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Ephrin-B2 and ephrin-B3 as functional henipavirus receptors

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Abstract

Members of the ephrin cell-surface protein family interact with the Eph receptors, the largest family of receptor tyrosine kinases, mediating bi-directional signaling during tumorogenesis and various developmental events. Surprisingly, ephrin-B2 and -B3 were recently identified as entry receptor for henipaviruses, emerging zoonotic paramyxoviruses responsible for repeated outbreaks in humans and animals in Australia, Southeast Asia, India and Bangladesh. Nipah virus (NiV) and Hendra virus (HeV) are the only two identified members in the henipavirus genus. While the initial human infection cases came from contact with infected pigs (NiV) or horses (HeV), in the more recent outbreaks of NiV both food-borne and human-to-human transmission were reported. These characteristics, together with high mortality and morbidity rates and lack of effective anti-viral therapies, make the henipaviruses a potential biological-agent threat. Viral entry is an important target for the development of anti-viral drugs. The entry of henipavirus is initiated by the attachment of the viral G envelope glycoprotein to the host cell receptors ephrin-B2 and/or -B3, followed by activation of the F fusion protein, which triggers fusion between the viral envelop and the host membrane. We review recent progress in the study of henipavirus entry, particularly the identification of ephrins as their entry receptors, and the structural characterization of the ephrin/Henipa-G interactions.

Emergence and outbreak

In the past decade, great attention has been drawn to two closely related, newly emerged paramyxoviruses, Nipah (NiV) and Henda (HeV). These viruses are clearly distinct from all other known genera within paramyxoviridae, both in virulence and molecular characters. Consequently, they were categorized into a new genus, henipavirus [1]. Both of them utilize ephrin-B2 and B3 as entry receptor [2–4].

HeV was named after the suburb in Queensland, Australia, where it was first discovered in 1994. The first outbreak occurred with the death of 13 horses and their trainer. The virus was transmitted from horses to human. There have been fourteen recorded outbreaks of HeV infections until 2010, with five of these events also involving a total of seven human cases, four of which were fatal. For unknown reasons, a sudden increase in the number of spillover events occurred between June and August 2011. The infection caused lethal respiratory

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disease and encephalitis in horses, and severe respiratory disease or late onset encephalitis in human [5–7].

NiV was discovered four years later and named after the village in peninsular Malaysia where the initial major outbreak started. Out of 265 human cases, 105 died during the initial outbreak, and more than 1 million pigs were culled. The principal clinical symptoms in humans were encephalitis with fever, headache, myalgia, drowsiness and disorientation. The outbreak recurred thereafter and spilled over Singapore, India and Bangladesh. There have been twelve recognized occurrences since 2001, the most recent in January 2010. During the initial outbreaks in Malaysia and Singapore, pigs appeared to serve as an amplifying host, with most human cases infected through contact with infected pigs. However, during the more recent outbreaks in India and Bangladesh caused by a new NiV strain (Bangladesh strain), human to human and nosocomial transmission was observed and even considered to be the primary mode of spread. The mortality of humans during the most recent outbreak reached 70% [8–13].

Serological surveillance and viral isolation studies indicate that the natural reservoir host of NiV and HeV are fruit bats belonging to the genus Pteropus, family Pteropodidae [14]. Their emergence as the cause of outbreaks is mainly attributed to deforestation, human intrusion into bat habitats and high-intensity livestock-farming practices [15–16].

Treatment

During a NiV outbreak an antiviral drug, ribavirin was used to treat the encephalitic patients in an open-label trial. Ribavirin has broad-spectrum activity against both RNA and DNA viruses. The ability to cross the blood-brain barrier following oral administration makes it convenient to treat NiV-encephalitis. However, the treatments were generally supportive with only 36% reduction of mortality. Other methods have also been attempted, but none have generated better results [17–18].

As one of the major approaches to antiviral drug design, targeting viral entry has been demonstrated to have therapeutic effect. Therefore, knowledge of the molecular characteristics and the mechanism of henipavirus entry can be the first step towards the development of effective antivirus drugs.

Molecular characteristics of henipavirus

Due to the broad species tropism and high morbidity, as well as high mortality in humans, henipavirus was classified as a BSL-4 (bio-safety level four) pathogen. Experimental growing and handling of the live virus is highly restricted [19]. Thus, molecular and structural biological studies are convenient approaches to characterize henipavirus-host cell interactions.

