Structure of the Ebola Virus Glycoprotein Bound to an Antibody from a Human Survivor

Ebolavirus: The *ebolavirus* causes a severe hemorrhagic fever with 50-90% lethality for which no vaccines or treatments are yet available. The more frequent re-emergence of the virus, its high prevalence among wildlife, and ease of importation of the virus make it a significant public health concern. A team of researchers have recently determined the crystal structure of the oligomeric, viral surface glycoprotein in complex with a rare antibody derived from a human survivor. This work explains how the glycoprotein, termed GP, mediates host recognition, drives fusion of the viral and host membranes and masks itself from immune surveillance. The structure also explains why antibodies that neutralize the virus are so rare, identifies the very few sites to which a neutralizing antibody might bind, and thus, provides templates for vaccines and antibodies against the virus.

The glycoprotein GP is the sole resident of the *ebolavirus* surface and is responsible for attaching to and entering new host cells, shielding of the viral surface from immune surveillance, and maintenance of viral stability between hosts (often in caves for long periods of time). Determination of the crystal structure of GP was critical for understanding these processes and in design and improvements of vaccines and therapeutics. However, structures of viral glycoproteins in their native, viral surface forms can be difficult to achieve as they are oligomeric, metastable, and heavily glycosylated. Indeed, half or more of the molecular weight of *ebolavirus* GP is comprised of heterogeneous carbohydrate and unstructured polypeptide. Through production of some 140 versions of the viral glycoprotein, alone or in complex with one of seven different antibodies, Jeffrey Lee, Marnie Fusco and Erica Ollmann Saphire of The Scripps Research Institute were able to crystallize the trimeric, prefusion form of GP, in complex with a neutralizing antibody derived from a human survivor of the 1995 Kikwit, Zaire outbreak. The researchers had to grow ~50,000 crystals for this project, and screen the 800 largest crystals over ~30 trips to ALS and SSRL in order to find one crystal that would diffract to 3.4 Å (collected on ALS BL5.02) and permit structure determination. Importantly, the GP crystallized retains all regions required for attachment, fusion and entry. Viruses pseudotyped with the crystallization construct plus the transmembrane domain are functional in infectivity assays and exhibit antibody neutralization profiles identical to wild-type GP.

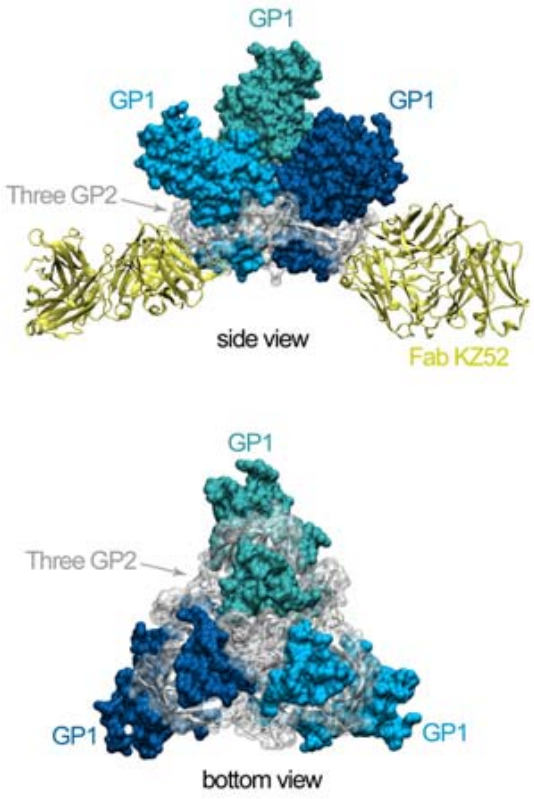

Figure 1. The crystal structure of *ebolavirus* GP reveals a three-lobed chalice-like structure. The three GP1 subunits (colored blue and green), mediate attachment to new host cells, and are tethered together by the three GP2 subunits (white). GP2 forms the protein machinery which drives fusion of the viral membrane with the host cell. The human antibody KZ52 (yellow) binds an epitope at the base of the GP chalice where it bridges GP1 to GP2.

Structural arrangement and rearrangement: *Ebolavirus* GP is cleaved by furin to yield two subunits termed GP1 and GP2 with separate structural and functional roles. Of these, GP1 is responsible for receptor engagement while GP2 mediates fusion of viral and host membranes. The crystal structure illustrates that the 450 kDa GP forms a three-lobed chalice shape with the bowl of the chalice assembled by the three GP1 subunits (Figure 1). The stem of the chalice is formed by three GP2 subunits that cradle and encircle the GP1 trimer. Here, the internal fusion loop and heptad repeat region of GP2 together wrap around GP1, and in turn, hydrophobic residues of GP1 clamp the heptad repeat of GP2 into its metastable, prefusion conformation (Figure 2). This clamp is released in entry through an as yet unidentified process, allowing GP2 to spring into its more stable, six-helix bundle conformation and trigger fusion of virus and host membranes.

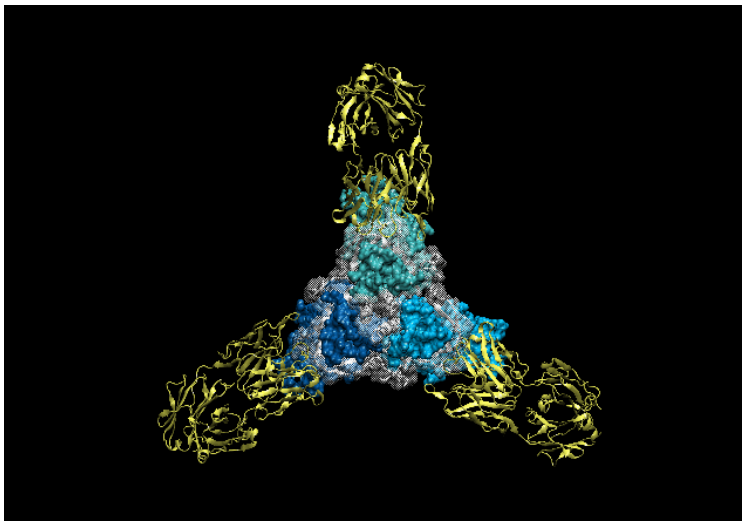

Figure 2. The GP-KZ52 complex. Here, one can see how the GP2 subunits (white) are wound around GP1 (blues) like thread around a spool. GP1 forms a hydrophobic clamp on GP2, holding it in this metastable, prefusion conformation on the viral surface.

Insight into receptor binding and entry: This structure, the first near-complete structure of any filovirus glycoprotein, allowed identification of a putative receptor-binding site on GP. This site is sequestered in the bowl of the GP trimer, further masked by a novel glycan cap domain and a heavily glycosylated, unstructured mucin-like domain. GP was known to be cleaved by cathepsin proteases as an essential step in entry, but the precise site or role of cleavage was unknown. Importantly, the crystal structure identifies the probable cleavage site of GP and illustrates how cleavage at this site uncaps the receptor binding regions freeing them for interaction with host cell receptor(s). Thus, the crystal structure of GP suggests that initial cellular attachment occurs *via* interactions of cell surface lectins with the mucin-like domain or other glycosylated regions on GP, and that the receptor-binding site is revealed later in the endosome upon proteolytic processing.

Templates for vaccines and immunotherapeutics: The crystal structure also reveals that most of GP is shielded by a thick cloak of carbohydrate and identifies the very few sites left exposed and available for antibody binding. Hence, this structure is now serving as a template for vaccines and antibodies to target these newly revealed slits in *ebolavirus*'s cloak.

Primary Citation

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References

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For SSRL

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