

## In-silico structural insights of Dengue 4 NS3 protease: homology modeling and structural validation

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### Abstract

Dengue is causing significant morbidity and mortality worldwide. In poor and underdeveloped countries, the disease is spreading at an alarming rate due to a rise in population density and a decline in environmental cleanliness. Due to the mutation and variety of distinct dengue virus species, the disease is difficult to cure with standard techniques. In addition, there is still a need for effective vaccination against this fatal virus. Designing a vaccine needs a full explanation of the structural characteristics of the NS3 protease, the primary antigenic component of the virus. Several bioinformatics methods were utilized in this study to characterize the NS3 protease of the dengue virus utilizing data from various public databases. Different physio-chemical properties were determined using the ProtParam tool. Secondary structure and motifs were predicted using the SOPMA server and MEME suit. Finally, homology modeling of the selected protein was conducted using the PHYRE2 server. Quality assessment of the predicted structures was performed by employing Ramachandran plot, ERRAT, RAMPAGE, verify 3D, and RMSD scores to establish and suggest one best model for further experimentation. A satisfactory validation score in all those quality assessments implies the proposed model to be a good fit for the future experiment on this protein. Such homology modeling of the viral protein paves the way to a successful protein model and consequently leads to efficient vaccine design.

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## 1. Introduction

Dengue has become a major health concern since second world war and is common in Asia and South America [1-2]. Dengue fever is caused by dengue virus which belongs to Flavivirus family and is spread by the female mosquito of *Aedes* type, specifically *A. aegypti* [3]. Flaviviruses including dengue virus are important human pathogens due to their mode of disease causing and lack of any functional vaccine or specific antiviral therapy against it [4-5].

The dengue virus enters the host cell through fusion process while viral protein binds with the host cell membrane protein [6]. Consequently, the flavivirus

RNA genome is translated into a polyprotein followed by the cleavage of that polyprotein into several components which include nonstructural protein 3 (NS3) [7]. The NS3 protein play vital role in viral replication which involves the cleavage of viral polyprotein through the action of its N-terminal region and its cofactor NS2B that together form the protease. In addition, C-terminal domain is involved in RNA helicase, viral RNA replication and virus particle formation. Another domain of NS3, the serine protease domain plays central role in the replication of dengue virus [8].



There is a dire necessity for new treatment method and novel therapeutic agents against dengue fever cases for it is becoming the cause of death of a huge population in endemic countries as well as amongst a large number of travelers from non-endemic countries. And no current antiviral therapy or vaccine is efficient enough to prevent dengue fever. Since, the NS3 protein seems to be very important in viral replication and infection, it can be a major target for designing new antiviral drugs or therapeutic agents specific for dengue virus [9-10]. Hence, the structural characterization and analysis of NS3 protease molecule by In silico approach along incorporating cutting edge network pharmacology [11-12] and molecular docking assay [13], can aid the design and development of novel dengue virus specific antiviral drug targeting dengue fever [14].

## 2. Materials and methods

### 2.1 Data acquisition

Whole protein sequence was downloaded from National Center for Biotechnology Information (NCBI). The amino acid sequence of Dengue 4 NS3 Full-length Protein (Chain A) was retrieved from NCBI database (<http://www.ncbi.nlm.nih.gov/>) submitted under the name of protein of Dengue virus 4. They are (+) ssRNA viruses under Flaviviridae family also known as Flavivirus with accession no 2VBC\_A having total sequence length 618 amino acid residues. This sequence was retrieved in FASTA format and further exploited for structural characterization and functional analysis.

### 2.2 Physico-chemical characterization

Examination of the physicochemical properties of the studied protein such as Molecular weight, theoretical pI (isoelectric point), AI (aliphatic index), II (instability index), +R and -R (total number of positive and negative residues) and GRAVY (grand average hydropathicity) of Dengue 4 NS3 full-length protein were calculated using ExPASy's ProtParam (<http://web.expasy.org/protparam/>) webserver tool [15]. The PredictProtein server was used for ascertaining various molecular functions and biological processes of ontology.

### 2.3 Secondary structure analysis

The server SOPMA was applied for secondary

structure calculations (helix, sheets, and coils) of the hypothetical proteins [16]. SOPMA remains for a self-optimized prediction method with alignment for the prediction of helix, strands, and coils of the protein sequence.

