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Comparative modeling and docking analysis of envelope protein from yellow fever virus

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Abstract

Yellow fever is an acute viral disease caused by Yellow fever virus. It has a high morbidity and mortality of global importance with an annual incidence rate of 200,000 infections and death toll of over 30,000. It is imperative to restrict the spread of this disease and to prevent huge human and economic losses. So far, no treatment or cure exists for yellow fever, there is great interest in developing strategies to control the disease. The viral genome encodes many proteins out of which, the E protein is involved in initiation of infection. We performed the comparative modeling and docking analysis of envelop gene (*1YFE*) from yellow fever virus. Homologous sequences were searched for the query *1YFE*), Based on high sequence similarity and lowest E-value, the envelope protein of dengue fever virus (*3G7T*) was chosen as template. Homology modeling was performed using "EasyModeller 4.0". The model was assessed by program "Procheck" and ProSA" web server resulting in 0.7% disallowed residues and Z score -6.18 respectively. 'Moe' was used for the superposition of the protein structure with the template and the superposed model was then subjected to ligand interaction analysis using Ds viewer. The docking of drugs was performed using docking server. In short, this study can be a good initiative towards finding a good cure for the unchecked yellow fever infections with global applicability.

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Introduction

Yellow fever is an acute viral infection caused by the Yellow Fever Virus (YFV) and is spread primarily by female mosquitoes of the *Aedes aegypti* (Cathey and Marr 2014). It infects humans, other primates, and several species of mosquitoes. YFV causes 200,000 infections and 30,000 deaths annually across the globe (Cathey and Marr 2014) with about 90% of these occurring in Africa (Tolle 2009). The virus is an RNA virus of the genus Flavivirus (Bryant *et al.,* 2007: Auguste *et al.,* 2010).

YFV is a positive-sense, single-stranded RNA virus with around 11,000 nucleotides long genome and has a single open reading frame encoding a polyprotein. Host proteases cut this polyprotein into three structural (C, PrM, E) and seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5); the enumeration corresponds to the arrangement of the protein coding genes in the genome (Heinz *et al.,* 2012: Mutebi *et al.,* 2002). Receptor binding, as well as membrane fusion, are catalyzed by the protein E, which changes its conformation at low pH, causing a rearrangement of the 90 homodimers to 60 homotrimers). After entering the host cell, the viral genome is replicated in the rough endoplasmic reticulum (ER) and in the so-called vesicle packets. At first, an immature form of the virus particle is produced inside the ER, whose M-protein is not yet cleaved to its mature form and is therefore denoted as PrM (precursor M) and forms a complex with protein E. The immature particles are processed in the Golgi apparatus by the host protein furin, which cleaves PrM to M. This releases E from the complex which can now take its place in the mature, infectious virion (Chastel, 2003).

Till now there are no specific antiviral agents for the treatment of YF virus (YFV), and despite a commercial YFV vaccine, there are still approximately 30,000 deaths worldwide each year and cases have been increasing in the last 20 years. The virus is endemic in Africa and South America, but cases of YFV have been reported in non-endemic areas also. YFV is related to hepatitis C, dengue, West Nile and other viruses of human concern. Mosquito species of *Aedes* and *Haemogogus* transmit YFV and serve as a reservoir for the virus; humans and monkeys are the primary hosts for viral infection. The disease may be limited to a mild febrile illness or may be more severe, including jaundice, renal failure, vascular instabil- ity and shock. There is an approximately 50% case fatality rate in severe YFV cases (Singh *et al.*, 2012).

Safe vaccines against the YFV exist, but the availability of vaccines is often limited, and people sometimes reluctant to get vaccinated. Therefore, outbreaks of YFV infection and exposure to the disease could be controlled by antiviral agents as a treatment strategy. The E protein plays a multifunctional role during virus replication in susceptible host cells and is a critical factor for viral pathogenesis because of its importance for virus infectivity, cellular tropism and host range, and its capacity to elicit virus-specific neutralizing antibodies (Monath, 2008).

