

Pharmaco-Informatics: Homology Modelling of the Target Protein (GP1, 2) for Ebola Hemorrhagic Fever and Predicting an Ayurvedic Remediation of the Disease

Preenon Bagchi*, Mahesh. M, Somashekhar.R

Azyme Biosciences, #1188/20, 3rd Floor, 26th Main, Jayanagar 9th Block, Bangalore-560069, India

*Corresponding author: Preenon Bagchi, Azyme Biosciences, #1188/20, 3rd Floor, 26th Main, Jayanagar 9th Block, Bangalore-560069, India, E-mail- preenonb@yahoo.com

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Abstract

Ebola hemorrhagic fever (Ebola HF) is caused by infection with Ebola Virus. Ebola virus, a member of the family *Filoviridae*, causes one of the most severe forms of viral hemorrhagic fever. In the final stages of the disease, symptoms progress to hypotension, coagulation disorders, and hemorrhages, and there is prominent involvement of the mononuclear phagocytic and reticulo-endothelial systems. It is assumed that the functions of the envelope glycoprotein are likely to play important roles in the pathogenicity of Ebola virus and the interactions of some viral proteins with the immune system are likely to play important roles in the extraordinary pathogenicity of this virus. Ebola virus (EBOV) entry requires the surface glycoprotein (GP) to initiate attachment and then fusion occurs between viral and host membranes. All glycoprotein forms are encoded by gene 4 of the EBOV genome. The strain selected is VGP_EBOSU with accession number Q7T9D9 of Sudan Ebola Virus - Uganda (2000) from NCBI'S entrez database. The 3D structure of Ebola Virus Protein was generated using Homology Modelling. For a predicted evaluation Andrographolide is used (the compound needs clinical trials to prove its efficacy in treatment). The 3D structure of Andrographolide was generated and was converted to *.pdb file which now docks with the *.pdb file of Ebola Virus Protein.

Keywords: Andrographolide; *Andrographis panicula*; Anti-Viral; Ayurveda; Ebola hemorrhagic fever; Ebola virus; Filoviridae; Glycoprotein; Kalmegh; RNA viruses; Siddha; Zoonotic

Introduction

Ebola hemorrhagic fever (Ebola HF) is a severe, often-fatal disease in humans and nonhuman primates (monkeys and chimpanzees) that has appeared sporadically since its initial recognition in 1976 (19). All virions classified as hemorrhagic are enveloped (covered) RNA viruses, whose survival is dependent on an animal reservoir. Viral hemorrhagic fever commonly describes a medical scenario in which multiple organ systems of the body are affected as well as extensive internal hemorrhaging (bleeding) The disease is caused by infection with Ebola Virus which is The World's Deadliest Virus (24) (named after a river in the Democratic

Republic of the Congo (formerly Zaire) in Africa, where it was first recognized). The virus is one of two members of a family of RNA viruses called the Filoviridae (19) (24). Three of the four subtypes of Ebola virus identified so far have caused disease in humans: Ebola-Zaire, Ebola-Sudan, and Ebola-Ivory Coast. The fourth, Ebola-Reston, has caused disease in nonhuman primates, but not in humans (24). The exact origin, locations, and natural habitat (known as the "natural reservoir") of Ebola virus remain unknown. However, on the basis of available evidence and the nature of similar viruses, researchers believe that the virus is zoonotic

(animal-borne) and is normally maintained in an animal host that is native to the African continent (19). Ebola itself has an average length 920 nm and a diameter of 80 nm. The virus is considered a level 4 biohazard and is only handled in the most sterile environments in full protective suiting. Ebola is spread through direct contact with blood or other bodily secretion of infected people (CDC, 2002; WHO, 2000).