### 2.4 Motif analysis

Protein motifs were the vital signs of the belonging domain, are very important in the case of predicting likely domain of Dengue 4 NS3 Full-length Protein. To predict the motifs of that protein, the online platform MEME suite was used [17].

### 2.5 Homology modeling of the Dengue 4 NS3 Full-length protein

Homology modeling of the Dengue 4 NS3 Full-length protein was conducted by using PHYRE2 server [18]. Full length of Dengue 4 NS3 protein sequence was uploaded to the PHYRE2 (Protein Homology/Analogy Recognition Engine V2.0) server (<http://www.sbg.bio.ic.ac.uk/~phyre2/html/page.cgi?id=index>) for three-dimensional structure predictions with the full amino acid sequence of the protein in FASTA format. The protein model was built from multiple sequence alignment of the query protein sequence with the template sequence with known structure and functions [19]. The modeled structure was elected on the ground of sequence correspondence with the Protein Data Bank.

### 2.6 Quality assessment of modeled structure

Homology models generally contain errors in their initial structures. An essential part of homology modeling is the verification or validation of the model. Several procedures were used to estimate the mistakes in the 3D models [20]. The predicted Models were furthermore deliberated for exact validation and verification by PROCHECK server [21] (<http://www.ebi.ac.uk/thorntonsrv/software/PROCHECK/>) for stereo-chemical analysis of dihedral angles in modeled protein structure. PROCHECK analyzes overall residue by residue/structural geometry, as determining by Ramachandran plot. VERIFY 3D ([http://services.mbi.ucla.edu/Verify\\_3D/](http://services.mbi.ucla.edu/Verify_3D/)) [22] explores the similarity of the model with its amino acid sequence (1-D) by allocating structural class based on its location and environment, thus comparing results of superior structures. The

Ramachandran plots for the model was prepared using the RAMPAGE server [23], viewing the percentage of protein residues in the favored, allowed, and outlier sections. ERRAT (<http://nihserver.mbi.ucla.edu/ERRAT/>) [24] is an another protein structure verification algorithm for assessing the progression of crystallographic model building and refinement. Therefore, the root-mean-square deviation of atomic positions (root-mean-square deviation, RMSD) is the measure of the average distance between the atoms (usually the backbone atoms) of superimposed proteins to validate the protein, which was performed using PyMOL software.

### 3. Results and Discussion

#### 3.1 Sequence retrieval

The entire amino acid sequence of Dengue 4 NS3 full-length protein was obtained from the NCBI database, with its 618 amino acids and stored in FASTA format which shown as below-

>pdb|2VBC|A Chain A, Dengue 4 Ns3 Full-length Protein

SGALWDVPSPAATQKATLSEGVYRIMQRGLFGKT  
QVGVGIIHMEGVFHTMWHVTRGVSICHETGRLEPS  
WADVRNDMISYGGWRLGDKWDKEEDVQVLAI  
EPGKNPKHVQTKPGLFKLTGEIGAVTLDFKPGTS  
GSPINKKKGKVIKGLYNGVVTKSGDYVSAITQAERI  
GEPDYEVDIEDFRKKRLTIMDLHPGAGKTKRILPSI  
VREALKRRRLRTLILAPTRVVAEMEEALRGLPIRY  
QTPAVKSDHTGREIVDLMCHATFTTLLSSTRVFN  
YNLIVMDEAHFTDPCSVAARGYISTRVEMGEAAA  
IFMTATPPGSTDPFPQSNPIEDIEREIPERSWNTGF  
DWITDYQGKTVMFVPSIKAGNDIANCLRKSGKRV  
IQLSRKTFDTEYPTKLTLDWDFVVTDDISEMGANF  
RAGRVIDPRRCLKPVLTDGPERVILAGPIPVTPAS  
AAQRRGRIGRNPAQEDDQYVFSGDPLKNDEDHA  
HWTEAKMLLDNIYTPEGIPTLFGPEREKTQAIDGE  
FRLRGEQRKTFVELMRRGDLPVWLSYKVASAGISY  
KDREWCFTGERNNQILEENMEVEIWTREGEKKKL  
RPKWLDARVYADPMALKDFKEFASGRK

