

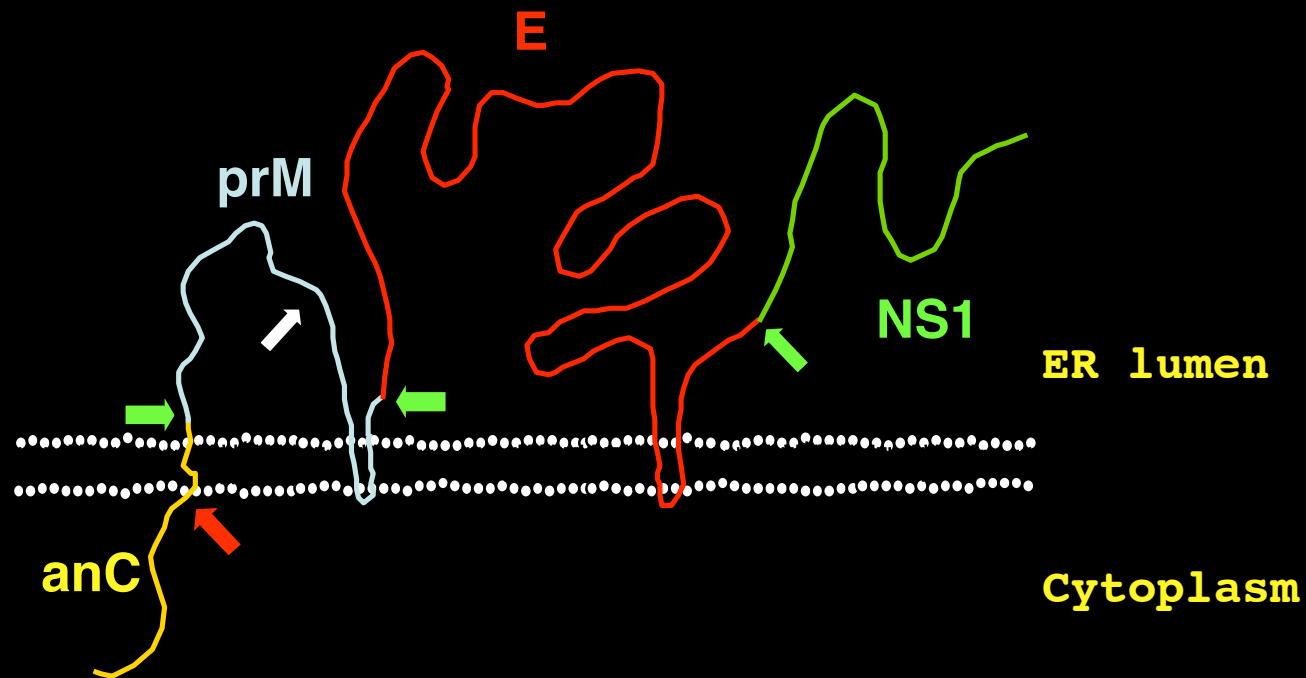
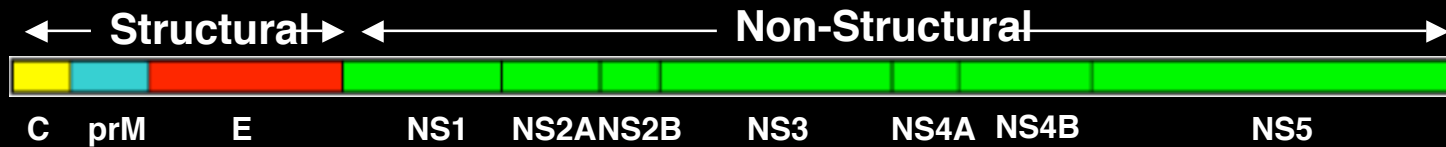
West Nile Virus

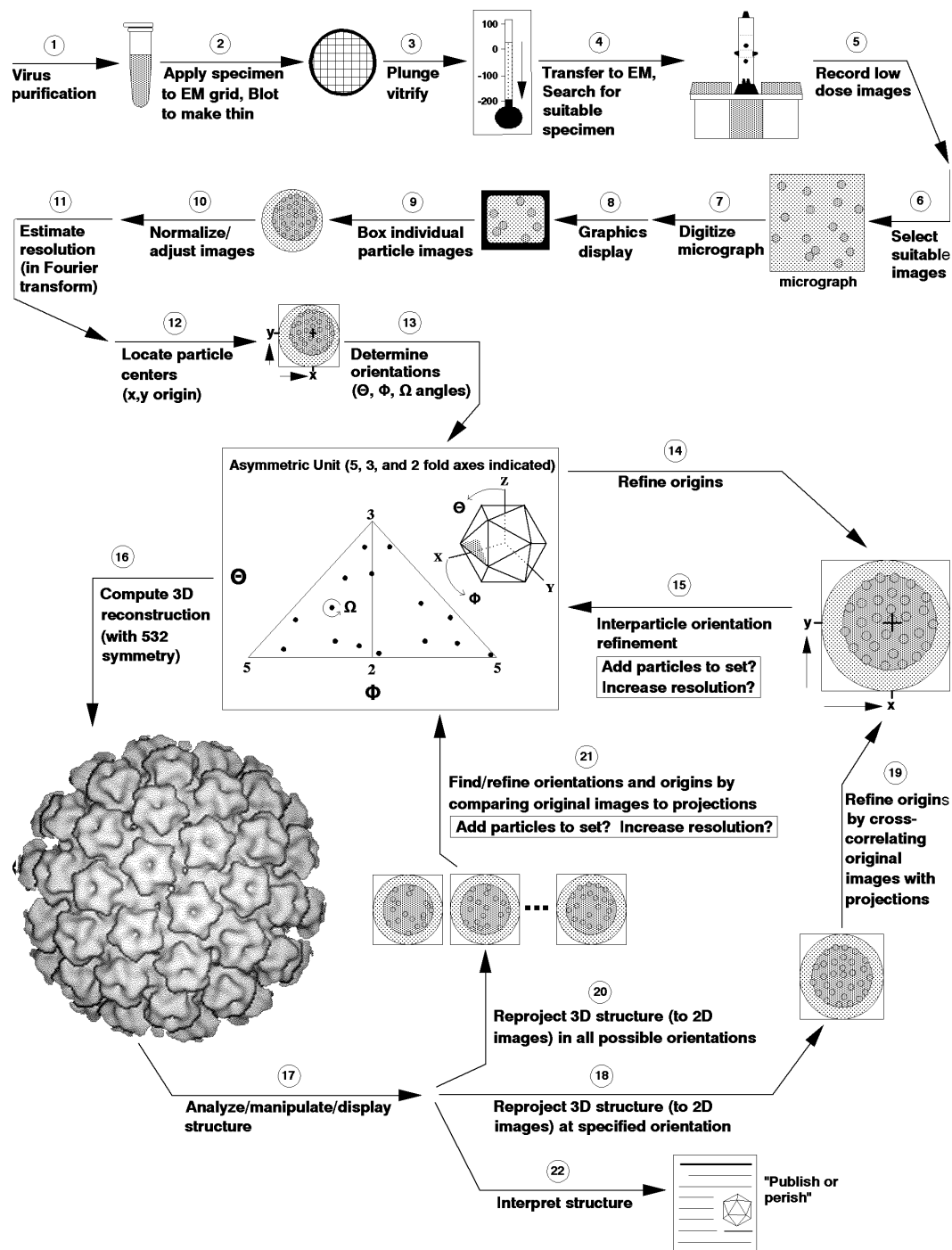
- *Flaviridae* family; flavivirus genus
other members: Yellow Fever, Dengue
- Closely related to Kunjin and Japanese encephalitis
- Transmitted by mosquitoes
- Plus strand RNA virus 11 kB genome
- Virus particle contains a lipid bilayer and three viral proteins:
 1. E - envelope protein
 2. M - membrane protein
 3. C - capsid or core protein

Flavivirus Structural Studies

- Structure of the WNV NY99 determined by cryo-EM and image reconstruction
- Determination of the organization of the E protein ectodomain into the density
- High resolution structure of dengue virus showing the topology of the membrane components
- Structure of immature prM-containing dengue particles, WNV particles have been collected and are being processed
- Solution structure of the dengue capsid protein using NMR; WNV is in progress

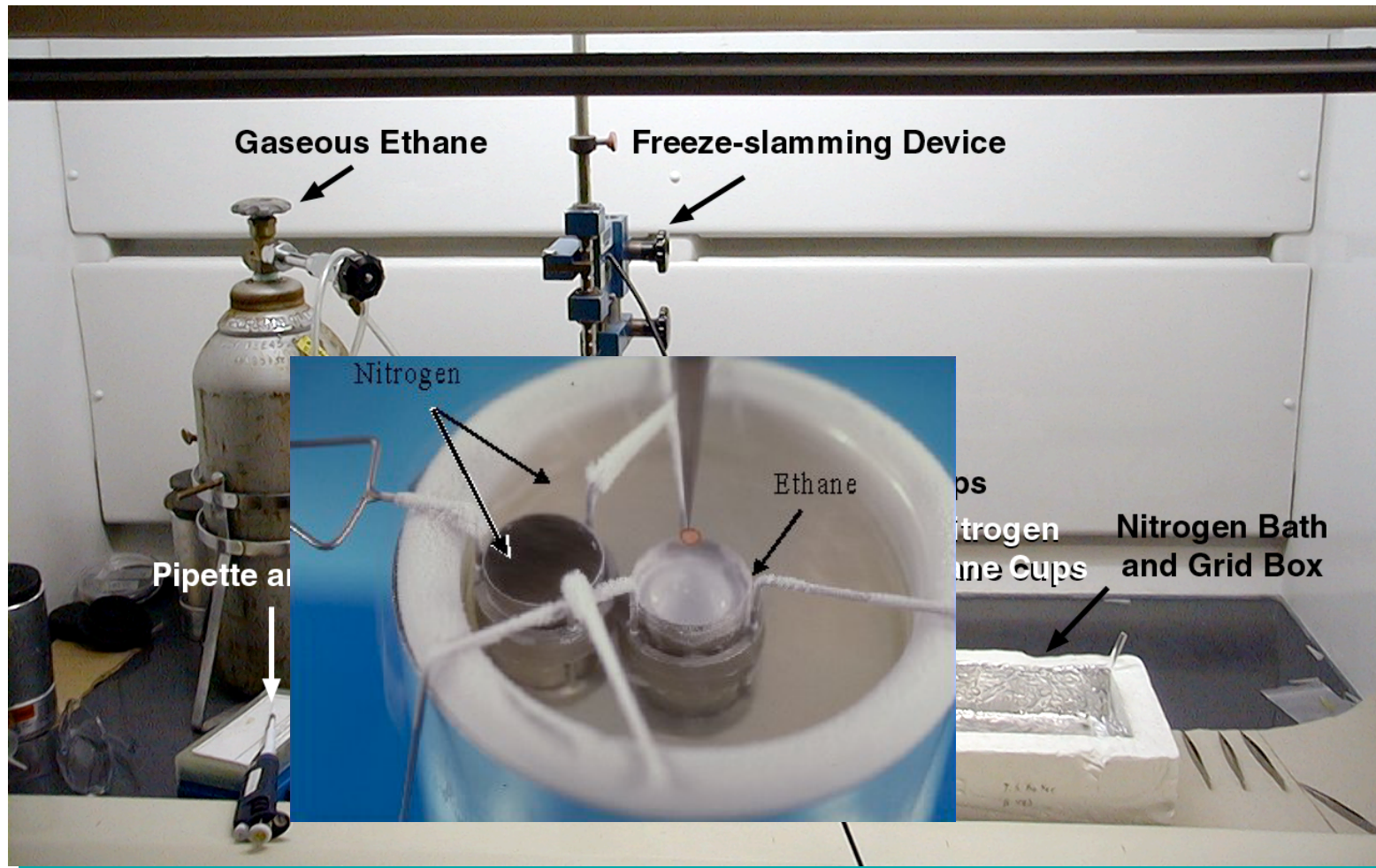
Flavivirus Polyprotein / Processing of the Structural Proteins



