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Molecular Modeling, Simulation and Studies on Interactions of Three Major Proteins Encoded by the *Ebola* Genome

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Abstract
Ebola genome encodes a number of proteins which interact with host partners and enable the virus to initiate the disease cascade in the host cell. VP24, VP30 and VP40 are key players in this disease incidence pathway as they are involved in interactions with Alpha Karyopherin 1, TARBP2, NEDD4, TSG 101 respectively; Structural studies have identified the nature of interactions between most of the interacting partners, however, detailed analyses of their interaction sites and druggable pockets of these proteins have not been reported till date. This work generates comparative models of the structures of the three proteins and analyses the druggable pockets of the proteins. It further studies the interactions of the proteins with their host partners and identifies the key interacting residues which regulate their interactions.

Keywords
Ebola, Interacting Proteins, Druggable Pockets

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INTRODUCTION
Ebola is a member of the genus *Ebola* virus comes under the family Filoviridae, order Mononegavirales[1]. However, the first known Filovirus was the Marburg virus which was discovered in 1967 [2]. Ebola was discovered later in 1976 when it caused severe hemorrhagic fever in the Democratic Republic of Congo and Sudan [3]. There are five known virus species in this genus: *Bundibugyo Ebola virus, Reston ebolavirus, Sudan ebolavirus, Taï Forest ebolavirus* (Côte d'Ivoire ebolavirus), and *Zaire ebolavirus* [4].

All five viruses cause Ebola Virus Disease (EVD) a type of hemorrhagic fever which has a very high fatality rate. The Reston virus has been reported outside Africa, in the Philippines and China, which causes fatal hemorrhagic fever in some nonhuman primate species, however does not seem to affect humans. The Reston Ebola virus was discovered in a facility in US where monkeys that were imported from a Philippines facility were infected. Out of the five, the *Zaire Ebolavirus* has the highest mortality rate [5]. It is also responsible for the Zaire outbreak of 1976 and the most recent outbreak in 2014 [6]. The countries that were most affected in the 2014 outbreak were Guinea, Liberia and Sierra Leone [7].

Signs and symptoms of EVD include headaches, fever, sore throat and muscular pain followed by rash, diarrhoea, and vomiting and ultimately decreases liver and kidney function. Both internal and external bleeding occurs in some people. Low white blood cell, platelet counts, and elevated liver enzymes are also found. EVD has a high risk of death, killing average of 50% of those infected, often resulting from the hypovolemic shock (six to sixteen days after symptoms manifest). *Ebola* spreads through direct contact with body fluids (blood and other secretions) of infected people. Fruit bats are thought to be natural hosts of the virus and may have been introduced to the human population by close contact with an infected or dead animal in the forest and then subsequent transmissions between humans. Infection does not generally spread through inhalation in humans; however, it is possible in the case of non-human primates [6]. Due to the severity of the infection by Ebola it has garnered a lot of attention and studies into it's genome, structure, transmission and pathophysiology are underway all over the world.

Ebola like all Filoviruses are enveloped and non-segmented and contain a negative sense RNA genome. The viral particles are 80 nm in diameter but vary in length. The genome consists of seven genes. The order of the genes are- 3' leader, nucleoprotein, virion protein 35 (VP35), virion protein 40 (VP40), glycoprotein, virion protein 30 (VP30), virion protein 24 (VP24), RNA dependent RNA polymerase (L) - 5' trailer [8]. The RNA genome is encapsulated by the nucleoprotein and thus constitutes the inner ribonucleoprotein complex [9]. The virus targets a broad range of cell types that includes monocytes, dendritic cells, endothelial cells, macrophages, fibroblasts, adrenal cortical cells, hepatocytes, and other types of epithelial cells. Monocytes, macrophages, and dendritic cells seem to play a major role in the spread of the virus from its initial site of infection to the whole body by the lymphatic system as well as through blood eventually reaching the liver and spleen [10]. The virus requires certain host cell proteins for its entry. The cholesterol transporter protein, Niemann–Pick C1 (NPC1) appears to be vital for the entry of the virions into the host cell [11]. Viral particles are believed attach to host cells by various proteins that are expressed on the surface of the plasma membrane like integrins, Axl, C- type lectins [12] [13]. After the attachment several viral glycoproteins induce its uptake by micropinocytosis. These particles then enter lysosomes and endosomes, where endosomal cysteine proteases (cathepsin B and L) cleave the viral glycoprotein envelope (GP) removing a heavily glycosylated region from it [14]. This removal exposes a domain that binds directly to NPC1[11] [15].