Functional characterization of a protein sequence is one of the most frequent problems in biology. This task is usually facilitated by accurate three-dimensional (3-D) structure of the studied protein. In the absence of an experimentally determined structure, comparative or homology modeling can sometimes provide a useful 3-D model for a protein that is related to at least one known protein structure. Comparative modeling predicts the 3-D structure of a given protein sequence (target) based primarily on its alignment to one or more proteins of known structure (templates). The prediction process consists of fold assignment, target-template alignment, model building, and model evaluation. This unit describes how to calculate comparative models using program easy modeler. Three-dimensional protein structures are invaluable sources of information for the functional annotation of protein molecules. These structures are best determined by experimental methods such as X-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy. However, the experimental methods cannot always be applied. In such cases, prediction of the protein structure by computational methods can frequently result in a useful model (Bhusan *et al.,* 2010).

Molecular modeling has become an integrated part of investigating, explaining, and predicting the properties of small organic molecules as potential drug candidates. Modeling techniques are applied in the fields of compound synthesis (conformational analysis and reaction planning), drug discovery (virtual screening), activity rationalization (docking and molecular dynamics simulations), and lead optimization including the prediction of antitarget effects (Umamaheswari *et al.,* 2011)

To predict protein structure by comparative modeling, two conditions have to be met. First, the sequence to be modeled (i.e., the target sequence) must have detectable similarity to another sequence of known structure (i.e., the template). Second, it must be possible to compute an accurate alignment between the target sequence and the template structure. The whole prediction process consists of fold assignment, target–template alignment, model building, and model evaluation. A simple predictor of the overall model accuracy is the degree of sequence similarity between the target and the template. The higher is the sequence similarity to the template, the more accurate is the modeler (Manjasetty *et al.,* 2008).

As structural genomics (SG) projects continue to deposit representative 3D structures of proteins, homology modeling methods will play an increasing role in structure-based drug discovery. Although computational structure prediction methods provide a cost-effective alternative in the absence of experimental structures, developing accurate enough models still remains a big challenge. In this contribution, we report the current developments in this field, discuss in silico modeling limitations, and review the successful application of this technique to different stages of the drug discovery process. (Cavasotto *et al.*, 2009)

Modeller is one of the most widely used tools for homology or comparative modeling of protein three-dimensional structures. MODELLER stands apart from other packages due to its free availability, powerful features and reliable results. But most users find a bit difficult to start with MODELLER as it is command line based. Hence a freely available GUI for MODELLER would thus be very helpful to exploit the powers and advantages of this package more effectively. EasyModeller is a graphical user interface to MODELLER program (Webb *et al.,* 2014).

Procheck checks the stereo chemical quality of a protein structure, producing a number of PostScript plots analyzing its overall and residue-by-residue geometry. It includes PROCHECK-NMR for checking the quality of structures solved by NMR (Laskowski *et al.,* 2001). This is online software that can specify a structure by entering its PDB code, chain identifier and NMR model number and leave the fields for chain id or model number blank, the first chain of the first model found in the PDB file will be analysed (Wiederstein and Sippl, 2007).

If someone do not need access to the expert-level analysis tools in Discovery Studio, but do need a commercial-grade graphics visualization tool for viewing, sharing, and analyzing protein and modeling data, complete the form below to receive the free DS Visualizer and ActiveX Control for interactive 3D visualization (Qing, 2015).

For docking analysis online tool PATCHDock was used. PATCHDock algorithm is inspired by object recognition and image segmentation techniques used in Computer Vision. Docking can be compared to assembling a jigsaw puzzle. When solving the puzzle we try to match two pieces by picking one piece and searching for the complementary one. We concentrate on the patterns that are unique for the puzzle element and look for the matching patterns in the rest of the pieces. PATCHDock employs a similar technique. Given two molecules, their surfaces are divided into patches according to the surface shape. These patches correspond to patterns that visually distinguish between puzzle pieces. Once the patches are identified, they can be superimposed using shape matching algorithms (Mutebi *et al.,* 2004).