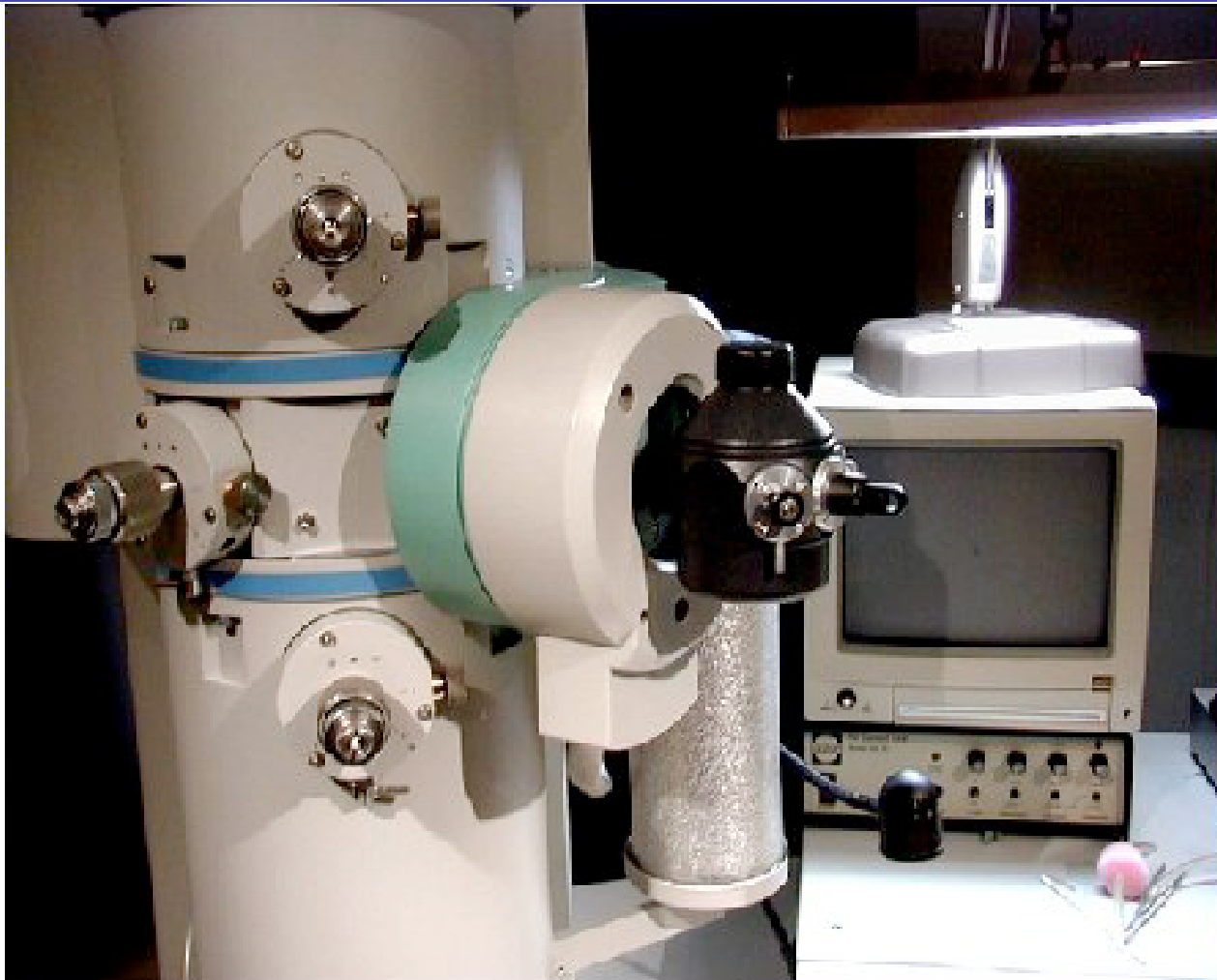


Cryo-electron Microscopy & Image Reconstruction

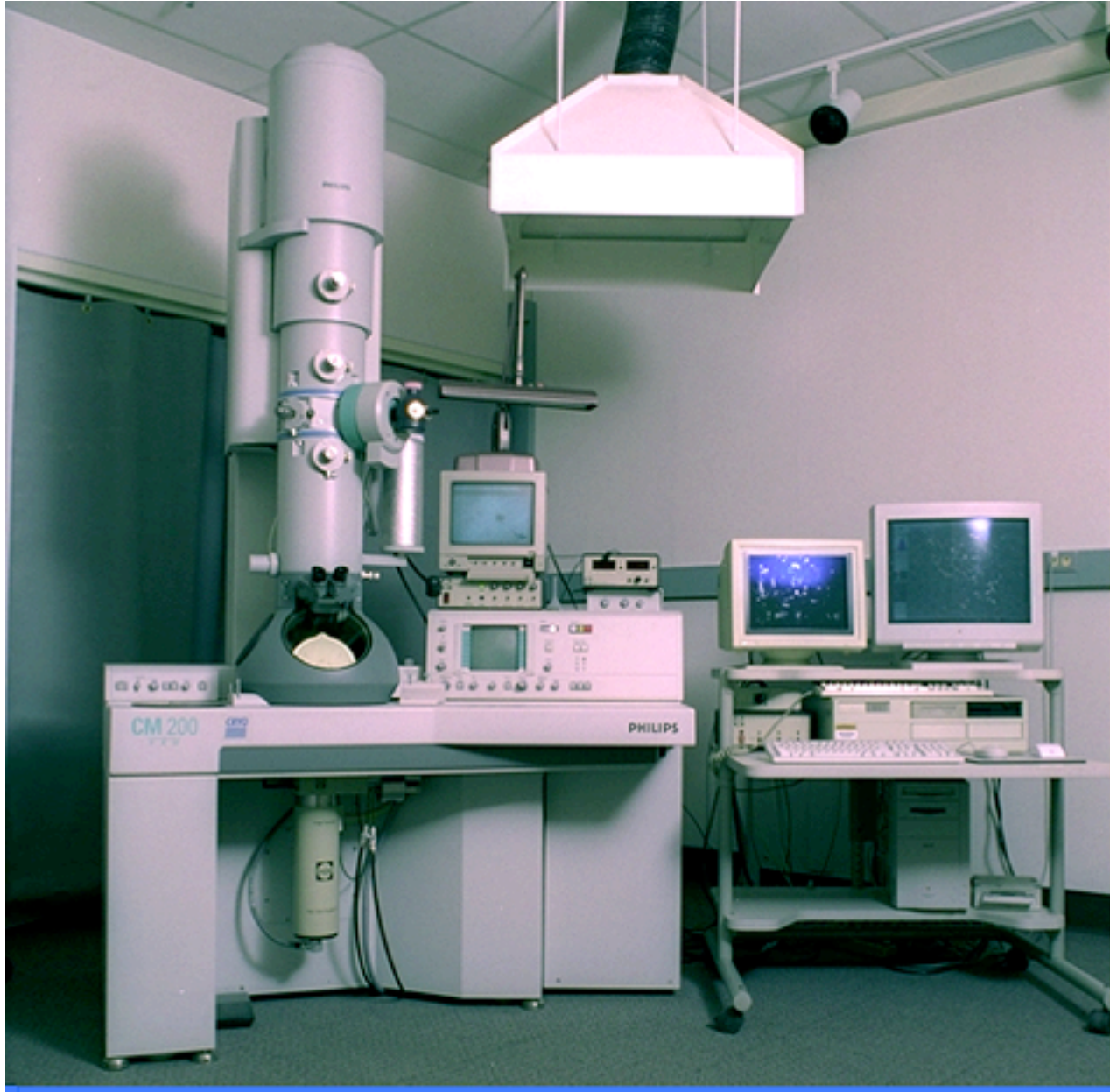
SAMPLE PREPARATION



HOLDER IN MICROSCOPE



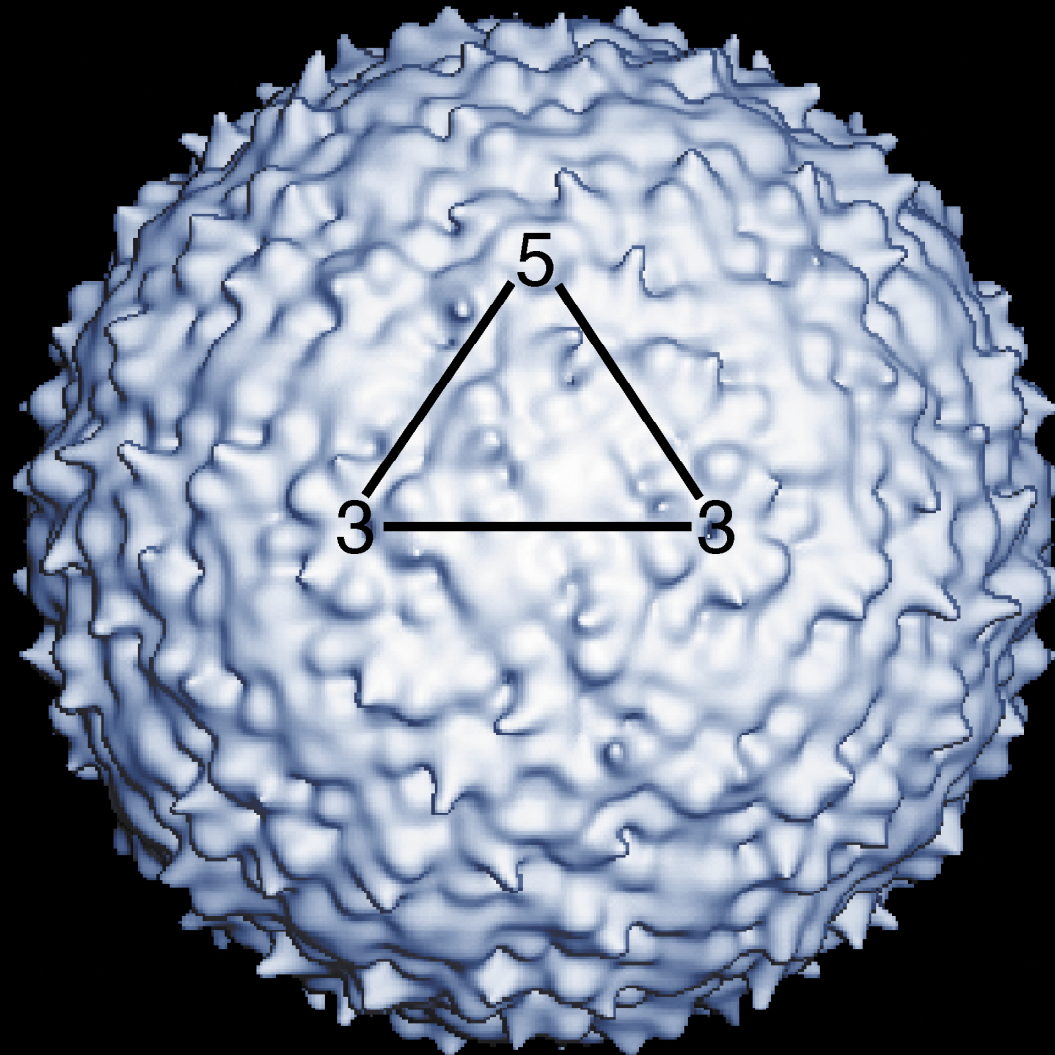
Philips CM200 FEG



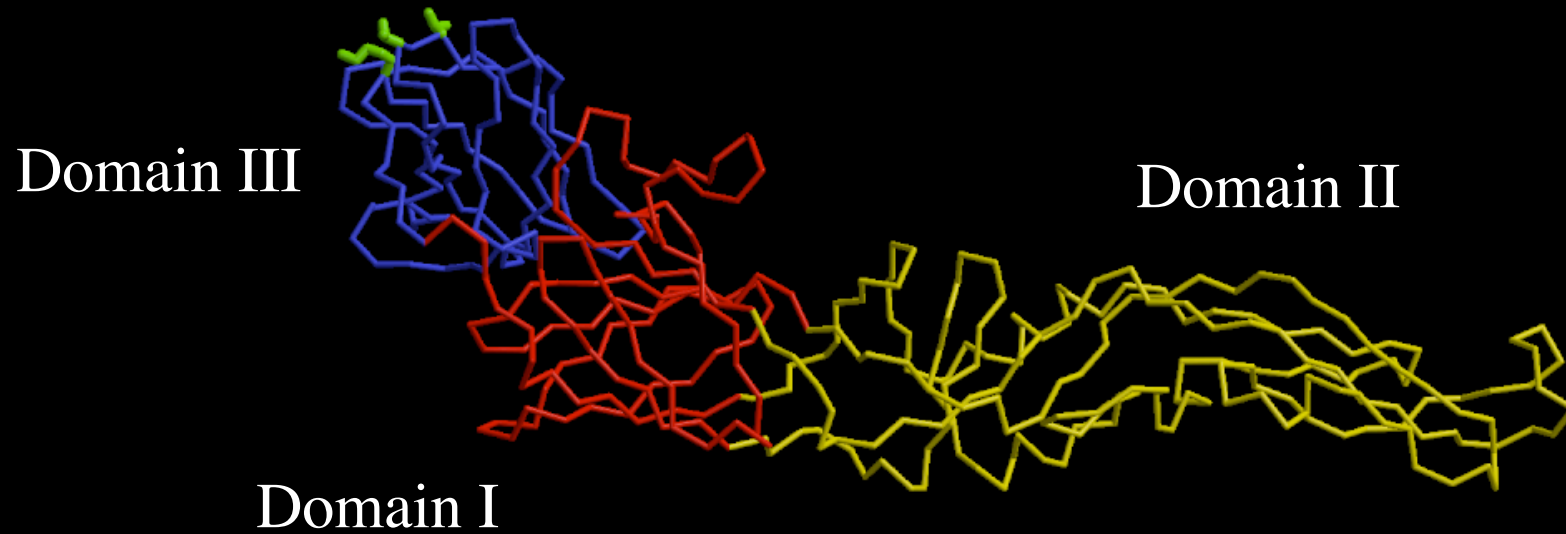
A cryo-electron micrograph showing a dense field of spherical West Nile Virus particles. The particles are approximately 100 nm in diameter and exhibit a characteristic outer shell with a distinct surface texture. They are distributed throughout the field of view, with a central rectangular box containing the title text.