Ebola envelope glycoproteins consist of a GP1 protein and membrane-bound GP2 protein that are covalently linked by a disulfide bond (Xin et al., 2005; Sanchez et al., 1996). Although the causes of filovirus virulence are not well defined, there is evidence that glycans on the viral glycoproteins play distinct roles in pathogenesis (Takada et al., 2001). For example, expression of Ebola glycoprotein in cells causes a reduction in host cell-surface protein expression that is associated with cell rounding and detachment (Xin et al., 2005; Simmons et al., 2002; Sullivan et al., 2005). Andrographis compounds have shown antiviral properties which appear to inhibit glycoprotein's in the virus. This impedes the virus's ability to invade cells in the body and replicate. The signs and symptoms of Ebola HF are not the same for all patients. Symptoms characterizing Ebola are unspecific in the first few days of the infection, making the virus even more dangerous. Infection is marked by initial signs of fever, fatigue,

exhaustion, muscle aches, and dizziness (WHO, 2000). As the disease progress bleeding under the skin, in internal organs, and from the eyes, ears, and mouth are seen. Patients with severe progressions of the disease express symptoms of shock, delirium, coma, seizures, and nervous system malfunction (CDC, 2002). Patients surviving infection by Ebola virions were found to develop stronger antibody responses in the early stages of infection than patients who eventually succumbed to the disease (Takada et al., 2001). The role of the innate immune response in the first few days of infection is considered very important in control of viral replication. Conversely up regulation of interleukin 2, 10, tumor necrosis factor, and interferons are associated with infection of the Ebola virus (Takada et al., 2001). Although their role is poorly understood, antibodies are thought to play an essential role in inhibiting infection of Ebola (Maruyama et al., 1999). Antibodies have been found that bind to the nucleoprotein, the envelope protein, and the secreted envelope glycoprotein. Studies have shown that neutralizing antibodies made in response to these glycoproteins are effective against the Ebola virus and show some promise in designing a vaccine (Maruyama et al., 1999). The table below outlines symptoms of the disease, according to the frequency with which they have been reported in known cases (24).

Time Frame	Symptoms that occur in most Ebola patients	Symptoms that occur in some Ebola patients
Within a few days of becoming infected with the virus:	high fever, headache, muscle aches, stomach pain, fatigue, diarrhea	Sore throat, hiccups, rash, red and itchy eyes, vomiting blood, bloody diarrhea
Within one week of becoming infected with the virus:	chest pain, shock, and death	Blindness, bleeding

Known Epidemics

Known human cases and deaths during outbreaks of *Sudan ebolavirus* between 1976 and 2003 (29).

Sudan ebolavirus was the second strain of Ebola reported in 1976. It apparently originated amongst cotton factory workers in Nzara, Sudan. The first case reported was a worker exposed to a potential natural reservoir at the cotton factory. Scientists tested all animals and insects in response to this, however none tested positive for the virus. The carrier is still unknown (ES, 1976).

A second case involved a nightclub owner in Nzara, Sudan. The local hospital, Maridi, tested and attempted to treat the patient; however, nothing was successful, and he died. The

hospital did not advocate safe and practical procedures in sterilizing and disinfecting the medical tools used on the nightclub owner, likely facilitating the spread of the virus in the hospital (29).

The most recent outbreak of *Sudan ebolavirus* occurred in May 2004. As of May 2004, 20 cases of *Sudan ebolavirus* were reported in Yambio County, Sudan, with five deaths resulting. The Centers for Disease Control and Prevention confirmed the virus a few days later (18) (30). The neighbouring countries of Uganda and the Democratic Republic of Congo have increased surveillance in bordering areas, and other similar measures have been taken to control the outbreak (29). The average fatality rates for *Sudan ebolavirus* were 54% in 1976, 68% in 1979, and 53% in 2000/2001 (21).

Ayurveda ('science of life') is a system of traditional medicine native to India and practiced in other parts of the world as a form of alternative medicine. In Sanskrit, the word Ayurveda comprises the words *āyus*, meaning 'life' and *veda*, meaning 'science' (Department of Ayurveda) (23). The earliest literature of Ayurveda appeared during the Vedic period in India. The *Sushruta Samhita* and the *Charaka Samhita* were influential works on traditional medicine during this era. Ayurvedic practitioners also identified a number of medicinal preparations and surgical procedures for curing various ailments and diseases (Department of Ayurveda).