Recent studies of the structure of E protein revealed that the transition of the dimeric form to the fusion-active trimeric form and the subsequent post-fusion

form involve conformational changes in both domains II and III (Bressanelli *et al.*, 2004; Modis *et al.*, 2003; Modis *et al.*, 2004). Hydrophobic ligand-binding pocket (BOG pocket) of flaviviral E proteins remains as an attractive target for novel antiviral agent discovery (Goncalves *et al.*, 2007). Thus, designing antiviral agent targeting BOG pocket residues of YFV E protein would be highly effective in controlling yellow fever. Unavailability of competent drug against yellow fever motivated us to analyze YFV proteome for functional assignment using support vector machine. Further, E protein was selected as molecular target for potential YFV antiviral drug discovery.

The present project aims towards the study of Envelope Protein of Yellow fever virus (YFV) in order to have a better understanding of its pathogenesis and responses along with its activity which causes Yellow fever using the Bioinformatics techniques. The proposed work is carried out via different software which makes the modelling and docking analysis of this diseases possible. The structure of Envelope Protein is studied All the steps were carried out in dry lab which helps us to save time and study the variations along with the changes in interaction. The study was carried out with special references to the cofactor binding sites. Ligand-inhibitor modelling was done separately in order to dock with assumed drugs to study breakage or binding of interactions. The present project aims towards better understanding of various aspects of structural features when targeted by proposed drugs

Materials and method

System specifications

This project were performed at IBGE, The University of Agriculture Peshawar. All the steps were carried out in dry lab using Dell laptop model 3420 with 2.40 GHz processor 8GB RAM, 500 GB Hard drive and an NVidia FX 1700 graphics card running in Window operating system was set as experimental environment for the present study. The present study focuses on structure prediction studies of envelope protein of Yellow fever virus by comparative or Homology modeling techniques. All steps of comparative modeling including sequence alignments, model building and evaluation were performed on a laptop. EasyModeller was used for model building. Ds-Viewer was used for graphical display.

Functional assignment of YFV proteome

In this study, the primary sequence of YFV envelope protein was retrieved from the SWISSPROT (http://www.us.expasy.org) (Gasteiger *et al.*, 2003). From PDB databases sequence, homologous to the target sequence, were extracted. BLAST against protein Data Bank (PDB) was used to carry out the sequence homology searches. Three templates i.e. 3G7T, 4B03 and 4C2I were selected on the basis of highest sequence identities between the target and template. The template was used to construct the three dimensional homology model of Envelope protein of Yellow fever virus by using the program Easy Moeller (version 4.0) (Kuntal *et al.,* 2010)

Procheck

Procheck checks the stereo chemical quality of a protein structure, producing a number of PostScript plots analyzing its overall and residue-by-residue geometry. It includes PROCHECK-NMR for checking the quality of structures solved by NMR (Laskowski *et al.*, 2001). The model was finalized by program Procheck and selected on the basis of the best and accurate analysis. It gave 10 files that opened with ghost studio viewer. First file contain Ramachandran plot which contain disallowed region and favored region. Select model with minimum residues or disallowed percentage (Lang, 2002)

ProSA

This is online software that can specify a structure by entering its PDB code, chain identifier and NMR model number and leave the fields for chain id or model number blank, the first chain of the first model found in the PDB file will be analysed (Wiederstein and Sippl, 2007). Then analyze with ProSA webserver. The ProSA (protein structure analysis) was used to highlights the problematic segments. It calculates a score for input-structure. Scores of native protein folds are in distinctive range. If score is outside this range the structure may have problems. The input files were the target and template models in PDB format.