Henipavirus belongs to the subfamily Paramyxovirinae of the family Paramyxoviridae, order Mononegavirales. Its unusually large genome (>18k nucleotides) encodes six major structural proteins, namely nucleocapsid (N), phosphoprotein (P), matrix (M) protein, fusion (F) protein, glycoprotein (G) and large (L) protein or RNA polymerase. The large genome is partially due to a long untranslated regions at the 3’ end of most transcription units. Similar to respironviruses and morbilliviruses, the henipavirus genome is arranged as 3’-N-P-M-F-G-L-5’. The entry process of henipavirus, which has been studied intensively in the past several years, is mediated by the two surface glycoproteins, F and G. They will be discussed in detail in this review [20–21].

Identification of ephrin-B2 and ephrin-B3 as host-cell receptors

Initial studies indicated that HeV and NiV use cell surface proteins as attachment targets based on the observation that virus infection is sensitive to protease treatment [22]. Great efforts were spent on identifying the functional receptors for henipavirus. The mystery was solved by two groups using distinct approaches:

The first approach can be summarized as immunoprecipitation followed by mass spectrometry analysis [2]. First, a recombinant NiV-G protein was generated by fusing the ectodomain of NiV-G with the Fc region of human IgG1. Immunoprecipitation assay using this Fc tagged NiV-G was able to pull down a 48 kDa surface protein from a fusion-permissive cell line, but not from non-permissive lines. The 48 kDa protein was identified as ephrin-B2 by tandem mass spectrometry. Its ability to mediate NiV infection and NiV-F/G induced fusion confirmed that ephrin-B2 is a functional henipavirus receptor [2].

The second approach was a comparison of NiV permissive and non-permissive cell lines using microarrays [3]. Two closely related human cell lines were identified previously: one (HeLa-USU) was non-permissive for henpavirus fusion and infection, while the other (HeLa-CCL2) was permissive. With the presumption that the difference might be attributed to the surface receptor, a microarray analysis was performed to compare the difference in surface gene expression between the permissive and non-permissive cell lines. The candidate genes were then transfected into the non-permissive HeLa-USU cell line. One of them, ephrin-B2 was able to transform this non-permissive cell line into a permissive one. In addition, soluble, recombinant ephrin-B2 could bind tightly the HenipaV-G protein and block fusion and infection. These results confirm that ephrin-B2 serves as a receptor for HeV and NiV [3].

Ephrin and Eph protein families and signaling

Eph is the largest family of receptor tyrosine kinases (RTK). Ephrins and Ephs can serve both as each other's receptors and ligands, mediating bi-directional signaling pathways that are involved in neuronal growth guidance, vascular formation, cancer development and other processes. Both Ephs and ephrins are highly conserved among homologues and across species. Despite their homology in primary sequence and 3D structure, the different Eph and ephrin family members interact selectively with their counterparts. According to their binding preference, Ephs and ephrins can be divided into 2 classes, A and B. Interestingly, B-class ephrins are transmembrane proteins, while A-class ephrins are attached to the membrane via a GPI anchor [23–24].

Henipavirus tropism and pathogenesis in relation to their ephrin receptors

The tissue tropism of henipavirus is correlated with the expression patterns of ephrin B2 and -B3. Eprin-B2 is expressed in neurons, endothelial cells, smooth muscle surrounding arteries, placental tissue, spleen and sinusoidal lining of lymph nodes. All of these tissues have been reported to be targets of henipavirus infection. Ephrin-B3 is expressed in lymphoid cells, which may account for the NiV-infection induced acute lymphoid necrosis [22, 25–27]. The broad species tropism of henipavirus is largely due to the protein sequence conservation of ephrin-B2 and -B3 across many species. Besides humans and pigs, cats, dogs and horses have also been infected by NiV [28]. Experimental infections extended the number of susceptible species even more [29–31]. As expected, ephrins cloned from several different species support NiV infection with very similar efficiencies in *in vitro* experiments [32].

Entry mechanism

For many enveloped viruses, entry is mediated by a single viral fusion glycoprotein that contains two distinct activities: receptor attachment and membrane fusion. The fusion activity is triggered either by receptor binding or by exposure to an acidic environment following endocytosis [33]. In the paramyxovirus family, the attachment and membrane fusion activities are performed by two separate envelope glycoproteins, with the attachment (G) and fusion (F) proteins working in concert to mediate virus attachment and entry using a yet poorly understood triggering mechanism [34–36].

The henipavirus G glycoproteins have type-II transmembrane topology containing a short N-terminal cytoplasmic tail and a large C-terminal extracellular globular head. Linking them are a transmembrane helical region and an extracellular stalk region. Membrane anchored G-proteins are tetrameric most likely due to disulfide linkage and stalk region bundling [20, 37]. Distinct from most other members within the Paramyxovirinae subfamily, the henipavirus attachment glycoprotein does not hemagglutinate, and neither binds sialic acid, nor retains neuraminidase activity. Instead it binds cell surface protein receptors on the target cells, namely ephrin-B2 and B3 [2–4].