After the fusion of the viral and internal cell membrane, the viral genome is transcribed which is facilitated by VP30, VP35 and RNA Polymerase (L) [16]. The signal to switch from transcription to replication is generated when VP30 is phosphorylated and it dissociates from VP35/L complex.NP, VP24, VP30 and VP35 then coat the newly replicated virus genomes [17]. The viral particles are then forced through the plasma membrane within lipid raft microdomain regions [18].

Many viruses have evolved mechanisms to avoid innate immunity. Ebola inhibits Interferon responses in target cells (both type I and II) resulting in defective dendritic cell maturation and T-cell maturation. Recent studies indicate VP35 and VP24 might play a vital role in this evasion [19] [20]. While VP35 is a polymerase cofactor playing important role in RNA synthesis it is also vital in inhibiting interferon induction through various mechanisms. It disrupts the function of RIG-I and MDA-5 which are innate pattern recognition factors that detect foreign RNA in the cytosol. RIG-I detects 5'-triphosphates of blunt-ended RNA and MDA-5 detects long double stranded RNA (dsRNA). Then they both have downstream reaction cascades via IPS-1 (MAVS, VISA, Cardif), NF-κB, IRF-3 and IRF-7 that lead to the expression of type I interferon and pro inflammatory cytokines [21]. Experiments show that VP35 prevents the phosphorylation of IRF-3 thus disrupting the RIG-I pathway. VP35 also interacts with dsRNAs to prevent RIG-I and MDA-5 responses [22].

In healthy cell antiviral responses are carried out by interferons. They bind to interferon receptors which via adapter proteins phosphorylates and dimerizes signal transducer and activator of transcription

(STAT) proteins which then travels to the nucleus to induce gene expression [23]. VP24 protein of Ebola viruses prevents the dimerized and phosphorylated STAT-1 to accumulate [24]. It also blocks transport of phosphorylated STAT-1 dimers to the nucleus by binding to karyopherin-α (member of the nucleoprotein interactor 1 family) [25]. Thus VP24 plays a vital role in blocking both Type I and Type II interferon signaling. Comparison of the structures of VP24 was conducted from the Sudan and Reston virus which revealed two conserved structural pockets that could provide the key to the pathogenesis of the protein VP24. Further solvent exchange studies suggest that these regions are important for the binding of the protein to STAT-1 [26].

The RNA-specific inhibition (RNAi) pathway inhibits viral gene expression. It cleaves viral RNAs into small interfering RNAs (siRNA) resulting in disruption of complementary RNA transcripts [27]. VP35 of Ebola suppresses RNAi silencing via dsRNA binding domains [28]. Another protein, VP30 binds to the components of RISC complex and also acts as a suppressor of RNAi silencing [29]. Under attack from viral infection, host cells decrease protein synthesis. It is achieved by double stranded RNA-dependent Protein Kinase, PKR. PKR binds to dsRNA which is then activated. The alpha subunit of the eukaryotic translation initiation factor- 2 (eIF-2α) is then phosphorylated by PKRVP35 interferes with the phophorylation of both PKR and eIF-2α, thereby blocking the PKR activity [30].

Early failure of host innate immune responses are thus initiated by VP24, VP30 and VP35 which is an essential part that eventually leads to the catastrophic Ebola Virus disease. Thus a complete structural and functional understanding of these proteins could provide the key to combat this deadly disease. Present treatments are mainly symptomatic and supportive which includes isolation, broad spectrum antibiotics, antipyretics, malaria treatment and most importantly intravenous administration of fluids. No present strategy has proven successful in pre and post exposure treatment of Ebola [10].

