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The Structural Basis for Filovirus Neutralization by Monoclonal Antibodies

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Abstract

Filoviruses, including ebolaviruses and marburgviruses, are the causative agents of highly lethal disease outbreaks. The 2013–2016 Ebola virus outbreak was responsible for >28,000 infections and >11,000 deaths. Although there are currently no licensed vaccines or therapeutics for any filovirus-induced disease, monoclonal antibodies (mAbs) are among the most promising options for therapeutic development. Hundreds of mAbs have been isolated from human survivors of filovirus infections that target the viral spike glycoprotein (GP). The binding, neutralization, and cross-reactivity of many of these mAbs has been determined. Several mAbs have been characterized structurally, and this information has been crucial for strategizing therapeutic and vaccine design. Here we present an overview of the structural features of the neutralizing/protective epitopes on filovirus glycoproteins.

Filoviruses cause severe disease in both humans and nonhuman primates. Outbreaks are unpredictable and occur with mortality rates between 25–90% [1,2]. Three genera comprise the family *Filoviridae*: *Ebolavirus* [which includes Ebola virus (EBOV), Sudan virus (SUDV), Bundibugyo virus (BDBV), Taï Forest virus, and Reston virus], *Marburgvirus* [which includes Marburg virus (MARV) and Ravn virus (RAVV)], and *Cuevavirus* [which includes Lloviu virus]. Ebolaviruses and marburgviruses cause the clinically similar Ebola Virus Disease (EVD) and Marburg Virus Disease (MVD), respectively.

Filoviruses form extended filamentous virions surrounded by a membrane envelope that is studded with copies of the surface glycoprotein (GP). GP is the only protein expressed on the viral surface, and serves to mediate entry into the target cell. Through GP, the virions first interact with target cells via lectins [3], membrane phosphatidylserine, or TIM-1 family members [4]. After internalization by macropinocytosis [5–7], the virions enter the endosome, where host cathepsins proteolytically process GP to remove the glycan cap and mucin-like domain, leaving behind GP cleaved (GP_{CL}) [8–10]. In GP_{CL}, the core of the

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protein is exposed and allows the receptor binding site (RBS) to recognize and engage domain C of the cholesterol transporter Niemann-Pick C1 (NPC1-C) [10–15]. Currently, GP is the primary target for antibodies and vaccines due to its prevalent exposure on the viral surface and its critical role in viral entry [16]. Given the complexity of antibody recognition and neutralization of filoviruses, analysis of structural differences in antibody-GP complexes and mechanisms of neutralization across the filovirus family is important for understanding antibody-mediated inhibition.

In the infected cell, GP is post-translationally processed by furin cleavage into GP_1 and GP_2 subunits [17]. The GP_1 subunit facilitates host cell attachment and receptor recognition, whereas GP_2 mediates fusion of the virus and host membranes [18–21]. Three GP_1–GP_2 heterodimers assemble into a trimeric peplomer, or “spike” on the viral surface [22–24]. The RBS is located beneath the glycan cap towards the top of the GP_1 subunit and contains a hydrophobic pocket into which loop 2 of NPC1-C binds [11,12,15]. The C-terminus of GP_1 has a heavily glycosylated mucin-like domain that is situated on the upper and outer portions of the peplomer [22]. The GP_2 subunit contains an N-terminal peptide (released from GP_1 by furin cleavage), an internal fusion loop (IFL), two heptad repeats (HR1 and HR2), a membrane proximal external region (MPER), and a C-terminal transmembrane domain [19,23]. HR1 wraps around the base of the GP_1 receptor-binding core while HR2 forms a “stalk” that connects the GP core to the viral membrane [23]. Many portions of GP_2 including the fusion loop, HR1, and the HR2 stalk are organized similarly between ebolaviruses and marburgviruses (Figure 1) [23,25].