The fusion F protein is a type-I transmembrane glycoprotein, initially synthesized as a precursor F0, which forms homo-trimers, and is later proteolytically processed into disulfide-linked subunits F1 and F2 [22, 38]. The direct association between paramyxovirus attachment glycoproteins and their respective F proteins has been reported, and potential interface regions have been mapped to sites on both the stalk regions and the globular head domains of several paramyxovirus species [39–48].

The least understood step in paramyxovirus entry is the link between virus attachment via the G protein and F protein activation. There are two current models: a “clamp” model where the F protein is activated by removal of an inhibition exerted via clamping of the attachment protein oligomer; and a “provocateur” model where F protein activation relies on a positive input conveyed via the attachment protein. Regardless of the differences, both models emphasize the importance of the interactions between the fusion, F, and attachment, G, proteins. Indeed, it is largely accepted in the viral-fusion field that in cases where attachment and fusion are functionally separated in two distinct proteins, a pre-fusion trimeric F glycoprotein exists in a “metastable” conformation that is stabilized through association with an oligomeric attachment glycoprotein partner. Upon cell-surface receptor binding, the F glycoprotein is somehow activated, presumably involving direct contacts between the attachment and fusion glycoproteins, and inserts its fusion peptide into the host cell membrane. The activation process facilitates a series of conformational changes in the F glycoprotein, which transitions into its post-fusion, six-helix-bundle conformation, concomitant with the merging of the viral membrane envelope and the host cell plasma membrane [34]. As already mentioned, many of the details of receptor binding and fusion initiation in henipaviruses have yet to be defined, and the structural characterization of the henipavirus F and G glycoproteins across the various stages of these processes is essential for understanding this critical step in the virus life-cycle.

Structures of the Henipa-G proteins

Consistent with the 81% identity in primary sequence, the structures of the G glycoproteins of HeV and NiV are very similar. The globular head domains of HeV G and NiV G can be superimposed with 0.5 A r.m.s.deviation in their Cα atoms. As with all other paramyxovirus attachment proteins, HeV G folds into a six-blade (B1–B6) β-propeller (Fig. 1A). Each blade is composed of four or five anti-parallel β-strands. Loops and helices form the connections between the strands and the blades. The N-terminal two strands join the C-

terminal three strands to form the last blade (B6) of the propeller. Surrounded by these six blades is a funnel-shaped central cavity that constitutes the receptor-binding site. The β-propeller architecture is reinforced by a massive hydrogen-bonding network, Van der Waals interactions, and seven disulfide bonds formed either inside or between adjacent blades [49–52]. Such an assembly doesn't allow much conformational change, even during receptor attachment. The rigidity of the G protein structure provides a sturdy base for cell attachment and may be important for the fusion activation mechanism. For example, two mutants (K443A and K465A), although located far away from the receptor-binding site, impair the stability of the β-propeller fold, and hence greatly diminish viral attachment and fusion [53].

Several structural studies reveal that the head domain of NiV-G exists as monomer both in solution and in the crystals, while the head domain of HeV-G exists as monomer in solution but is packed as a dimer in the crystals (Fig. 1B). A very similar homodimeric interface is also observed in other paramyxovirus attachment proteins, but the HeV-G homodimer interface area is the smallest. This may be related to the fact that HeV-G attaches to cell surface proteins and not carbohydrates. In the HeV-G dimer, the axis of the two propellers form an acute angle, which presumably serves to avoid steric clashes upon binding to two receptors simultaneously [52, 54–57]. The recently published structure of a tetrameric NDV (New Castle Disease Virus) attachment protein, including both head domain and stalk region, confirmed the presence of this homodimeric interface in the context of a full attachment protein ectodomain assembly [58]. Moreover, the HeV-G dimer can be superimposed upon the NDV-G dimer with only a small angle adjustment. Therefore, the observed homodimeric interface in HeV-G may very well be physiologically and functionally relevant. The absence of this dimeric interface in the NiV-G crystal may be due to the crystallization condition or to the fact that the interaction is weak in the absence of the disulfide-linkage and the stalk region. Interestingly, in the structures of receptor bound G protein, this homodimeric interface is also absent (Xu et. al manuscript submitted), which might provide insight into the G-induced F protein activation and will be discussed in the following paragraphs.

