other factors responsible for the activation and inactivation of IFT motors is an attractive hypothesis. Alternatively, one component of IFT trains could play a role for dynein-2 analogous to the role that dynactin plays for dynein-1. In such a model, the activating protein might occupy a position within the anterograde train that is inaccessible to the dynein. Binding of this activating complex to the motor would then become possible only upon structural reorganization of the anterograde train into its retrograde form. A more detailed understanding of the structure of anterograde and retrograde IFT trains and of the ciliary tip complex is likely to be crucial to full understanding of the mechanism of dynein activation in IFT.

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# Human antibody pieces together the puzzle of the trimeric Lassa virus surface antigen

Antra Zeltina & Thomas A Bowden

**The envelope glycoprotein spike, the sole antigen on the Lassa virus (LASV) surface, constitutes the focal point of the host neutralizing immune response. A high-resolution structure of the trimeric LASV glycoprotein in an antibody-bound form illuminates the molecular architecture of the antigen and reveals the mode of action of the most abundant known class of Lassa-specific human neutralizing antibodies.**

The World Health Organization has included LASV, a rodent-borne hemorrhagic fever agent, in the list of top emerging diseases likely to cause severe outbreaks in the near future[1]. LASV is an arenavirus responsible for thousands of deaths each year in western Africa, and there is a paucity of medical countermeasures to treat LASV infection. The envelope-displayed LASV glycoprotein spike complex (GPC) drives host cell entry and is a key target for antiviral and vaccine development. Each protomer of the mature trimeric spike consists of a membrane-inserted stable signal peptide (SSP), a host cell receptor-attachment subunit (GP1), and a transmembrane fusion subunit (GP2)[2]. A major consideration for immunogen and antiviral design is the higher-order assembly of this multisubunit complex. Until now, the construction and characterization of an intact GPC ectodomain have been hindered by the metastability of the complex, a property that is important in driving energetically favorable conformational changes within and between noncovalently associated subunits during pH-mediated host cell entry.

In a manner reminiscent of the genetic stabilization and enhancement of proteolytic maturation of the HIV-1 envelope glycoprotein[3], Hastie *et al.*[4] have executed a comprehensive mutagenesis approach to lock the LASV GPC ectodomain in a prefusion conformation, creating a soluble homogeneous sample suitable for structural analysis. This approach includes the introduction of a disulfide bond to covalently link the GP1 and GP2 subunits, a 'helix-breaking mutation' (E329P) in GP2, and the replacement of the native site 1 protease cleavage site between GP1 and GP2 with an RRRR furin site to facilitate efficient processing of the overexpressed protein. This so-called 'GPCysR4' construct is recognized by human-derived neutralizing antibodies specific to the prefusion GP1–GP2 complex. However, GPCysR4 forms only monomers of the GP1–GP2 heterodimer in solution and not the native trimer.

Hastie *et al.*[4] circumvent this conundrum by piecing the trimeric LASV GPC ectodomain together using a multisubunit-targeting Fab fragment from a recently isolated human neutralizing antibody 37.7H[5]. Consistent with a previous low-resolution EM analysis of virion-displayed GPC[6], the ensuing crystallographic investigation confirms that the heavily glycosylated LASV glycoprotein spike has a tripodal organization (**Fig. 1**). Interestingly, the high-resolution analysis by Hastie *et al.* reveals features not observed in the glycoproteins of viruses that also have class 1 fusion machinery, such as Ebola virus, HIV-1, and influenza virus[7–9]. For example, LASV GPC lacks a central three-helix fusion core, and the GP1 subunits contribute extensively to the trimeric interface. Thus, this structure not only clarifies the higher-order assembly of arenaviral glycoproteins but also expands our knowledge of the structural diversity amongst viruses bearing class 1 fusion glycoproteins.

Prior to pH-induced conformational changes of the LASV GPC in endosomal compartments during viral fusion, host cell entry is instigated by an initial attachment interaction with the cell surface receptor α-dystroglycan (α-DG)[10]. Mapping analysis reveals that residues involved in α-DG binding are located near the trimeric interfaces of the GP1 subunit, rationalizing previous observations that recombinantly produced LASV GP1 monomers are incapable of interacting with α-DG[11] and that an intact trimer is likely required for receptor recognition (**Fig. 1**)[12]. This contrasts with the mode of cell attachment by transferrin-receptor-binding arenaviruses from the Americas (for example, Bolivian hemorrhagic fever virus), which

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encode the entire receptor-binding site on a single GP1 monomer[13]. The differential use of the GP1 glycoprotein, as revealed by this investigation, provides an initial structural rationale for the differential host receptor entry pathways used by these geographically and genetically distinct groups of arenaviruses.