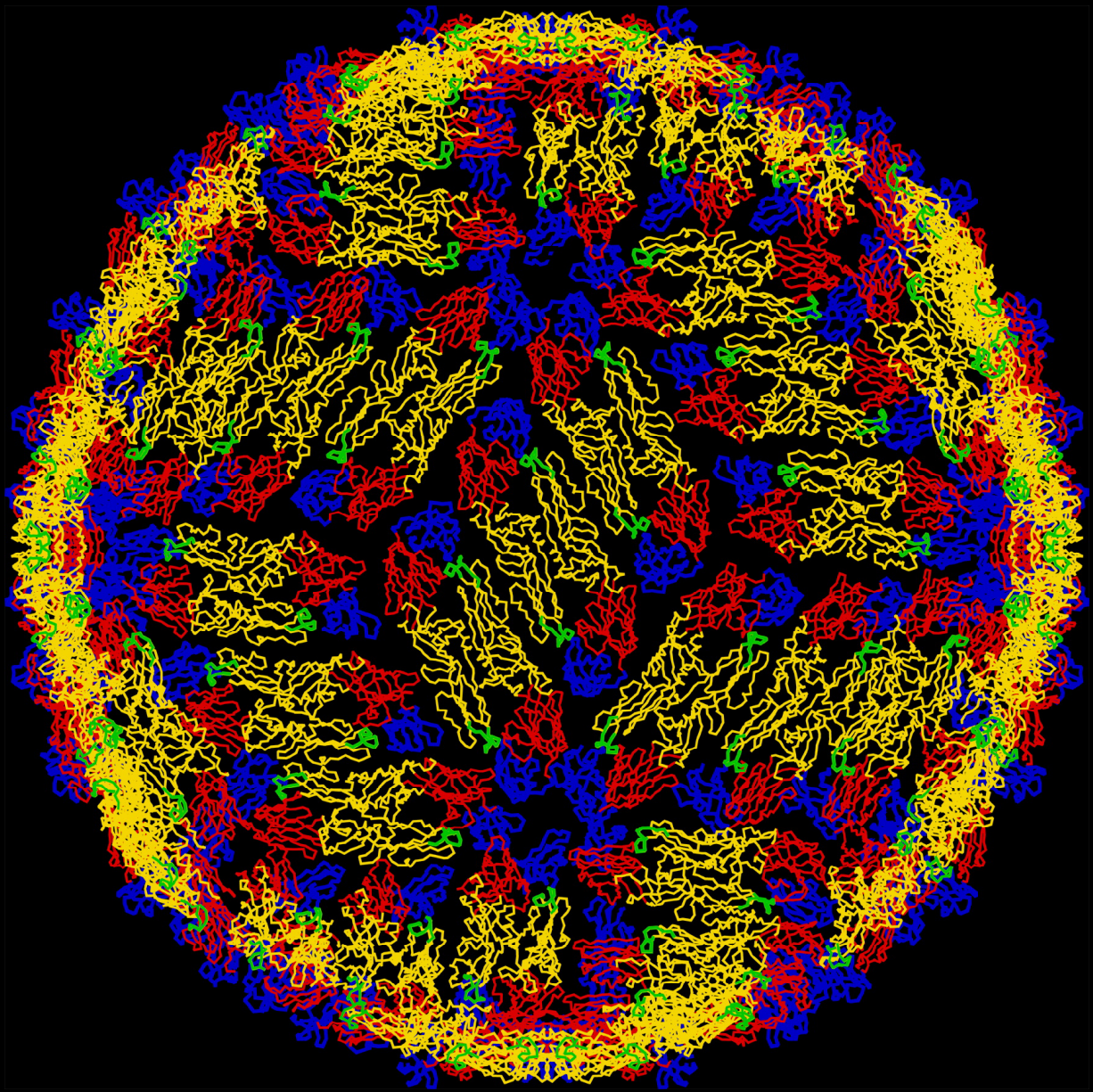
Cryo-EM of West Nile Virus

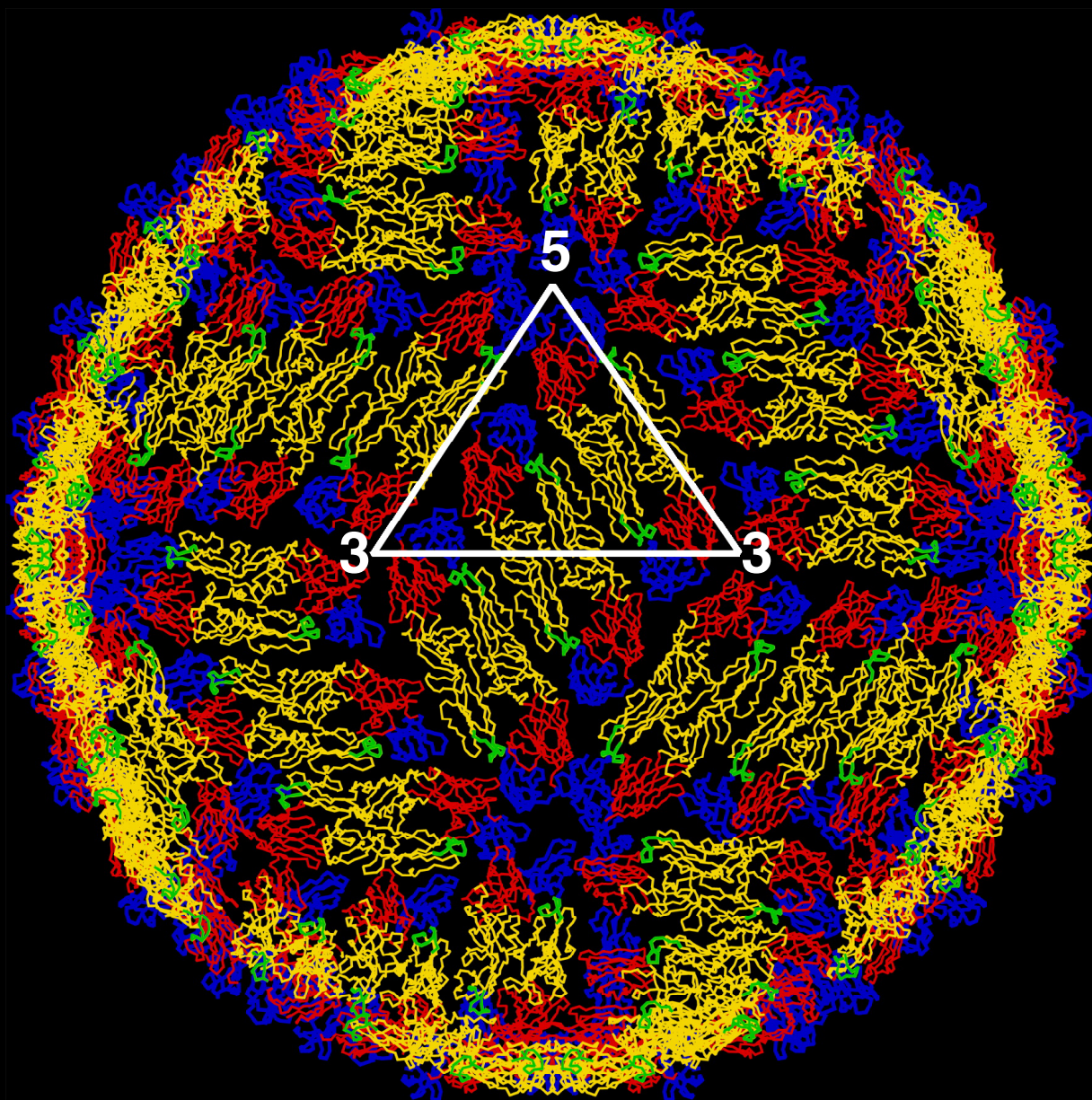
West Nile Virus

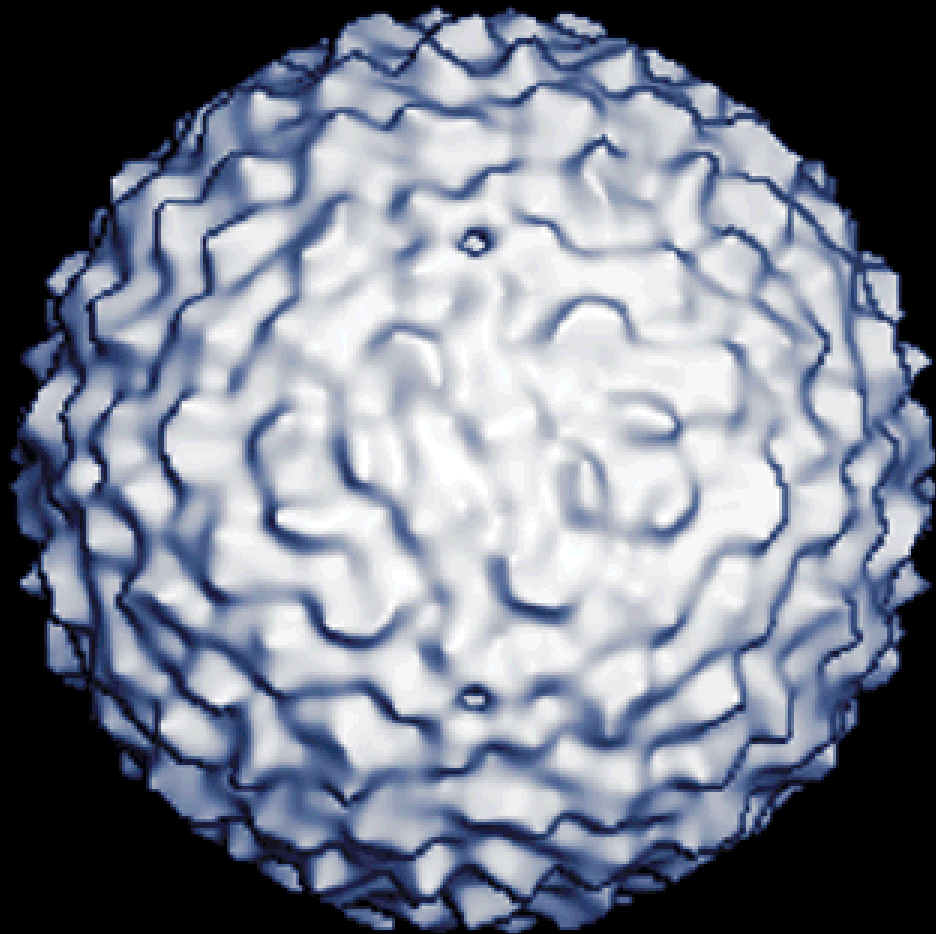


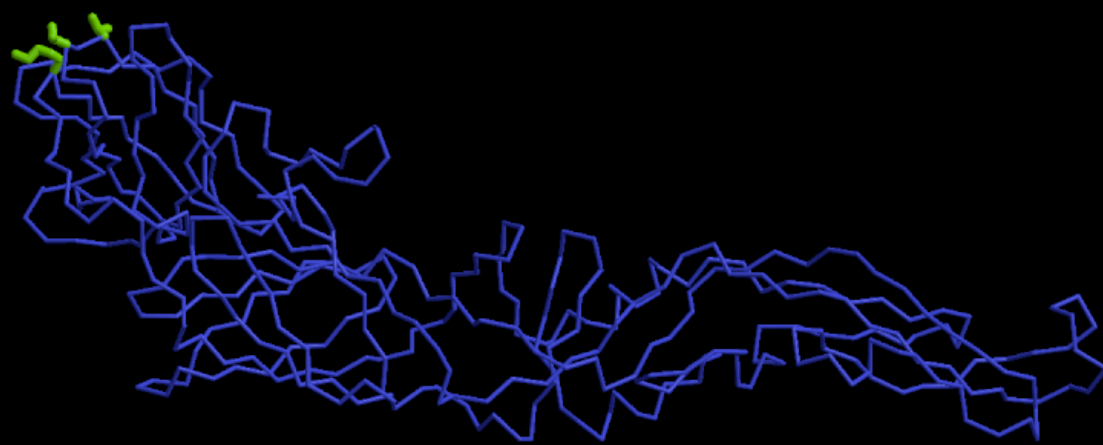
Dengue E Protein Atomic Structure

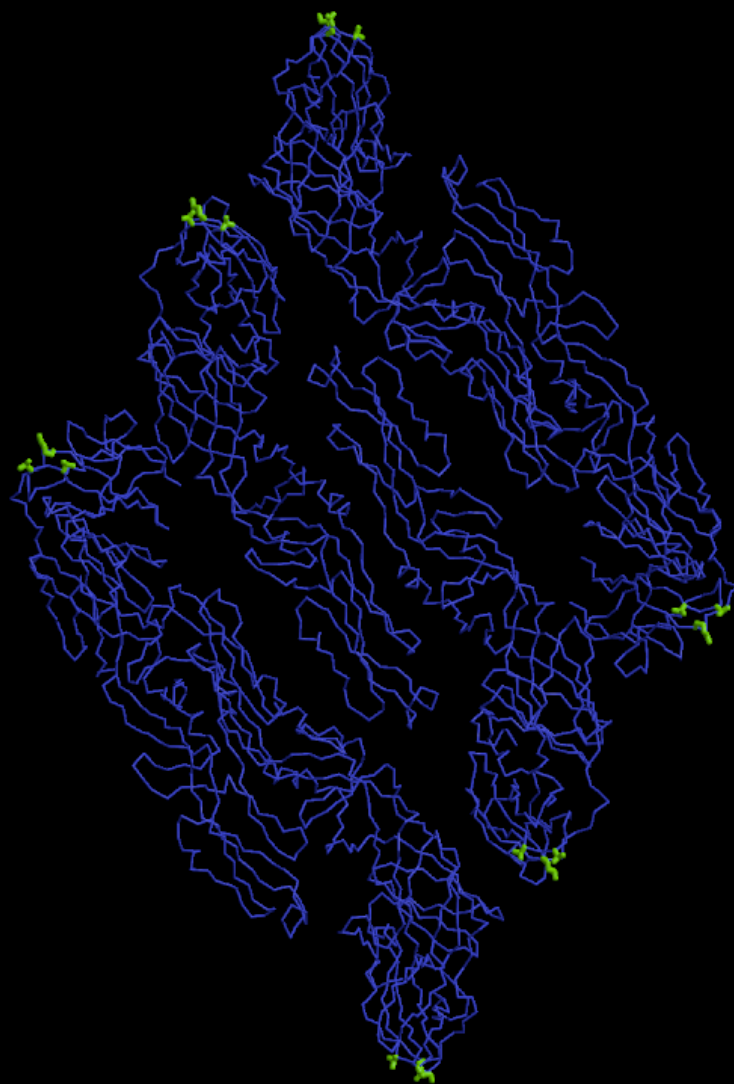


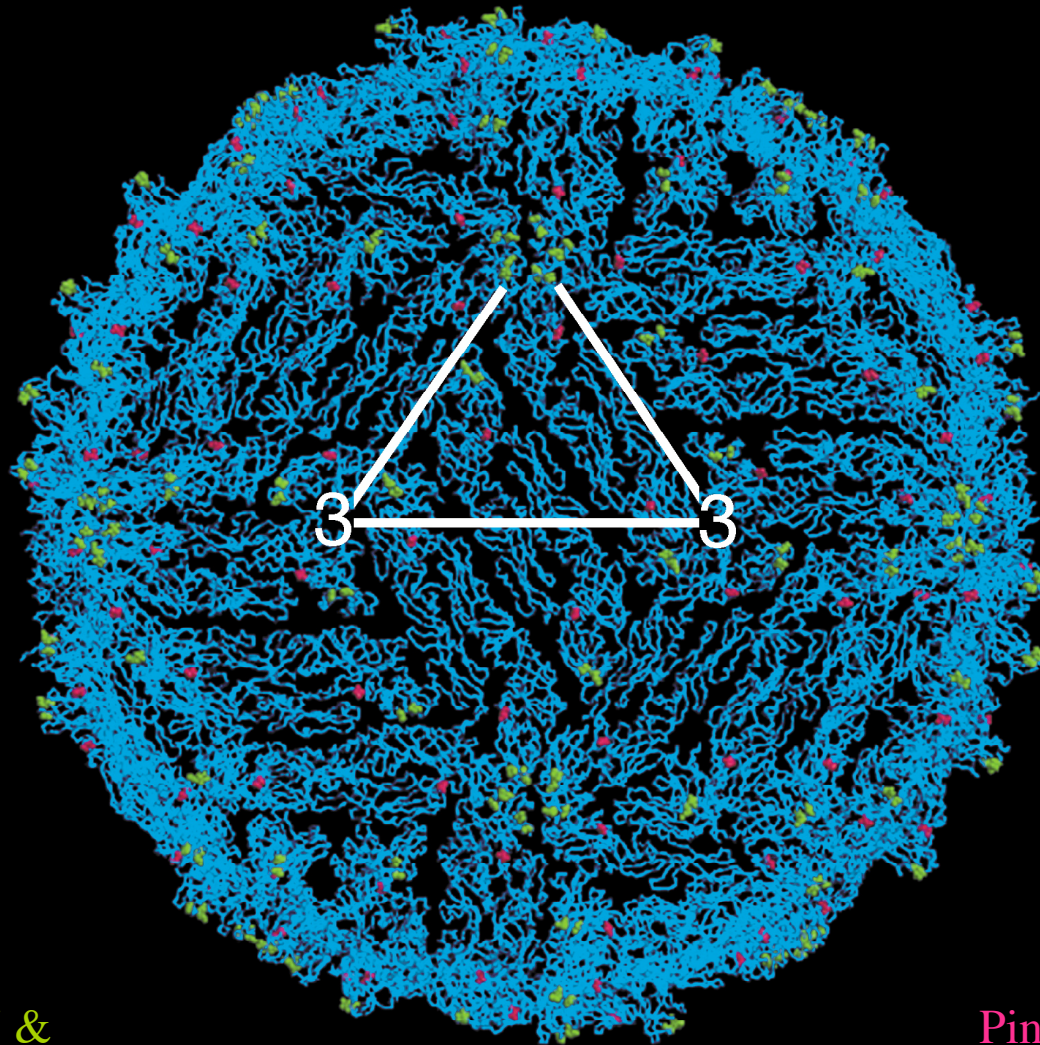






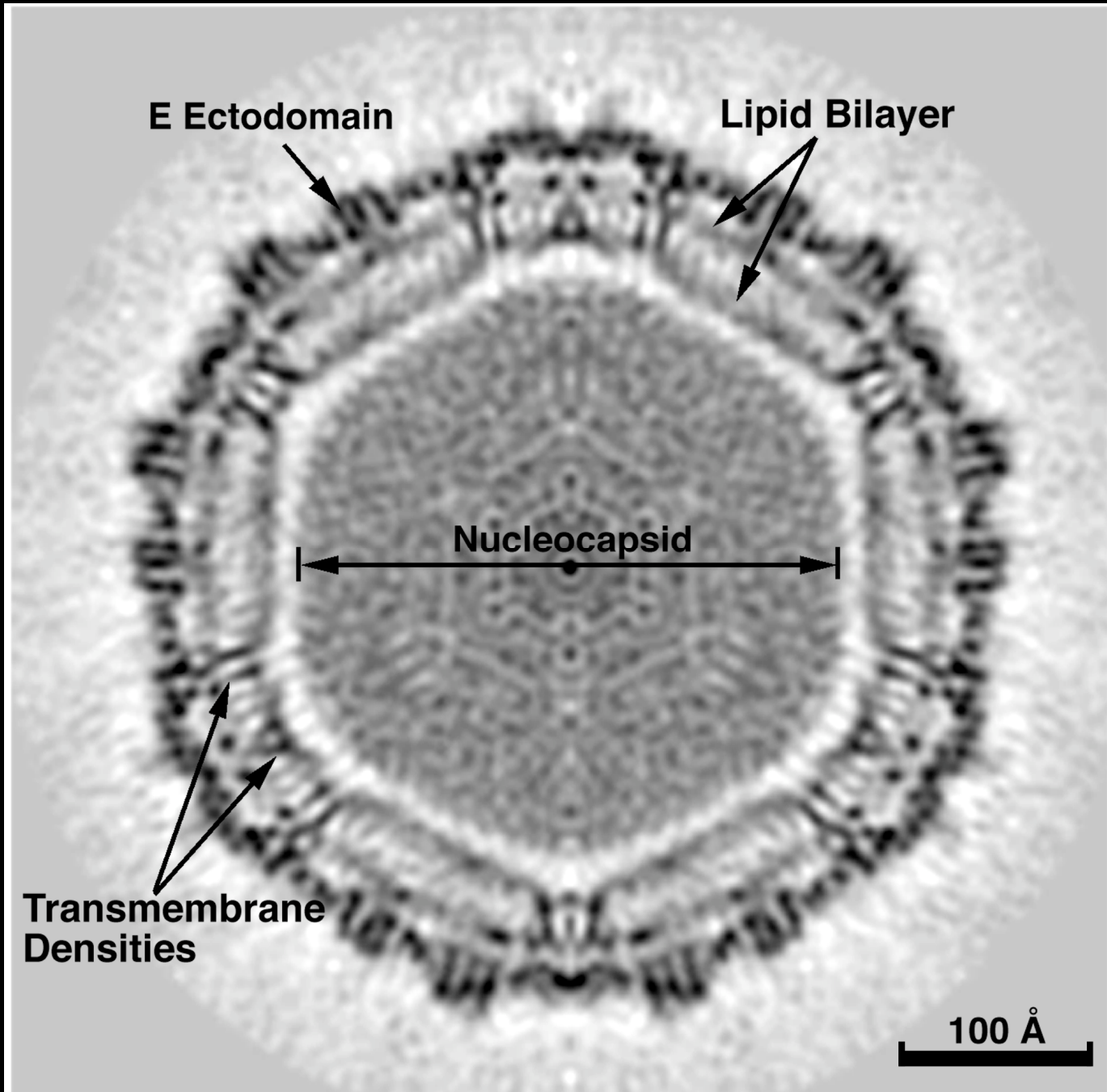


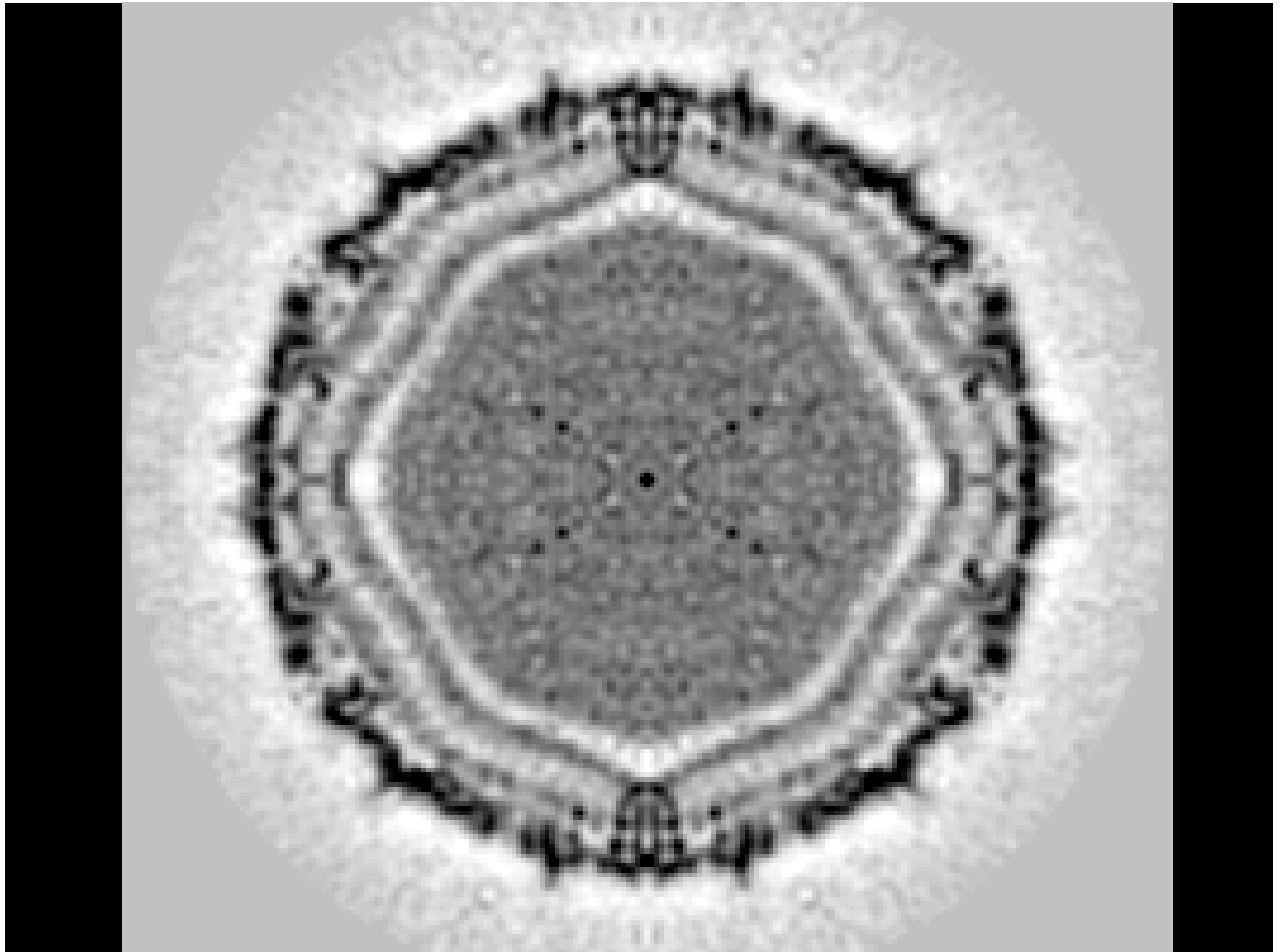


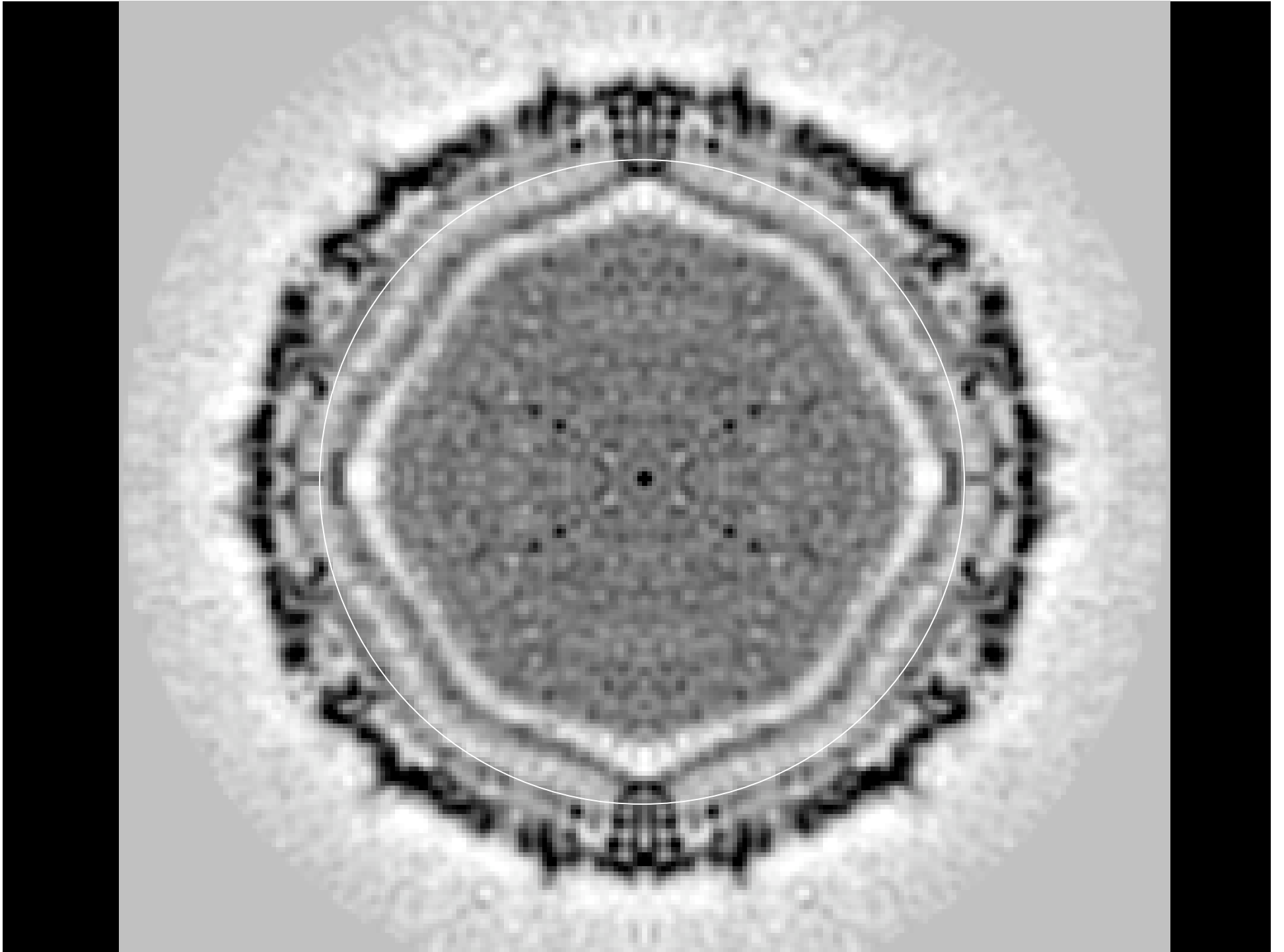


Green: residues 307 &
330 that bind neut.
antibodies

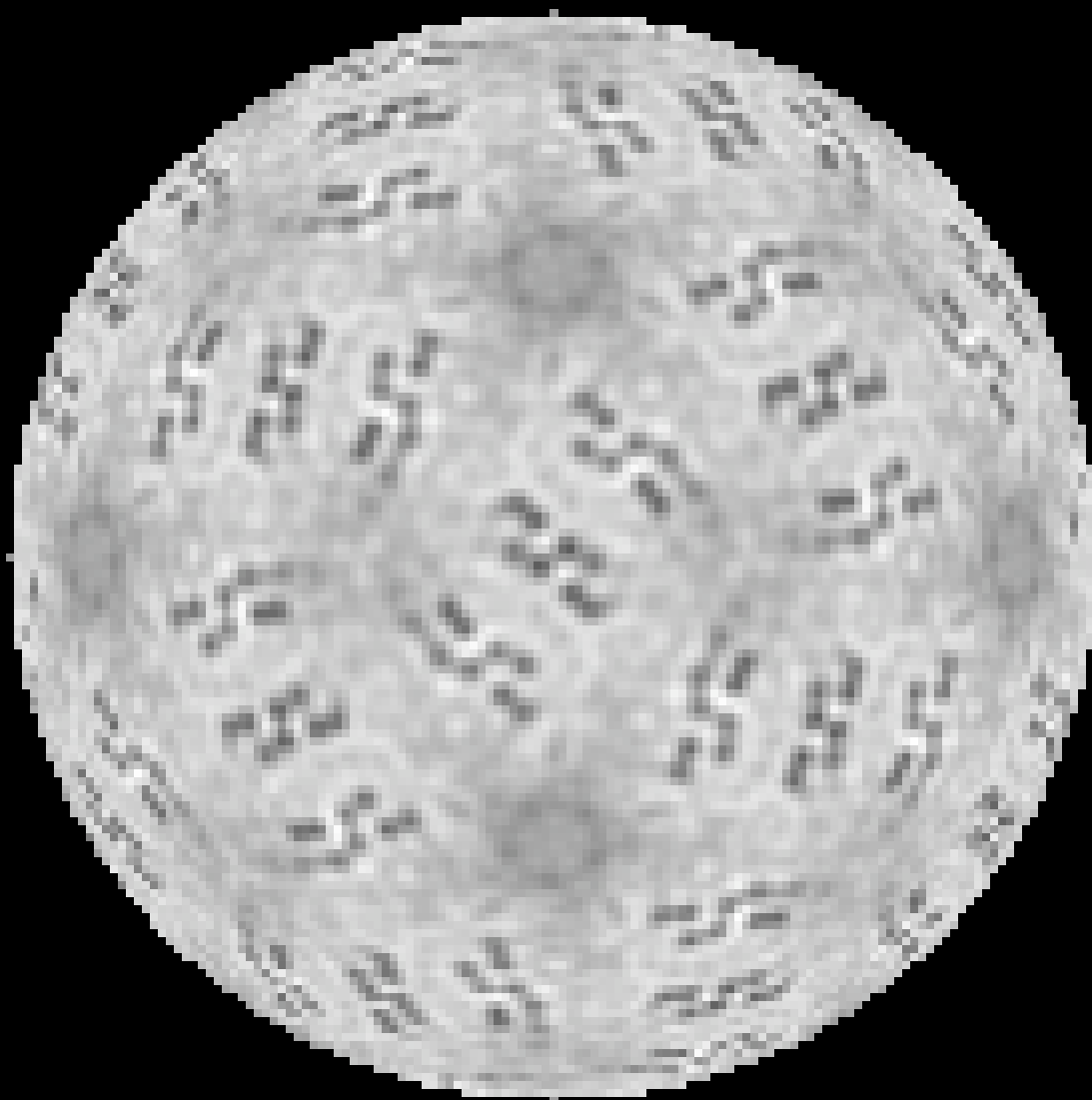