Structures of henipa-G/ephrin complexes

Ephrin-B2 and ephrin-B3 share about 40% sequence identity and the overall structures of the G/ephrin complexes are very similar. The G protein/receptor interacting interface is composed of a central hydrophobic area and surrounding polar area. The hydrophobic part of the interface is between the ephrin G-H loop and the central cavity of the G protein propeller (Fig. 2A). There are four residues on the tip of the G-H loop (Trp125, Leu124, Pro122 and Tyr/Phe120) inserting into four preformed hydrophobic pockets in the cavity of the G protein. Surrounding the hydrophobic interface core are mostly hydrogen bonds formed by polar and charged residues (Fig. 2B). The binding of the G protein to its receptor buries an area of 1272 $Å^2$ in the complex between HeV-G/ephrin-B2, 1354 $Å^2$ in the complex between NiV-G/ephrin-B3 and 1393 $Å^2$ in the complex between NiV-G/ephrin-B2 [49, 51]. Of the interacting residues, those forming salt-bridges and those in the hydrophobic core contribute mostly to the binding affinity. For instance, Glu533 in NiV-G forms two salt-bridges with Arg57 and Lys116 in ephrin and mutations in Glu533 abrogate viral attachment and fusion [59–60]. Another example is F120 of ephrin-B2, which deeply inserts into one of the pockets in the NiV-G central cavity, and mutations in this residue partially or completely abolish the binding [49]. With the assistance of the currently available high-resolution structural information, specific chemical compounds or peptides could be designed to inhibit the G protein-ephrin interactions, either by occupying the central G-protein cavity or by targeting the surrounding areas.

Conformational changes in ephrin upon viral attachment

The only ephrin region that undergoes major conformational changes upon G-protein binding is the G-H loop (Fig. 3A). Interestingly, compared to Eph binding, the G-H ephrin loop transforms more dramatically upon henipa-G association. For instance, Trp125 at the tip of this loop shifts by more than 8 Å upon viral attachment, while upon binding EphB2 and EphB4 it shifts by less than 4 Å [24, 49, 61–62]. This conformational rearrangement is crucial for the snugly fitting of the four G-H loop hydrophobic residues (Trp, Leu, Pro and Tyr/Phe) into the four preformed pockets in the G-protein. The G-H ephrin loop is at the center of the binding interface not only in the G-protein/ephrin complexes, but also in all Eph/ephrin complexes. The plasticity of this ephrin region is essential for its ability to recognize and bind various structurally distinct interaction partners.

The overall rigidity of the ephrin molecule, on the other hand, provides a sturdy scaffold for efficient viral attachment. The ephrin structures in nine crystallographically different G protein complexes, including six ephrin-B2/HeV-G complexes in two different crystals forms, two ephrin-B3/NiV-G complexes and one ephrin-B2/NiV-G complex, superimpose very well. The G protein-contacting regions, including the C, F and G ephrin strands overlap, while the rest of the molecules differ only by small-angle rotations (Fig. 3B).

Thus, in addition to their wide expression, high expression levels and sequence conservation, the overall architecture of the ephrins provides an extra advantage for their utilization as viral attachment proteins. Indeed, the fact that the ephrins (much like antibody variable domains) contain a flexible loop region embedded in a rigid protein frame, allows for high affinity virus attachment and a very stable and sturdy attachment-protein/cellular-receptor complex serving as foundation for the subsequent membrane fusion events.

An interesting question is why, in light of the high sequence conservation within the ephrin family, only ephrin-B2 and B3 are used as henipavirus entry receptors? The structures of the G protein/receptor complexes provide the explanation. The A-class ephrins have a G-H loop that is quite different both in structure and in sequence from that of the B-class ephrins, and none of the A-class ephrin loops can fit into the rigid G-protein cavity. In case of the third B-class ephrin, ephrin-B1, the large side chains of the ephrin-B1 specific residues Tyr124 and M125 would cause steric collision when placed into the G protein cavity and therefore prevent it from serving as a henipavirus attachment protein. Indeed, replacing these two residues with Leu and Trp allows ephrin-B1 to support henipavirus entry [4].

Comparing ephrin binding of G proteins vs. Ephs and biological implications

Strikingly, the Ephs and the henipavirus G proteins bind the same regions of ephrins, including the G-H loop and strands C, F and G. There are slight differences however in the exact interacting ephrin residues as shown on Fig. 4. It has been reported that soluble ephrin-B2 has an inhibitory effect on henipavirus attachment and infection, presumably by competing for G-protein binding with the cell-attached ephrins [3]. Thus it is conceivable that, for therapeutic purpose, soluble ephrin-based proteins might be engineered that specifically inhibit henipavirus entry, without disturbing the normal ephrin-Eph signaling events. Indeed preliminary unpublished results in the authors' laboratory suggest that mutations can be designed in ephrin-B2 that significantly decreases its affinity to EphB4, while retaining its affinity to the henipavirus G proteins.

