Asymmetric Reconstructions of Immature Tick-borne Encephalitis Virus Particles Reveal Defects Caused by the Assembly Process

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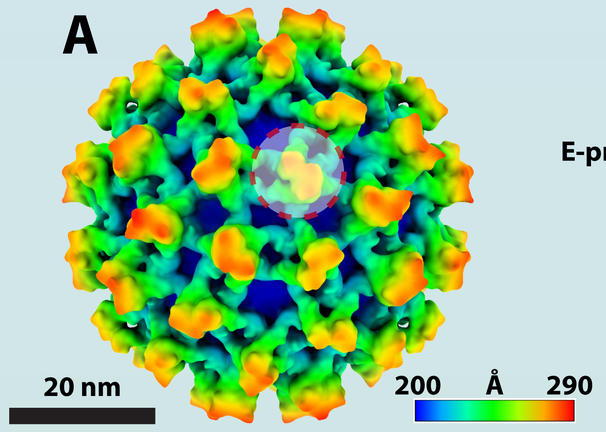
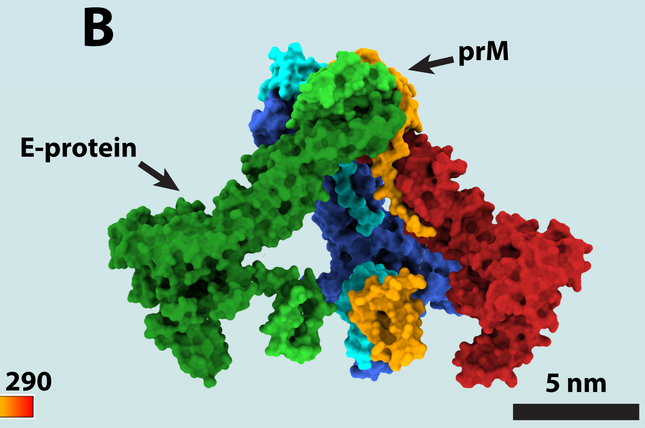
Introduction

Tick-borne encephalitis virus (TBEV) is an enveloped virus belonging to the family *Flaviviridae*. It is mainly transmitted by ticks and causes severe disease of central nervous system in humans. Virion surface is covered by envelope proteins (E-protein), that are together with the membrane proteins (M-protein) anchored in virus lipid bilayer. During the viral life cycle, the immature non-infectious virus undergoes a maturation process. This process includes proteolytic cleavage of prM and major reorganization of the envelope proteins on the viral surface.