Andrographis paniculata, the Kalmegh of Ayurveda (23) is an erect annual herb extremely bitter in taste in each and every part of the plant body (27) (26). When in bloom, *Andrographis* exhibits small white flowers (16) (28). The plant is known in north-eastern India as 'Maha-tita', literally 'king of bitters'. Scientists have studied this herb for nearly thirty years (25) (22). Since ancient times, *A. paniculata* is used as a wonder drug in traditional Siddha and Ayurvedic systems of medicine as well as in tribal medicine in India and some other countries for multiple clinical applications. The therapeutic value of Kalmegh is due to its mechanism of action which is perhaps by enzyme induction. The plant extracts exhibit antityphoid and antifungal activities (22). Kalmegh is also reported to possess antihepatotoxic, antibiotic, antimalarial, antihepatitic, antithrombogenic, antiinflammatory, antisnakevenom, and antipyretic properties to mention a few, besides its general use as an immunostimulant agent. A recent study conducted at Bastyr University, USA confirms anti-HIV activity of andrographolide (22) (Calabrese et al., 2000).

Andrographolide is a labdane diterpenoid that is the main bioactive component of the medicinal plant *Andrographis paniculata* (20) (Chakravarti et al., 1951) (28). Andrographolide is an extremely bitter substance extracted from the stem and leaves of the Kalmegh (*Andrographis paniculata*) (28).

Methodology

The following work utilizes the protocol involved in Computer Aided Drug Designing. The Ebola Virus selected is Sudan Ebola Virus - Uganda (2000).

Softwares/Web Servers used

1) modeller9v5 MODELLER (copyright © 1989-2008 Andrej Sali) <http://salilab.org/index.html> {The most widely used software for homology (theoretical) modeling; also has option to take three templates as input for model generation}.

- 2) Swiss-PdbViewer v4.01 by Nicolas Guex, Alexandre Diemand, Manuel C. Peitsch, & Torsten Schwede (Swiss Institute of Bioinformatics) <http://spdbv.vital-it.ch/index.html> {Here used for visualization, has option to provide distinct color to protein & molecule}.
- 3) ACD/ChemSketch Freeware, version 11.00, Advanced Chemistry Development, Inc., Toronto, ON, Canada, www.acdlabs.com, 2008. {Widely used academic software for chemical structure drawing, software like hyperchem available}.
- 4) RAMPAGE: Assessment of the Ramachandran Plot MolProbity | Crystallography and Bioinformatics Group <http://mordred.bioc.cam.ac.uk/~rapper/rampage.php> {A trusted server for Ramachandran Plot analysis only to identify the best protein generated by homology modeling in the article (also servers like VADAR, & softwares like SPDBV & PROCHECK can be used for the same purpose)} S.C. Lovell, I.W. Davis, W.B. Arendall III, P.I.W. de Bakker, J.M. Word, M.G. Prisant, J.S. Richardson and D.C. Richardson (2002) Structure validation by C α geometry: phi,psi and C β deviation. *Proteins: Structure, Function & Genetics*. 50: 437-450.
- 5) ArgusLab 4.0.1, Mark A. Thompson, Planaria Software LLC, Seattle, WA <http://www.arguslab.com> {particularly used for molecule format converter}.
- 6) HEX_SERVER http://www.csd.abdn.ac.uk/hex_server/ {A good & trusted software for docking and free for academic usage} High Order Analytic Translation Matrix Elements For Real Space Six-Dimensional Polar Fourier Correlations, D.W. Ritchie (2005) *J. Appl. Cryst.* 38, 808-818.

The strain selected is VGP_EBOSU with accession number Q7T9D9 of Sudan Ebola Virus - Uganda (2000) from <http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=protein&id=75559166>.