Interestingly, compared to the Ephs, the henipavirus G-proteins have well-structured binding pockets even in the unbound state. In addition, the G protein/ephrin complexes have larger

interface areas than the Eph/ephrin complexes, all of which is consistent with the generally higher binding affinities observed between G proteins and ephrins that might be important for efficient viral entry. Indeed, unlike the tightly regulated (and often competing because of multiple Eph/ephrin expressed within the same cell) interactions between Ephs and ephrins, that might be important for fine-tuning the various downstream signaling events, viral fusion likely depends on tight and irreversible G-protein attachment. The high G-protein/ephrin affinity also allows for the henipavirues to effectively compete with endogenous Ephs for ephrin binding.

Ephrin reverse signaling and henipavirus infection

Endogenous Eph-ephrin signaling is triggered by clustering of both Eph and ephrin molecules upon cell-cell contact. Since the henipavirus G proteins interact with ephrins in a similar way as Ephs, and since the G proteins exists as tetramers (possibly even as higher-order oligomers) on the viral envelope, it is likely that viral attachment results in ephrin oligomerization and/or clustering. Thus the question arises whether henipavirus attachment can induce ephrin reverse signaling, and what a role such ephrin reverse signaling could play during henipavirus infection. It was shown that a truncated ephrin, lacking a cytoplasmid region, can still function as entry receptor, suggesting that ephrin reverse signaling is not required for henipavirus infection [63]. Nevertheless, ephrin clustering might still play a role in viral entry, for example by contributing to the pathogenesis of henipavirus infection. Indeed, it has been suggested that NiV infection alters ephrin expression patterns in the CNS and affects the endogenous Eph-ephrin signaling, resulting in neuropsychiatric or neuropathologic sequelae during NiV infection [64].

Conformational changes in the G glycoprotein and implications for the entry mechanism

The local conformational changes within a single G protein head domain upon receptor association are minor and mostly localized to the ephrin-contacting loops. The only exception is a strand movement within blade 6 of the G protein propeller, resulting from the remodeling and enlargement of the Phe120 (Tyr120 in ephrin-B3) binding pocket [65]. In the HeV-G/ephrin-B2 structure this remodeling effects subtle rearrangements that could be traced to a small but significant conformational change in the previously discussed HeV-G homodimeric interface. Whether this subtle change is sufficient to disrupt the G-protein homodimer upon ephrin binding or, in another word, whether the absence of a homodimeric interface in all known G protein/ephrin complexes can be attributed to this subtle structural change, requires further investigation.

Significant global rearrangements, on the other hand, including relocation between the head and stalk G-protein domains, as well as rearrangement of the tetrameric G-protein architecture, could (and probably do) also occur upon ephrin attachment, but the current structural studies involve only head-domain fragments and, therefore, have not been able to identify them. Theoretically, both local conformational changes and global rearrangement could trigger F protein activation depending on the detail of the association between the G the F proteins. Thus, future structural studies should focus on G-protein constructs containing both the head and stalk regions and their interactions with the F-glycoprotein.

Structure of a neutralizing monoclonal antibody/HeV-G complex

A series of cross-reacting (against both HeV and NiV) human monoclonal neutralizing antibodies were recently developed using the G protein as an antigen to screen a Human B cell naïve antibody library [66]. One of these antibodies and its derivatives were found to be

very potent in henipavirus neutralizing assays [67]. Experimental treatment during a HeV outbreak also showed promising results [68]. The structure of the Fab fragment of this antibody complexed with the HeV-G protein (to be published) revealed the mechanism of the antibody's neutralizing effect. A striking similarity was discovered upon comparison of the binding between the Fab and G with that between ephrin and G. The high-affinity Fab/HeV-G interface also contains two regions (Fig. 5): one involving the hydrophobic loop on the tip of Fab's CDR (complimentary determining region)-H3, which inserts in the same HeV-G hydrophobic cavity as ephrin; and one involving CDR-H2 and L1, which form polar contacts, including multiple hydrogen bonds, with the rim of HeV-G cavity. The Fab-binding area on the HeV-G surface mostly overlaps with the ephrin-binding area, which is consistent with the ability of the neutralizing antibody to compete with ephrin for G protein binding.