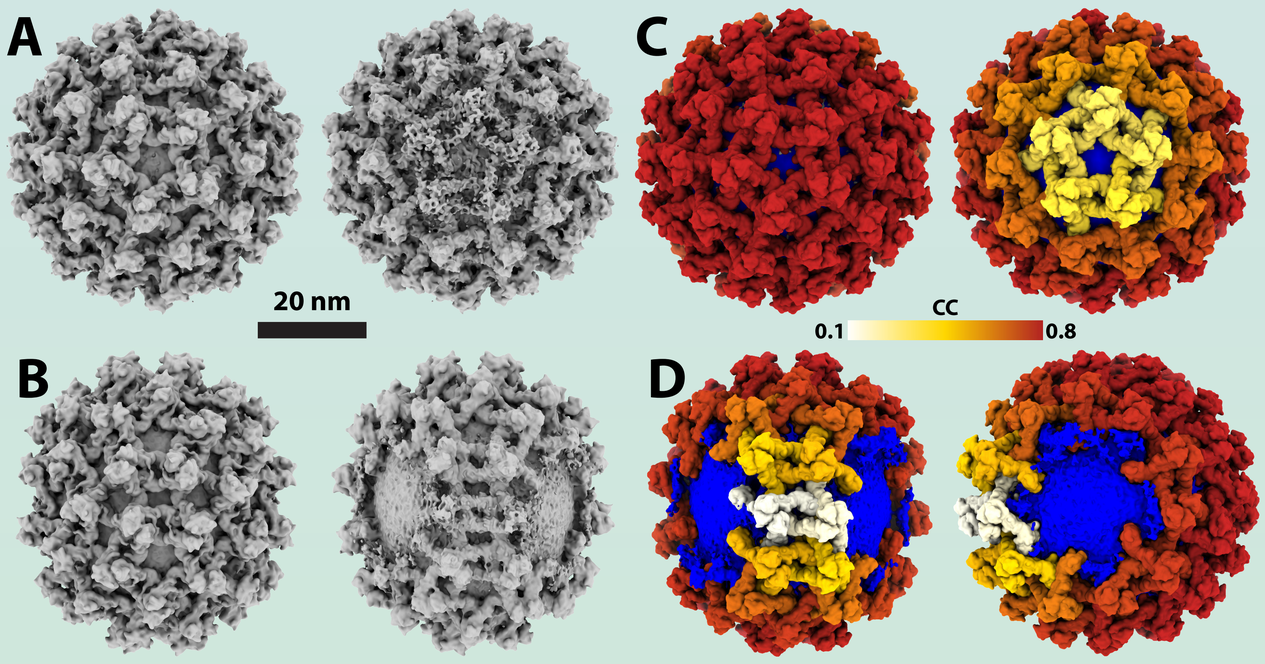
iTBEV

Immature TBEV particle

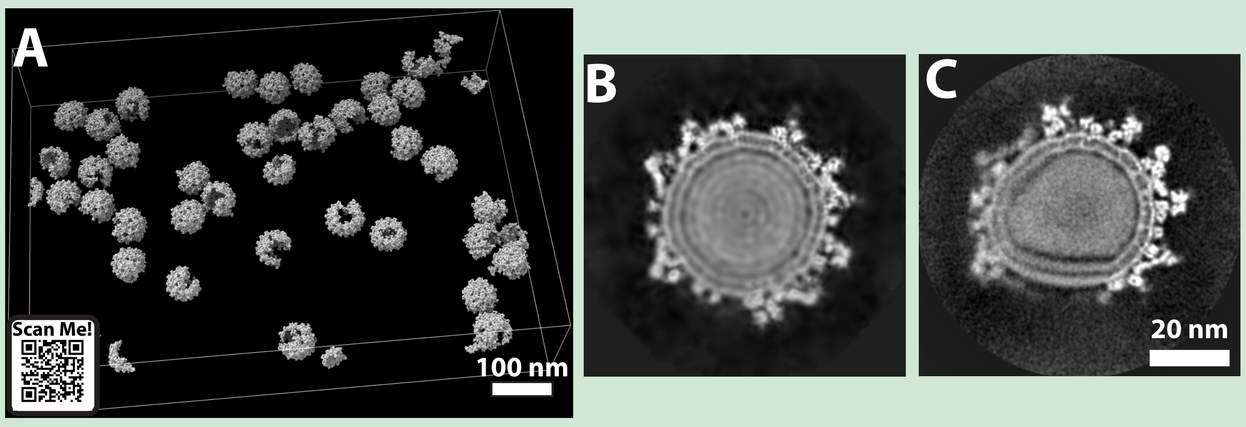




(A) Radially colored, icosahedrally averaged reconstruction of whole immature TBEV particle resolved to resolution 7.1 Å. The circle shows the asymmetric unit extracted for sub-particle reconstruction and sub-tomogram template matching. **(B)** Molecular surface representation of immature TBEV asymmetric unit molecular model. E-protein in dark colors and prM in light colors.



Asymmetric reconstructions of immature TBEV particles revealed classes of **(A)** "near complete" particles and **(B)** "aberrant" particles. Both types of particles feature well-organized side that comply with the icosahedrally averaged particle while the other side features distortions from the symmetry. **(C, D)** Molecular surface of E3prM3 "spikes" fitted into asymmetrically reconstructed maps colored by correlation.



(A) Placed-back refined sub-tomograms of the iTBEV "spikes" into the original tomograms show that the irregularities are not correlated with the "air-water" interface of the sample. Central slice of the reconstructed maps of **(B)** "near complete" and **(C)** "aberrant" particles shows density corresponding to the genome of the virus.

