Characterizing alpha helical properties of Ebola viral proteins as potential targets for inhibition of alpha-helix mediated protein-protein interactions [version 3; referees: 2 approved]

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Abstract

Ebola, considered till recently as a rare and endemic disease, has dramatically transformed into a potentially global humanitarian crisis. The genome of Ebola, a member of the Filoviridae family, encodes seven proteins. Based on the recently implemented software (PAGAL) for analyzing the hydrophobicity and amphipathicity properties of alpha helices (AH) in proteins, we characterize the helices in the Ebola proteome. We demonstrate that AHs with characteristically unique features are involved in critical interactions with the host proteins. For example, the Ebola virus membrane fusion subunit, GP2, from the envelope glycoprotein ectodomain has an AH with a large hydrophobic moment. The neutralizing antibody (KZ52) derived from a human survivor of the 1995 Kikwit outbreak recognizes a protein epitope on this AH, emphasizing the critical nature of this secondary structure in the virulence of the Ebola virus. Our method ensures a comprehensive list of such `hotspots'. These helices probably are or can be the target of molecules designed to inhibit AH mediated protein-protein interactions. Further, by comparing the AHs in proteins of the related Marburg viruses, we are able to elicit subtle changes in the proteins that might render them ineffective to previously successful drugs. Such differences are difficult to identify by a simple sequence or structural alignment. Thus, analyzing AHs in the small Ebola proteome can aid rational design aimed at countering the `largest Ebola epidemic, affecting multiple countries in West Africa' (http://www.cdc.gov/vhf/ebola/outbreaks/2014-west-africa/index.html).

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**Introduction**

The Ebola virus was first discovered in 1976, and has been since known as a rare, but deadly disease. However, the current outbreak in West African countries (Guinea, Liberia, Nigeria, Sierra Leone and Senegal) has rapidly deteriorated into a full blown epidemic, and poses grave humanitarian dangers to these countries. Ebola, along with the Marburg virus, belongs to the *Filo*viridae family, and causes haemorrhagic fever by quickly suppressing innate antiviral immune responses to facilitate uncontrolled viral replication.

Interestingly, the genome of the Ebola virus encodes seven proteins, although their extreme ‘plasticity allows multiple functions’. Protein structures are formed by well ordered local segments, of which the most prevalent are alpha helices (AH) and β sheets. AHs are right-handed spiral conformations which have a hydrogen bond between the carbonyl oxygen (C=O) of each residue and the alpha-amino nitrogen (N-H) of the fourth residue away from the N-terminal. AH domains are often the target of peptides designed to inhibit viral infections. Recently, we have provided open access to software that has reproduced previously described computational methods to compute the hydrophobic moment of AHs (PAGAL).

In the current work, we characterize the helices in the Ebola proteome using PAGAL, and demonstrate that the helices with characteristically unique features are involved in critical interactions with the host proteins. The PDB database is queried for the keyword ‘Ebola’, and the structures obtained are analyzed using DSSP for identifying AHs. We process all PDB structures, and do not filter out redundant structures based on sequence. These helices are analyzed using PAGAL, and the results are sorted based on three criteria - hydrophobic moment and high proportion of positive or negative residues. The helices that are ranked highest in these sorting criteria are involved in critical interactions with either antibodies or host proteins. For example, the Ebola virus membrane fusion subunit, GP2, from the envelope glycoprotein ectodomain has an AH with the largest hydrophobic moment in all helices analyzed. This helix has part of the epitope recognized by the neutralizing antibody (KZ52) derived from a human survivor of the 1995 Kikwit outbreak, emphasizing the critical nature of this helix in the virulence of Ebola. Another example, obtained by choosing the helix with the highest proportion of negatively charged residues, is the interaction between the human karyopherin alpha nuclear transporters C terminus and the Ebola virus VP24 protein (eVP24), which suppresses tyrosine-phosphorylated STAT1 nuclear import.

These helices probably are, or can be, the target of molecules designed to inhibit AH mediated protein-protein interactions. Our method provides a comprehensive list of such targets. Further, each protein can be individually queried using PAGAL, and thus identify helices that might have a poor global rank, but still be critical in the particular proteins context.