Notably in the Fab/HeV-G complex, three of the four pockets of the HeV-G ephrin-binding cavity, those for ephrin residues Phe/Tyr120, Pro122 and Leu124, are occupied by similar residues of the CDR-H3 loop (Leu, Pro, Pro). Moreover, the three-dimensional shape and insertion angle of the antibody CDRH3 loop is similar to that of the ephrin G-H loop. The difference is that, the CDR-H3 forms a β-hairpin secondary structure while the G-H loop does not. In addition, the surrounding hydrophobic antibody residues interact with the aromatic residues on the stem of the CDR-H3 β-hairpin providing rigidity for its shape, unlike the ephrins where the G-H loop is much more flexible. The residues at the tip of the β-hairpin adapt a conformation that completely fills the existing HeV-G cavity without expanding it. Thus, the potency of this antibody is closely related to its affinity for HeV-G, which is the result of its molecular characteristics: it is relatively rigid, complimentary to the HeV-G protein target in both shape and chemical properties, and does not cause any conformational changes in the G glycoprotein upon binding. The identification of this antibody further confirms the feasibility of inhibiting henipavirus infection by inhibiting viral attachment. Using antibodies to compete with ephrin for G protein binding provides new avenues for anti-virus treatment. Structure-based optimization of the target recognition residues within the antibody can potentially increase the effectiveness of the neutralization.

Conclusion

Henipaviruses, which cause (so far) incurable infectious disease, have put a constant threat to human life and health in those regions where outbreaks occur frequently. A recent film "Contagion" even imagined a disastrous situation of this virus spreading to US territories. Understanding the mechanism of the infection and developing a cure in timely manner can prevent world-wide spread and help the people in the epidemic areas. The identification and study of the ephrins as viral entry receptors has significantly advanced our knowledge of these viruses. The recently determined high-resolution structures of the henipavirus G proteins, and their complexes with ephrins and neutralizing antibodies, provide great insight into the mechanism of henipavirus entry, as well as a molecular template for antivirus drug development. Future study should focus on computationally assisted drug design and screenings using currently available structural data, as well as on understanding the mechanism of activation of membrane fusion and the interplay between the viral G and F glycoproteins.

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Highlights

We review the usage of ephrin-B2 and ephrin-B3 as entry receptors by henipaviruses.

We review recent structures of henipavirus attachment glycoprotein/ephrin complexes.

We examine how ephrin binding by the G glycoproteins might trigger membrane fusion.

We examine potential antiviral strategies targeting henipavirus cell entry.

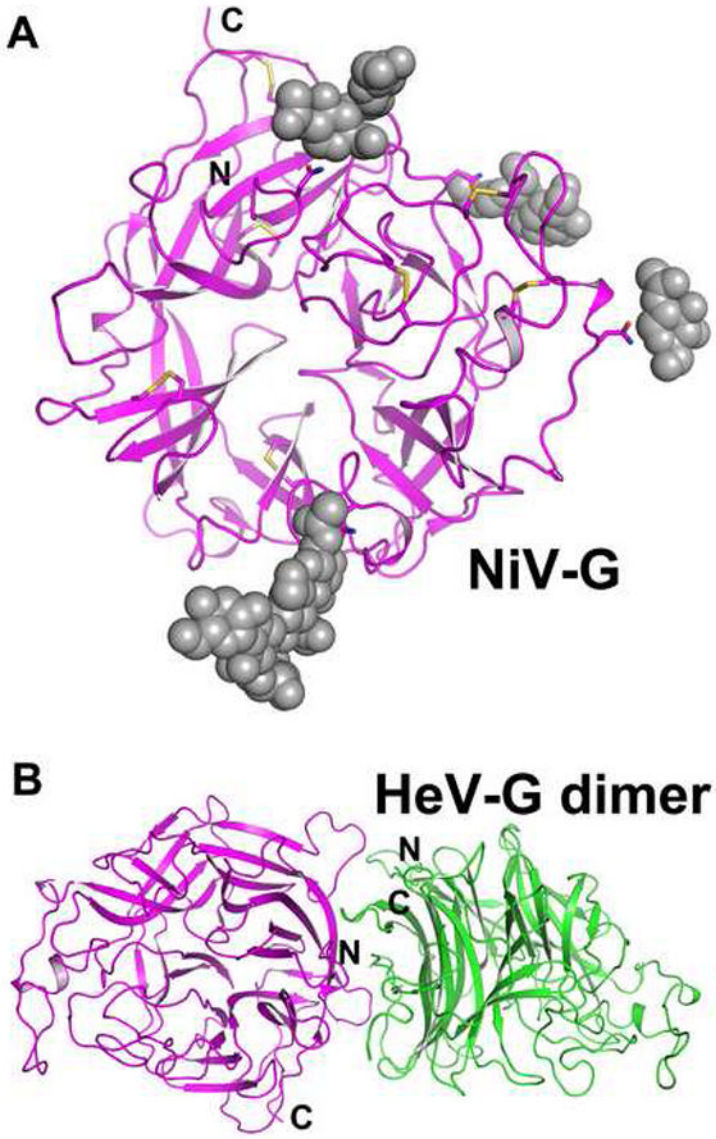

Figure 1. Structures of Henipavirus G glycoprotein head domains

A. The structure of the Nipah virus G glycoprotein monomer. Disulfide bonds are presented as yellow sticks. Glycosylation residues are illustrated as grey spheres.