Conclusion

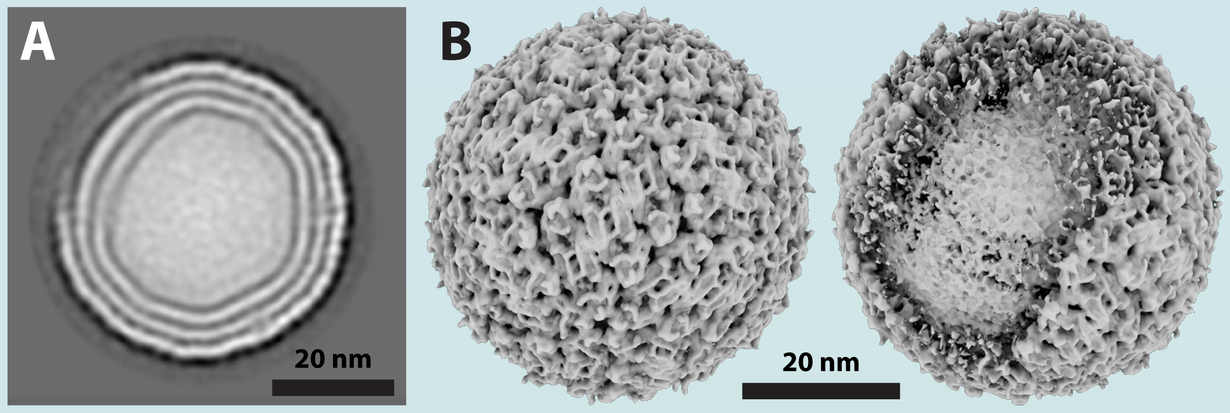
- TBEV forms "near complete" or "aberrant" immature particles.
- The "aberrant" assembly of the particles does not inhibit:
  - » genome packaging
  - » scission of the endoplasmic reticulum during budding of the particle
  - » maturation process of the particles
- Incomplete mature TBEV particles:
  - » follow the "herringbone" organization of the surface proteins

Methods

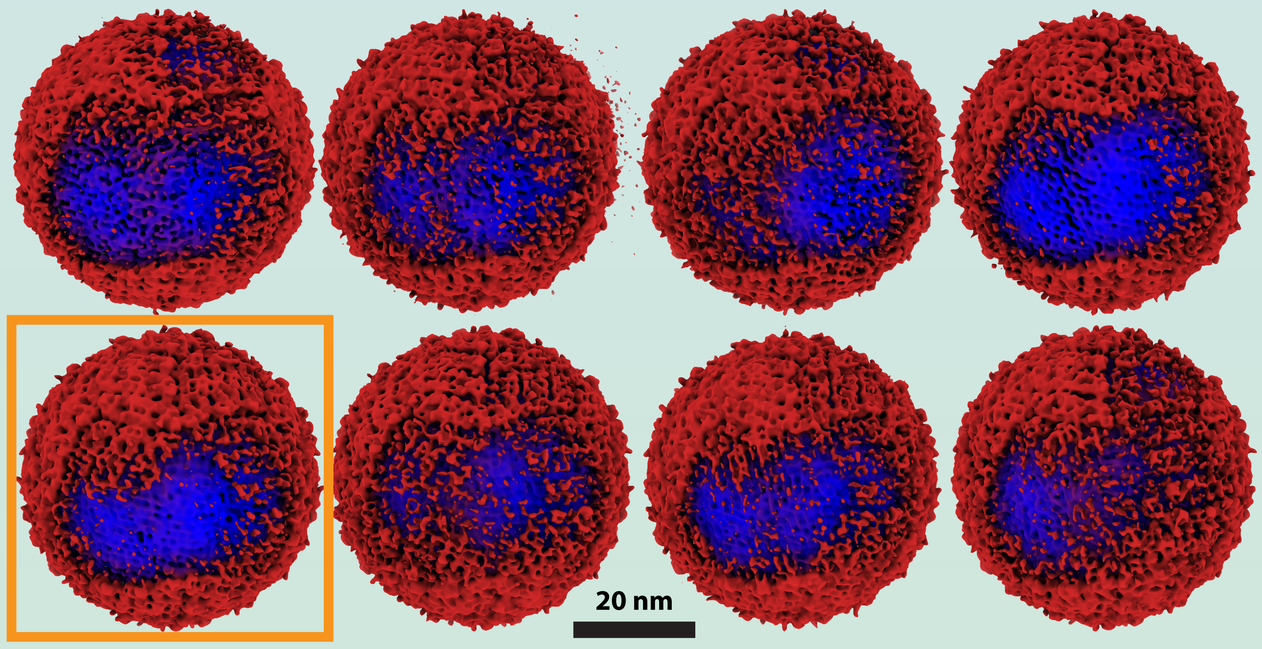
The immature and mature TBEV particles, were purified from infected tissue culture cells and visualized by cryo-electron microscopy (Cryo-EM), using Titan Krios microscope equipped with K3 detector behind energy filter (10 eV slit). Set of micrographs and tilt-series were collected for the subsequent analysis. Single particle analysis and sub-tomogram averaging were performed on extracted particles. Classifications were performed using Bayesian approach and neural network approach implemented in cryoDRGN.