Although, Ebola and Marburg viruses are members of the *Filo*viridae family, they have different antigenicity of the virion glycoprotein. By comparing the AHs in proteins of Marburg and Ebola viruses, we are able to elicit subtle changes in the proteins that might render them ineffective against previously successful drugs. These differences are not apparent from a simple sequence or structural alignment. Thus, in the current work, we elucidate a simple methodology that can aid rational design of drugs and vaccine, an important aspect of the global effort to counter the deadly Ebola epidemic.

**Materials and methods**

We searched for the keyword ‘Ebola’ in the PDB database (Table 1). Subsequently, each protein was split based on the chain id, resulting in 146 single chained proteins (See ALPHA.zip in Dataset 1). We have not reduced the set based on sequence similarity since the proteins might have different conformations based on their ligands. Note, this list might include non-Ebola proteins which might have been co-crystallized with the Ebola protein. However, they have been put through the same analysis since they might provide insights into the Ebola proteins themselves.

These proteins were then analyzed using DSSP, and resulted in 758 helices in all (See ALPHA.zip in Dataset 1). These helices were then analyzed using PAGAL. The PAGAL algorithm has been detailed previously. Briefly, the Edmundson wheel is computed by considering a wheel with centre (0,0), radius 5, first residue coordinate (0,5) and advancing each subsequent residue by 100 degrees on the circle, as 3.6 turns of the helix makes one full circle. We compute the hydrophobic moment by connecting the center to the coordinate of the residue and give it a magnitude obtained from the hydrophobic scale (in our case, this scale is obtained from ). These vectors are then added to obtain the final hydrophobic moment.

The color coding is as follows: all hydrophobic residues are colored red, while hydrophilic residues are colored in blue: dark blue for acidic residues and light blue for amides.

The raw file generated by analyzing all 146 proteins through PAGAL is provided as PAGAL_RAWDATA.txt (Dataset 1), and contains the hydrophobic moment, percent of positive charges and

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**Table 1. PDB ID of Ebola proteins analyzed.**

<table>
<thead>
<tr>
<th>PDB ID</th>
<th>Description</th>
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<tr>
<td>1EBO, 2EBO, 3VE0, 3CSY..</td>
<td>Ebola virus envelope protein</td>
</tr>
<tr>
<td>218B, 3V7O</td>
<td>Minor nucleoprotein VP30</td>
</tr>
<tr>
<td>3FKE, 3L25, 4LG2, 4IBK..</td>
<td>Polymerase cofactor VP35</td>
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<tr>
<td>3VNE, 4D9O, 4M0Q, 4U2X..</td>
<td>Membrane-associated protein VP24</td>
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<td>4OAZ, 4OAZ</td>
<td>Nucleoprotein</td>
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<tr>
<td>1ES6, 1H2D, 3TCQ, 4LDM..</td>
<td>Matrix protein VP40</td>
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</table>
the total number of charged residues for every helix. These are then sorted based on the charge (negative or positive) or the hydrophobic moment. We ignore the helices that have none or a single charged residue, and those that are smaller than 10 residues in length. The proportion of charged residues is computed based on the total number of charged residues, and not the length of the helix.

All protein structures were rendered by PyMol (http://www.pymol.org/). The sequence alignment was done using ClustalW. The alignment images were generated using Seaview. Protein structures have been superimposed using MUSTANG.

Results and discussion

Dataset 1. PAGAL analysis of Ebola-related alpha helices

http://dx.doi.org/10.5256/f1000research.5573.d37453

A PDB database search using the keyword 'Ebola' generated 146 single chained proteins, which were analyzed using Define Secondary Structure of Proteins, resulting in 758 alpha helices (ALPHA.zip). Note, this list might include non-Ebola proteins which might have been co-crystallized with the Ebola protein. These helices were analyzed using PAGAL (PAGALRAWDATA.txt), which details the hydrophobic moment, percent of positive charges and the total number of charged residues for every helix.

