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Comparing EM Approaches for Studying Filoviral Glycoproteins

Amanda Paz Herrera, Nguyen Nguyen, Tommi White and Donald Burke

University of Missouri, Columbia, Missouri, United States

Filoviruses are enveloped viruses containing three highly dangerous genera, Ebolavirus, Marburgvirus, and Cuevavirus. The Ebolavirus genus is composed of five species, Bundibugyo (BDBV), Reston (RESTV), Sudan (SUDV), Tai Forest (TAFV), and Zaire ebolavirus (EBOV). The Zaïre Ebola Virus species (EBOV) was responsible for largest Ebola outbreak in history, which lasted from 2013-2016 and led to approximately 28,000 reported cases of infection and 11,000 deaths (according to World Health Organization [WHO] Ebola Situation Reports). Since then, two main vaccines have been developed, and one has been officially approved, but their efficacy and long term effects are still under study(1). At the same time, EBOV diagnostics in primary health care facilities are still a challenge, as they are limited to antigen detection tests, which often result in false positive results and require specialized equipment (2). Current diagnostics and therapeutics for EBOV are insufficient for combating future outbreaks. Thus, it is necessary to continue to investigate novel reagents that can help us learn about the interactions between the virus and its host cells.

An attractive target for therapeutic and diagnostic purposes is the EBOV glycoprotein (GP), which is the only protein on the surface of the virus and is critical for tropism and infection. Following cell entry, EBOV GP undergoes enzymatic processing by host cathepsins that cleave the glycoprotein (GP_{CL}) and expose the Receptor Binding Site (RBS), which interacts with the endosomal receptor Niemann-Pick C1(NPC1) (3). Efforts in therapeutic development that target EBOV GP have primarily utilized neutralizing antibodies (Abs) extracted from survivors. Epitope mapping, was very helpful for designing these antibody cocktails and for understanding GP vulnerability areas for Abs (4). However, because of the size of Abs (50-150 kDa), they are unable to bind to "hard-to reach" or hidden epitopes on the GP due to GP's structure (trimeric and highly glycosylated). Thus, both diagnostics and therapeutics would benefit from exploiting smaller affinity reagents that could penetrate into those "hard-to-reach" epitopes. Aptamers are small (~30 KDa), short structured oligonucleotides that show high affinity and specificity for their molecular targets. Our lab has generated single stranded DNA (ssDNA) aptamers against EBOV GP, and we are currently characterizing these aptamer-GP interactions. Due to their flexible nature, nucleic acids pose a challenge for structural characterization. Currently, the few published structural aptamer-target studies have used X-ray crystallography to characterize the molecular details of these interactions, however, crystallization of nucleic acids is a bottleneck for structural analysis via crystallography(5). Thus, using Cryo-Electron Microscopy, a structural technique that allows characterization of macromolecules in native-like conditions enables thorough, quicker studies on aptamer-target complexes**.** Here, we present various potential strategies to study viral glycoprotein using different EM approaches, including negative stain Transmission Electron Microscopy, Single Particle Analysis, and Cryo-Electron Tomography. Our strategy includes obtaining low resolution, preliminary information from recombinant EBOV GP, as well as studying the EBOV GP, in a more biologically relevant context, displayed on the membrane of a chimeric virus. Using different microscopy techniques to study the GP by itself will provide insights into the best methods to study aptamers in complex with their target GP, and their potential for becoming therapeutic and diagnostic tools for EBOV.

Investigating at molecular detail the interactions of aptamers and their EBOV GP target is crucial for understanding the nature and requirements for binding and can eventually also provide insights on neutralization. Although aptamers have been generated against a wide variety of targets, aptamer-target

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complexes are scarcely available through literature. Therefore, thorough biochemical analysis of aptamer-GP complexes coupled with a structural platform for studying them at molecular levels will further our understanding of the aptamers' abilities to reach hidden epitopes, and vulnerability.

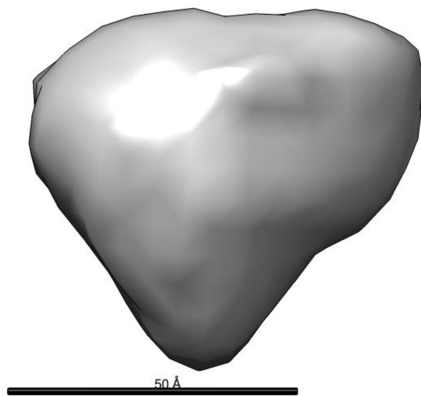

Figure 1. Initial low resolution model of recombinant EBOV GP lacking the transmembrane and Mucin-Like Domain. The model represents the expected chalice-like morphology of the GP trimer. The reconstruction was generated via negative stain (nW) single particle analysis.

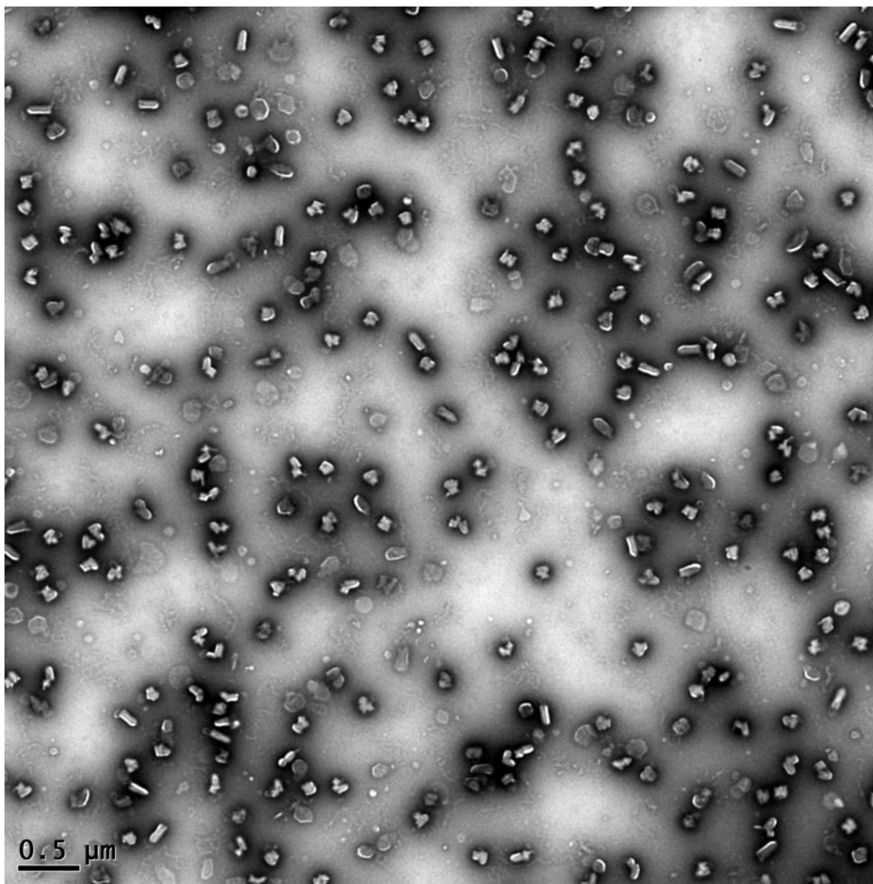

Figure 2. Fig. 2: Representative micrograph of chimeric rVSV-EBOV GP (Vesicular Stomatitis Virus expressing Ebola Glycoprotein) embedded in negative stain (nW).

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