mTBEV

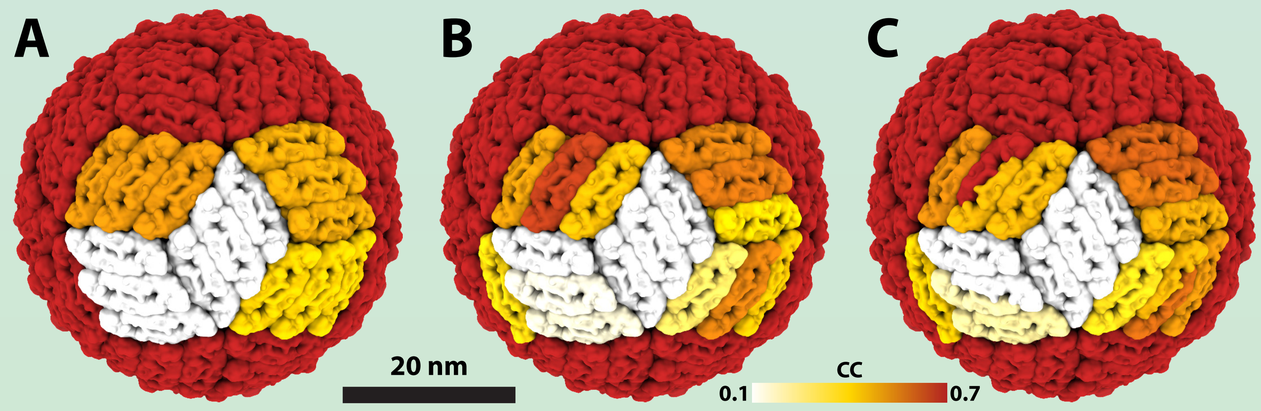
Mature TBEV particle



(A) Two-dimensional classification of mature TBEV particles revealed classes with particles featuring defects indicating missing layer of E-proteins. **(B)** Asymmetric reconstruction of these particles leads to 3D particles that have "herringbone" organization of E-proteins on one side, while missing E-proteins on the other side. Isosurface representation of the electrostatic potential map is show in the panel.

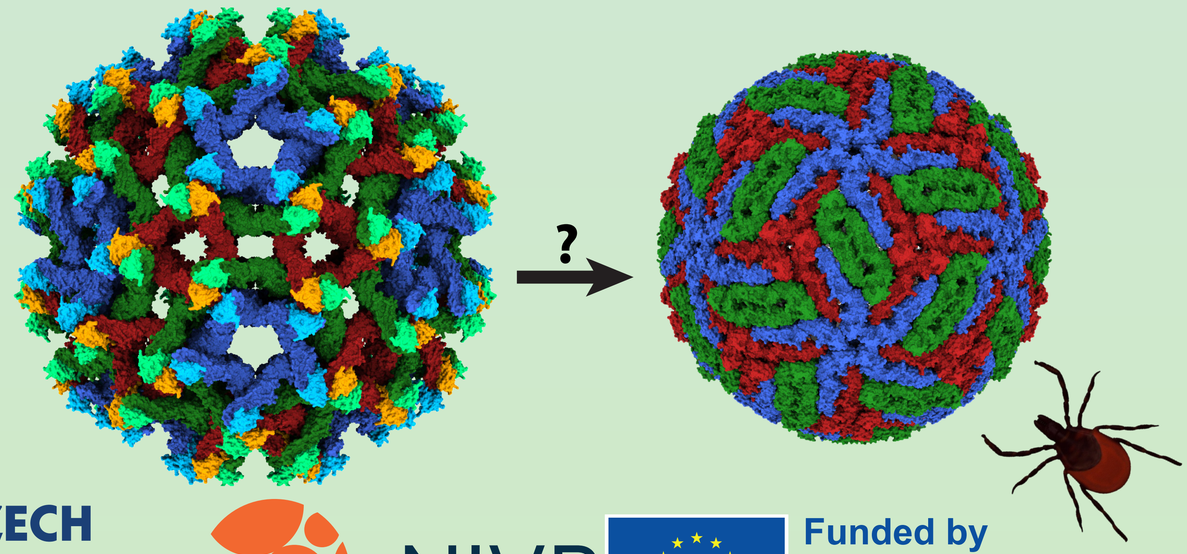


Radially colored isosurface representations of electrostatic potential maps of classes of irregular mature TBEV particles. In red the surface layer of E-proteins is depicted, blue parts correspond to the underlying viral membrane. Correlation analysis of the map in orange frame is shown below.



(A) Molecular surface of "Herringbone" models of E/M-proteins fitted into selected map colored by correlation coefficient. **(B)** Same colored by individual E/M-protein hetero-tetramers or **(C)** E/M protein dimers.

Maturation



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