The GP_2 of marburgviruses contains an additional domain, absent in ebolaviruses, termed the “wing” due to its outward projection and flexibility [26]. The wing results from an N-terminal shift in the relative position of the furin cleavage site between marburgviruses and ebolaviruses [27]. In marburgviruses, the mucin-like domain is attached to the C terminus of GP_1, whereas the wing domain is attached to the N terminus of GP_2 (Figure 1) [25,26]. Although the marburgvirus wing domain was thought to be analogous to the C-terminal portion of the ebolavirus mucin-like domain, recent structural information revealed otherwise. Part of the marburgvirus wing (residues 469–478 and 487–498) anchors itself to the GP core through a pair of beta strands that hug GP_1 in an organization that is analogous to β1–β2 of ebolavirus GP_1 [23,25]. The remaining portions of the marburgvirus wing, including residues 436–469 and those that connect the beta strand pair to the IFL, have yet to be defined structurally. Antibodies directed against the wing domain show neutralizing activity in cell culture and are protective in the mouse model [26]. However, whether the wing domain is involved in virus fusion and entry is unclear and is an important question for further investigation.

A broad array of neutralizing antibodies against ebolavirus GPs have been isolated from animal immunization studies as well as from human survivors of EVD [28–42]. Structural studies of such antibodies revealed neutralizing epitopes across the surface of GP [43,44]. Sets of antibodies can be grouped into epitope classes that are known to react to a GP_1/GP_2 containing region at the bottom of the GP core termed the “base”, the glycan cap of GP_1, the head/apex of GP_1, the IFL of GP_2, the HR2 stalk of GP_2, or to several linear epitopes within

the mucin-like domain (Figure 1) [45]. At least one member of each epitope class offers *in vivo* protection in the mouse model of EBOV infection [29–32,35,42,44,45].

The mechanism of protection can be roughly divided according to the physical location of the epitope on the GP surface. Antibodies that recognize epitopes contained within GP_{CL} (e.g., non-glycan cap or mucin-like domain) are typically neutralizing [11,29,30,33,35,37,39,41,43–46] and likely block infection by physically impeding virus entry through prevention of conformational changes required for fusion [8,47,48], GP cleavage [41], or receptor binding [25,49]. Meanwhile, antibodies targeting domains removed by cathepsin cleavage are less likely to be neutralizing [11,30,32,41,45,46], although exceptions exist [30,34].

Interestingly, some antibodies with limited neutralization capacity are nonetheless highly protective in an animal model [32,42,45,50], likely via immune-mediated clearance of the pathogen and infected cells [45,51,52]. The upper and outer location of these epitopes on the GP molecule could provide enhanced accessibility to FcR-bearing cells [22] and/or binding to these conformationally mobile parts of GP may facilitate IgG-IgG associations (Figure 2).

The primary product of the ebolavirus GP gene is not GP, but rather an abundantly produced soluble protein dimer termed secreted glycoprotein (sGP) [53,54]. The N-terminal 295 amino acids of GP and sGP are identical and include the RBS and the glycan cap domains (Figure 3). The C termini, however, differ. Only GP encodes the mucin-like domain and GP_2. sGP instead ends in a short peptide with a cysteine that mediates a disulfide bond [54,55]. Antibodies that target the RBS and/or the glycan cap generally also bind sGP, sometimes with higher affinity than GP [28,29,52,55–61]. sGP has been hypothesized to serve several roles, including as an immune decoy, although its precise role in infection has not yet been fully elucidated [62]. Notably, the antibody termed mAb 114, which recognizes both GP and sGP, protected non-human primates against EBOV challenge when administered as a monotherapy [31,43], and 13C6, a key component of the ZMapp cocktail, also cross-reacts between sGP and GP [32,42]. However, in the mouse model, sGP cross-reactivity appears not have a significant impact on protection [45].

Antibodies that cross-react among multiple pathogenic filoviruses would allow rapid treatment mobilization prior to the identification of the infecting strain. Moreover, which virus strain will emerge cannot be predicted, and production and stockpiling of distinct antibody therapies against each of the antigenically distinct filoviruses is cost-prohibitive. However, antibodies that cross-react with multiple filovirus GPs remain rare. The five known ebolavirus GPs differ by ~50% at the amino acid level, and EBOV and MARV GP sequences differ by ~70%. Much of the sequence variation, however, is concentrated in the mucin-like domain with less variation in the GP core, although conservation in the core does vary by epitope. For example, the IFL of GP2 is more conserved, and three broadly reactive antibodies have been identified with overlapping epitopes involving the tip or stem of the fusion loop [29,35,44]. In contrast, most antibodies against the base are virus-specific likely due to differences in amino acid composition and conformation in the GP_2 N-terminal peptide [23,33,55]. Other broadly cross-reactive antibodies map to the glycan cap and the HR2 stalk [30], while broadly reactive *non*-neutralizing antibodies have been mapped to

sites around the GP_1 head and glycan cap [52,59]. High-resolution structures of the broadly reactive antibodies are needed to illuminate the determinants of their reactivity and to inform vaccine design.