The 3D (*.pdb) structures of VGP_EBOSU were generated by the process of Homology Modelling (using modeller 9v5) using the templates: 2EBOA (Identity=91%) (Source Strain: Ebola Virus Zaire Mayinga), 2RLJA (Identity=78%), (Source Strain: Zaire ebolavirus (ZEBOV)) and 3CSYA (Identity=64%) (Source: Zaire Ebola Virus Glycoprotein in complex with a neutralizing antibody from a Human Survivor of the 1995 Kikwit). The best templates were selected by submitting VGP_EBOSU protein coordinates to HHpred - Homology detection & structure prediction by HMM-HMM comparison @ <http://toolkit.tuebingen.mpg.de/hhpred> and to BLAST search engine. Five *.pdb structures were gener-

ated by modeler. These structures were submitted to Ramachandran Plot Server and best protein (*.pdb) was selected.

For treatment we have chosen a remedy from Indian traditional Ayurvedic practice. We have chosen Kalmegh (*Andrographis paniculata*) (based on ref. no.: 1 [a study by Ajoy Basak *et. al.*] which determine the anti-viral property of Kalmegh and also from the recent study conducted at Bastyr University, USA confirming anti-HIV activity of Andrographolide (Calabrese *et al.*, 2000).

The leaves contain the highest amount of andrographolide. It is the primary medicinal component of Kalmegh. It has a very bitter taste, is a colorless crystalline in appearance, and is called a “diterpene lactone” - a chemical name that describes its ringlike structure. Besides the related bitters cited above, other active components include 14-deoxy-11,12- didehydroandrographolide (andrographolide D) (15).

The chemical structure of andrographolide was derived with ACD/Chemsketch software and is converted to *.pdb file (andrographolide.pdb) with the help of Arguslab software. Then finally it is found that VGP_EBOSU.pdb docks with andrographolide.pdb (by submitting the *.pdb files to hex server).

Results

The VGP_EBOSU.pdb protein obtained after homology modeling was analyzed with Rampage Ramachandran Plot server. The results obtained:

Model 1:

Number of residues in favoured region (~98.0% expected): 634 (94.1%)

Number of residues in allowed region (~2.0% expected): 26 (3.9%)

Number of residues in outlier region : 14 (2.1%)

Model 2:

Number of residues in favoured region (~98.0% expected): 627 (93.0%)

Number of residues in allowed region (~2.0% expected): 37 (5.5%)

Number of residues in outlier region : 10 (1.5%)

Model 3:

Number of residues in favoured region (~98.0% expected): 635 (94.2%)

Number of residues in allowed region (~2.0% expected): 30 (4.5%)

Number of residues in outlier region : 9 (1.3%)

Model 4:

Number of residues in favoured region (~98.0% expected): 634 (94.1%)

Number of residues in allowed region (~2.0% expected): 32 (4.7%)

Number of residues in outlier region : 8 (1.2%)

Model 5:

Number of residues in favoured region (~98.0% expected): 628 (93.2%)

Number of residues in allowed region (~2.0% expected): 32 (4.7%)

Number of residues in outlier region : 14 (2.1%)

Model 4 was selected as the best model as per Ramachandran Plot analysis since it has least number of residues in outlier region.

Model 4 was found to dock successfully with Andrographolide.pdb.

Conclusion

Since VGP_EBOSU.pdb was found to dock with andrographolide.pdb, it can be predicted that andrographolide can serve as a treatment for Ebola Hemorrhagic Fever.

Future Perspectives

The above work is solely a Bioinformatics work devel-



Figure 1: Sudan Ebola Virus

Source: http://www.earthopenetwork.net/Ebola_Outbreak_Suspected_in_Loss_of_Congo_Gorillas.htm

