Background

Alphaviruses are enveloped RNA viruses that include several human pathogens, such as Chikungunya Virus (CHIKV). Despite their significant impact on human health, high-resolution structures of alphavirus virions have been challenging to obtain due to the intrinsic heterogeneity of these particles. Recent advances in cryo-electron microscopy (cryo-EM) have enabled breakthroughs, as demonstrated by the 3.1 Å structure of the Sindbis virus (SINV) TE12 strain resolved by our group, achieved using a local-based reconstruction method to overcome particle heterogeneity. This structure revealed conserved residues critical to the viral life cycle, including a hydrophobic pocket in the E2 protein's subdomain D, stabilized by an unidentified pocket factor near the viral membrane. These findings highlight potential targets for antiviral interventions and provide insights into virus assembly and host-cell entry mechanisms. In this proposal, we aim to apply a similar strategy to CHIKV strain 181/25 (BSL-2) to obtain a high-resolution structure of the virion. The preliminary data in Figure 2 demonstrate the feasibility of this approach, showcasing the distribution, morphology, and structural features of CHIKV particles through negative staining and cryo-EM imaging.

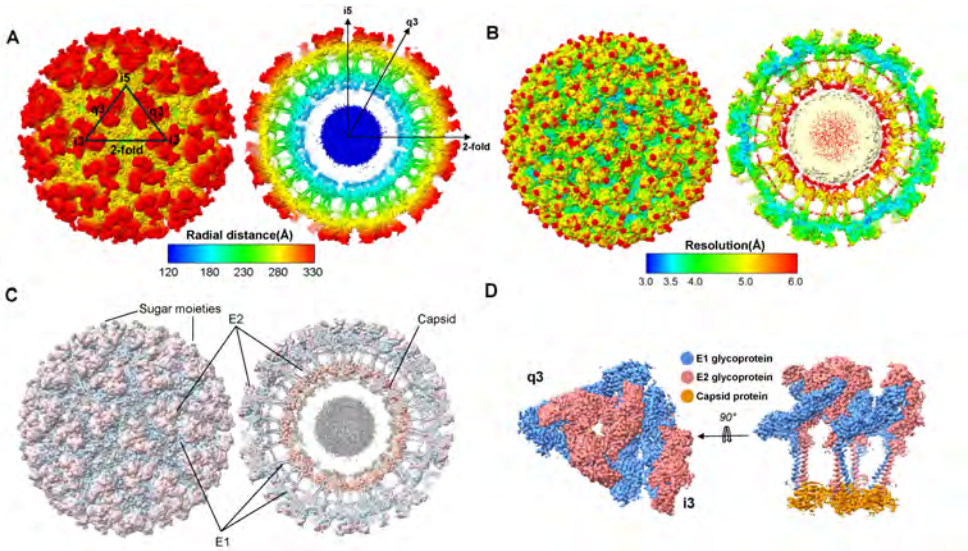

Figure 1: **Structural Analysis of Sindbis Virus (SINV) TE12 Strain Particles by Cryo-EM.** (A) Radial depth-colored surface representation of SINV particles, highlighting icosahedral symmetry axes (black arrows indicating two-, three-, and fivefold axes). (B) Resolution distribution of the cryo-EM density map, excluding RNA (light-yellow), depicted on the scale shown below. (C) Surface rendering showing detailed structural components of SINV, including glycoproteins E1 (blue), E2 (light pink), capsid proteins (dark pink), and sugar moieties (gray). (D) Two orthogonal views of the SINV asymmetric unit, formed by four E1–E2 heterodimers and capsids organized according to $T = 4$ icosahedral symmetry. E1, E2, and capsid proteins are displayed in blue, pink, and yellow, respectively, with quasi-threefold (q3) and threefold (i3) symmetry axes labeled.

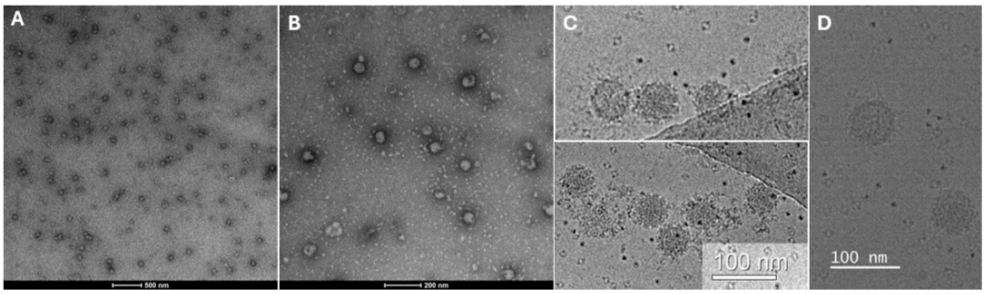

Figure 2: **Electron Micrographs of Chikungunya Virus (CHIKV) Strain 181/25 (BSL-2).** (A) Negative staining image showing the distribution of virus particles. Scale bar, 500 nm. (B) Higher magnification negative staining image revealing the spherical morphology of the virus particles. Scale bar, 200 nm. (C) Cryo-EM image of virus particles preserved in vitreous ice prepared on a QUANTIFOIL® (R2/1, 200 mesh) grid, displaying detailed structural features. Scale bar, 100 nm. (D) Cryo-EM image of virus particles preserved in vitreous ice prepared on a C-flat™ (R2/1, 200 mesh) grid, showing high-resolution morphology. Scale bar, 100 nm. Images in (A) and (B) were acquired using the FEI Tecnai Spirit Bio-Twin Transmission Electron Microscope, and images in (C) and (D) were acquired using the FEI Tecnai G2 F30 Field Emission Gun Transmission Electron Microscope, both at the Characterization Facility of the University of Minnesota.