Helices with large hydrophobic moment

We began by analyzing the helices which have a large hydrophobic moment (hydrophobic scale is obtained from13) (Table 2). The Edmundson wheel for the helix 1EBOE.HELI0X1 from the structure of GP2 from the Ebola virus membrane fusion glycoprotein (PDBid:1EBO)16 is shown in Figure 1a. Figure 1b shows the residues comprising these helices (in magenta) in the apo form (PDBid:1EBO)16. The neutralizing antibody (KZ52) derived from a human survivor of the 1995 Kikwit outbreak (PDBid:3CSY)17 recognizes an epitope on this AH, emphasizing the critical nature of this AH in the virulence of the Ebola virus (Figure 1c.d). The antibody most likely inhibits the rearrangement of GP2 segments, which abrogates the fusion of the internal loop in the host membrane17. Table 3 shows the residues in the specified helix (residues 553-597, chain J, PDBid:3CSY) making possible hydrogen bonds.

Table 2. Identifying helices with unique properties. Property based on which the sorting is done is either the Hydrophobic moment (HM) and the percentage of negative (NEG) or positive residues (POS). HM: Hydrophobic moment, RPNR: Ratio of the positive to the negative residues, Len: length of the helix, NCH: number of charged residues. GP: glycoprotein from Ebola, VP24: Membrane-associated protein from Ebola, VP35: Polymerase cofactor.

<table>
<thead>
<tr>
<th>Property</th>
<th>Protein</th>
<th>Helix</th>
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<th>HM</th>
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<th>NCH</th>
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Figure 1. Helix with large hydrophobic moment in GP2 from the Ebola virus membrane fusion glycoprotein. (a) Edmundson wheel for 1EBOE.HELI0X1. The hydrophobic moment vector is not to scale. The color coding is as follows: all hydrophobic residues are colored red, while hydrophilic residues are colored in blue: dark blue for positively charged residues and light blue for amides. (b) Structure of PDBid:1EBOE. 1EBOE.HELI0X1 is marked in magenta and the leucine zipper is in blue. (c) 1EBOE.HELI0X1 is disrupted by an antibody derived from a human survivor of the 1995 Kikwit outbreak (PDBid:3CSY). (d) Gly553/N on 1EBOE.HELI0X1 makes a possible hydrogen bond to Thr100/OG1 at a distance of 2.7 Å.
with different residues in the human Fab KZ52 heavy chain (resi-
dues 1-228, chain A, PDB:3CSY). Among all the interactions,
only Gly553 is on 1EBOE.HELIX1 (at a distance of 2.7 Å from
Thr100/OG1), although the others are sequentially proximal. These
few interactions are sufficient to bind to this helix, rendering the
virus non-virulent, and leading to human recovery. The importance
of interfacial hydrophobicity in viral proteins involved in host entry
through membrane fusion has recently been discussed in detail, and
remains ‘an underutilized therapeutic target’\textsuperscript{26}. 1EBOE.HELIX0
(Table 2) also has a high hydrophobic moment, but is actually an
isoleucine zipper derived from GCN4\textsuperscript{27} (Figure 1b).

**Helices with high proportion of negatively charged residues.**

Identifying difference among related species

We then analyzed the helices having a high proportion of nega-
tively charged residues, sorted based on the length of the helix
when the percentage of negatively residues are the same (Table 2).
Figure 2a shows the Edmundson wheel for the helix 4U2XA.
HELIX5 (which has only two charged residues - the basic E113 and
D124), while Figure 2b,c shows this helix in the protein complex
marked in magenta. Note, that we exclude AHs with either zero
or one charged residues (see Methods). Protein PDBid:4U2XD is
the human karyopherin alpha nuclear transporter (KPNA) C ter-
minus in complex with the Ebola virus VP24 protein (eVP24)\textsuperscript{18}.
eVP24 interferes with the immune response by selectively targeting
the interactions obtained from the crystal
structure of the Ebola virus glycoprotein in complex
with a neutralizing antibody from a human survivor.
The helix with a large hydrophobic moment, as
determined from PDBid:1EBOE, is disrupted in the
structure from PDBid:3CSY through possible hydrogen
bonds with different residues in the human Fab KZ52
heavy chain (antibody, chain A). The helix residues are:
553-597 in chain J, PDB:3CSY.