Pink: glycosylated
residue N154

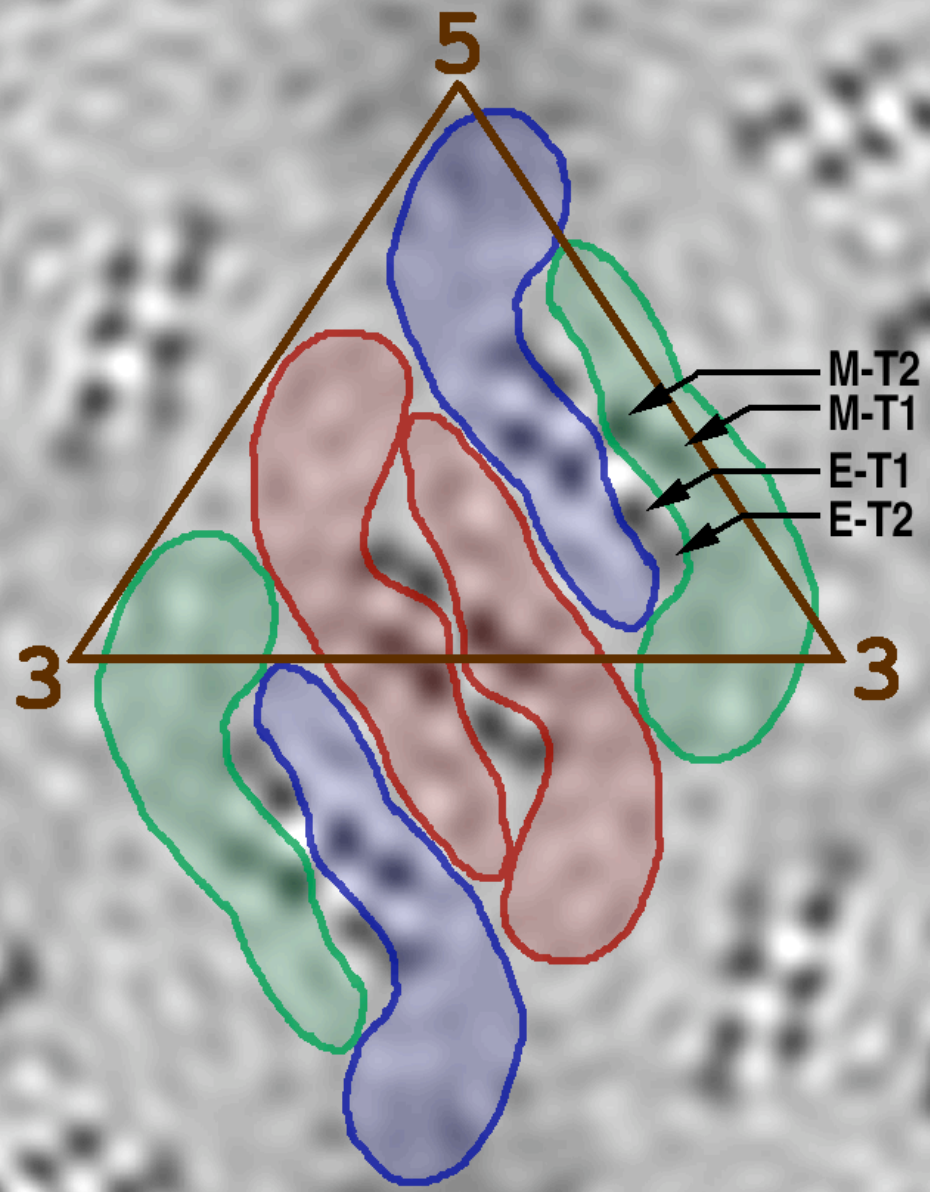


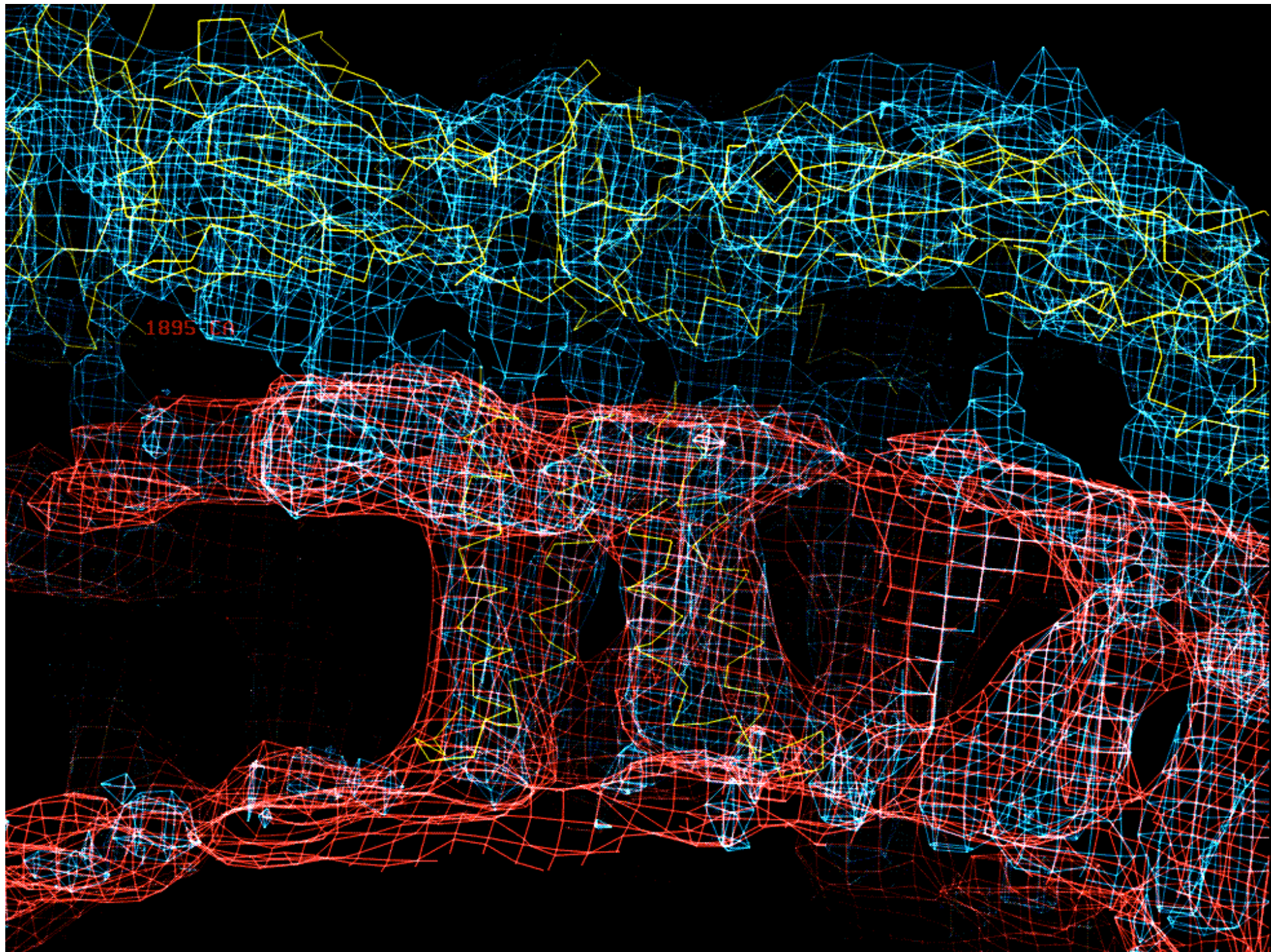


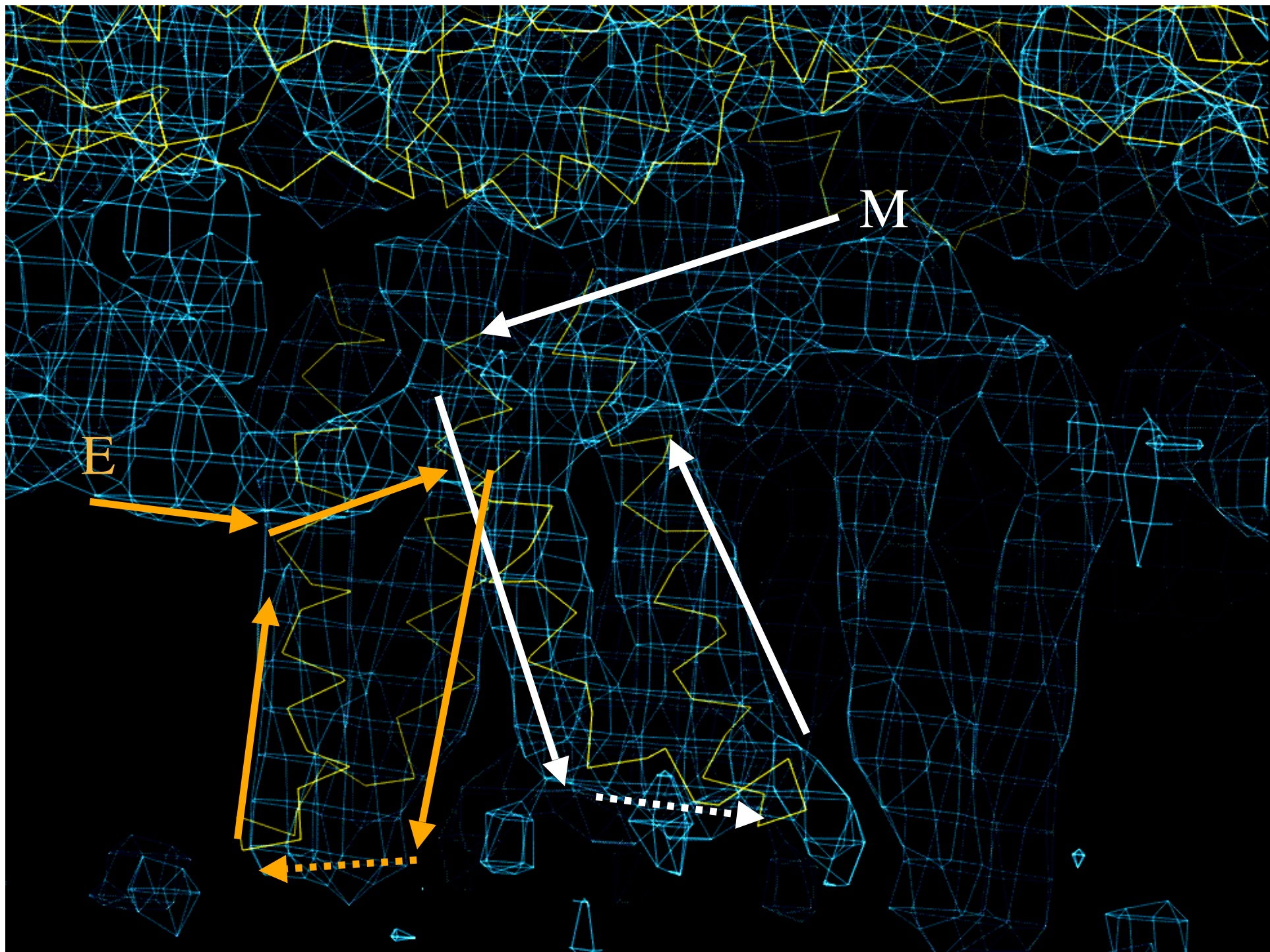


11 Å

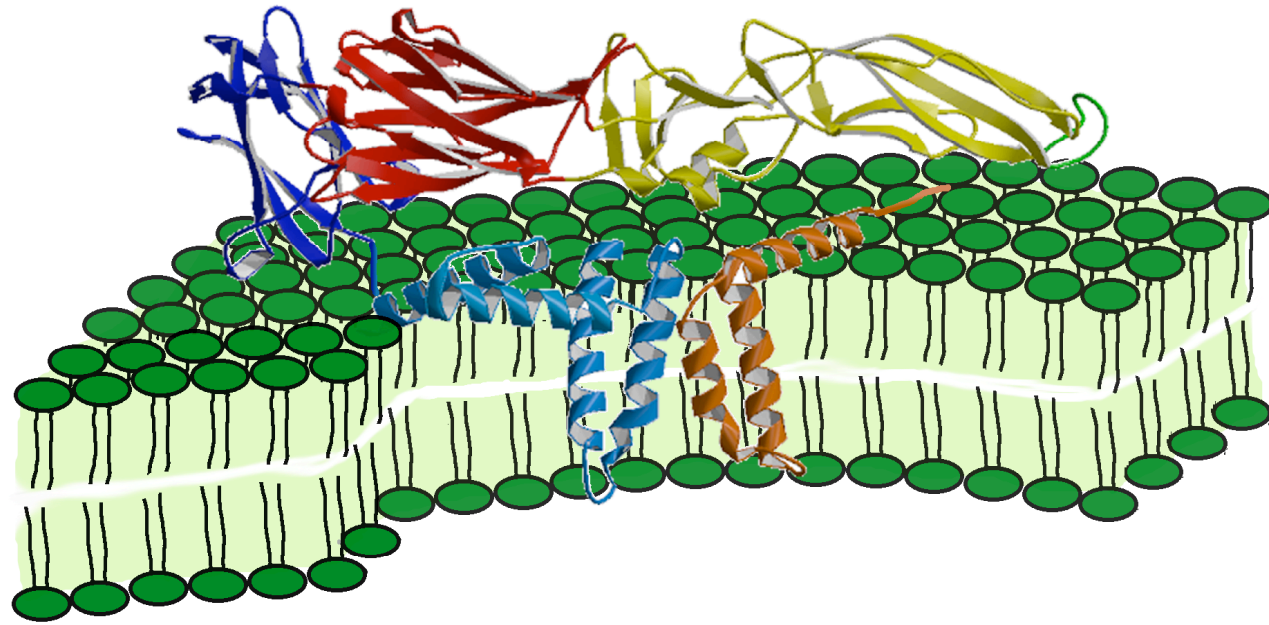




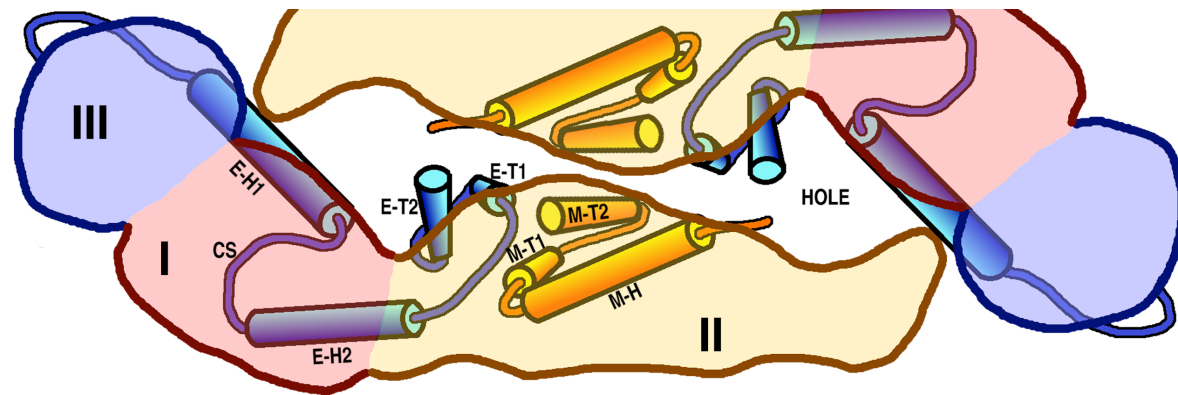




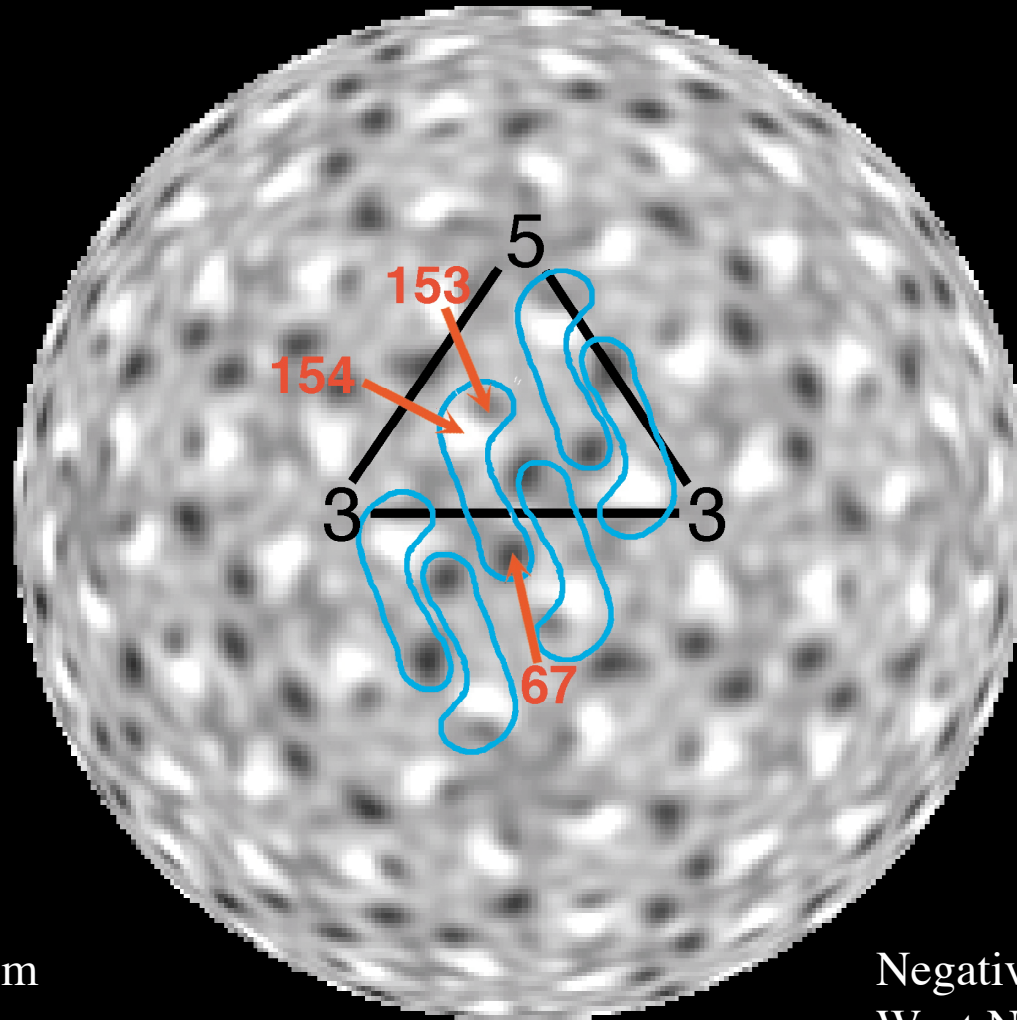
Side View



Top View

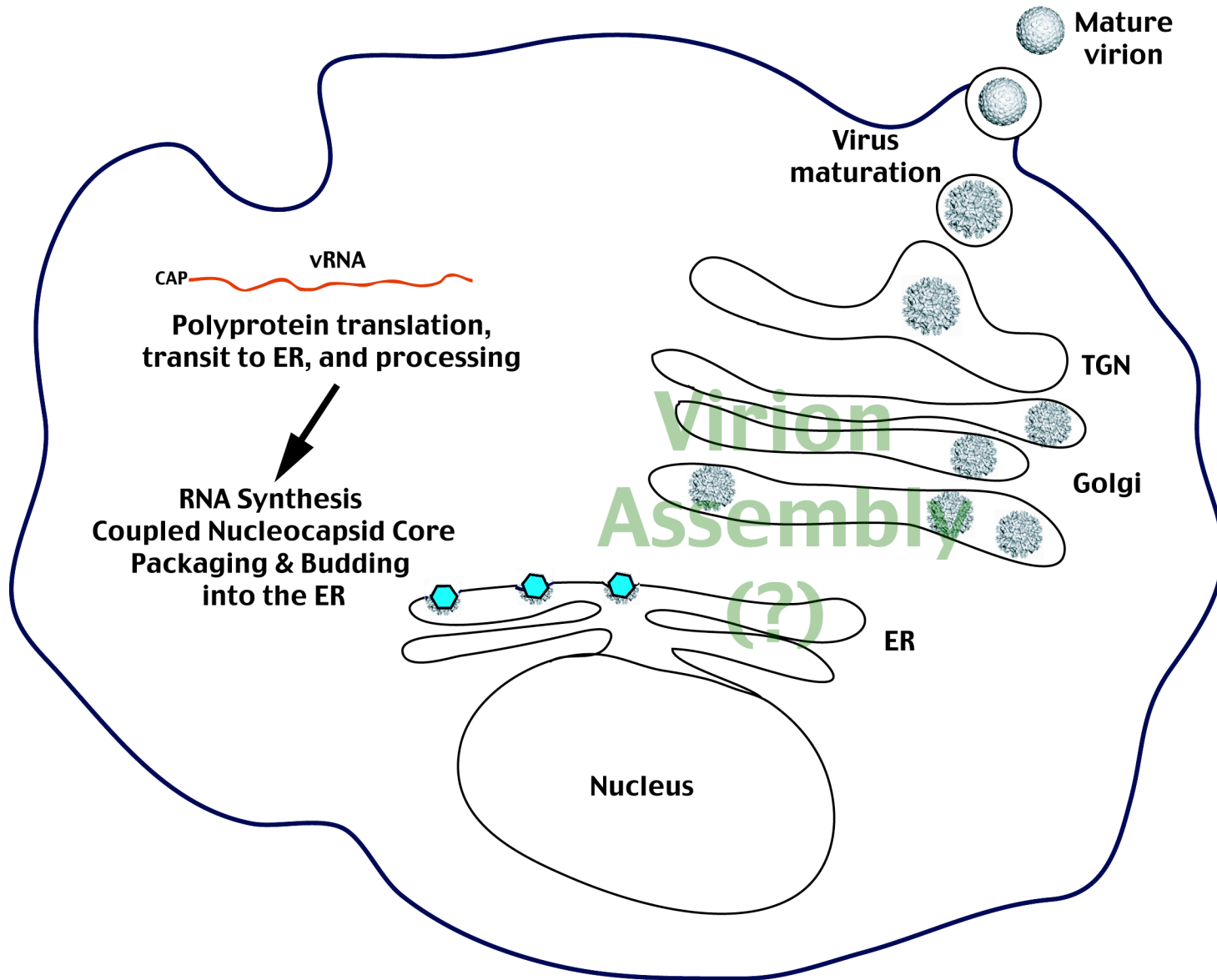


Difference Map between Dengue and WNV



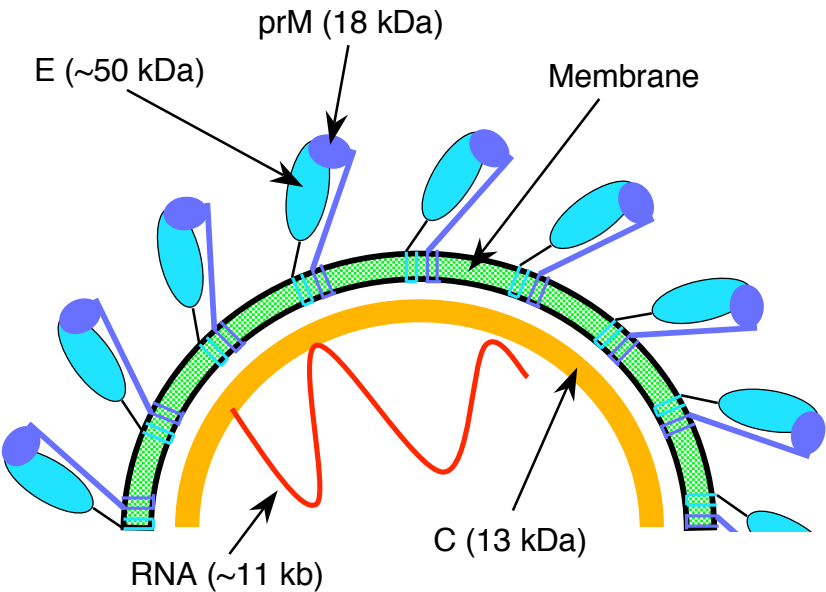
Positive density from
Dengue virus is shown
in black

Negative density from
