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Analysis Of Membrane Bilayers In Flavivirus And Alphavirus

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Viral membrane maintains the integrity of the virus structure and involves in many vital events in the virus life cycle. For example, enveloped viruses deliver the viral genome into the host cell through protein-mediated fusion of the viral and cellular membranes. This requires concerted conformational changes of both lipid molecules and the viral proteins. To understand the structural basis of the protein lipid interaction and the dynamic nature of the viral membrane, we performed quantitative measurements on the structures of the membrane envelopes in the cryo-electron microscopy (cryo-EM) reconstruction maps of immature and mature Flaviviruses (Dengue and West Nile viruses), and Alphavirus (Sindbis).

The structure of both immature, mature Flaviviruses and Sindbis virus were determined with cryo-EM reconstruction methods to 9-13-Å resolution [1-3]. Immature Flavivirus is 600-Å in diameter and is comprised of 60 protein spikes projecting from the viral membrane (radius 165-205-Å). Each spike is a trimer of the prM and E heterodimer. The immature flaviviruses transform into 500-Å smooth particles and subjected for furin cleavage in the low pH environment of trans-Golgi network. The surface of a mature Flavivirus particle is tightly covered by 90 E dimers with a set of three dimers arranged as a herringbone-like raft structure. The stem regions of E and M proteins consist of amphipathic α-helices interacting with the outer membrane layer. Alphaviruses have 700-Å diameter and the proteins are arranged in a T=4 icosahedral structure. The surface of the virus is comprised of 80 trimeric organization of E1 and E2 heterodimers. Viral membrane occurs at the radii of 217 to 261-Å. The transmembrane domain of E1 and E2 form a parallel coiled-coil structure that traverses the viral membrane and registers into one nucleocapsid protein.

We analyzed the radial positions of the membrane leaflets in West Nile virus (WNV) reconstruction map (10-Å), immature dengue virus map (13-Å) and Sindbis virus reconstruction map (9Å). Fig.1 displays the computational method and the results for WNV. The position of each membrane leaflet correlates the local protein organization. The Gaussian and average curvatures were calculated for individual membrane leaflet. The curvature maps reflect changes of the radial position of the lipid bilayers. The membrane thickness varies 15-Å (center to center distance) across the virus particle. This analysis suggests that the protein organization can influence the structure and curvature of each viral lipid membrane leaflet. We propose that re-organization of the viral fusion proteins and its interaction with the target membrane induce sequential changes in the outer and inner leaflets in both viral and target membranes during membrane fusion.

References:

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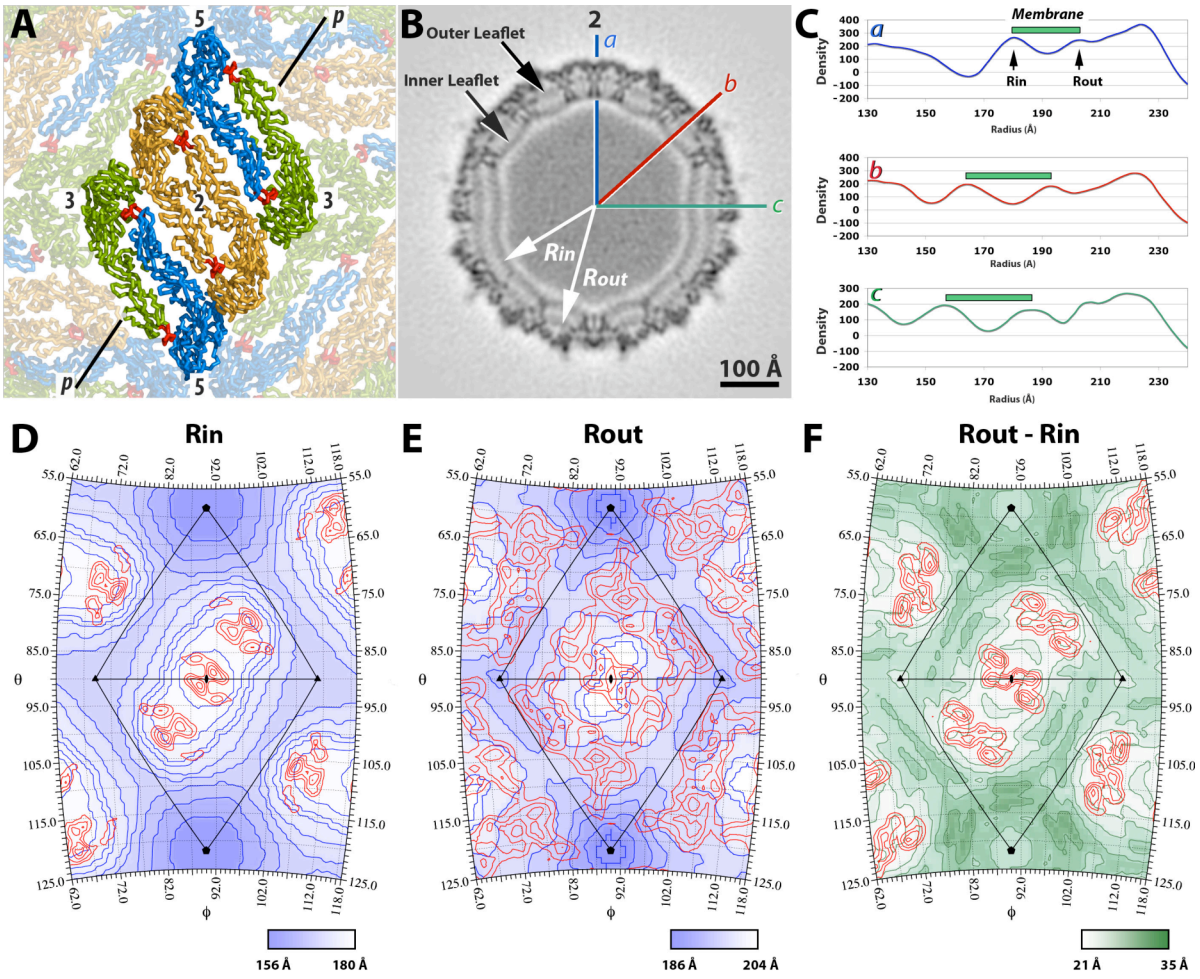

Fig.1 Positions of membrane leaflets in mature WNV. (A) The fitted E dimers arranged as a herringbone-like raft structure on the surface of WNV. The icosahedral two-fold dimer is in orange, the two other quasi-related E molecules in the same asymmetric unit are colored in blue and green. The fusion peptide is colored in red. The numbers demark the icosahedral five-, three-fold axes. The line "p" represents the position of the cross section in B. (B) Cross section of the WNV reconstruction map. The dark densities represent protein, lipid and nucleocapsid. "a" (icosahedral two-fold), "b" and "c" (~2° away from the icosahedral five-fold axis) label the directions of radial density plots in C. The scale bar is 100-Å. (C) Radial density plots at icosahedral two-fold axis (a), at a direction between two neighboring E rafts (b), and at icosahedral five-fold axis (c). The green bar represents the membrane bilayer based on the centers of the membrane leaflets. (D) The distribution of the inner leaflet radius. The icosahedral axes are labeled in closed shapes: pentagon (five-fold), triangle (three-fold), oval (two-fold). The open triangles represent the icosahedral asymmetric units. The radius of the inner membrane leaflet (156-180-Å) is represented as blue shades contoured in 2.5-Å intervals (applicable to D-F). The red lines are protein components in the inner membrane leaflet contoured at 1-σ level intervals (applicable to D-F). (E) The distribution of the outer leaflet radius (blue) superimposed with protein distribution (red) densities that are attributable to the E/M stem region. (F) The distribution of the membrane thickness, defined by (R_{out}-R_{in}) (green), superimposed with the protein densities (red) of the E/M transmembrane helices.

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