Superposition

The structural superposition was performed by using Molecular operating environment software (MOE). From structure superposition knowledge about the structural design similarity of the protein was obtained.

Visualization

After the model is superposed then find ligand interaction in the superposed model using Ds viewer. With the help of Ds viewer we can find ligand interaction, such as hydrophobic interaction, hydrophilic interaction and the interaction with ordered H_2O molecules.

Docking Analysis

Docking of drugs has been done via docking server PATCHDock but before docking it is necessary to search drugs for the protein which obtained from online tool Drug databank. The predicted structure is docked with three predicted drugs (FLUORESCIN, GLUTATHIONE and IMIDIZOL). Docking has been carried on server and then viewed by Ds-viewer.

Results

Comparative Modelling

Sequence homology searches of for the query envelope protein (*1YFE*) was carried out by using BLAST against protein data bank (PDB) the envelope protein of dengue fever virus (*3G7T*) was chosen as a template on the basis of highest sequence similarity score and lowest E-value for constructing the 3D structure of Yellow fever envelope protein. With the help of EasyModeller the target and template sequences, as a result the gaps in *1YFE* (Fig. 1) while adding the gaps in the template (*3G7T*). Comparison of multiple sequence alignment (3 sequences) shows considerably sequence similarity among primary structures of all known envelop protein is conserved throughout the whole family. (Fig. 2) After model building the model is check by program Procheck and final model was selected on the basis of the best and accurate model (Fig. 4). It gaves 9 files that open with ghost studio viewer. First file contain Ramachandran plot which contain disallowed region and favored region. Select model with minimum residues or disallowed percentage i.e. the lowest residue in this research work was *3G7T*-8 whose value was 0.7% (Fig. 3a). Then the model is analyzed by ProSA web server. It gives the Z-score and number of residues present in sequence. The Z-score will be good if it is smaller than zero. The Z-score of *3G7T*-8 was found to be -6.18 (Fig. 3b and 3c).

Fig. 1. Target (1YFE) and template (3G7T) alignment by using EasyModeller. Conserved active site (red) residues are highlighted. The region show that is thought to be the active site show strong amino acids homologies.

Fig. 2. Indicating the Alignment of the template sequences with query sequence.