West Nile virus is shown
in white

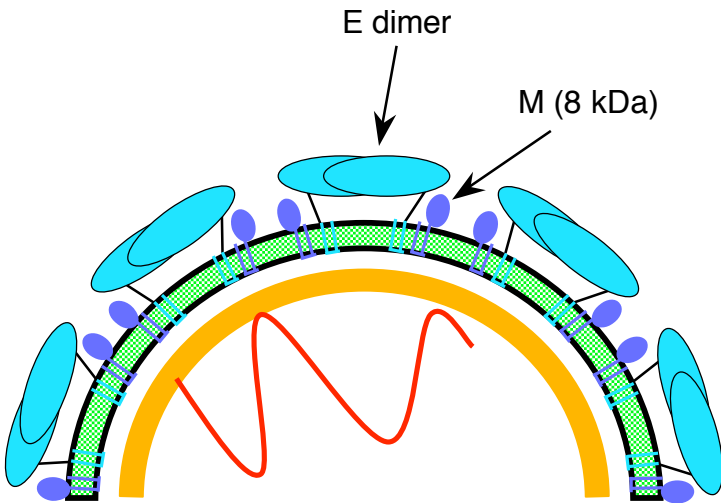


Flavivirus Maturation

Immature Virion



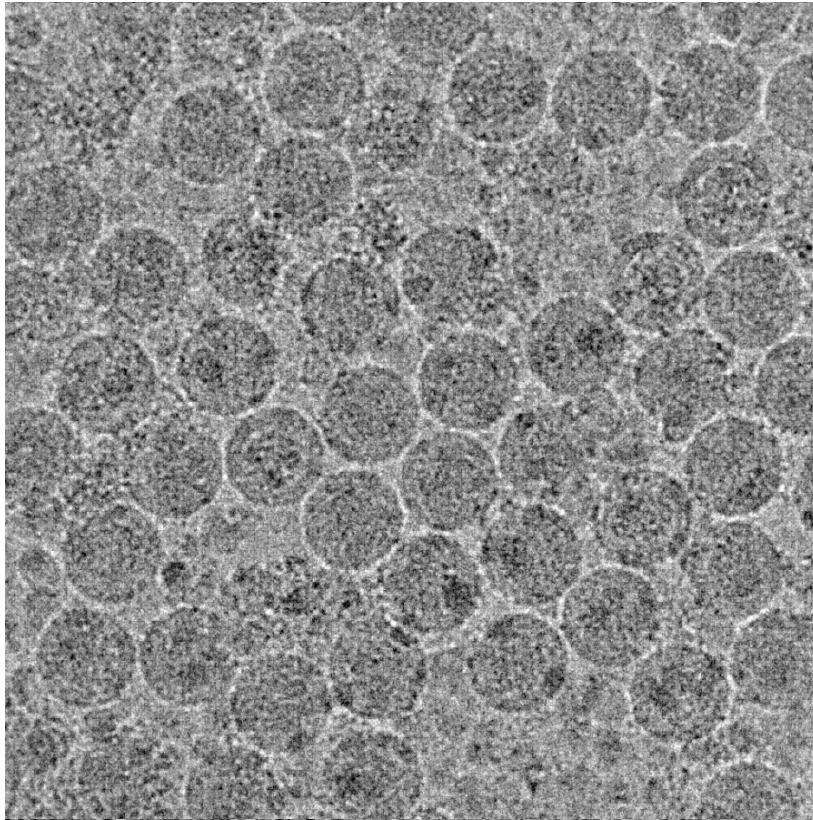
Mature Virion



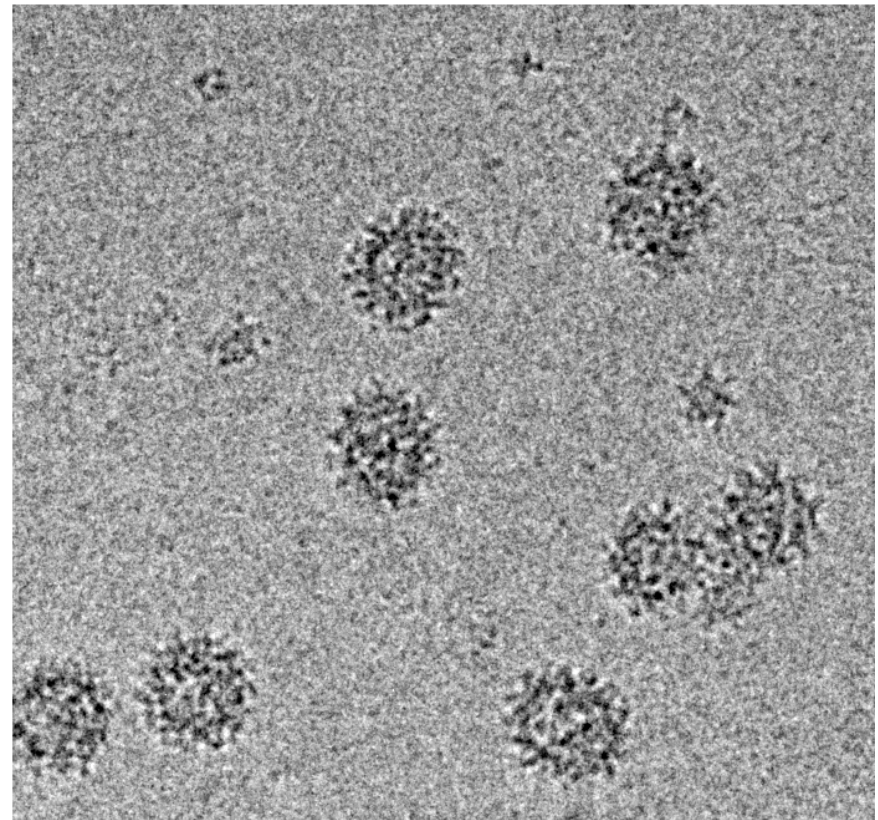
prM $\xrightarrow[\text{(Trans Golgi Network)}]{\text{furin}}$ M (75aa) + pr (90aa)

prM/E heterodimer $\xrightarrow{\hspace{10em}}$ E-E homodimer

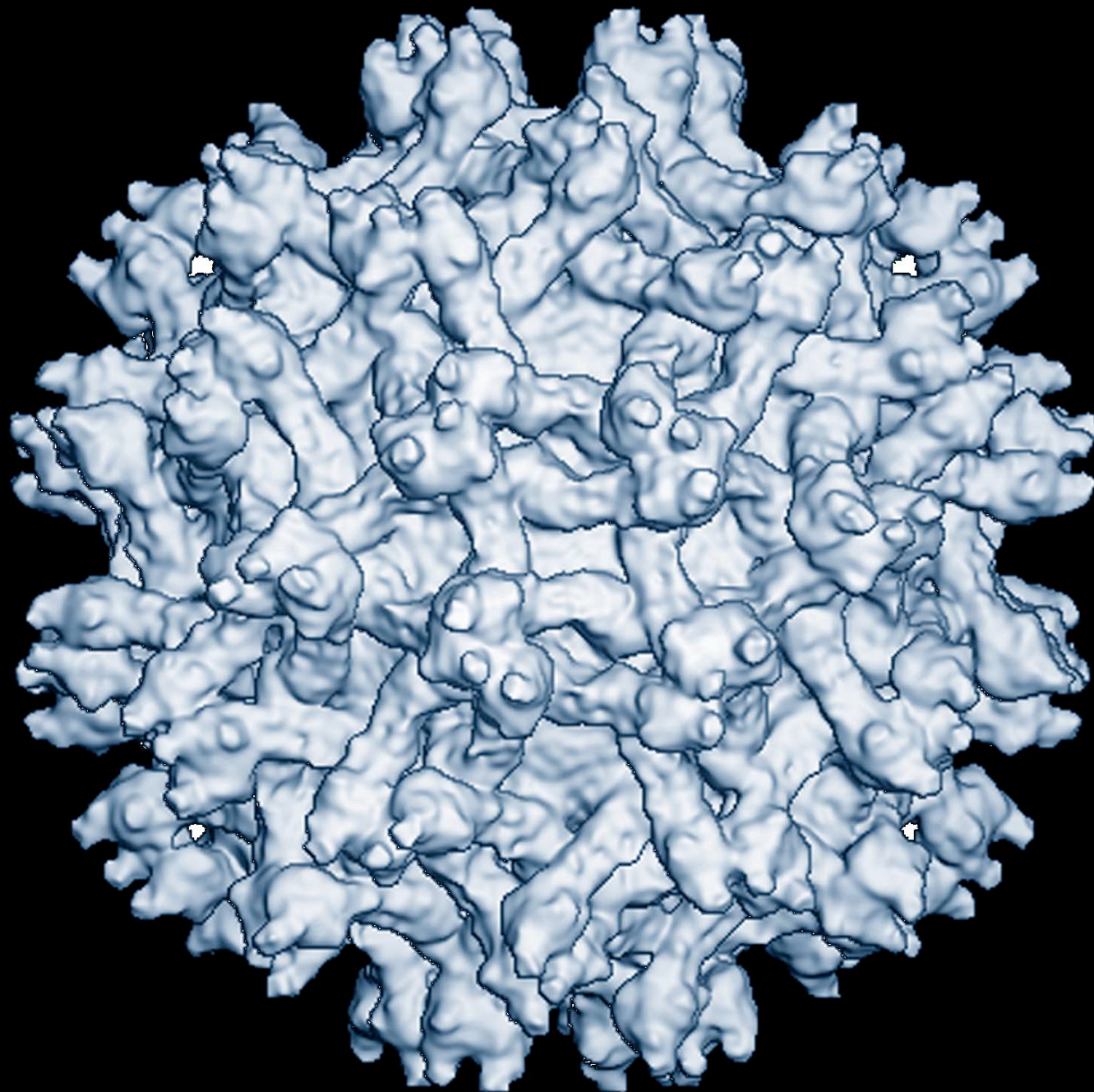
Cryo-EM Micrographs

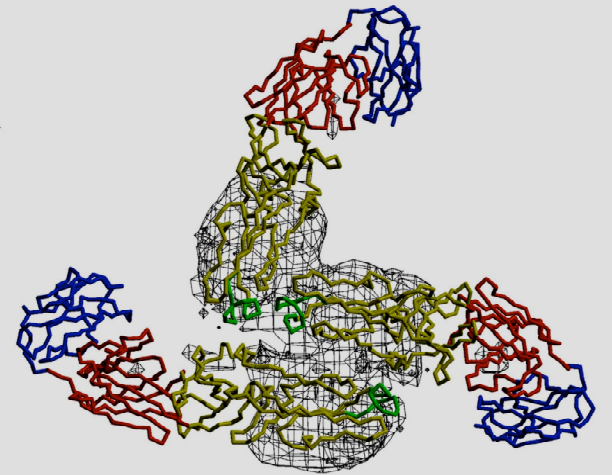
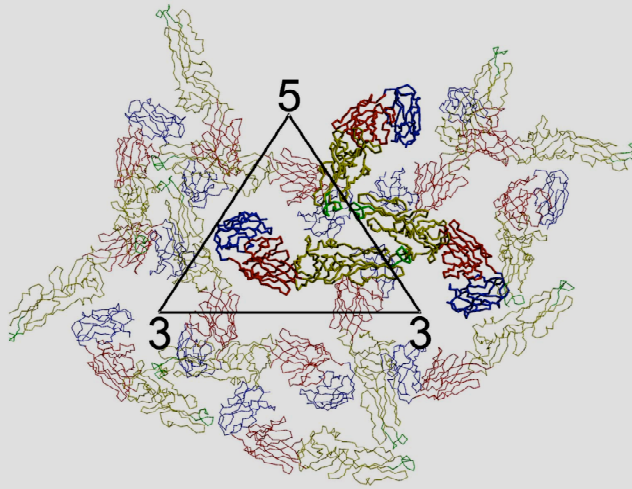
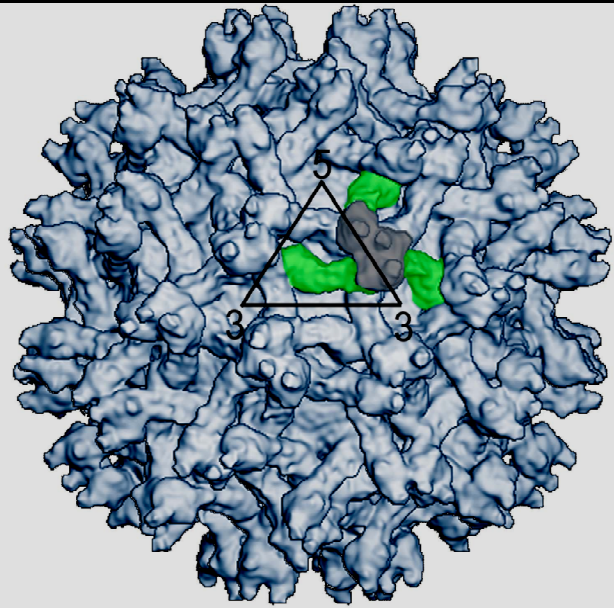