In marburgviruses, the glycan cap appears to be less effective at shielding the RBS. Unlike survivors of ebolavirus infection, the majority of antibodies identified in a human survivor of MARV infection recognize the RBS, and can bind whether or not the GP is cleaved (i.e., whether or not the glycan cap is present) [63]. Two crystal structures of antibodies bound to the marburgvirus GP RBS are available [25,49] as are several low-resolution negative stain EM structures of other antibody complexes with this site [49,63] (Figure 4). Recent work describes a high resolution structure of a marburgvirus GP containing the glycan cap (PDB: 6BP2) [25]. In this structure, although the GP was intact, the cap was disordered and could not be observed structurally.

The fact that anti-MARV antibodies can bind the RBS of uncleaved marburgvirus GP suggests that the RBS of marburgviruses may be transiently exposed. We believe that the marburgvirus RBS is transiently rather than completely exposed because NPC1-C can not bind uncleaved GP, and instead only reacts with GP_{CL} [25,26,49,63]. High-affinity antibodies may be able to displace the glycan cap, whereas lower affinity NPC1-C interactions may not; the affinity of NPC1-C for MARV GP_{CL} at pH 7.4 is ~150 μM [12]. It is also possible that is that low levels of non-specific proteolysis of the glycan cap and mucin-like domain *in vivo* could facilitate exposure of the RBS to immune recognition.

For marburgviruses, protective antibodies have only been identified thus far against two epitopes on the GP: the RBS [25,49,63] and the marburgvirus-specific wing [26]. Murine antibodies against the wing are protective in the mouse model, but have not yet been evaluated in larger animal models [26]. The human antibody MR191 directed against the RBS has been evaluated in non-human primates and offered complete post-exposure protection five days after virus exposure [64]. Interestingly, escape mutants of MR191, uncovered using MARV GP-pseudotyped recombinant VSV, do not exist in the antibody's RBS footprint, but instead, are in the glycan cap and the wing domains [25,63], suggesting that conformational adjustments in these flexible domains may affect the RBS through a mechanism that is not yet understood. Identification of additional protective antibodies against other sites will allow development of treatment cocktails to mitigate escape.

Antibodies that cross-react among multiple pathogenic filoviruses would allow rapid treatment mobilization prior to the identification of the infecting strain. Moreover, which virus strain will emerge cannot be predicted, and production and stockpiling of distinct antibody therapies against each of the antigenically distinct filoviruses is cost-prohibitive. However, antibodies that cross-react with multiple filovirus GPs remain rare. The five known ebolavirus GPs differ by ~50% at the amino acid level, and EBOV and MARV GP sequences differ by ~70%. Much of the sequence variation, however, is concentrated in the mucin-like domain with less variation in the GP core, although conservation in the core does vary by epitope. For example, the IFL of GP2 is more conserved, and three broadly reactive antibodies have been identified with overlapping epitopes involving the tip or stem of the fusion loop [29,35,44]. In contrast, most antibodies against the base are virus-specific.

Although these antibodies may bridge the anchor point of the fusion loop, they also recognize other residues in GP that differ in sequence and conformation, such as the GP_2 N-terminal peptide [23,33,55]. Other broadly cross-reactive antibodies map to the glycan cap and the HR2 stalk [30], while broadly reactive non-neutralizing antibodies have been mapped to sites around the GP_1 head and glycan cap [52,59]. High-resolution structures of the broadly reactive antibodies are needed to illuminate the determinants of their reactivity and to inform vaccine design.