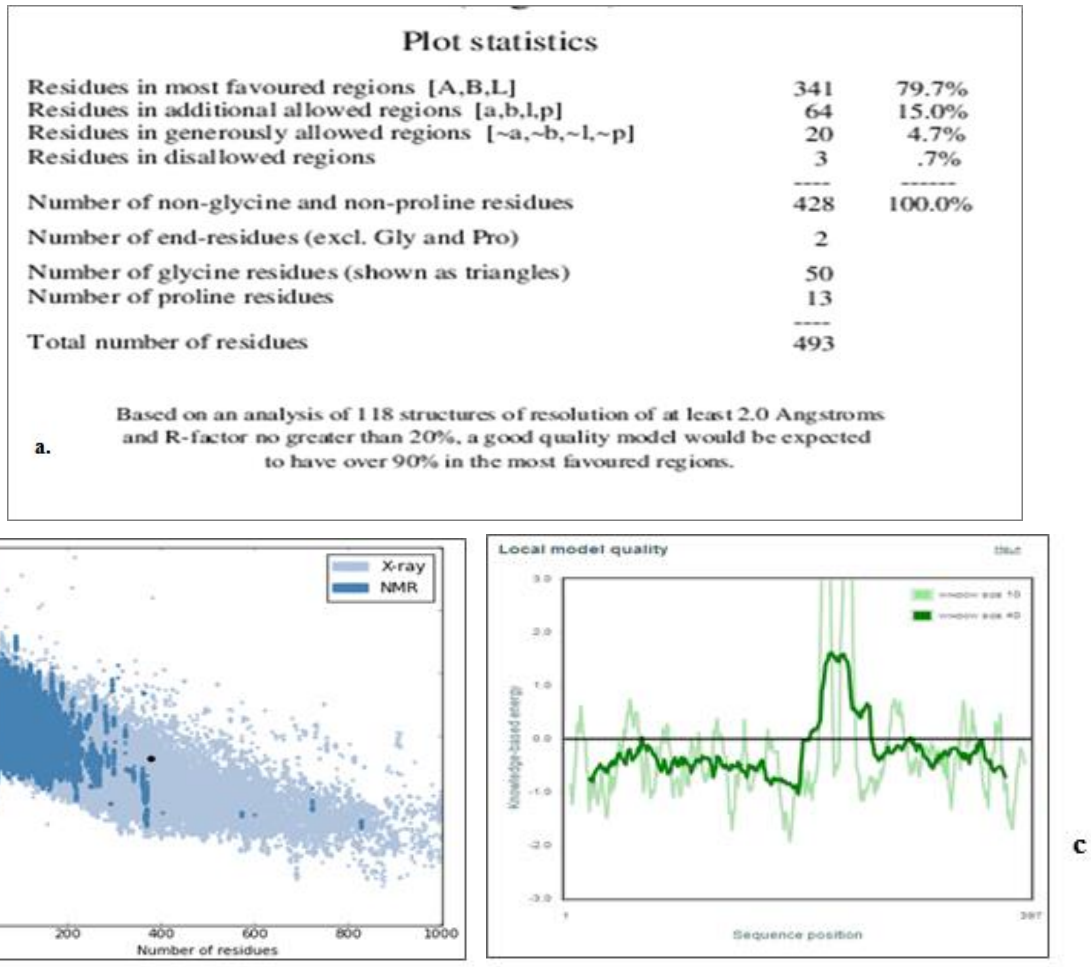

Fig. 3 (a, b & c). These three residues Val 205, CYS 116 and ILE 355 are present in disallowed regions with 0.7% value and 341 residues are present in most favoured region with 79.7% value and ProSA plot shows then Z-score (-6.18) and energy graph of residues scores of a native protein structure.

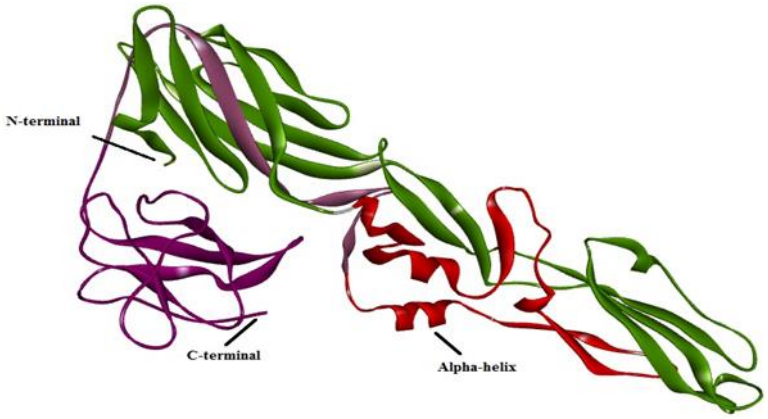

Fig. 4. Schematic representation of homology model of envelop protein showing arrangement of secondary structure of *1YFE*.

Superposition

The Moe is used for the superposition of the protein structure with the template (Fig. 6). After the model is superposed then ligand interaction was find in the superposed model using Ds viewer (Fig. 5).