Various investigational treatments are underway. Ribavirin which interferes with capping of viral mRNAs in by arenaviruses and bunyaviruses has no effect (in-vitro and in-vivo) on filoviruses. In RNA based treatments the aim is to interfere or disrupt transcription and replication with the use of antisense oligonucleotides or RNA interference. This approach is showing promise which is based on results in rodents and non-human primates infected with Zaire Ebola virus. However a serious drawback of this approach is prior knowledge of the sequence of a particular Ebola virus species which is difficult to obtain in an early stage of an outbreak [10]. Recombinant vaccines based on vesicular stomatitis virus have shown positive results in post exposure treatment in non-human primates infected with Zaire Ebola virus and Sudan Ebola virus.

Human-adenovirus-type-5 vectors have been successful in protecting non- human primates from the virus challenge. Another successful approach in the replication deficit platform was Ebola virus-like particles generated by co-expression of the viral matrix protein (VP40), nucleoprotein, and glycoprotein. However, a drawback for this approach is its potential need of an adjuvant and booster immunization in non-human primates making it unsuitable for emergency use [10].

Until now most of the studies regarding the biology and pathogenesis of the virus has been bases only on the strain Zaire Ebola Virus. Further efforts are needed to understand the other species and primary transmission from bats to humans. In this work we focus on studying the interactions of three major proteins encoded by the Ebola genome - VP24, VP30, and VP40 along with their interacting partners of the host - alpha karyopherin 1, TARBP2, NEDD4 and TSG101 respectively using molecular modeling, simulation and docking.

2. MATERIALS AND METHODS

1.1 Retrieval of Protein Sequences

The primary sequences of Ebola virus proteins and its interacting partners (Table 1) were obtained from the NCBI (https://www.ncbi.nlm.nih.gov/) in FASTA Format [31].

Ebola Virus Proteins	Interacting Partners
VP24(AAD14588.1)	Alpha Karyopherin 1 (AAH90864.1)
VP30 (AAG40169.1)	TARBP2 (NP_599150.1)
VP40 (AAD14583.1)	NEDD4 (AAI44285.1)
	TSG 101 (AAC52083.1)

Table 1: List of proteins with their corresponding ids.

2.2 Sequence Based Analyses

Secondary structures of the viral proteins and its interacting partners were predicted using the online tool PSIPRED (http://bioinf.cs.ucl.ac.uk/psipred/) [32] . Conserved domain prediction of the protein sequences were performed using DomPred

(http://bioinf.cs.ucl.ac.uk/psipred/) [32] and Interpro (https://www.ebi.ac.uk/interpro/) servers [33].

1.1. Homology Modelling & Structural Analyses

Tertiary structure predictions of the proteins were done using comparative modelling approach. Template selection was done using PSI- BLAST and BLAST [34] (https://blast.ncbi.nlm.nih.gov) for similarity against Protein Databank [35], keeping all the parameters at default. The models were produced using MODELLER 9.18 [36], a programme forcomparative structure modelling.

1.2. Molecular Dynamics Simulation

The modeled structure was then subjected to molecular simulation for 10ns using a GROMOS96 43a2 force field in Gromacs. The B factor calculations were expressed in the form of residue fluctuations following a validation run at the CABS flex server.

1.3. Validation

MOLPROBITY (http://molprobity.biochem.duke.edu/) server [37] was used to assess and validate the generated structures. Quality estimation of the models was performed using QMEAN (https://swissmodel.expasy.org/qmean/) Server [38].

1.4. Analyses of Binding Pockets

After the validation step, pocket detection and drug ability assessment was performed using the tool, DOGSITE SCORER [39] (http://proteinsplus.zbh.uni-hamburg.de/).

1.5. Molecular Docking and Analysis of Interactions

In order to study the interactions between the Ebola viral proteins and its interacting partners docking was performed using the software, Patch Dock [40] [41] (https://bioinfo3d.cs.tau.ac.il/PatchDock/) and the optimum docked complexes were obtained using the tool FIREDOCK [42] (http://bioinfo3d.cs.tau.ac.il/FireDock/). The docked complexes were validated using the standalone tool HEX 8.0 [43].

The docked complexes were analysed using PDBSUM and LigPlot+ [44] to study the hydrogen bonds, common interacting residues,

RESULTS AND DISCUSSION:

The modeled and simulated structure (Fig 1a, 2a, and 3a)was found to be stable stereochemically as revealed by the Ramachandran plot analyses. The radius of gyration over the 10 nano second duration.