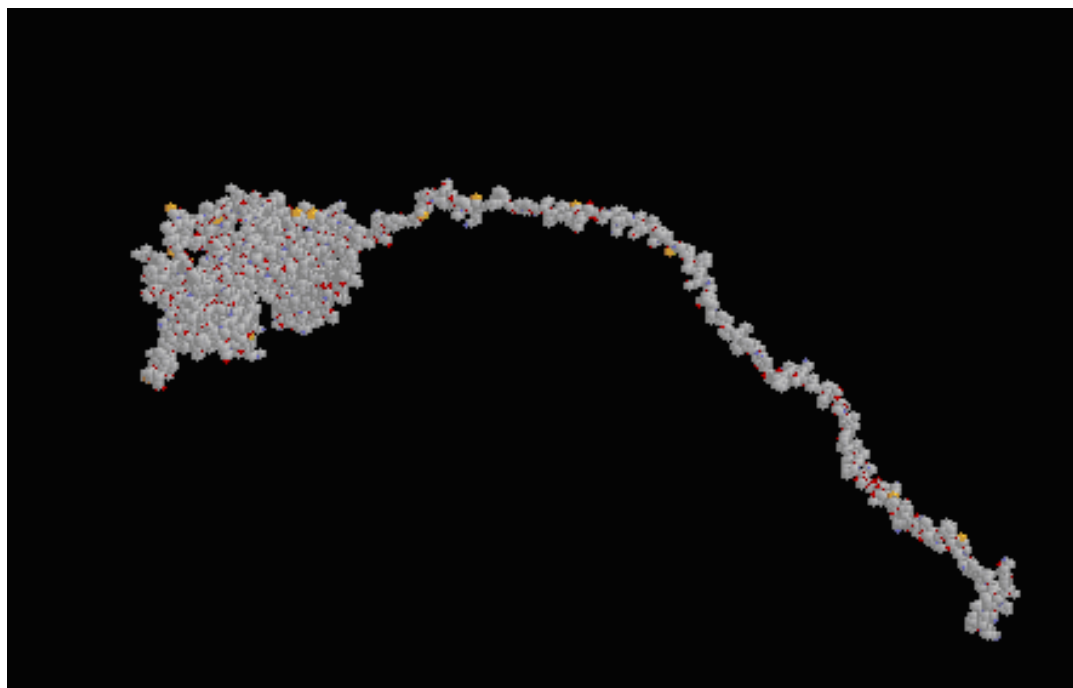


Figure 2: Best modelled structure of Ebola Virus Protein (VGP_EBOSU.pdb) (Visualization in Rasmol).