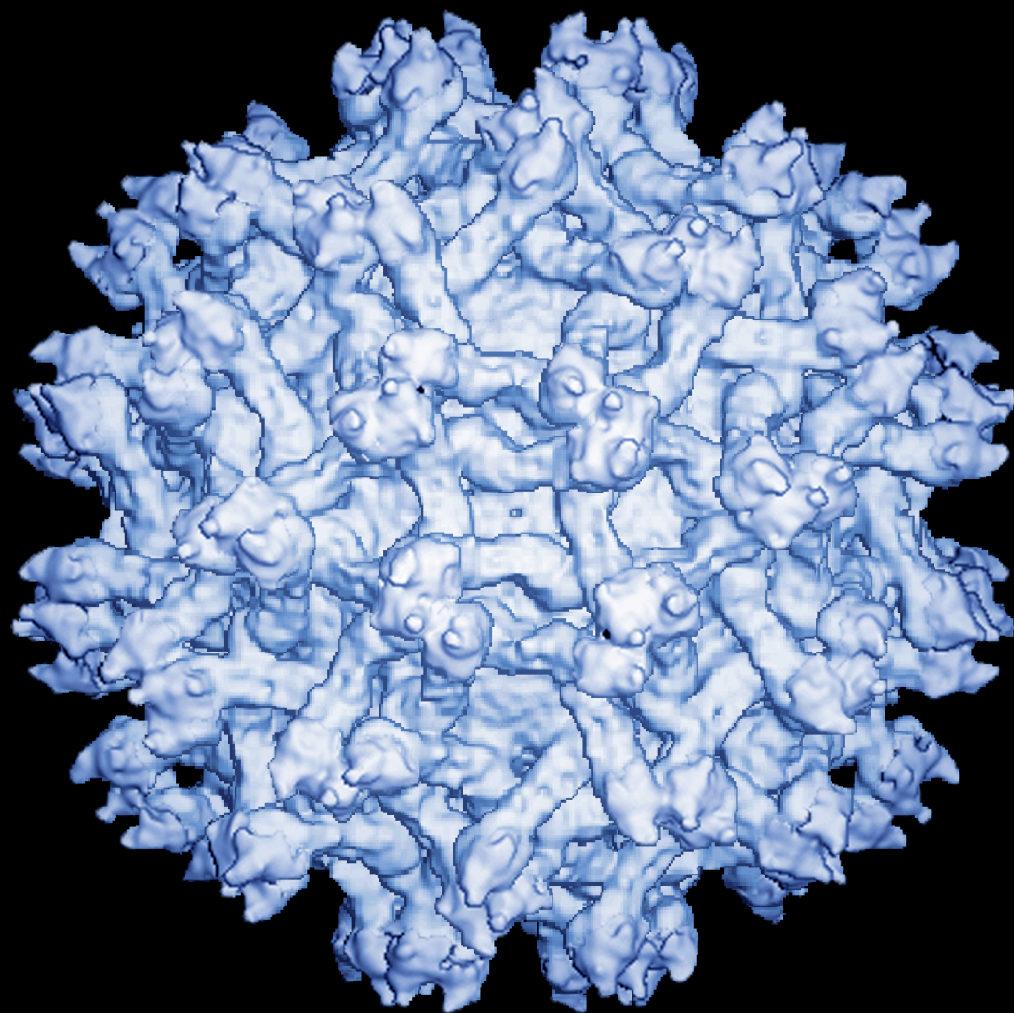
Native Den-2

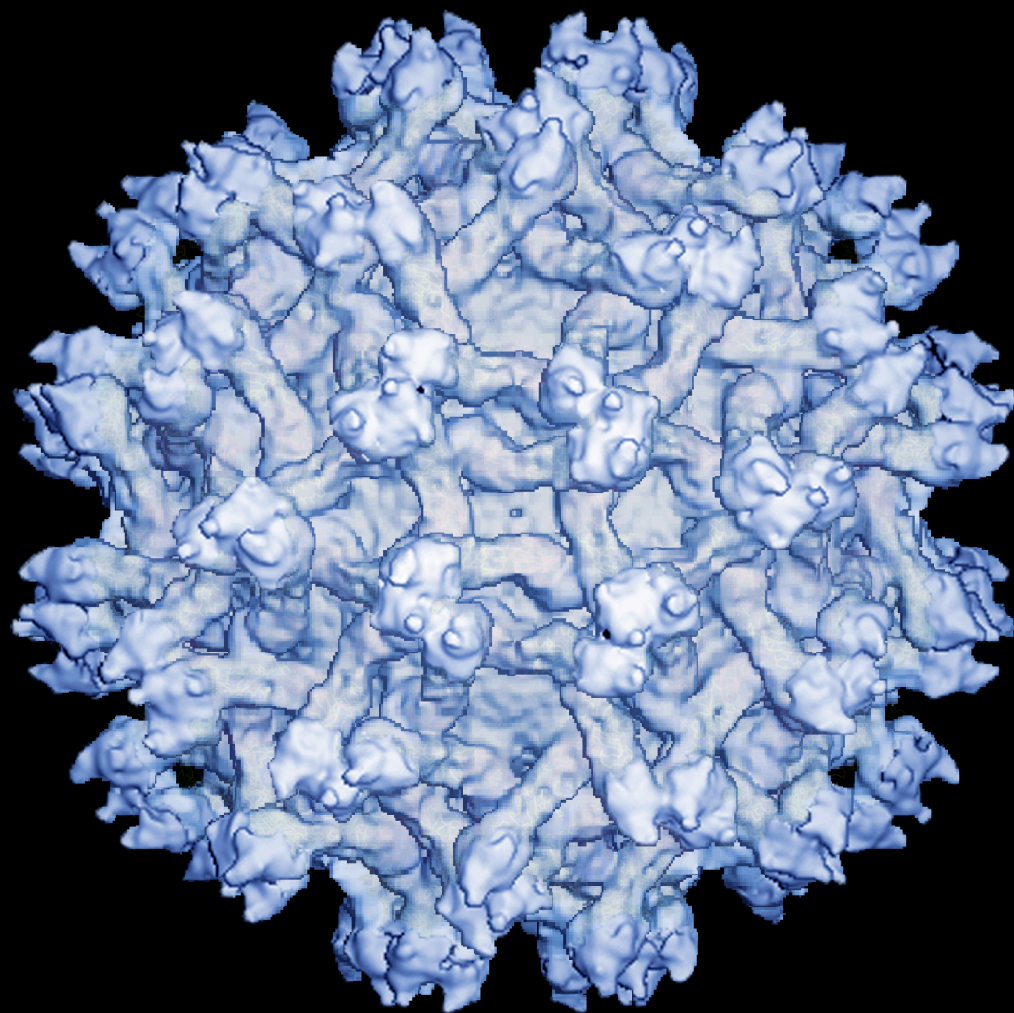


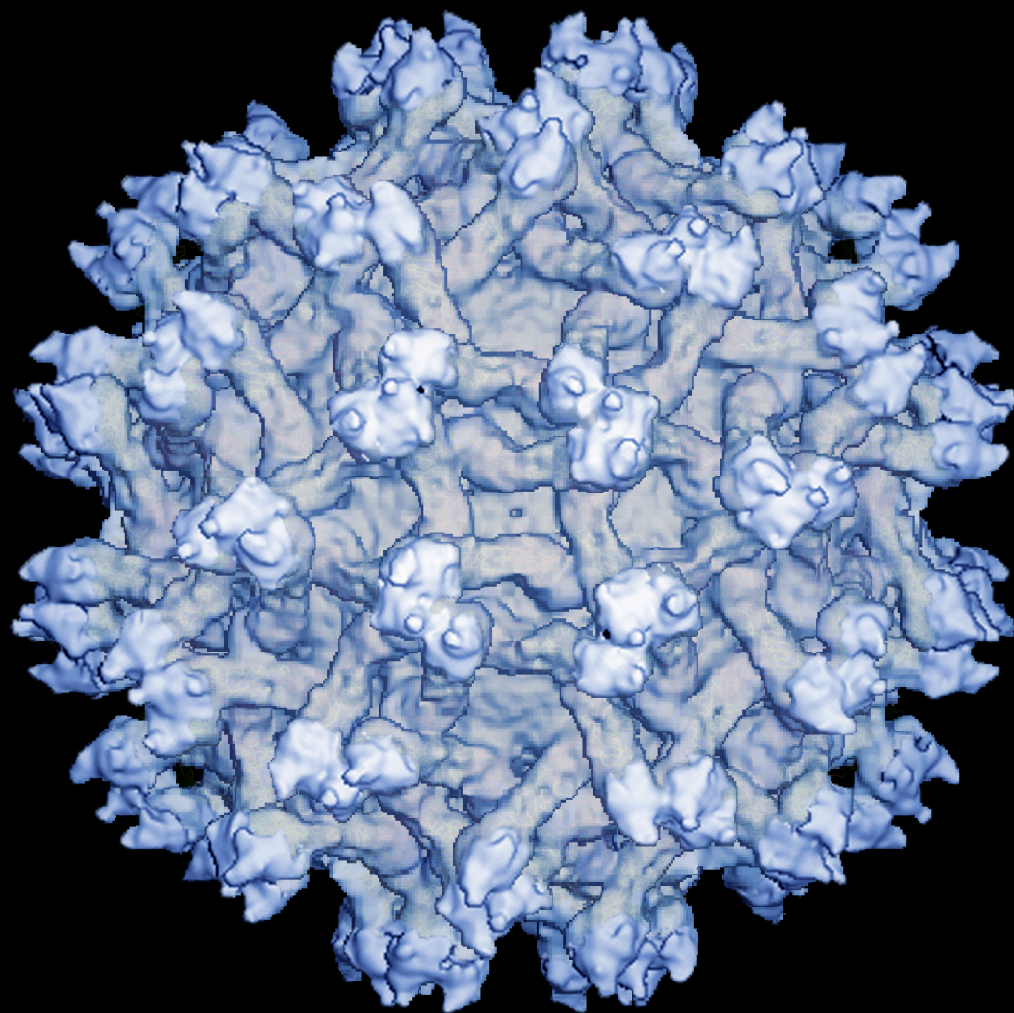
prM-containing
Den-2

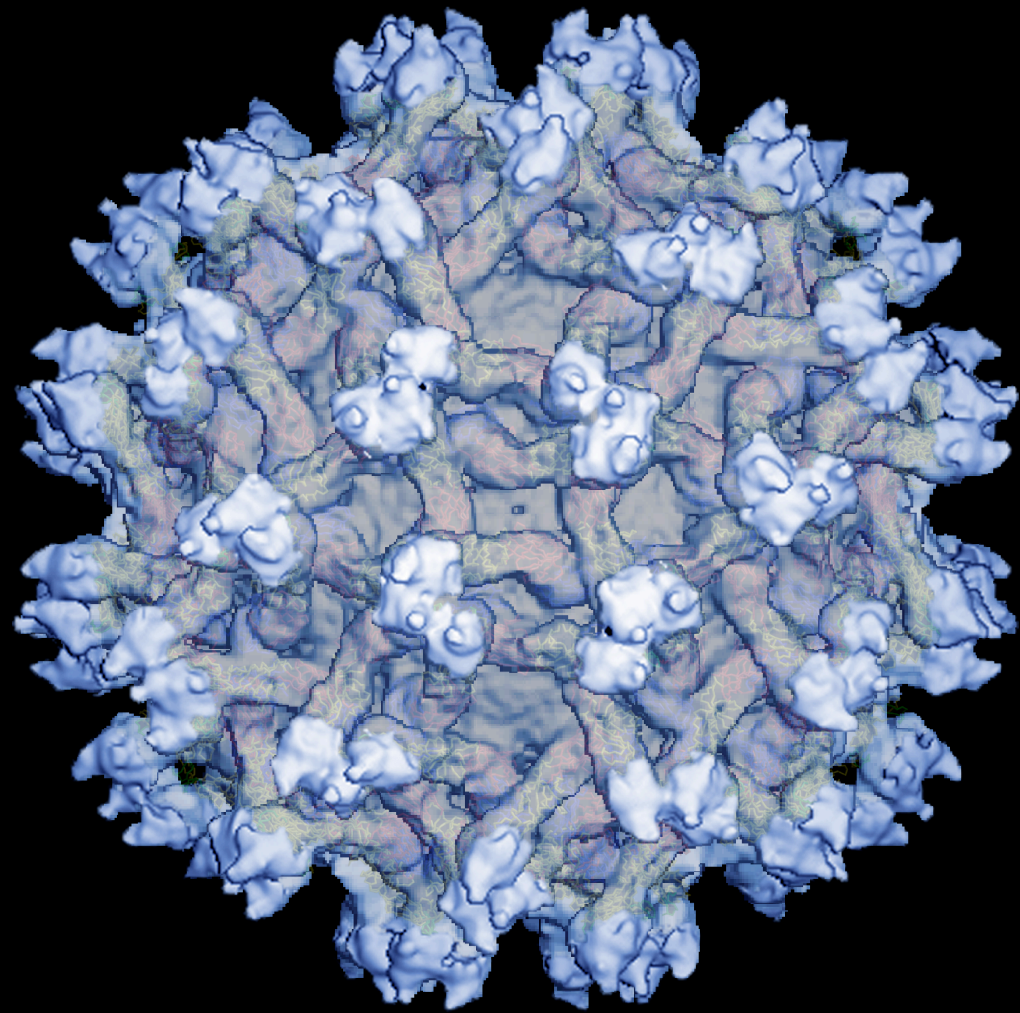


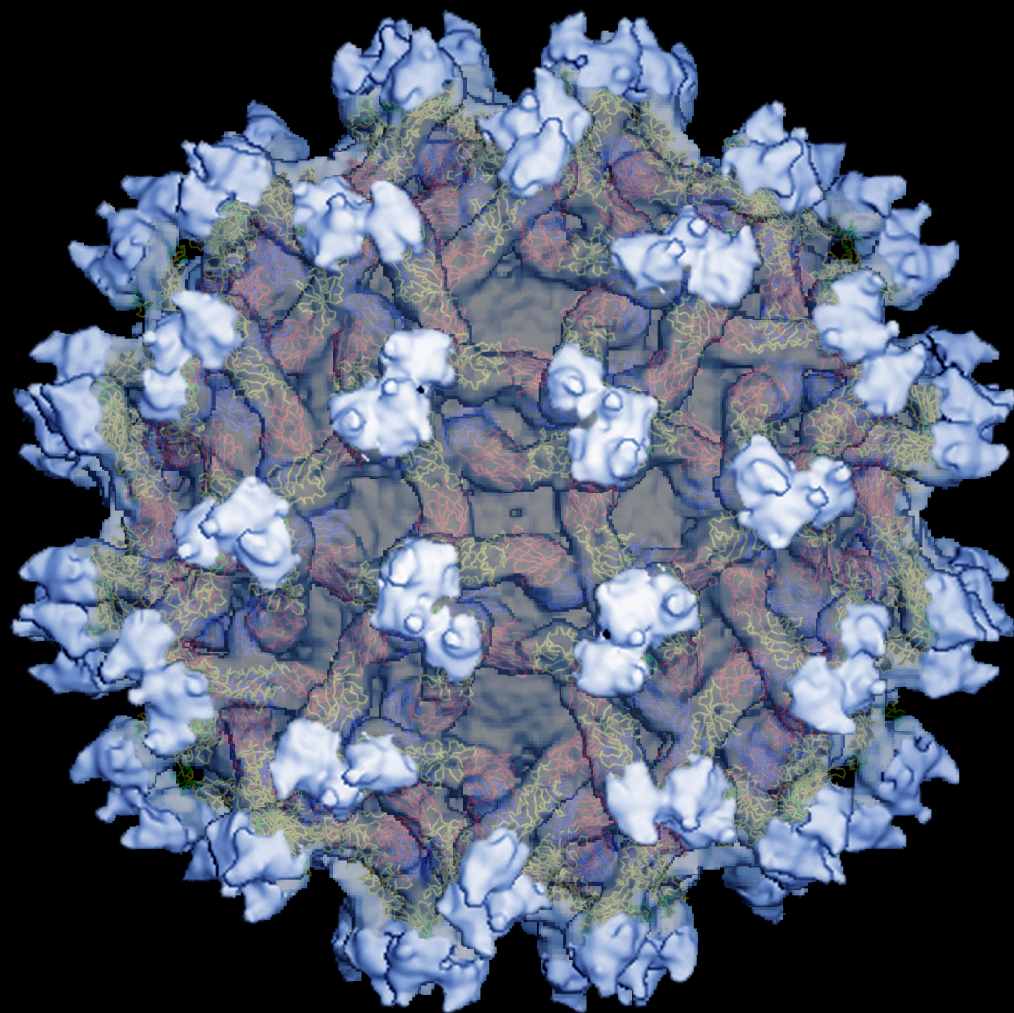


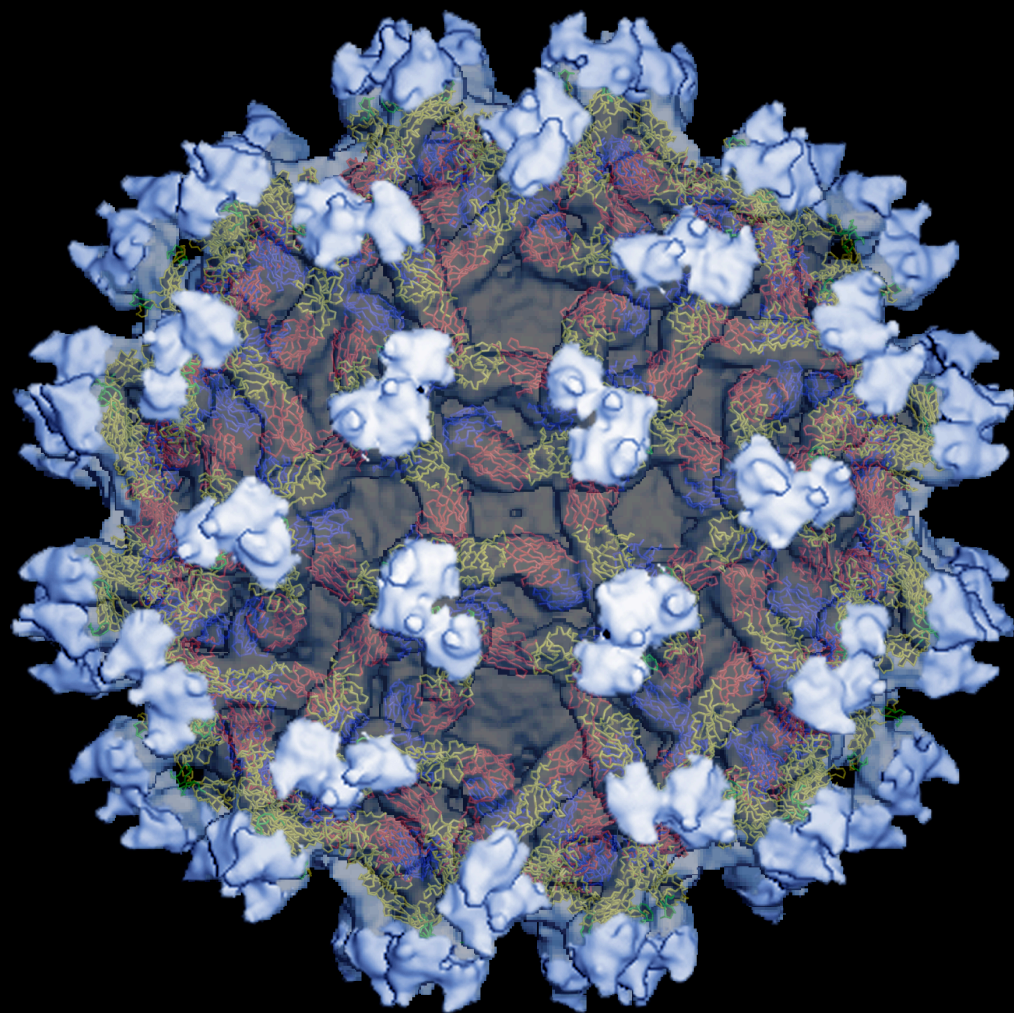


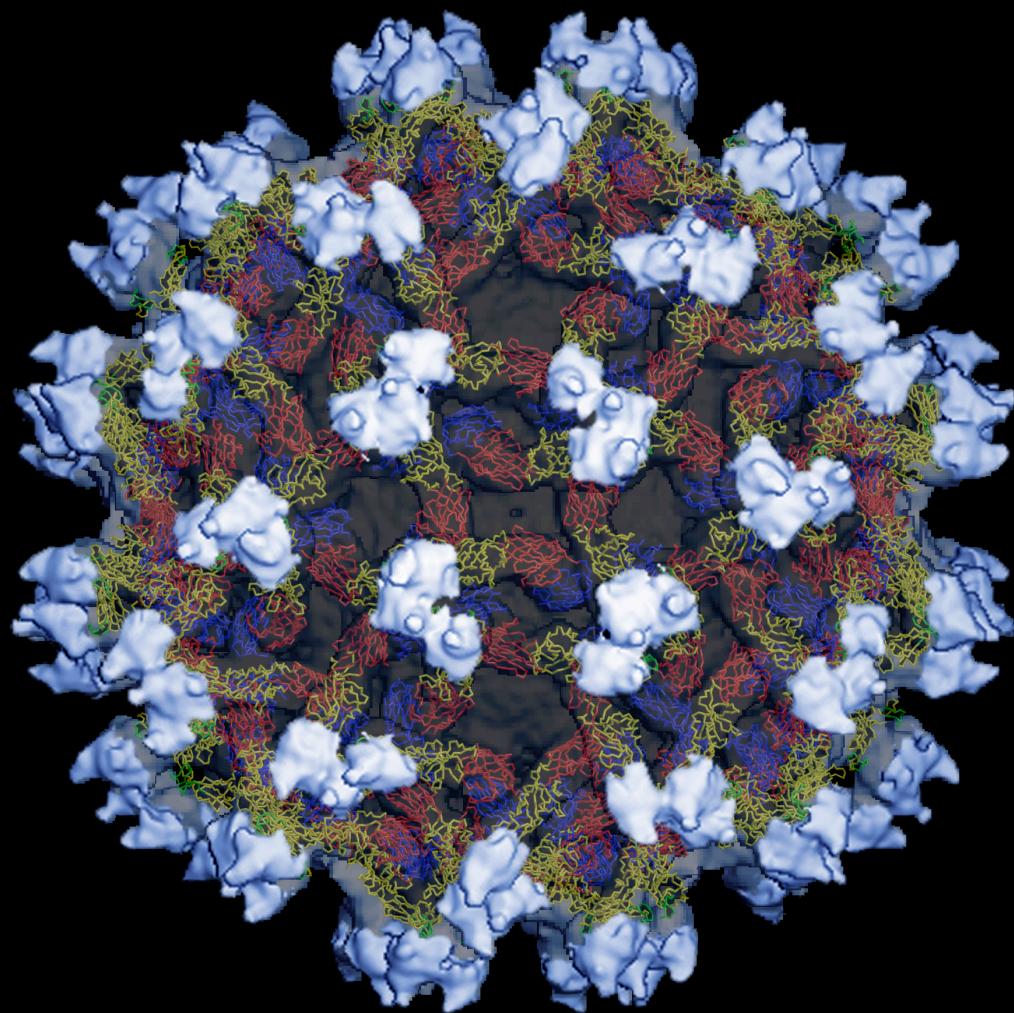


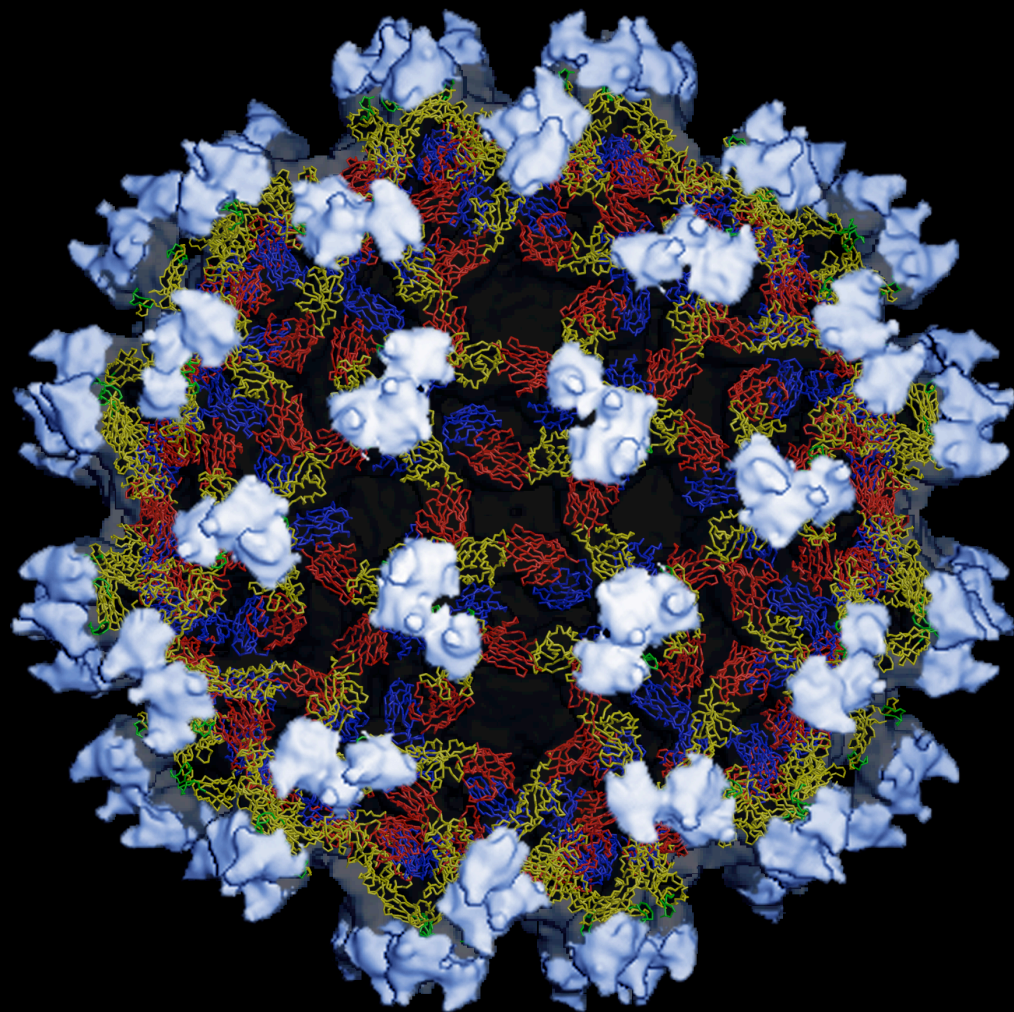


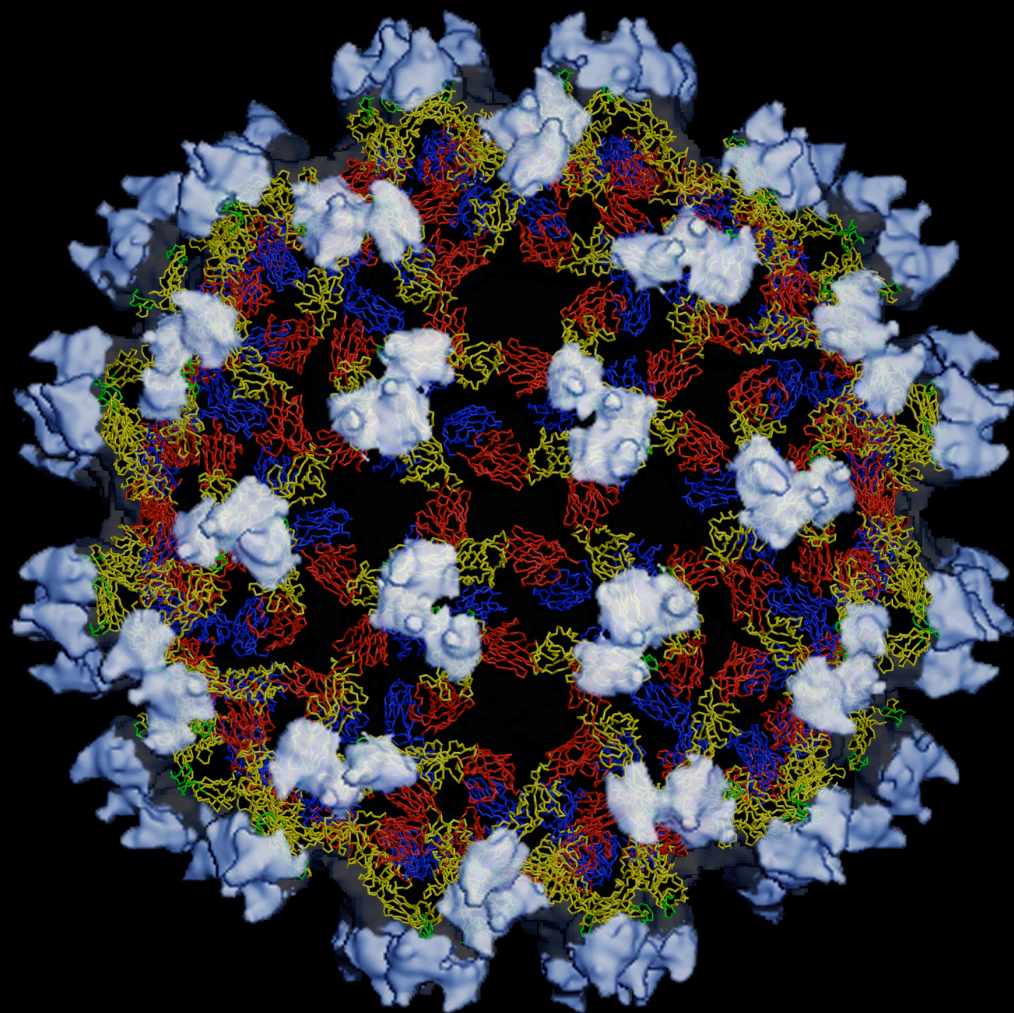


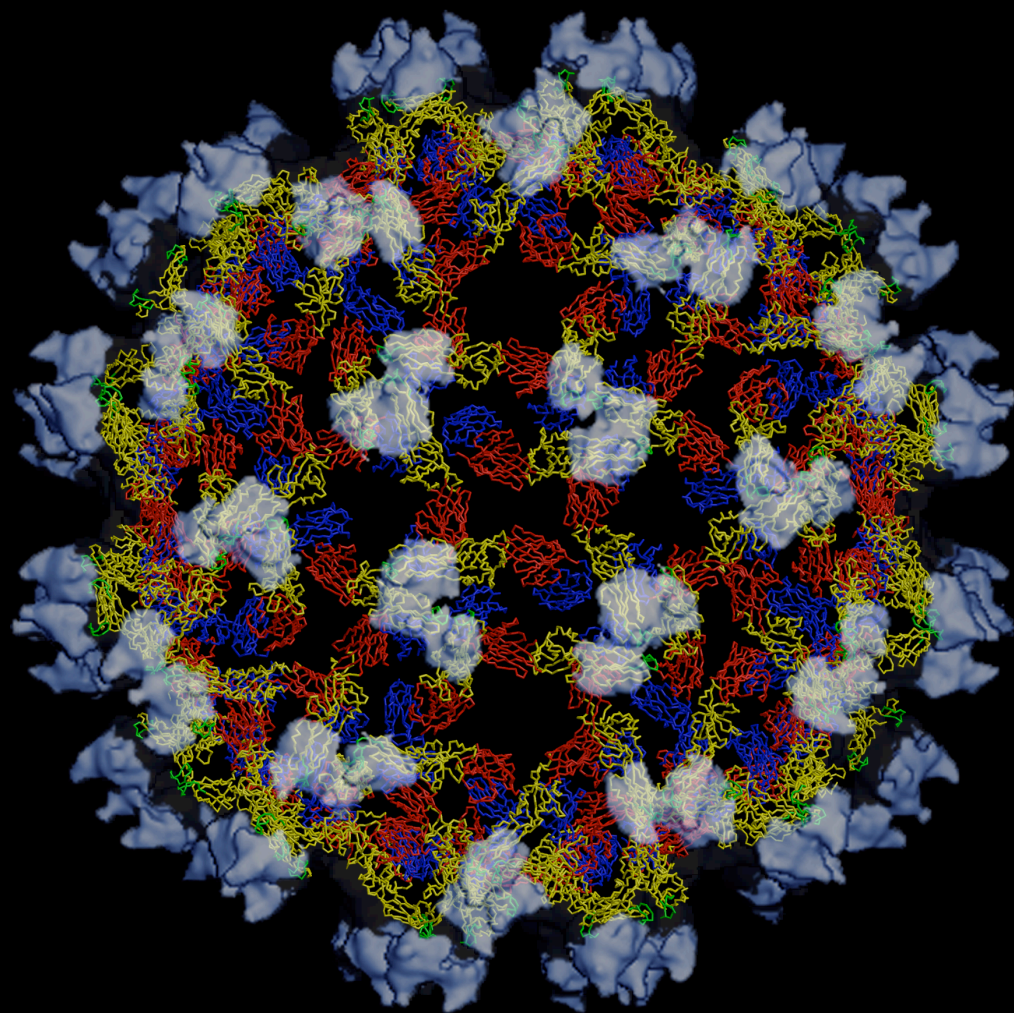


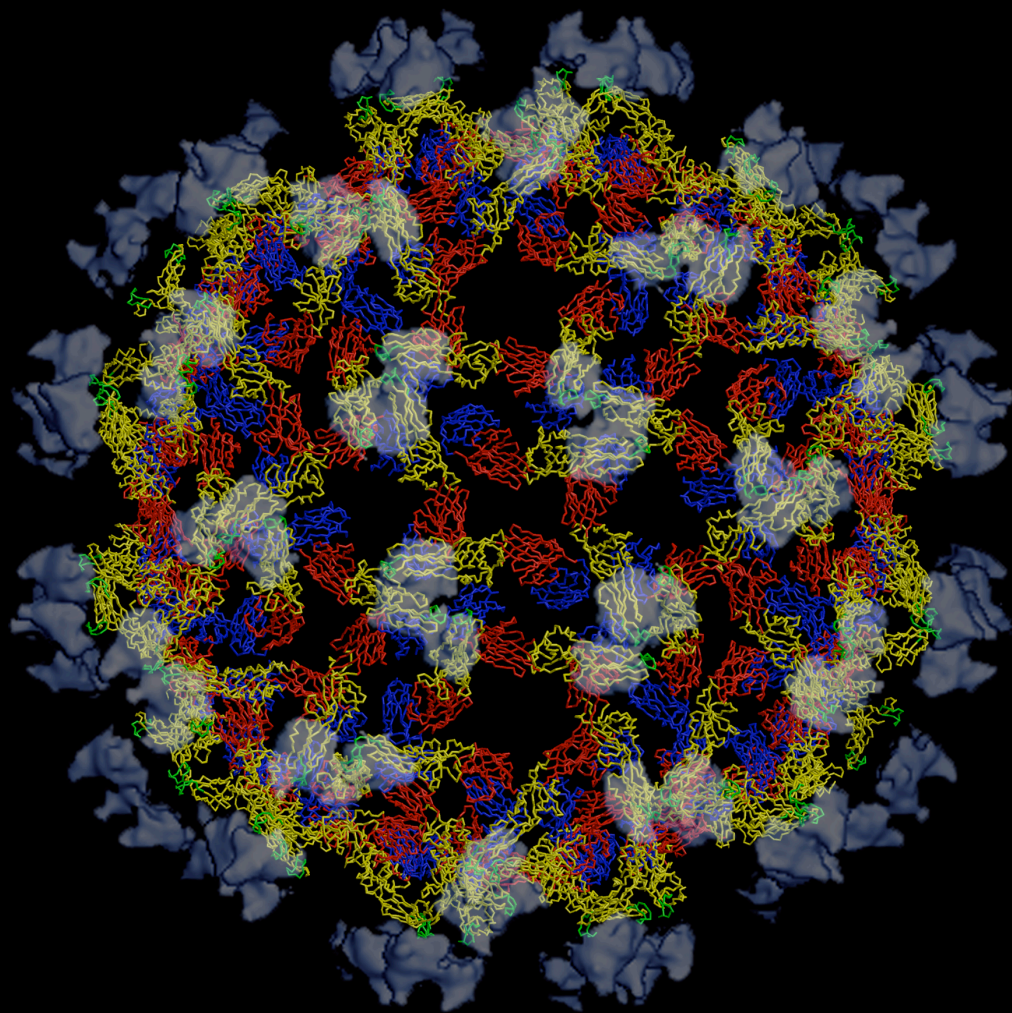


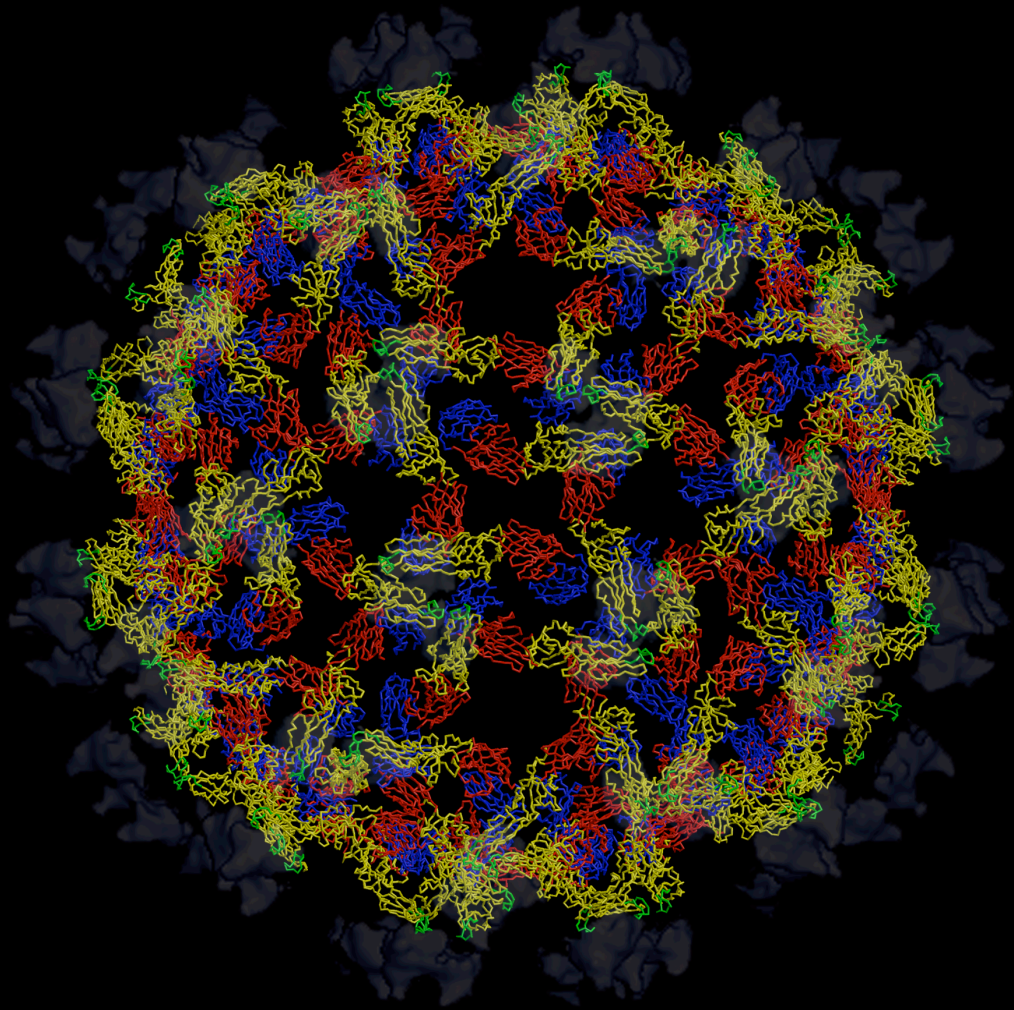


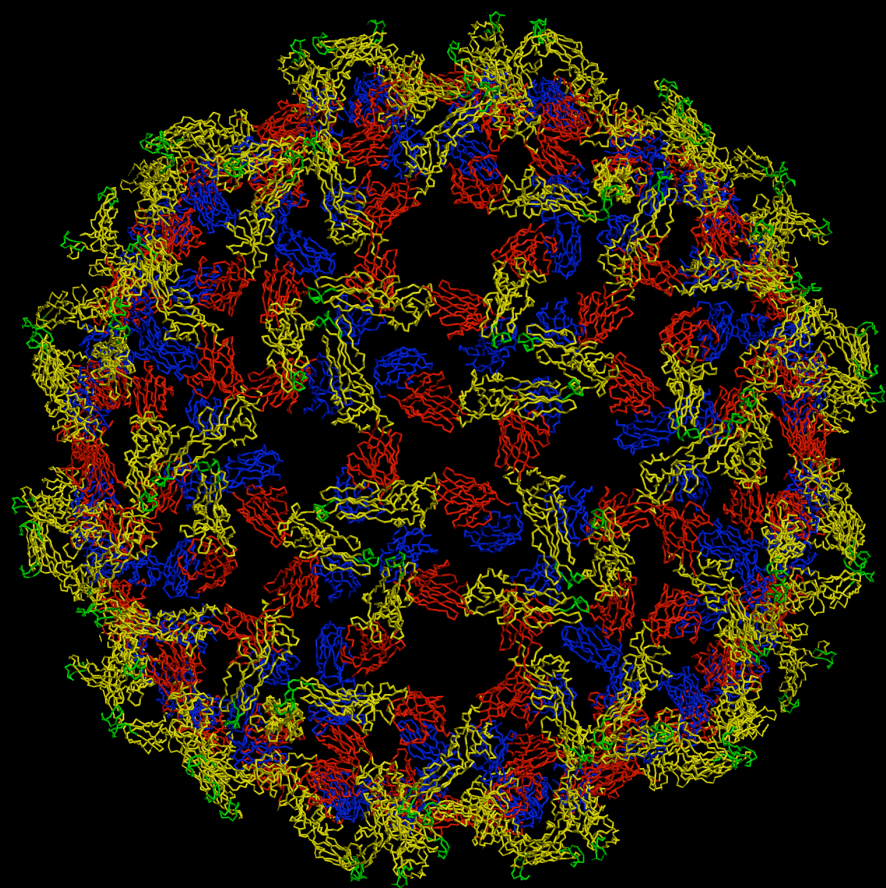


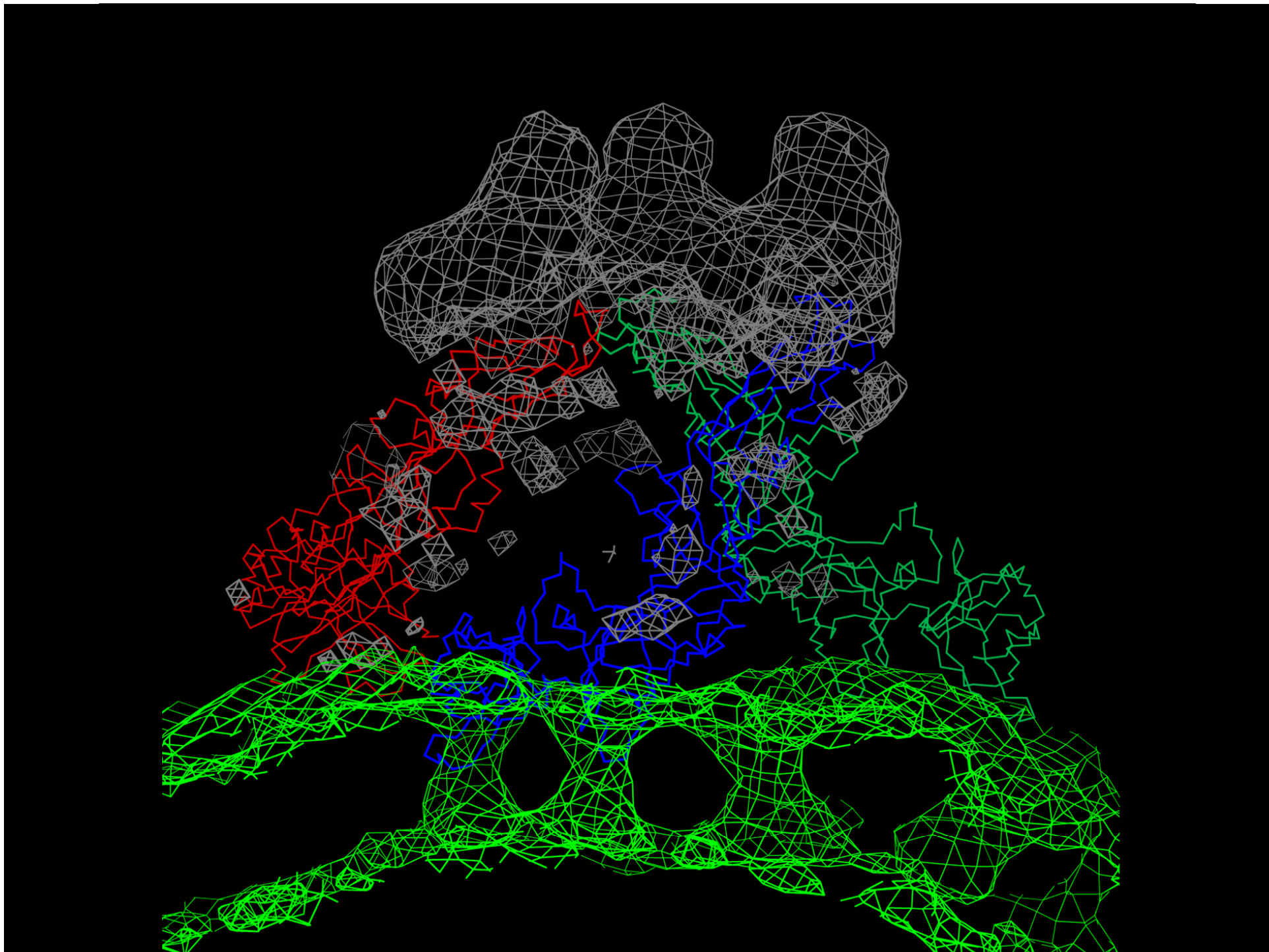






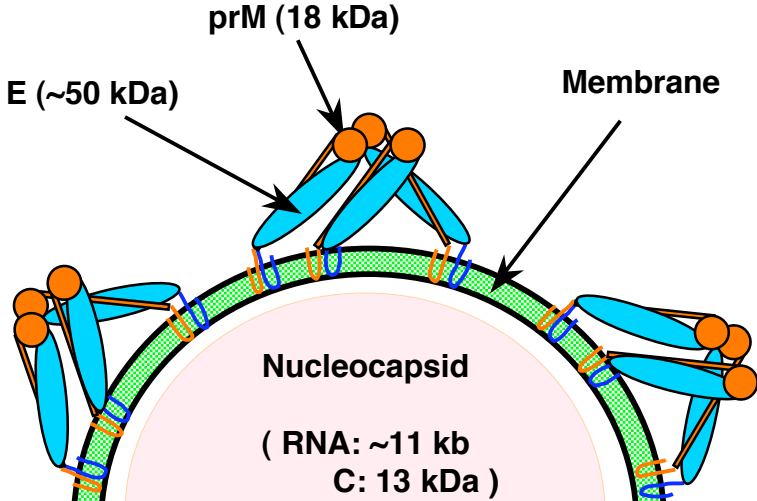




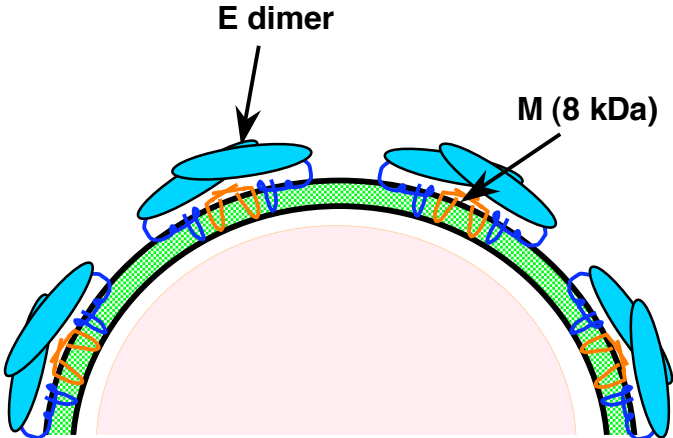


Flavivirus Maturation

Immature Virion



Mature Virion



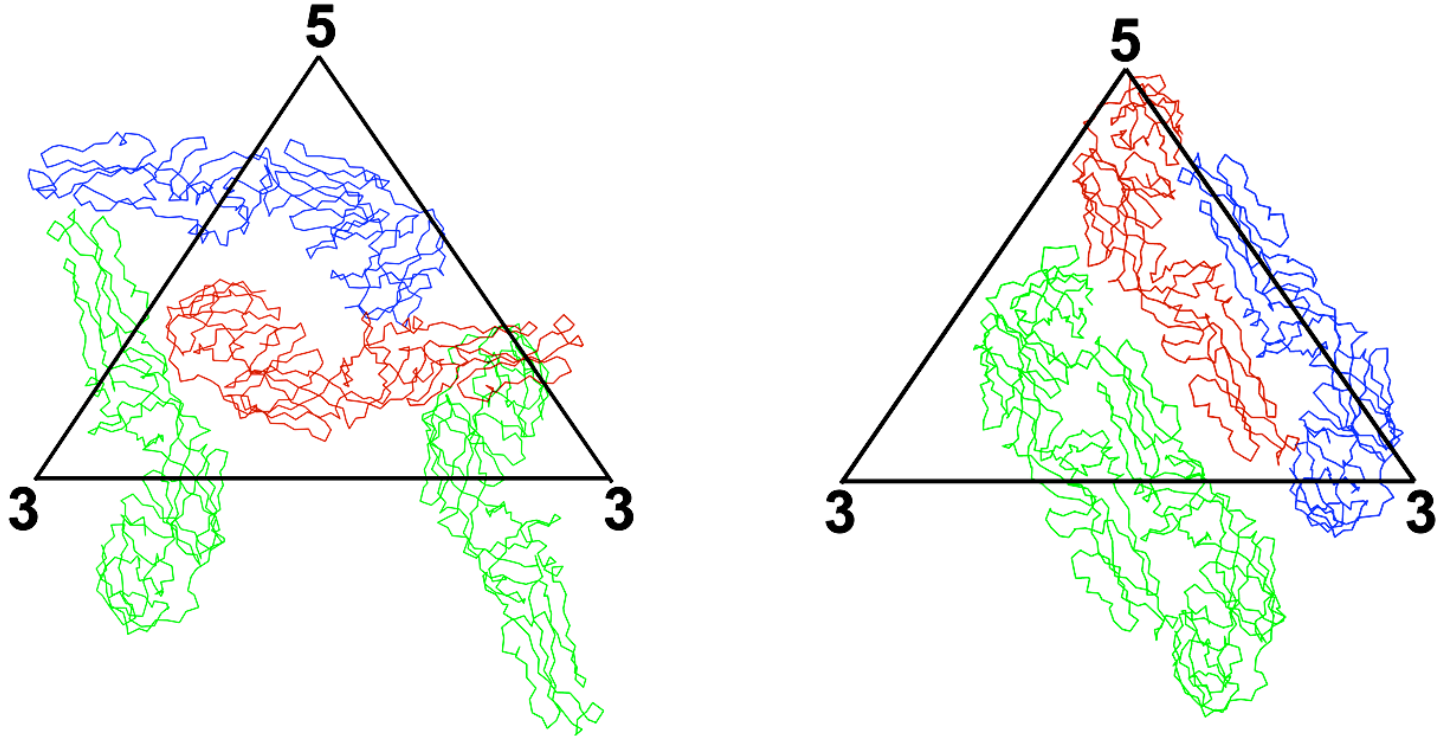