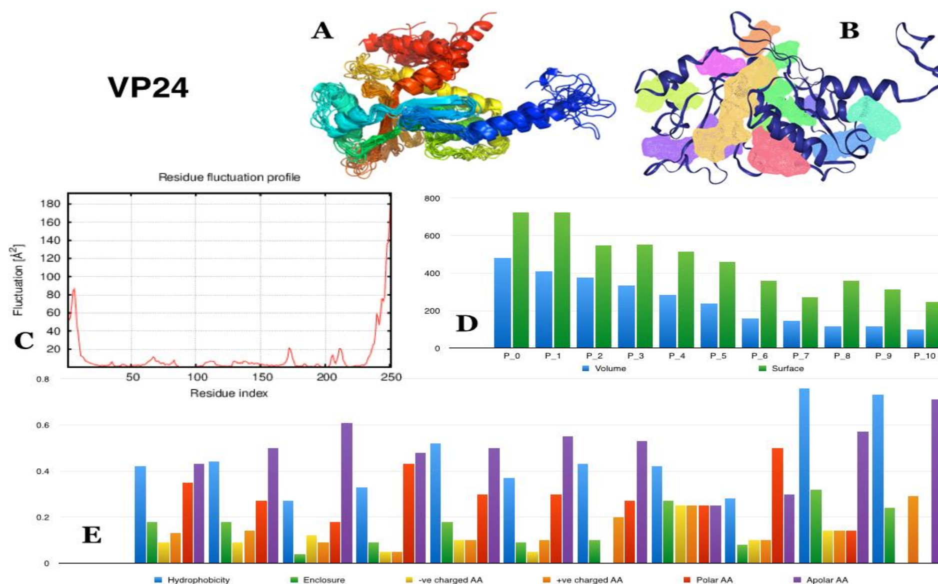

Fig 1: Structural Analyses of VP24 protein. A: Molecular Structure (Superimposed) of the Ebola VP24 protein; B: Predicted binding pockets. C: The residue fluctuation profiles as revealed using molecular simulations. D and E: Properties of the individual pockets as predicted.

along with the RMSD values over time showed that there were conformational states of the structure which were further established by the analyses of B -factors [Supplementary File 1] and RMSD values in a

residue specific manner. VP24 appeared to be the most energetically favourable and stable protein according to its fluctuation profiles followed by VP40 and VP30 respectively (Fig 1c, 2c, and 3c). Disopred predictions did not reveal any significant disordered regions in the three *Ebola* proteins under study.

Numerous binding pockets were identified in the structure and their properties were calculated including their volumes and surfaces (Fig 1b, d and e; 2b, d and e and 3b, d and e) (Supplementary file 2). The interactions studied using molecular docking revealed stable complexes with numerous interaction sites. VP24 and alpha karyopherin interacted with each other with one salt bridge, 10 hydrogen bonds and 726 non bonded interactions, of which 33 residues of VP24 and 38 residues of alpha karyopherin were involved in the protein - protein interface (Fig 4 A and E). In case of the interactions of VP30 and TARBP2, 8 salt bridges, 58 hydrogen bonds and 6780 non bonded contacts were noted in an interface spanning 198 residues of VP30 and 170 residues of TARBP2 (Fig 4 B and F). VP40 interactions with TSG101were comparatively weaker, with 80 non bonded contacts and 4 hydrogen bonds with a very small protein - protein interface of 11 residues for VP40 and 8 residues for TSG101 (Fig 4 C and G) while for VP40 - NEDD4 interactions there was 1 salt bridge, 3 hydrogen bonds and 125 non bonded