#### 3.2 Physicochemical properties

The primary query analysis was calculated using the ProtParam tool of ExPASy proteomics server and the physicochemical properties were analysed. Molecular weight of our target protein, Dengue 4 NS3 was found to be 69420.23Da, made up of 618 AA residues and determined pI value is 8.49 (pI<7) which

indicates basic characters of protein (Table 1). Whereby, this Isoelectric point (pI) was computed to determine the acidic or basic nature of protein [25]. The instability index provides an estimate of the stability of our protein. A protein whose instability index is smaller than 40 predicted as stable; a value above 40 predicts about the unstable protein [26]. The aliphatic index defines thermal stability based on the position occupied and the redundancy of amino acids alanine, valine, and leucine of globular protein [27]. The total number of residues positively charged (Arg+Lys) was larger than the total number of residues negatively charged (Asp+Glu). As per the table, instability index of our target protein was 32.48 which is below 40 that representing our protein to be stable. In case of Aliphatic Index (AI) the studied Dengue 4 NS3 full-length protein showed the tendency of having a wide variety of temperature as confirmed (AI= 77.78) Aliphatic Index (AI) above 70. The High Grand Average hydropathy (GRAVY) value of the protein was calculated to expect its solubility and a high-quality score indicates hydrophobicity while a negative rating indicates hydrophilicity. The very low GRAVY indices of both proteins indicate they may interact well with water. Our measured value was -0.479 which in fact implies to be hydrophilic. All the parameter values are represented in Table 1.

**Table 1.** Physicochemical Properties of Dengue 4 Ns3 Full-length Protein(PDB:2VBC\_A) derived from Protparam

| Parameters  | Values   |
|---|----------|
| Number of Amino Acids                                 | 618      |
| Molecular weight                                      | 69420.23 |
| Theoretical pi  | 8.49     |
| Total number of negatively charged residues (Asp+Glu) | 84       |
| Total number of positively charged residues (Arg+Lys) | 88       |
| Instability index                                     | 32.48    |
| Aliphatic index                                       | 77.78    |
| GRAVY   | -0.479   |

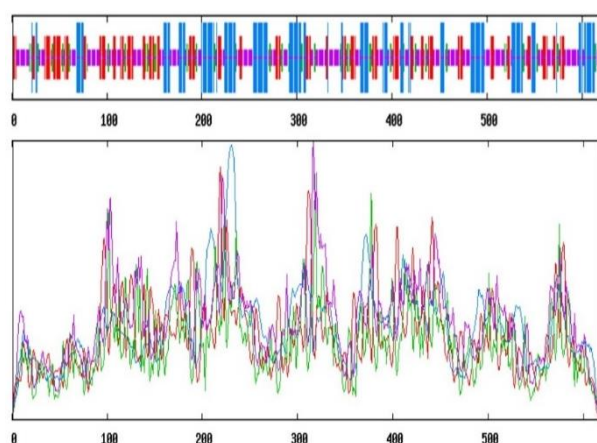
#### 3.3 Secondary Structure Prediction

SOPMA server was used to estimate the secondary structure of the Dengue 4 NS3 full-length protein. The percentage of secondary structural elements is tabulated in Table 2.

**Table 2.** Secondary structure of Dengue 4 Ns3 Full-length Protein(PDB:2VBC\_A) derived from SOPMA server

| Parameters      | Values |
|-----------------|--------|
| Alpha helix     | 25.73% |
| Extended strand | 22.65% |
| Beta turn       | 10.36% |
| Random coil     | 41.26% |

Random coil was found to predominate (41.26%), followed by alpha helix (25.73%), extended strand (22.65%) and beta turn (10.36%). Random coil acts as the primary secondary structural elements for a protein molecule, which plays an important role in protein structural stability (Fig. 1).

**Figure 1:** Secondary structure prediction of Dengue 4 Ns3 Full-length Protein (PDB: 2VBC\_A) from SOPMA

### 3.4 Motif prediction:

Dengue 4 NS3 full length protein motif was predicted using MEME suite. Motif enables to determine the functional area of proteins and motif also represents the conserved pattern in protein sequences through which we are able to construct degenerate primers of those protein sequences. According to Baker et al [28], by default, MEME looks for up to three motifs, each of which may be present in some or all of the input sequences. By default, it can predict up to three motifs and can be chosen in three different parameters for the distribution of the motifs. For this experiment, wide variety of motifs as three and placement distribution as any range of repetitions have been selected. All different parameters have been left as default value. MEME suite routinely predicts the width and occurrence quantity of motif, in order to minimize the E-value of predicted motif. This is properly-known

fact that e-value represents the motif's statistical significance.