prM $\xrightarrow[\text{(Trans Golgi Network)}]{\text{furin}}$ M (75aa) + pr (91aa)

prM/E heterodimer \longrightarrow E-E homodimer

(prM/E)₃ heterotrimer \longrightarrow E-E homodimer

Flavivirus Maturation

(involves major rearrangement of E glycoproteins)



Immature particle



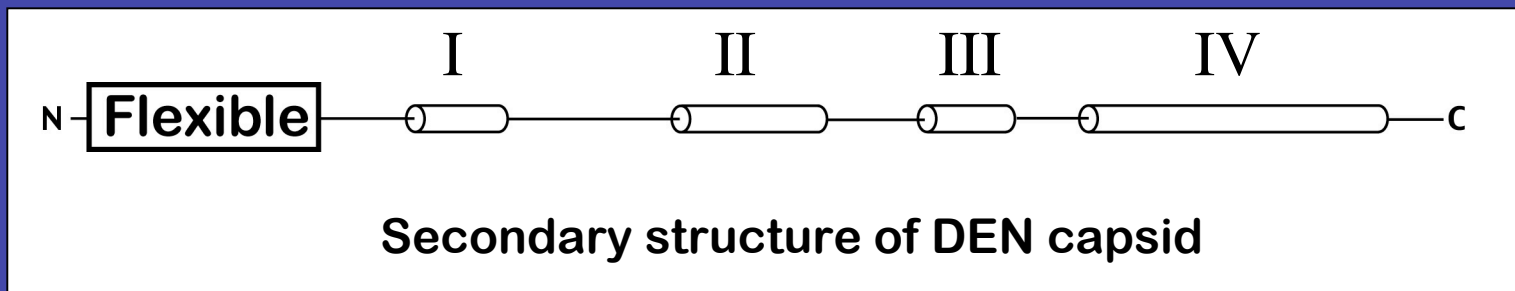
Virion

Flavivirus Capsid Protein

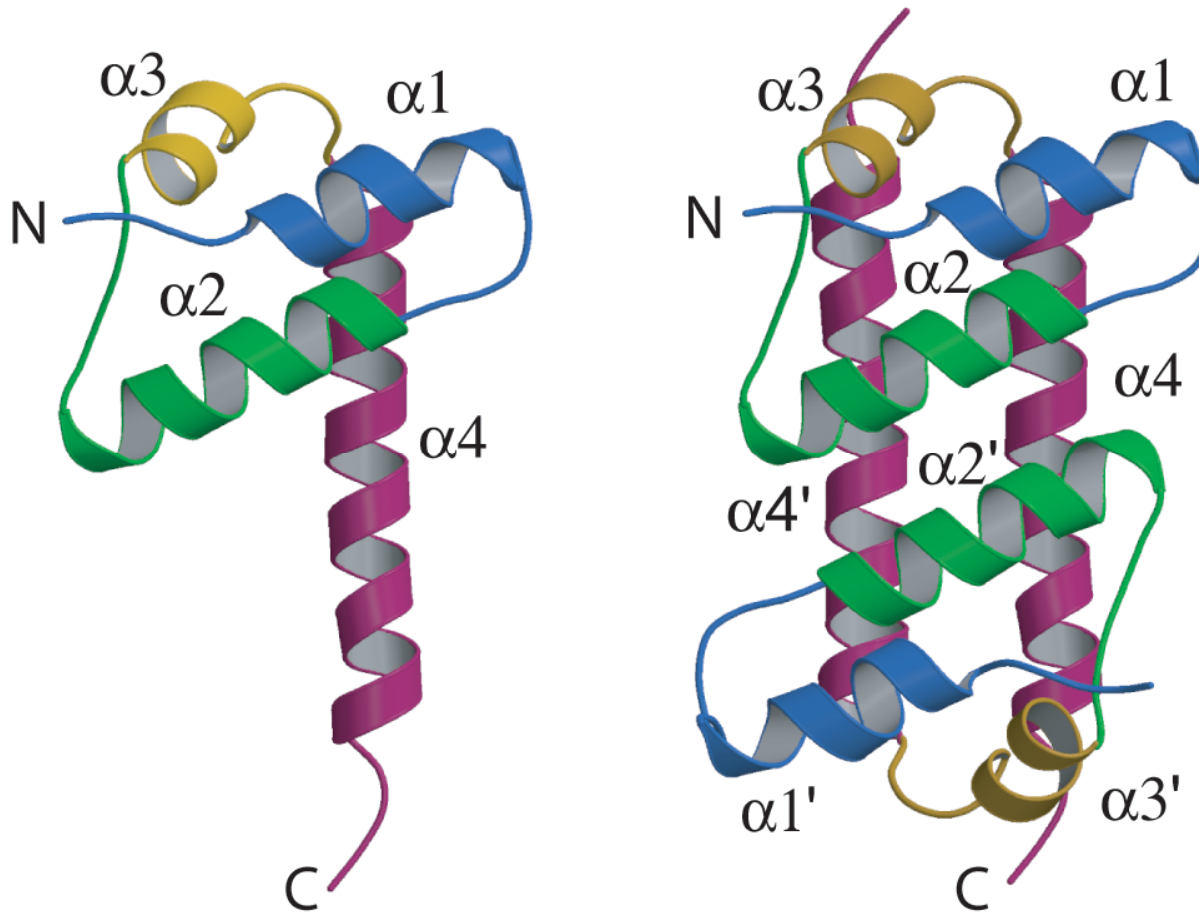
- **M.W. is approximately 10-12kDa**
- **Highly basic, over 20% lysine and arginine**
- **A carboxy-terminal hydrophobic sequence anchors capsid to membranes**
- **Internal hydrophobic sequence**
- **Specifically interacts with genome RNA during virus assembly**

WNV, YFV, DEN Capsid Proteins

- The capsid proteins were expressed in *E. coli* and purified
- The capsid proteins are dimers in solution
- The secondary structure of DEN capsid as determined by NMR is composed of four alpha helices
- The N-terminus (~20 residues) of DEN capsid is structurally flexible



Dengue Capsid Protein



Monomer

Dimer

Conclusions

- West Nile virus has an ordered arrangement of its E protein with 3 molecules in the asymmetric unit
- West Nile and Dengue are remarkably similar in their structure and organization of E proteins
- The transmembrane components of the dengue E and M proteins are ordered and visible in the lipid bilayer
- The immature prM-containing dengue virus contains trimeric spikes that are capped with prM, similar to the architecture seen in alphaviruses
- NMR studies indicate that the dengue capsid protein is organized into a series of four helices

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