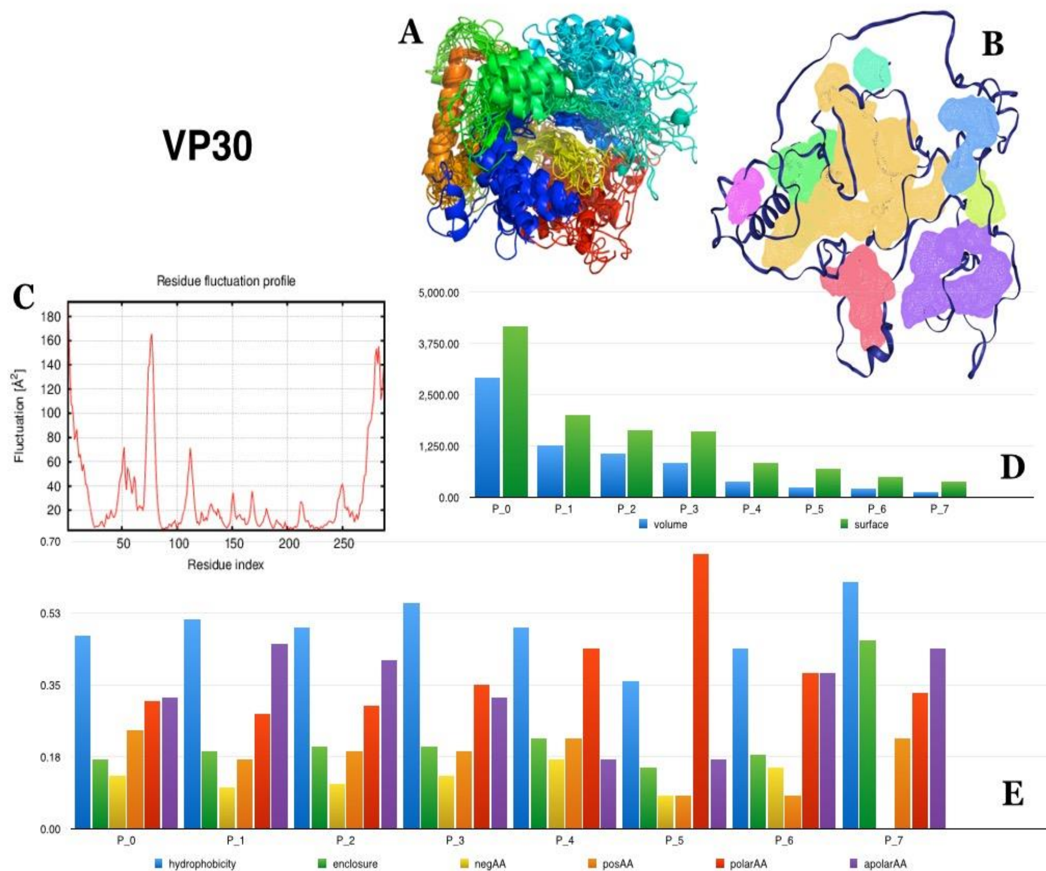

Fig 2: Structural Analyses of VP30 protein. A: Molecular Structure (Superimposed) of the Ebola VP24 protein; B: Predicted binding pockets. C: The residue fluctuation profiles as revealed using molecular simulations. D and E: Properties of the individual pockets as predicted.