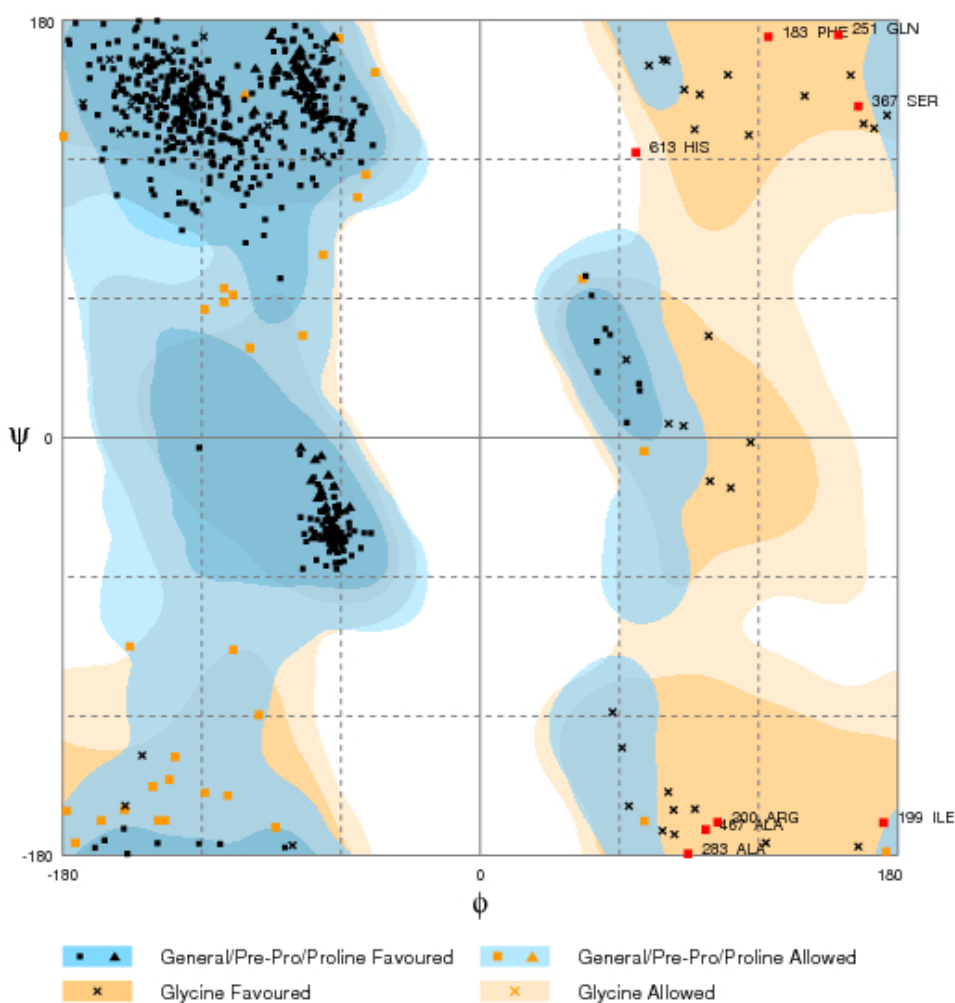


Figure 3: Ramachandran Plot of VGP_EBOSU.pdb (best model).



Figure 4: Kalmegh

Source: <http://www.biogreennutrachem.com/images/kalmegh.JPG>

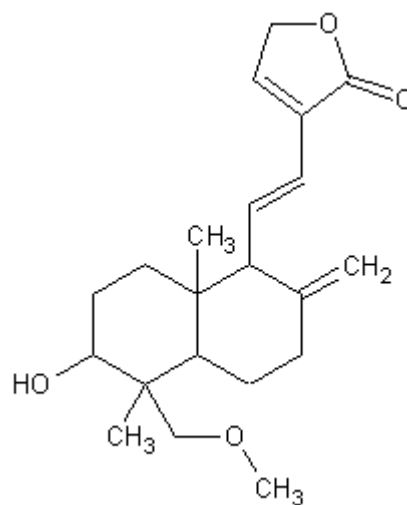


Figure 5: Drawing of andrographolide structure using ACD/Chemsketch.

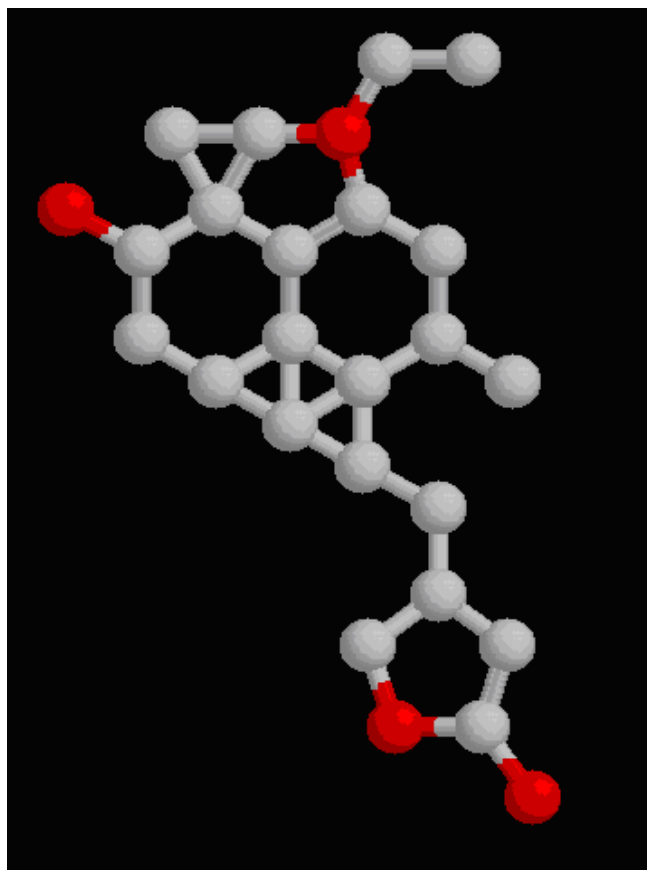


Figure 6: Andrographolide.pdb (Visualization in Rasmol).