**Table 3. Interactions obtained from the crystal structure of the Ebola virus glycoprotein in complex with a neutralizing antibody from a human survivor.**
The helix with a large hydrophobic moment, as
determined from PDBid:1EBOE, is disrupted in the
structure from PDBid:3CSY through possible hydrogen
bonds with different residues in the human Fab KZ52
heavy chain (antibody, chain A). The helix residues are:
553-597 in chain J, PDB:3CSY.

<table>
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<th>AtomEbola</th>
<th>AtomAntibody</th>
<th>Dist (Å)</th>
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<tr>
<td>ASP/552/OD1</td>
<td>SER/53/OG</td>
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</tr>
<tr>
<td>GLY/553/N</td>
<td>THR/100/OG1</td>
<td>2.7</td>
</tr>
<tr>
<td>ASN/550/O</td>
<td>ASN/31/O</td>
<td>2.9</td>
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<td>ASP/552/OD1</td>
<td>SER/53/CB</td>
<td>2.9</td>
</tr>
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<td>ASN/550/ND2</td>
<td>PRO/97/O</td>
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<tr>
<td>ASN/550/ND2</td>
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<tr>
<td>ASP/552/OD2</td>
<td>SER/53/OG</td>
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</table>

**Figure 2. Helix 4U2XA.HELIX5 from membrane-associated protein VP24 with a high proportion of acidic residues.**

(a) Edmundson wheel for 4U2XA.HELIX5. (b) Complex of VP24 (PDB:4U2XA) and human karyopherin alpha nuclear transporters (KPNA) C terminus (PDB:4U2XD). D124 from VP24 probably has an electrostatic interaction with K481 from KPNA. This interaction is sufficient to interfere with the immune response to Ebola infection.
tyrosine-phosphorylated STAT1 nuclear import. It does not hinder the transport of other cargo that may be required for viral replication. 4U2XA.HELIX5 is responsible for forming the complex with the KPNA protein through a helix (4U2XD.HELIX9, in blue), and K481 from KPNA is in contact with D124 from eVP24 (distance between K481/NZ and D124/OD2 is 3.98 Å). Their interaction is probably electrostatic, since the atoms have opposite charges. VP24 has also been shown to directly bind to STAT1, further compromising the immune response. Recently, KPNA was docked to Reston Ebola VP24 (PDBid:4D9OA) using the VP24 from Zaire Ebola (PDBid:4U2XA) as a template. The docked structure showed that a single mutation might be one of the critical factors responsible for the non-pathogenic nature of Reston Ebola in humans. Also, it was shown that the VP24 from Marburg virus (PDBid:3VNEA), which has a different immunosuppressive mechanism than the Ebola virus, has different properties in the helices responsible for binding KPNA in the Zaire Ebola VP24.

The next helix having a high proportion of negatively charged residues (3FKEA.HELIX2) is from a VP35, a classic example of a moonlighting protein, that can be a component of the viral RNA polymerase complex, a viral assembly factor, or inhibitor of host interferon production. This helix is part of the dsRNA-binding domain of VP35 that is involved in the formation of the asymmetric VP35 RBD dimeric interface in Reston Ebola virus through a hydrogen-bonding network of residues and a solvent molecule. Interestingly, this helix is homologous (100.0% similar and 78% identity in 9 amino acid overlap) to helix ‘1A’ of an ATP-dependent transcriptional activator. This helix interacts with another ‘1B’ helix from a different monomer in an anti-parallel fashion to facilitate dimerization.

VP35 consists of several helices, and is reasonably conserved in the Marburg virus from the same Filoviridae family (42% identity, 58% similarity) (Figure 3a). Often, it is difficult to identify the regions of the protein that differ from a sequence or structural