In our findings, lowest E value of 4.0e-001 for motif 1 with 3 sites and frame width of 6. Highest sites turned into observed in motif 1 and lowest become found in motif 3. These motifs consisted of six widths, which ranged from 4.0e-001 - 6.8e+001 to E values. In the Table 3; depiction of e-value, width, sites, sequence logo and regular expressions are documented.

### 3.5 Molecular Modeling Studies:

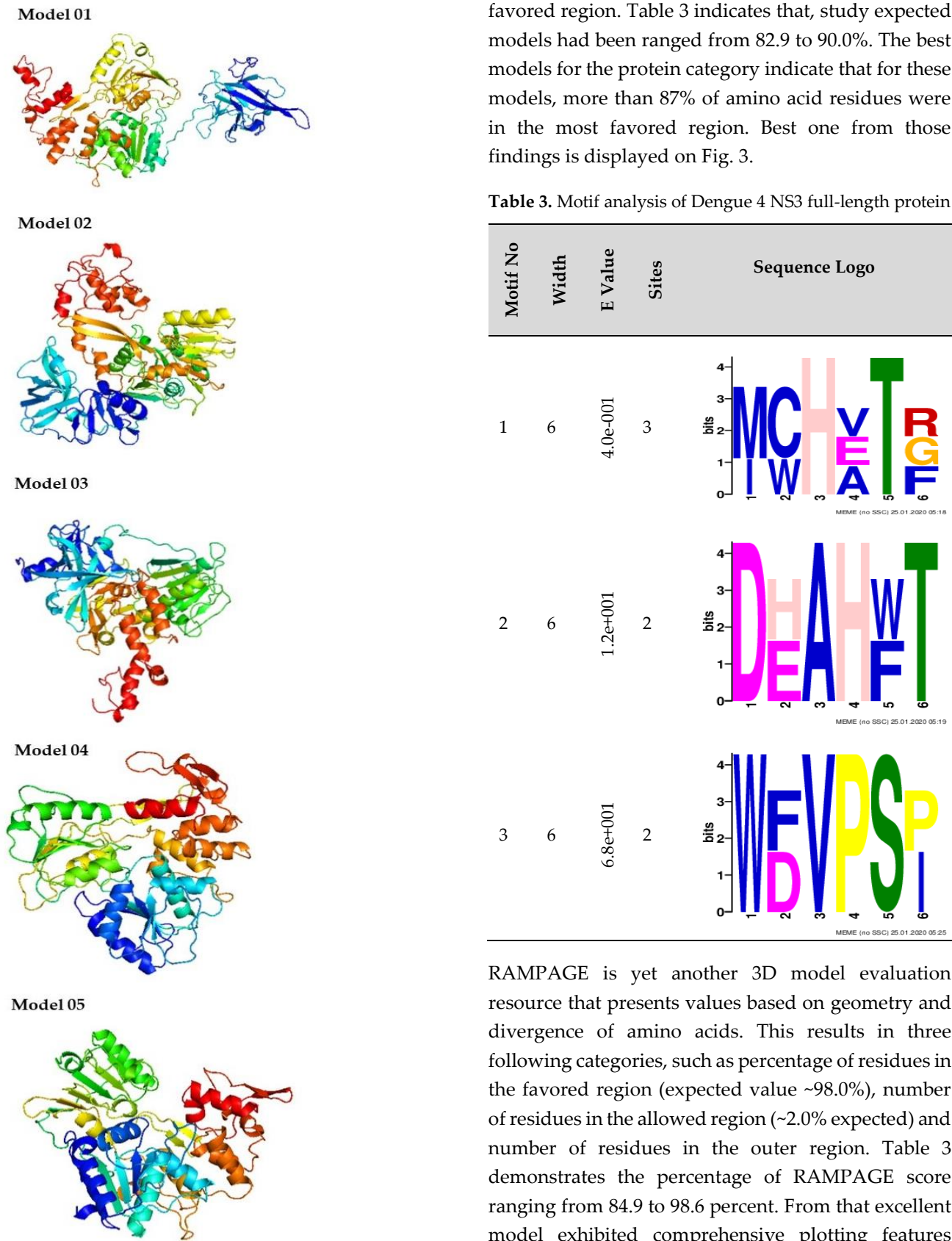
PHYRE2 (Protein Homology/Analogy Recognition Engine) was used to model our query protein sequence of Dengue 4 NS3 protein. PHYRE2 operates on the PSI-BLAST algorithm in which the target sequence is subjected to PSIBLAST iterations that detect the evolutionary relationships among the homologous sequences. Protein structure homology analysis of the proteins was performed in PHYRE2 using "automated mode". When an unknown sequence is submitted, a 3-D model is compared to the algorithm that has already rendered HMM of known structures. PHYRE2 offers accurate results even in sequence identification of > 15 percent. Predicted 3D protein structures, and findings in PDB format were available. The top ranked model categorized on the basis of number of aligned residues and alignment consistency. The 3D model was visualized by PyMOL and shown in Fig. 2.