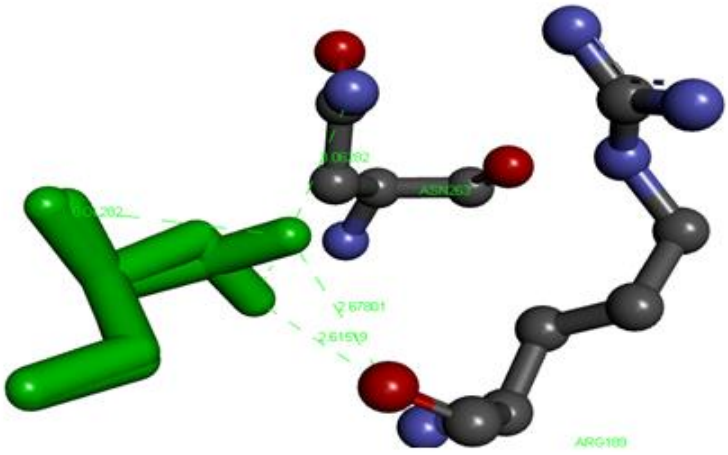

Fig. 5. The Ligand bind with the nitrogen of ASN263 and binds to the oxygen of ARG189 of *1YFV*.

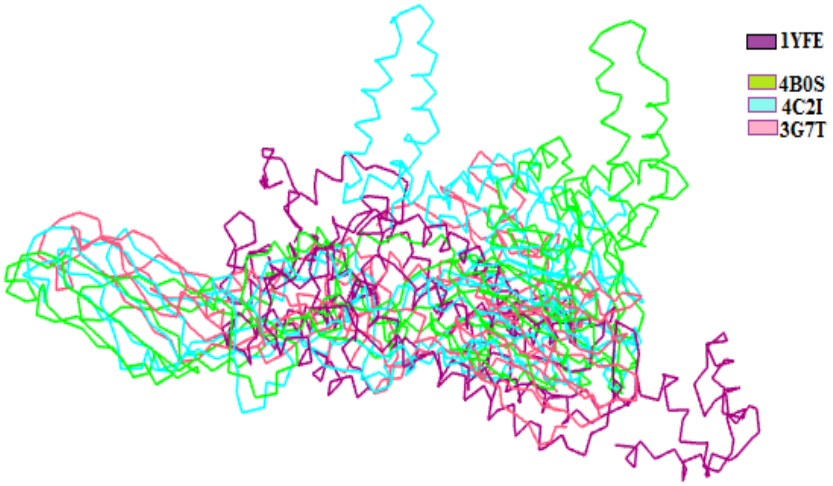

Fig. 6. Superposed protein structure of target *1YFE* and templates *3G7T*, 4C21 and 4B03.

Docking analysis

Docking of drugs has been done via docking server. PATCHDock is online server use for docking analysis first upload the receptor molecule in PDB format and then upload PDB format of ligand molecule that bind with the receptor and in the study the receptor molecule is *1YFE* and ligands molecule are 3 drugs (FLUORESCIN, GLUTATHIONE and IMIDIZOL) (Fig. 7 and 8). The output of PATCHDock is a list of candidate complexes between user specified receptor and ligand molecule. The list is presented to the user in the format of a table, (Table 1, 2 and 3) each table line represents one candidate complex. In addition, the server provides an option to download up to 100 top ranking candidate complexes in the PDB format in one zipped file. The user may specify the number of solutions and the server will prepare a Zip file for download. I Open the downloaded PDB file with Ds viewer and find Ligand interaction between the target and the drugs.

Table 1. Hydrogen bond interaction between target *1YFE* and drug GLUTATHIONE.

Residues	Protein	Drug	Bond distance	Type of Interaction
GLU	N1	OD1	2.5AO	H-Bond
GLU	N1	OD1	1.7AO	H-Bond

Table 2. Hydrogen bond interaction between target *1YFE* and drug IMIDAZOLE.

Residues	Protein	Drug	Bond distance	Type of Interaction
GLN25	N1	NE2	3.0AO	H-Bond

Table 3. Hydrogen bond interaction between target *1YFE* and drug FLUORESCIN.