![Figure 3. Polymerase cofactor VP35 (PDBid:3FKE). VP35 has several moonlighting functions related to immune evasion. (a) Sequence alignment of VP35 from Marburg (PDBid:4GHLA) and Ebola (PDBid:3FKEA). (b) Structural alignment using MUSTANG. The helices that have differing properties are marked in yellow. 3FKEA.HELIX1 spanning residues 238-252 is marked in magenta. This is a helix with a high proportion of positively charged residues that have been observed to have important interactions in the structure. (c) Edmundson wheel for 3FKEA.HELIX1. (d) 1D5 (in blue) in complex with VP35 (PDBid:4IBFA).](image-url)
alignment (Figure 3b), in case there is interest in understanding different responses of the proteins to known drugs or even the immune system. Table 4 compares the characteristics of the helices in the VP35 from Ebola and Marburg (the helix numbering is offset by one, due to a small N-terminal helix in the Marburg protein (which might be due to crystallization technique differences and probably is not critical). Thus, we have numbered these helices using alphabets. It can be seen that most of the helices have the same properties, barring helices E and F, where the acidic residue is present in the E helix in Marburg and in the F helix in Ebola. These helices are marked in yellow in Figure 3b. Also, it can be seen that helix C, which has a high proportion of acidic residues in VP35, has a fewer number of those residues in Marburg. The difference in the pathogenicity of these viruses are encoded in the structure of the expressed proteins, and the design of drugs and vaccines to counter virulence should take these differences into account.

Helices with high proportion of positively charged residues

4U2XA.HELIX7 from VP24 is a helix having a high proportion of positively charged residues (Table 2), and contains two (L147P and R154L) of three mutations (L147P, M71I and R154L) that sensitizes guinea pigs to the Zaire Ebola virus\(^6\). This helix is marked in yellow in Figure 2c. The second helix (3FKEA.HELIX1) is from VP35, which was discussed previously\(^13\). This helix spans residues 238–252 and includes Lys248 and Lys251, a basic patch which is VP35, which was discussed previously\(^13\). This helix spans residues 238–252 and includes Lys248 and Lys251, a basic patch which is

Table 4. Detecting differences in related proteins based on characteristics of alpha helices. Comparing the VP35 protein from Marburg (PDBid:4GHLA) and Ebola (PDBid:3FKEA). Note the helices are offset by one, due the presence of an extra helix in the Marburg VP35. Thus, we name the helices using alphabets. It can be seen that most helices have the same properties, barring helices E and F, where the acidic residue is present in the E helix in Marburg and in the F helix in Ebola. HM: Hydrophobic moment, RPNR: Ratio of the positive to the negative residues, Len: length of the helix, NCH: number of charged residues.

<table>
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<tr>
<th>Helix Name</th>
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<th>RPNR</th>
<th>NCH</th>
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Gln739 are partly disordered so that some or all of their atoms are not visible in the electron density\textsuperscript{41}. Glu645, His646, Glu649 are part of this helix, and are thus critical to the disorderedness of the protein, which is critical for its moonlighting roles. Note, that Glu has been observed to be the second most disorder promoting residue (after proline)\textsuperscript{42}. Furthermore, Tyr652 and Leu656, which lie in this helix, are residues that have been hypothesized to be part of the protein-protein interaction site involving this protein\textsuperscript{41}.

**Conclusions**

The ability of a genome as small as the Ebola virus to inflict a dishearteningly high percentage of mortality in human subjects is a humbling experience in the context of the tremendous technological advancements achieved in the last few decades\textsuperscript{3,4}. The Ebola virus potently suppresses the human immune response\textsuperscript{2,6} by binding with key human proteins involved in the immune pathway\textsuperscript{18}. These protein-protein interactions are often mediated through well structured secondary regions within the protein structures (alpha helices), and the design of molecules that inhibit these “hotspots”\textsuperscript{20,44} has been a well known strategy to develop drugs to counter bacterial and viral infections\textsuperscript{45,46}. For example, synthetic peptides derived from the oligomerization domain of polymerase subunits has been shown to inhibit viral proteins\textsuperscript{45,46}. In addition, there might exist other protein domains that might be exploited by non-native viral peptides to obstruct viral functionality. In the current work, we characterize alpha helices in the Ebola virus proteome using a recently
implemented open access software (PAGAL)\(^2\), thus identifying potential targets for inhibition of the helix mediated interactions. Through several examples, we demonstrate that helices with unique features are involved in interactions with host proteins (either antibodies from survivors, or proteins regulating the immune response). Further, we also provide an alternate way of analyzing differences in related proteins (from the Marburg virus) by focusing on the properties of corresponding helices. As future work, we intend to develop methodologies to design peptides that would target these ‘hotspots’\(^4\). It has to be kept in mind that it has been a challenge to design small ligands that disrupt protein-protein interactions, and designers resort to several innovative techniques to overcome thermodynamic instability or proteolytic susceptibility\(^5\). These helices can essentially be epitopes\(^5\) for developing antibodies against the virus\(^5\). Interestingly, ZMap, a cocktail of three antibodies has shown reversion of advanced Ebola symptoms in non-human primates\(^5\), and uses only glycoprotein-specific epitope generated antibodies\(^5\). It is interesting to hypothesize that additions to this cocktail with antibodies derived from other epitopes (for example, 4U2XA.HELIX5 from VP24 that is involved in immune response suppression) could prove more effective. Thus, we provide a comprehensive list of potential targets within the small proteome of the Ebola virus that can directed rational design to quickly innovate therapies.