The first EBOV GP-antibody structure was determined ten years ago. Over the ensuing decade, additional structures of EBOV-, SUDV-, and MARV-reactive mAbs in complex with their cognate GPs have showcased the variety of antibody epitopes that lead to neutralization and protection, as well as differences among GP targets. Still needed are structures of BDBV GP, RESTV GP, and discovery, characterization or engineering of more mAbs that broadly react and broadly neutralize among the different filoviruses. Further, a better understanding of the Fc-mediated functions of these antibodies would support ongoing development of effective immunotherapeutics and vaccines to prevent filovirus disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Filovirus neutralization and protection is related to epitope on filovirus GPs
- GP epitopes on marburgvirus and ebolavirus differ fundamentally at a structural level
- Ebolavirus and marburgvirus GP have six and two, respectively, antibody epitope classes
- Marburgvirus cleavage sites and glycosylation differ from those for ebolaviruses
- The wing domain is unique to marburgvirus GP

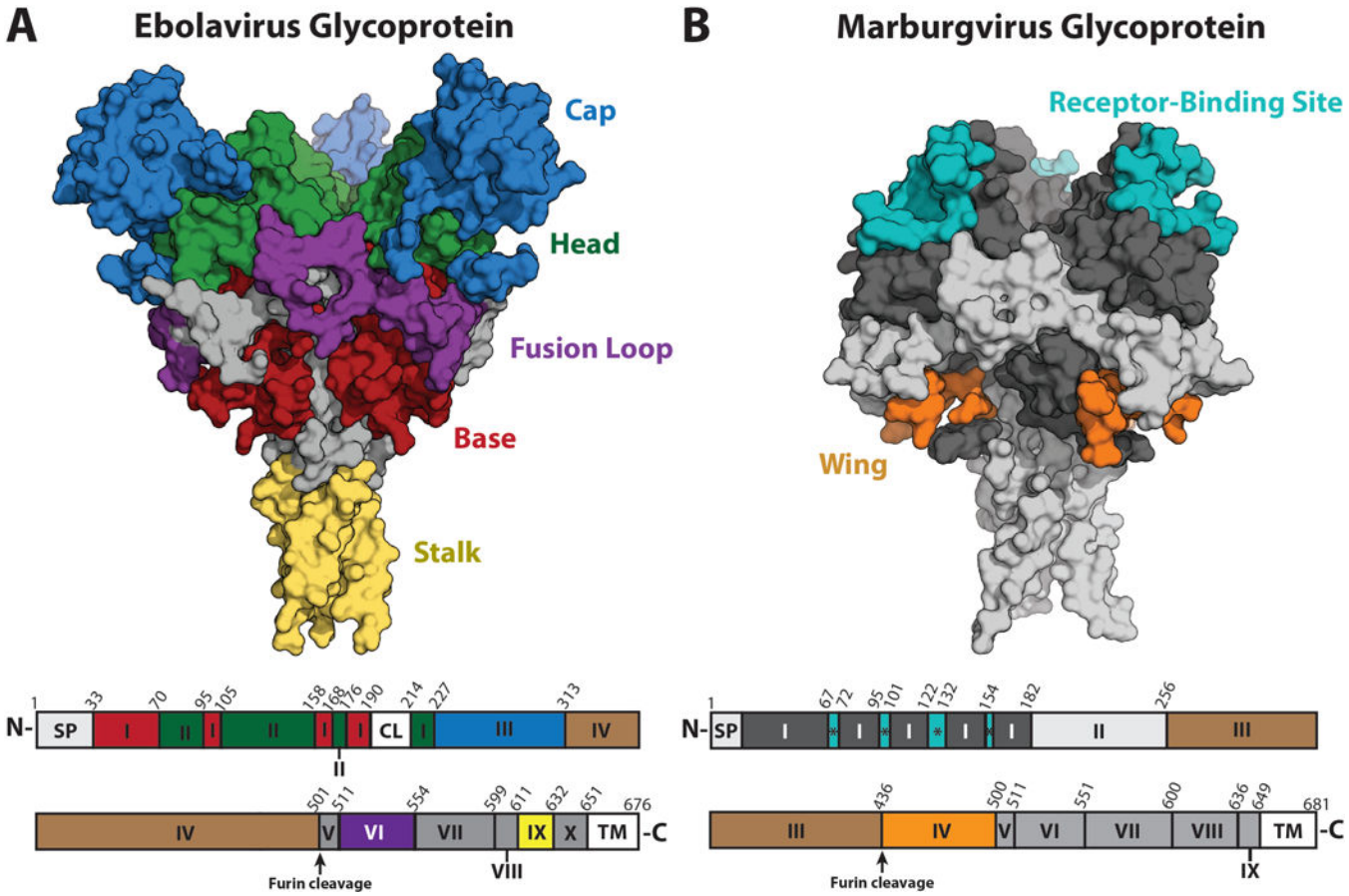

Figure 1. Antibody epitopes on filovirus GPs