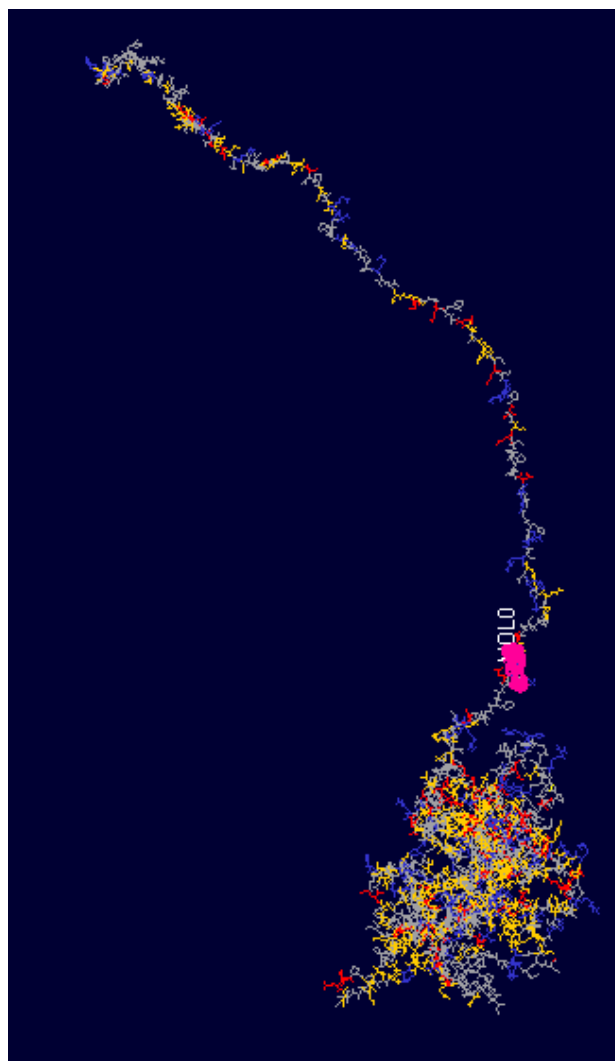


Figure 7: Docked structure of VGP_EBOSU.pdb and andrographolide.pdb (visualization in SPDBV).

oped using computational tools. Since without effective clinical studies, the compound can't be considered as treatment for Ebola Hemorrhagic Fever. So the authors would carry out the trails in a virology lab to establish the effectiveness of andrographolide in treating Ebola Hemorrhagic Fever and concentration and the dosage of the compound.

Discussion

Since the above work is an *in-silico* work, the predicted compound (which proves its efficacy after docking studies) has to go for clinical trials to establish its effectiveness in curing the disease. The above work aims to serve all those researchers and patients in African Continent who are currently experiencing this incurable disease.

The *in-silico* approach helps the researchers by giving them an in-hand idea so that they can happily advance towards the treatment of the disease. The *in-silico-herbal* work is presently an important subject of research since it is time saving, enables effective utilization of funds supplied and gives the best and well predicted results for effective utilization. Again, herbs have the least or no side effects and can be easily metabolized by the body. The study on virulence and extent to which this Ebola virus will infect human body was proved (Takada and Kawaoka, 2001).

The anti-viral property of the drug was reported (31) and (Yi-Feng et al., 2004). In the present study the Indian Ayurvedic herb, kalmegh (*Andrographis paniculata*), is used. It has been used for medicinal purposes for centuries in India but the anti-viral property of this herb was unknown to the world for over a decade. Also work aims to prove that no disease is incurable but the cure may be hidden in some other form. Also this work aims to highlight application of Bioinformatics in Drug Designing.

Dedication

This work is dedicated to Dr. Ajit Kar of Satsang Rasaisana Mandir (Satsang Chemical Works), Deoghar, who has followed the ideology and guidelines of Lord Sri Sri Thakur Anukulchandra and dedicated ayurveda to the service of mankind.

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