Author contributions
SC wrote the computer programs. All authors analyzed the data, and contributed equally to the writing and subsequent refinement of the manuscript.

Competing interests
No competing interests were disclosed.

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References
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Current Referee Status: ✅ ✅

Version 3

Referee Report 10 February 2015

https://doi.org/10.5256/f1000research.6510.r7576

Sean Ekins
Collaborative Drug Discovery, Burlingame, CA, USA

This publication is scientifically sound and has potential utility for those interested in identifying possible target binding sites and for molecular modification studies e.g. site directed mutagenesis.

It would also have been useful to test other computational methods besides PAGAL or even describe the pros/cons of these. Could they be used in consensus to improve predictions? Perhaps the authors can comment on this.

I would clarify that our docking and pharmacophore computations (Ekins et al ref) only suggests FDA drugs chloroquine/ amodiaquine may bind VP35. It is clear from the x-ray structures in the PDB that several drug-like molecules can bind. We also have yet to verify our predictions but thank you for citing this.

This study does make you wonder too what else could be done computationally for Ebola research, what should be most helpful for drug discovery?

Probably a logical step might be to identify potential binding locations on the alpha helices for small molecules and possibly dock compounds at these sites.

The authors clearly describe their approach and present results which can be tested by them or others in future. With limited published scientific knowledge on the Ebola virus it is important that such computational approaches are used.

**Competing Interests:** No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 2

Referee Report 19 December 2014
The authors have responded to my previous questions and improved the manuscript. Although, the described computational approach can identify alpha helices with unique features, the author’s proposal that the helical propensities can be linked to host protein interactions is rather weak and requires experimental data to validate the method.

There are still a conceptual error in the manuscript:

Page 4: "The antibody most likely inhibits the rearrangement of GP2 segments, which abrogates the fusion of the internal loop in the host membrane."

Do the authors mean that the antibody binding prevents Gp2 conformational changes required for membrane fusion?

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.
Because of the available structure? This helix contains only two charged residues and would not fall under the classification of carrying a high charge!

The third helices described in detail are from Vps35 and the authors identify several helices with carry charges, but no clear targets are discussed.

Page 6: The authors make a connection between the number of acidic residues in a helix from Ebola Vps35 compared to Marburg Vps35 and the frequency of outbreaks, which is a complete over interpretation of their data.

In summary the manuscript describes an interesting approach to identify or validate potential drug targets. However, the authors need to be more cautious in interpreting their results. Without any experimental validation their approach to link helical properties to protein interaction propensities is extremely weak.

**Competing Interests:** No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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**Discuss this Article**

**Version 3**

**Author Response 29 Mar 2016**

**Sandeep Chakraborty**, Tata Institute of Fundamental Research, India

Further corroboration that characteristically distinct alpha helices are critical for interactions. http://jvi.asm.org/content/early/2016/03/21/JVI.00322-16.full.pdf

"The loss of the alpha helix secondary structure at these epitopes between 1995 and 2004 is intriguing and may warrant experimental data to understand its implications. Nonetheless, our reconstructions support previous hypotheses that regions involved in critical interactions with the host proteins are often these unique alpha helices."

**Competing Interests:** No competing interests were disclosed.
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