(A) Ebolavirus GP with antibody binding epitopes shown as patches of color on the GP surface (PDB: 5JQ7) [65] and a corresponding sequence map below. Labels for ebolaviruses: SP = Signal Peptide, I = Base, II = Head, CL = Cathepsin Cleavage Loop, III = Glycan Cap, IV = Mucin-like Domain (MLD), V = N-terminal Loop, VI = Fusion Loop, VII = Heptad Repeat 1 (HR1), VIII and IX are together Heptad Repeat 2 (HR2), of which IX = Stalk, X = Membrane Proximal External Region (MPER), and TM = Transmembrane domain. **(B)** Marburgvirus GP with antibody binding epitopes shown as patches of color on the GP surface (PDB: 6BP2) [25]. Labels for marburgviruses: SP = Signal Peptide, I = GP_1, * = Receptor binding site, II = Glycan Cap, III = MLD, IV = Wing, V = N-terminal loop, VI = Fusion Loop, VII = HR1, VIII = HR2, IX = MPER, and TM = Transmembrane domain. The RBS is illustrated only on marburgvirus GP for clarity; on uncleaved ebolavirus GP, the glycan cap masks the RBS.

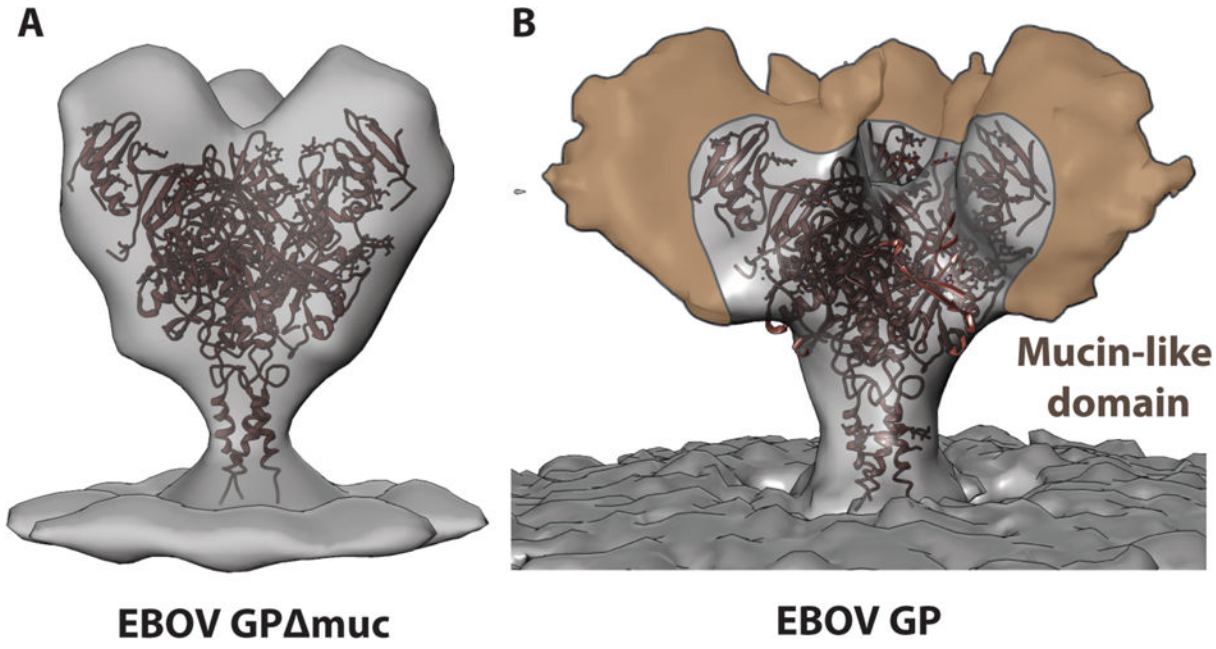

Figure 2. Visualization of the Mucin-Like Domain

(A) The crystal structure of the mucin-deleted EBOV GP (PDB: 5JQ3) [65] is shown docked into a subtomogram averaged map of mucin-deleted EBOV GP [66] and a single-particle generated map of intact, mucin-containing EBOV GP **(B)** [22]. Although the observation of density for mobile regions is limited by technical factors in single particle reconstruction, the regions of the mucin-like domain that are visible appear to extend upwards and outwards from the glycan cap and base region, thereby shielding much of the GP core.

