å resolution
(Zhang et al, NSMB, 2012)

Conclusions

- First thorough characterization of broadly neutralizing human antibodies against dengue virus
- These antibodies apparently point to the “Achilles heel” of the virus for neutralization escape
- The structure suggests that a single immunogen, in the form of a stabilized E dimer, should be used for subunit vaccine development

Alex
ROUVINSKI

Pablo
GUARDADO CALVO

Giovanna
BARBA-SPAETH

Marie-Christine
VANEY

Stéphane
DUQUERROY

UNITÉ DE VIROLOGIE STRUCTURALE, INSTITUT PASTEUR, PARIS

Gavin Screaton

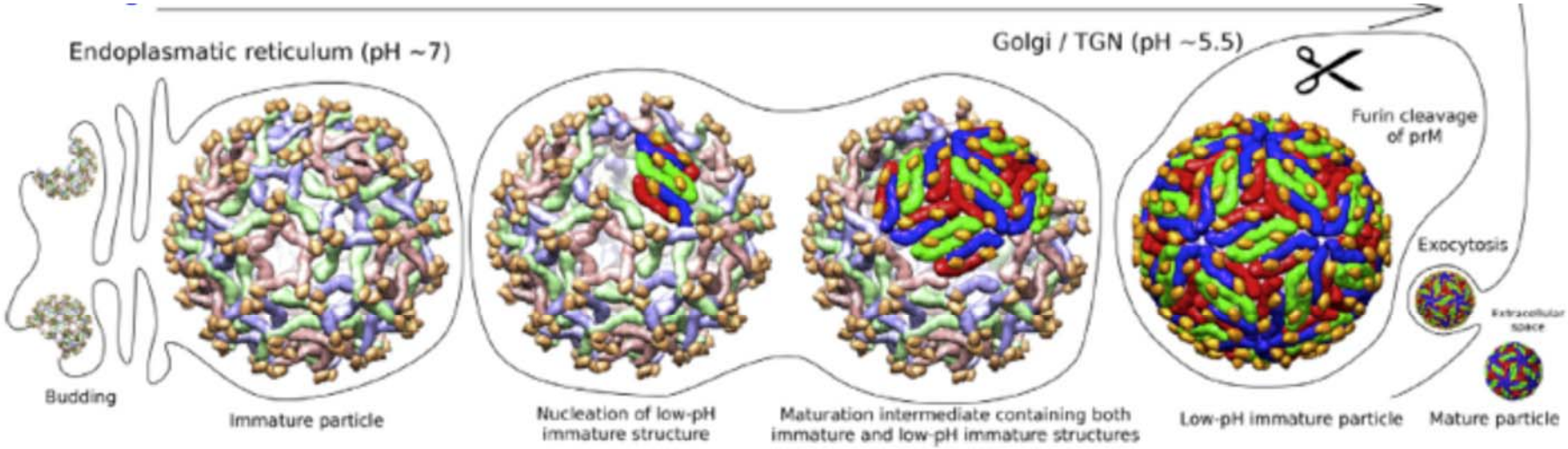
Juthathip Mongkolsapaya

IMPERIAL COLLEGE, LONDON

Xiaokang Zhang

Z. Hong Zhou
UCLA

Nui Dejnirattisai
Hua Wongwiwat



Plevka et al, JSB 2014