LASV also requires an intracellular receptor, the lysosome-associated membrane protein 1 (LAMP1), during endocytic uptake of the virus[12]. Previous crystallographic analysis of monomeric LASV GP1 (ref. 11) revealed a conformational state distinct from that of the GP1 of the genetically related lymphocytic choriomeningitis virus solved in the context of a GP1–GP2 protomer[14], an observation attributed to the isolated LASV GP1 forming a low-pH-induced conformation capable of binding LAMP1 (ref. 11). The LASV GPC ectodomain structure by Hastie *et al.* enables a molecular-level comparison of the prefusion and low-pH-induced states of LASV GP1, confirming that it is not only the GP2 subunit that undergoes extensive rearrangements to achieve host cell entry[4,11,14–16]. Although GP1-specific rearrangements have been suggested for other arenaviruses, particularly those found in the Old World[17], it will be of interest to determine whether such rearrangements are a conserved feature across the entire family.

The neutralizing monoclonal antibody 37.7H was derived from a human survivor and demonstrates promising protective properties *in vivo*[5,18]. The analyses by Hastie *et al.* provide a structural rationale for neutralization[4], revealing that 37.7H stabilizes the GPC by locking the complex in the prefusion conformation, preventing the conformational rearrangements required for both LAMP1 binding and membrane fusion. The 37.7H epitope is located at the membrane-proximal base of the trimer and includes two neighboring GP2s, as well as minor contacts with a single GP1 (**Fig. 1**). Given the importance of quaternary multisubunit epitopes in antibody-mediated neutralization of LASV[5], it becomes clear that future monoclonal antibody isolation and structural characterization will rely heavily on structure-guided stabilization of a trimeric and ligand-free soluble LASV glycoprotein.

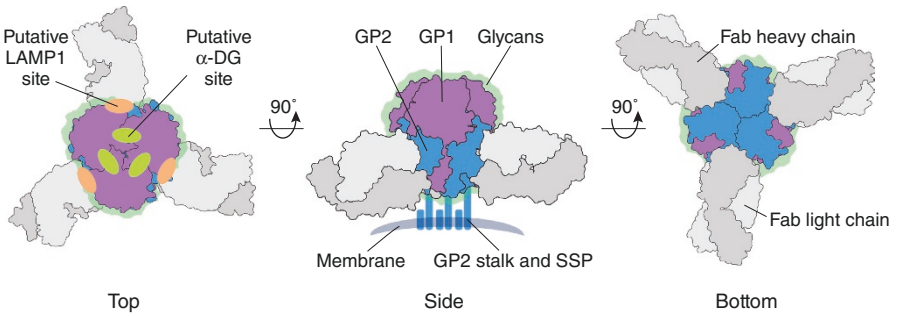


**Figure 1** Organization of the trimeric LASV GPC spike in complex with the Fab fragment of a multisubunit-targeting neutralizing antibody. The protein surface is based upon the recently determined crystal structure by Hastie *et al.*[4] (PDB 5VK2). GP1 and GP2 subunits, purple and blue, respectively; heavy and light chains of the Fab, dark gray and light gray, respectively. N-linked glycans (mint green) surround most of the protein surface. The approximate locations of the putative α-DG- and LAMP1-binding sites are labeled in lime green and orange, respectively. Blue cylinders show the expected location of the stable signal peptide (SSP) and GP2 stalk.

In summary, the work by Hastie *et al.*[4] represents a major milestone in our understanding of the architecture of the LASV surface antigen. Ingeniously, this breakthrough was achieved by gluing together genetically stabilized GP1–GP2 protomers into a native trimeric state with a multisubunit-targeting human neutralizing antibody. This work establishes a foothold in elucidating the structure and function of the LASV spike and provides insights into one of the several mechanisms by which the humoral immune system may target this deadly virus. Importantly, this work also provides a blueprint for the generation of structurally optimized arenaviral immunogens, which can be further used for the isolation of therapeutically promising monoclonal antibodies. While future structural studies will undoubtedly investigate the contribution of the GP2 transmembrane region and SSP to the functionality of the trimeric complex[2,19] as well as the mechanisms by which LASV GPC recognizes host cell receptors, this work pieces together the formerly elusive puzzle of the LASV glycoprotein spike architecture.

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