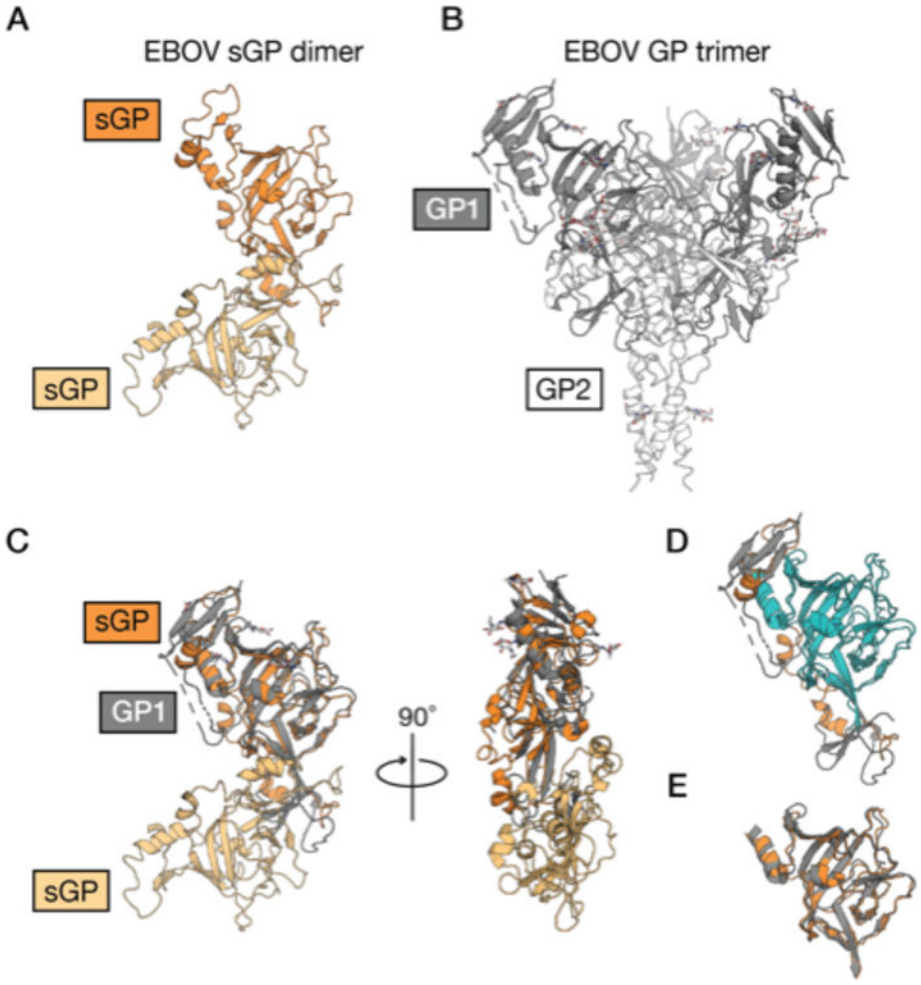

Figure 3. Structural similarities between sGP and GP

(A) The EBOV sGP dimer (PDB: 5KEM) [55] is shown as a cartoon with each monomer shaded orange. **(B)** The EBOV GP trimer (PDB: 5JQ3) [65] is shown as a cartoon with GP_1 (dark gray) and GP_2 (white). **(C)** GP_1 and a single monomer of sGP align with a C_α r.m.s.d. of 3.87 Å over 217 aligned residues. sGP and GP share 100% sequence identity for their first 295 amino acids. **(D)** They are structurally similar between the core residues 66–184 and 216–259 (blue), although parts of these regions are likely obscured from immune surveillance by the dimerization interface. **(E)** The isolated cores (same as the blue region in **(D)**) align with a C_α r.m.s.d. of 1.71 Å over 163 residues.