B. The structure of the Hendra virus G glycoprotein dimer

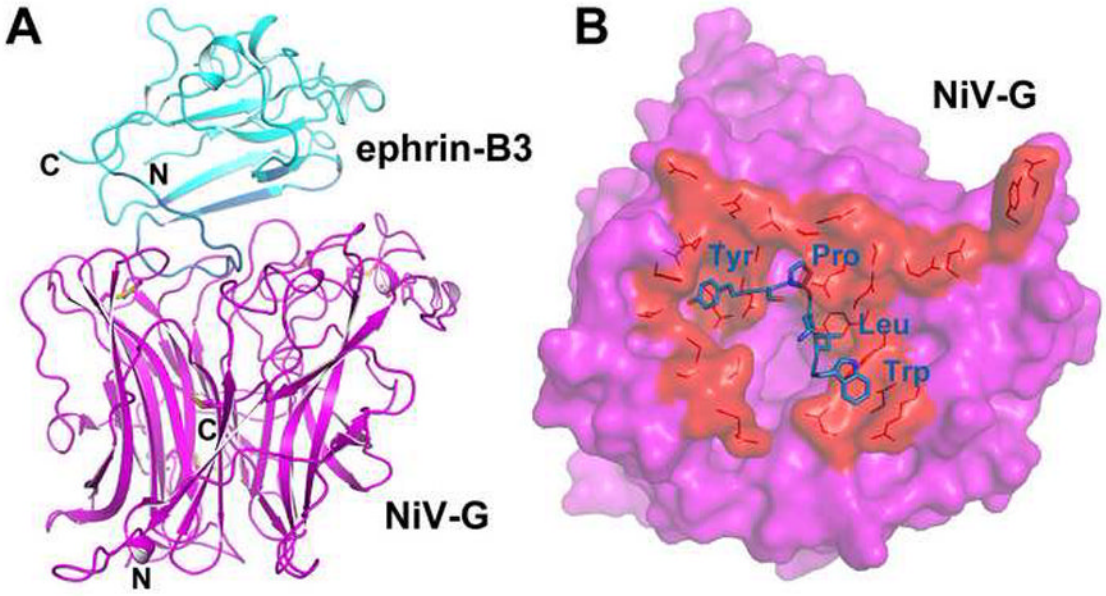

Figure 2. The structure of the Nipah G glycoprotein bound to ephrin-B3

A. Schematic representation of the NiV-G/ephrin-B3 complex structure. Ephrin-B3 is in cyan, NiV-G is in magenta. The G protein-contacting regions of ephrin-B3 are in blue. The N and C termini are indicated.

B. View of the surface of the Nipah G glycoprotein from the point of view of the incoming ephrin. The ephrin-B3 G-H loop is in blue, and the Nipah G protein surface, which contacts ephrin, is in red.

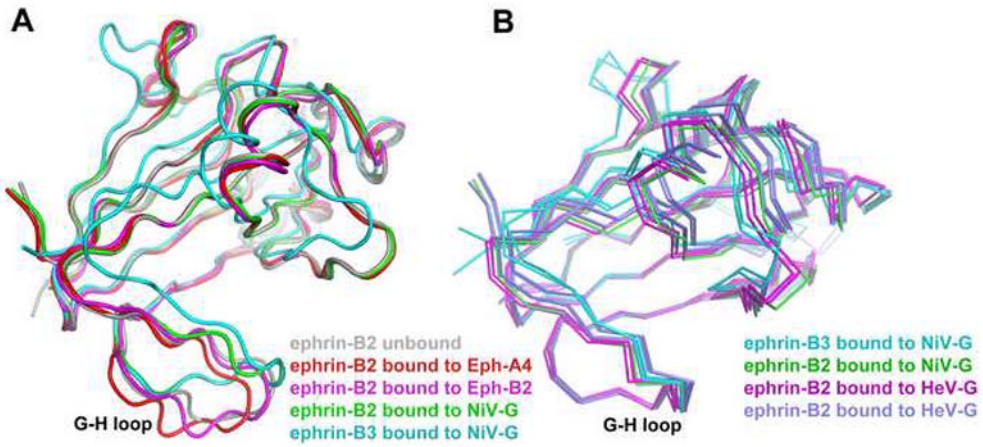

Figure 3. Different ephrin conformations observed in the complexes of ephrins with different binding partners