Residues	Protein	Drug	Bond distance	Type of Interaction
HOH478	O	O5	2.0AO	H-Bond
FLU	024	OD1	2.3AO	H-Bond
HIS188	N	O9	3.1Ao	H-Bond

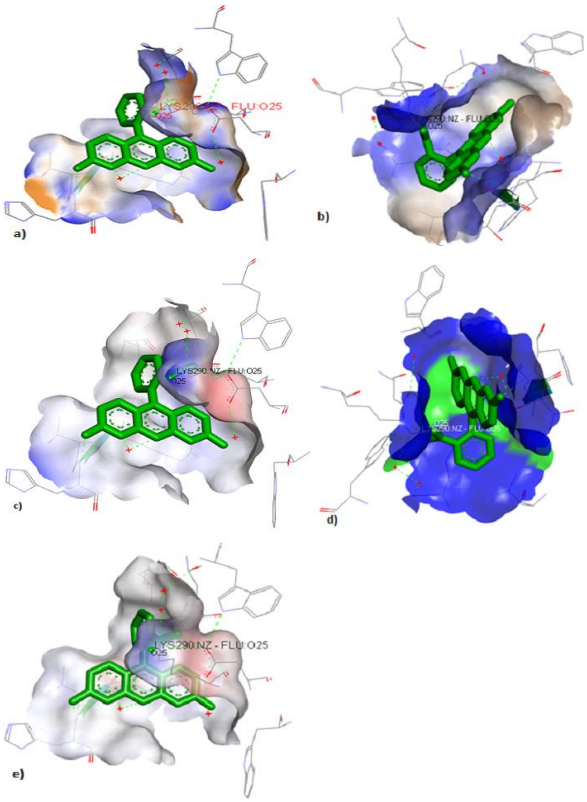

Fig. 7. Residues interacting with ligands are labeled. a) Aromatic interaction, brown color shows face to face interaction, blue color shows edges on interaction, O25 of drug FLUORESCIN interacts with NZ-LYS290 of template *1YFE* shows that interaction is on edges. b) Hydrophobicity, brown and blue colors shows that the pocket is hydrophobic at some places and hydrophilic at other respectively, O25 of drug FLUORESCIN interact with NZ-LYS29 of template *1YFE* show that the interaction is hydrophilic. c) Ionizabilty, red and blue colors shows that the pocket is acidic at some places and basic at other respectively, O25 of drug FLUORESCIN interact with NZ-LYS290 of template *1YFE* which show that the pocket is basic. d) SAS, blue and green color shows that pocket is interacting with salt blue color shows high interaction with salt and green color shows low interaction respectively, O25 of drug FLUORESCIN interact with NZ-LYS29 which shows high salt interaction. e) Charge, red color shows that pocket is negatively charged at some places and blue color show positively charged at some places, O25 of drug FLUORESCIN interact with NZ-LYS290 which shows that charge on the pocket is positive.