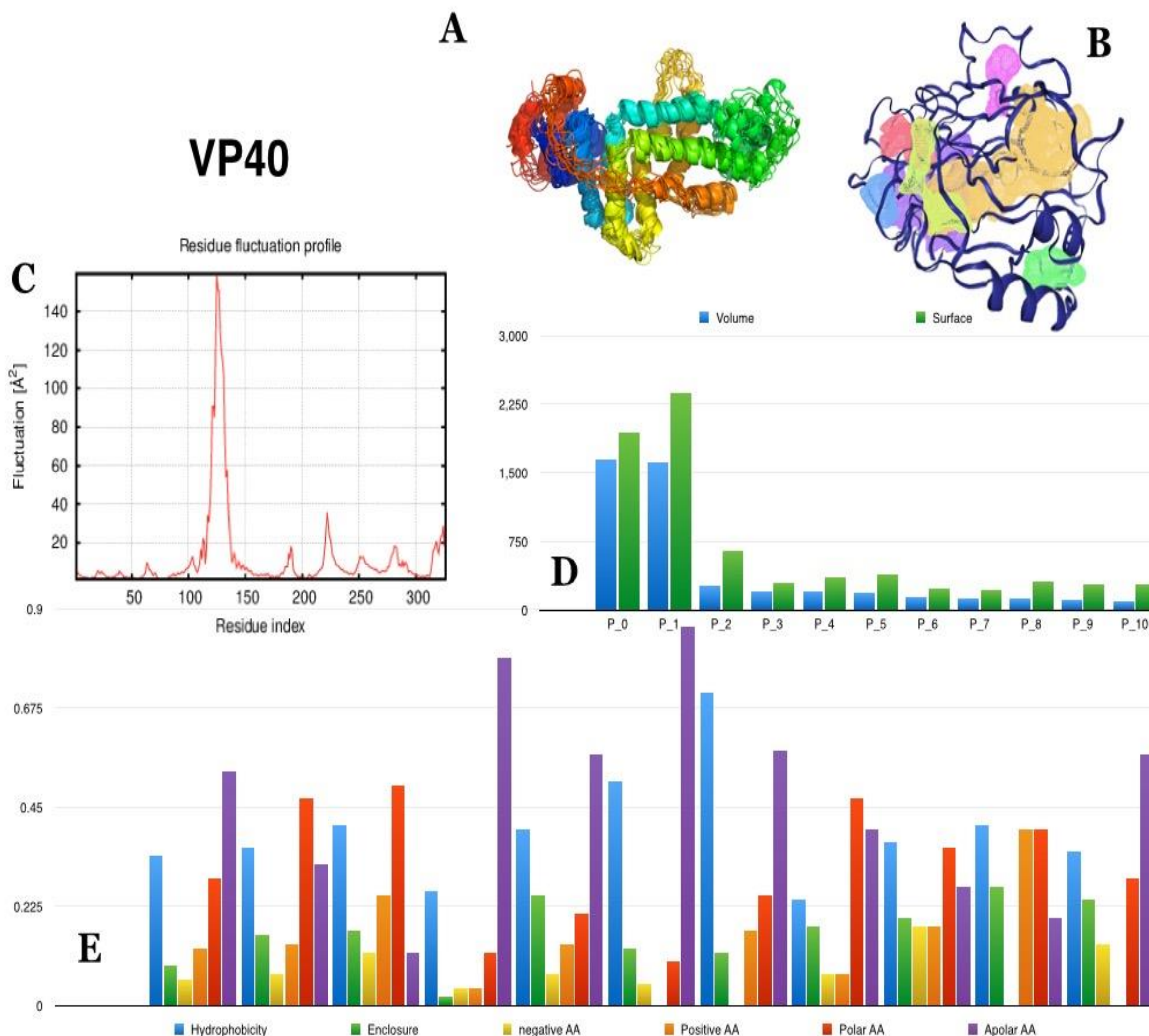

Fig 3: Structural Analyses of VP40 protein. A: Molecular Structure (Superimposed) of the Ebola VP24 protein; B: Predicted binding pockets. C: The residue fluctuation profiles as revealed using molecular simulations. D and E: Properties of the individual pockets as predicted.

contacts spanning a total interface size of 11 residues for VP40 and 13 residues for NEDD4 (Fig 4D and H). The ratio of hydrophilic:hydrophobic amino acids at the interacting interfaces were found to be 1.09, 3.2 and 1.63 respectively for VP30, VP24 and VP40 (Fig 4 I) [Supplementary File 3]. The complexes of VP40, were found to be the most stable of the four complexes that were analysed with identical relative entropy of complex formation at -642. 7; while complexes of VP30 and VP24 had minimum free energies of - 203.7 and -231.8 respectively (Fig 4J). Recent studies have reported the interactions of *Ebola* small glycoprotein to Dronedarone and its derivatives [45]; however, the interactions of *Ebola* virulent proteins with host partners have not been studied in detail. This work identifies important landmarks in the Ebola proteins by pinpointing the interaction sites with its host partners. A large number of interacting sites were identified including salt bridges, hydrogen bonds as well as non - bonded - Van der waal's interactions.

VP40 exhibited an unique mode of interaction in which the interacting interfaces of the protein with NEDD4 and TSG101 did not share a single common residue indicative of the dynamic nature of the protein in forming protein - protein complexes.