The different ephrin structures are color coded as indicated in the figure

A. The structural superimposition was performed using only the ephrin structures

B. The structural superimposition was performed using only the viral G glycoprotein structures

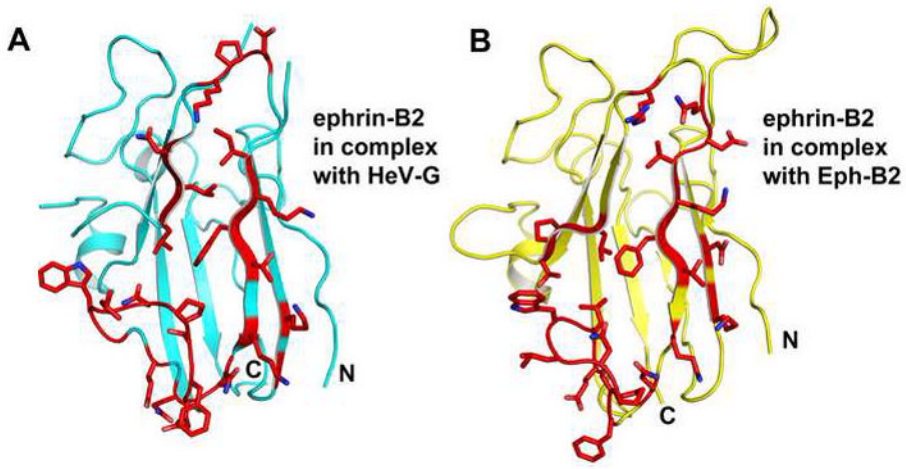

Figure 4. Comparison of the ephrin-B2 residues, which interact with the Hendra G protein and with EphB2

A. Structure of ephrin-B2 in its complex with the Hendra virus G glycoprotein. Ephrin-B2 is in cyan and the G protein-contacting residues are in red.

B. Structure of ephrin-B2 in its complex with EphB2. Ephrin-B2 is in the same orientation as in panel A. Ephrin-B2 is in yellow and the EphB2 contacting residues are in red.

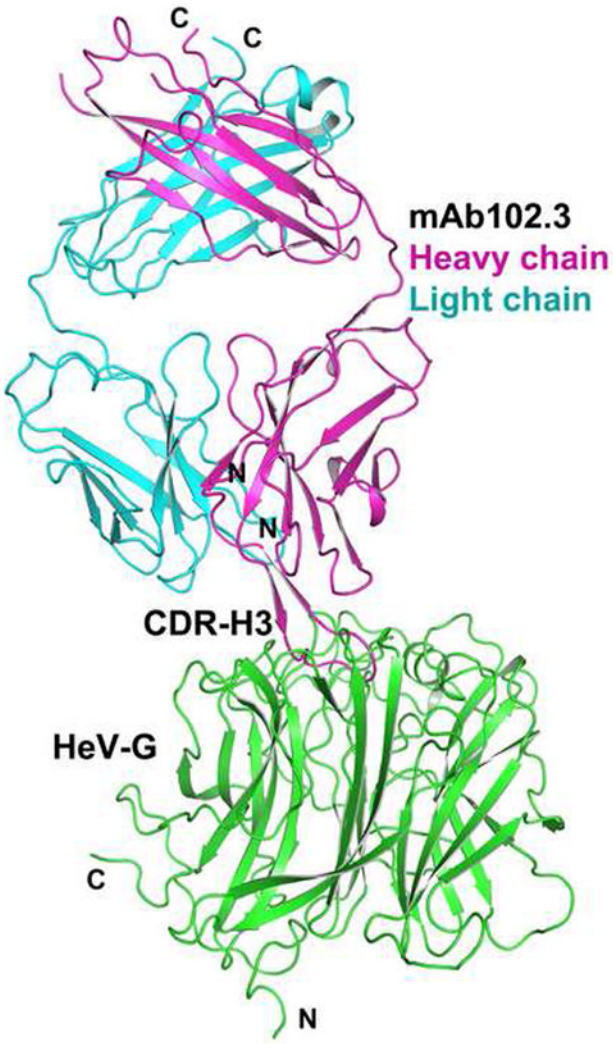

Figure 5.
Crystal structure of the Fab fragment of a neutralizing monoclonal antibody bound to the Hendra virus G glycoprotein