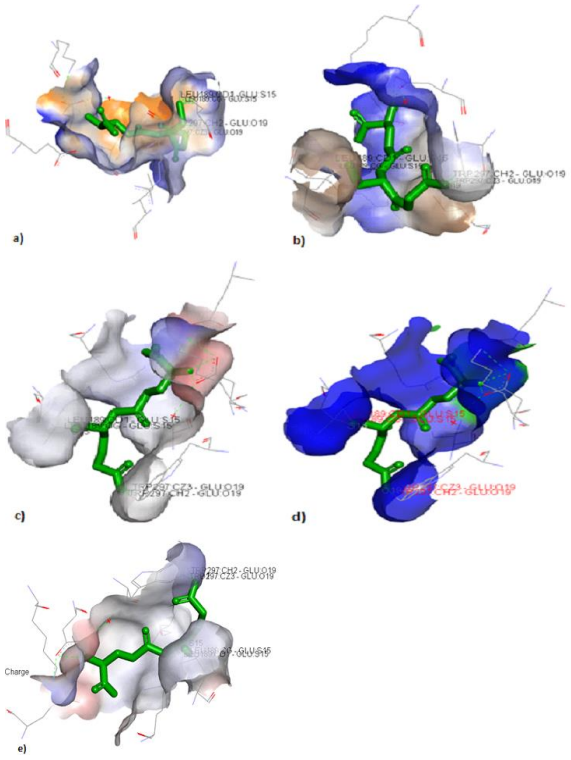

Fig. 8. Residues interacting with ligands are labeled. a) Aromatic interaction, brown color shows face to face interaction, blue color shows edges on interaction, LEW189:CD1 of template *1YFE* and also S15 with LEU189: CH, Which shows that interaction is on the edges. The O19 of Drug GLUTATHIONE interact with TRP29:CZ3 of template *1YFE* which shows face interaction. b) Hydrophobicity, brown and blue colors shows that the pocket is hydrophobic at some places and hydrophilic at other respectively, S15 of drug GLUTATHIONE interact with LEU189:CD1 of template *1YFE* and also S15 with LEU19: CG, shows that interaction is hydrophobic. The O19 of Drug GLUTATHIONE interact with TRP297:CH2 of template *1YFE* shows that interaction is hydrophilic. c) Ionizabilty, red and blue colors shows that the pocket is acidic at some places and basic at other respectively. O19 of drug GLUTATHIONE interact with TRP297:CZ3 similarly O19 with TRP297:CH2, S15 with LEU189:CD1 and S15 with LEU189: CG respectively with Drug GLUTATHIONE and template *1YFE*, which shows that the pocket is neutral. d) SAS, blue and green color shows that pocket is interacting with salt blue color shows high interaction with salt and green color shows low interaction respectively, O19 of Drug GLUTATHIONE interacts with TRP297:CZ3 of template *1YFE* similarly O19 with TRP297:CH2, S15 with LEU189:CD1 and S15 with LEU189: CG respectively with drug GLUTATHIONE and template *1YFE*, which shows high salt interaction. e) Charge, red color shows that pocket is negatively charged at some places and blue color show positively charged at some places, O19 of Drug GLUTATHIONE interacts with TRP297:CZ3 of template *1YFE* similarly S15 with LEU189:CD1 and S15 with LEU189:CG respectively with Drug GLUTATHIONE and template *1YFE* shows that the charge on the pocket is positive.

Discussion

Yellow fever, known historically as yellow jack, yellow plague (Nayak *et al.,* 2009) or bronze john (Bazin 2011) is an acute viral disease. In most cases, symptoms include fever, chills, loss of appetite, nausea, muscle pains particularly in the back, and headaches Symptoms typically improve within five days ,In some people within a day of improving, the fever comes back, abdominal pain occurs, and liver damage begins causing yellow skin. If this occurs, the risk of bleeding and kidney problems is also increased. Many protein structures have been successfully predicted using bioinformatics tools (Barros *et al.,* 2011).

In order to develop drugs for the infection we need the 3D structures of different viral proteins. Because no treatment or cure exists for yellow fever, there is great interest in developing strategies to control the disease. From the modeled structure of the *1YFV* gene of Yellow fever virus and its docking with the selected legends, it could be concluded that the *1YFV* can be targeted with different therapeutics and hence can be a potent future strategy.

YFV infection and pathogenicity. There are literature evidences for critical role of YFV E protein in spread, replication and pathogenesis of YFV in host cells (Anderson, 2003; Heinz and Allison, 2000). Homology search against human genome had revealed that YFV E protein was not having significant similarity with host genome. Hence, in the present study, YFV E protein was selected as molecular target for designing antiviral drug against yellow fever.

In summary, in the absence of crystal structures for any of the proteins comprising the *YFE*, we are left to attempt to construct homology models which we have done using the freely available SWISS-MODEL server. Further preparation of these models required freely available and commercial tools. In the case of the yellow fever E protein homology model, this has the added benefit of enabling the construction of a full virion. By comparing the yellow fever virion to the existing structures for other flaviviruses we can see similarities and differences on the surface

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