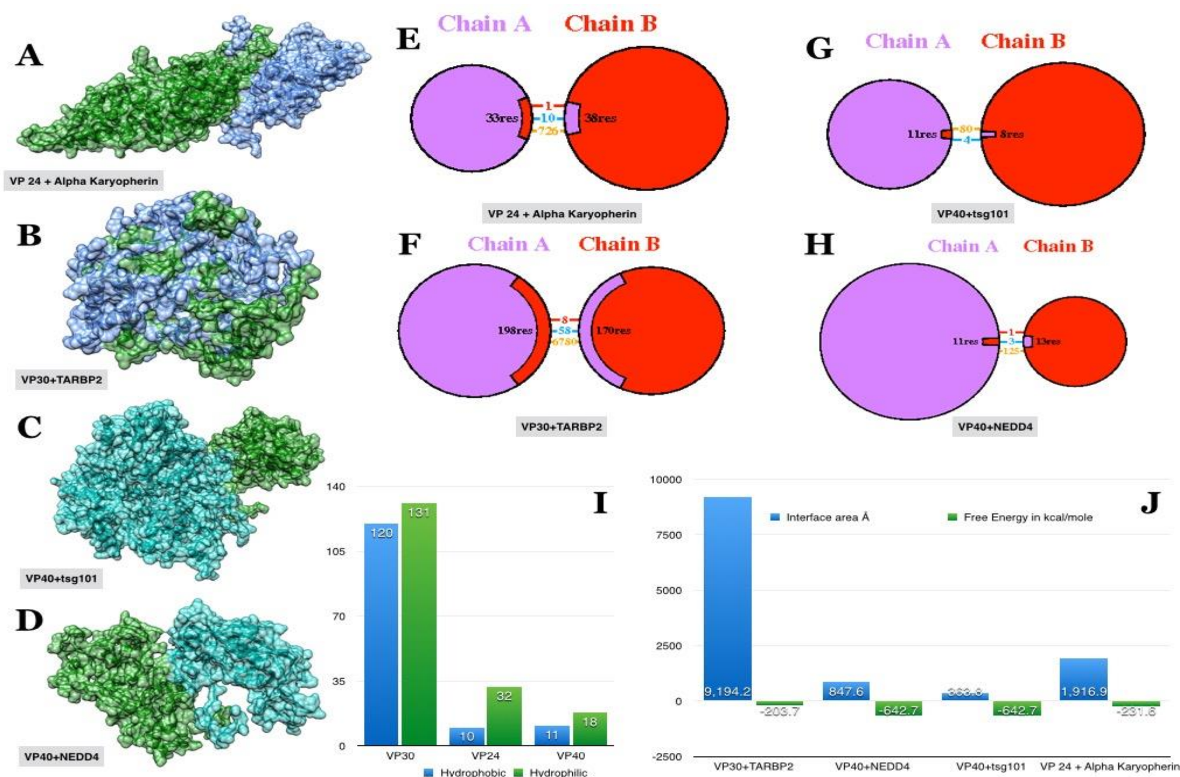

Fig 4: Results of interaction study. A: Surface view of docked complex of VP24 and alpha karyopherin 1; B: Surface view of docked complex of VP30 and TARBP2; C: Surface view of docked complex of VP40 and TSG101; D: Surface view of docked complex of VP40 and NEDD4. E: Schematic diagram showing the interacting interfaces of the docked complex between VP24 and alpha karyopherin 1 F: Schematic diagram showing the interacting interfaces of the docked complex betweenVP30 and TARBP2; G: Schematic diagram showing the interacting interfaces of the docked complex between VP40 and TSG101; H: Schematic diagram showing the interacting interfaces of the docked complex between VP40 and NEDD4; I: Ratio of hydrophilic and hydrophobic amino acids involved in complex formation; J: Total interface area and free energy (relative entropy) of the interactions.

CONCLUSION:

This study reports the modeling and structural analyses of three important *Ebola* virulent proteins with standard methods of comparative modeling, simulation, and validation of the structures. The results indicate the success in the process of molecular modeling and identifies the proteins structures to be suitable for downstream structure analyses. The analyses of potential binding pockets and their drug ability scores, should enable researchers working with Virtual Screening to design effective lead compounds so as to inhibit the possible sites of interactions of these proteins.

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