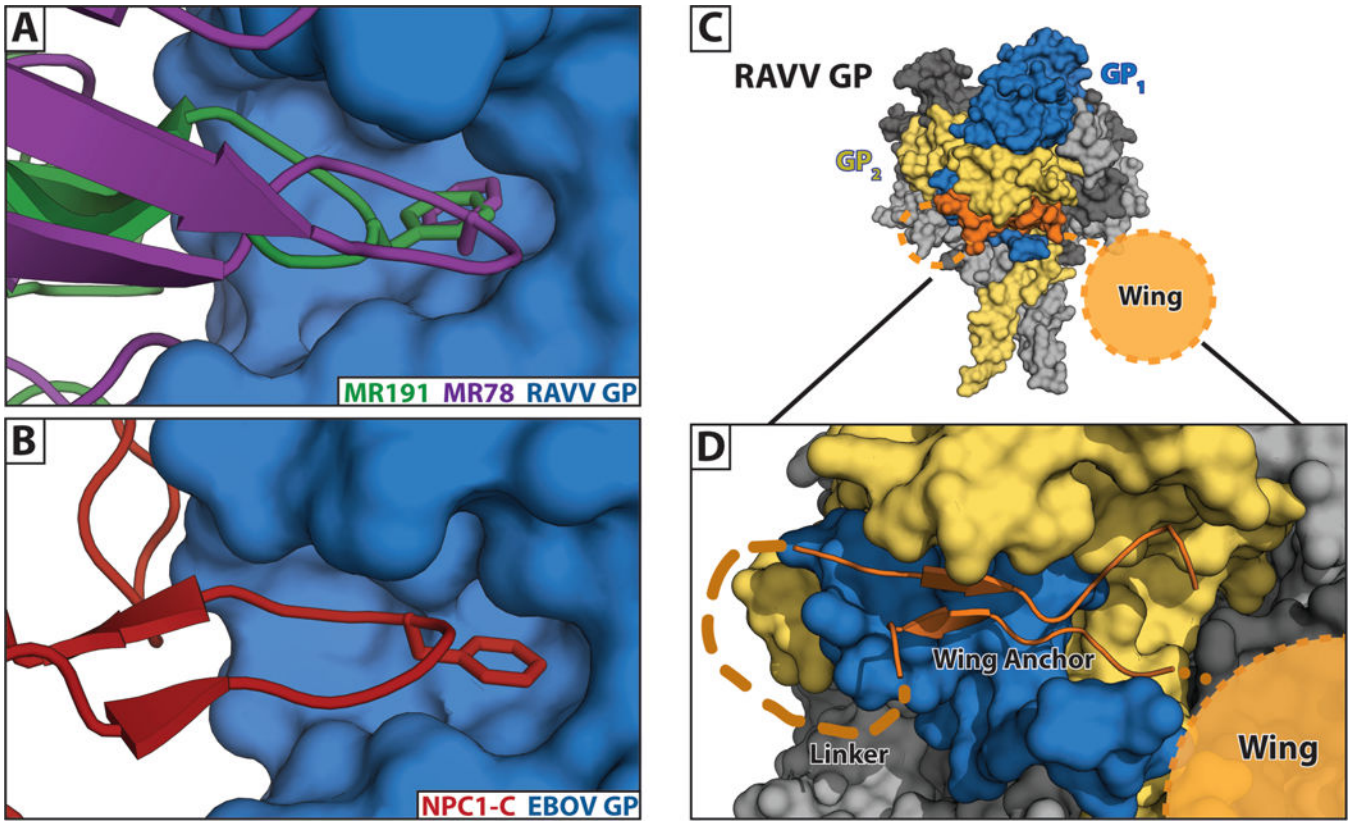

Figure 4. Neutralizing epitopes identified for marburgvirus

(A) Crystal structures of neutralizing antibodies MR191 [25] and MR78 [49] bound within the RBS of RAVV GP. **(B)** A phenylalanine at the apex of the CDR-H3s of both antibodies reaches into the hydrophobic pocket of the marburgvirus GP in a manner that structurally mimics interactions of ebolavirus GP both with its glycan cap and its host receptor, NPC1-C [12]. **(C)** A marburgvirus GP is shown with a single GP monomer colored in blue (GP_1) and gold (GP_2). The remaining two monomers are grey. The anchor of the wing domain (orange) is shown wedged underneath the base of GP. **(D)** Enlarged view of the wing illustrating the β-strand wing anchor region (connected by an 8 aa linker), and the relative position of the 33 aa wing targeted by antibodies.