### 3.6 Quality assessment

These models were then validated by using Ramachandran plot, ERRAT, RAMPAGE, Verify 3D and RMSD score. Validation scores of Ramachandran plot, ERRAT, RAMPAGE, Verify 3D and RMSD have been shown in Table 4.

PROCHECK evaluate stereochemical quality of modeled protein with the aid of comparing residue-by-residue geometry and general structural geometry. That provides transmission of amino acid residues on the Ramachandran plot grouped into four coated regions of color importantly residues in the most favored regions, residues in additional allowed regions, residues in generously allowed regions and residues in disallowed regions. In accordance with the PROCHECK standard, a good quality model should have more than 90% amino acid residing in the most

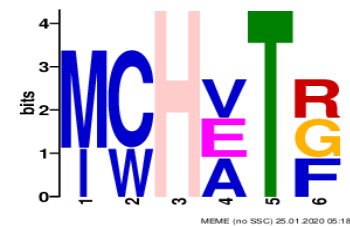
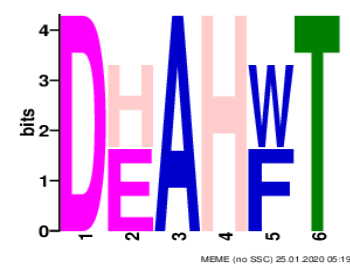
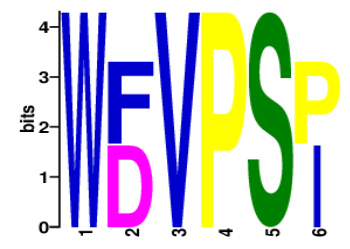




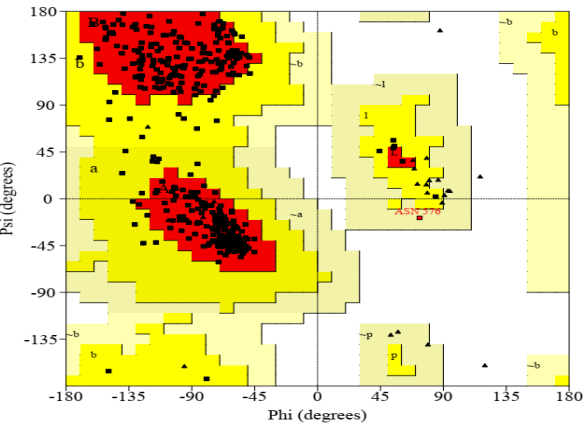
**Figure 2:** Homology modeling of Dengue 4 Ns3 Full-length Protein (PDB:2VBC\_A) from Phyre2

favored region. Table 3 indicates that, study expected models had been ranged from 82.9 to 90.0%. The best models for the protein category indicate that for these models, more than 87% of amino acid residues were in the most favored region. Best one from those findings is displayed on Fig. 3.

**Table 3.** Motif analysis of Dengue 4 NS3 full-length protein

| Motif No | Width | E Value  | Sites | Sequence Logo   |
|----------|-------|----------|-------|---|
| 1        | 6     | 4.0e-001 | 3     |    |
| 2        | 6     | 1.2e+001 | 2     |   |
| 3        | 6     | 6.8e+001 | 2     |  |

RAMPAGE is yet another 3D model evaluation resource that presents values based on geometry and divergence of amino acids. This results in three following categories, such as percentage of residues in the favored region (expected value ~98.0%), number of residues in the allowed region (~2.0% expected) and number of residues in the outer region. Table 3 demonstrates the percentage of RAMPAGE score ranging from 84.9 to 98.6 percent. From that excellent model exhibited comprehensive plotting features illustrates in Figure 4. Initially, ERRAT tested the reliability of the generated model, which examined the statistics of non-bonded interactions between different types of atoms, primarily based on feature



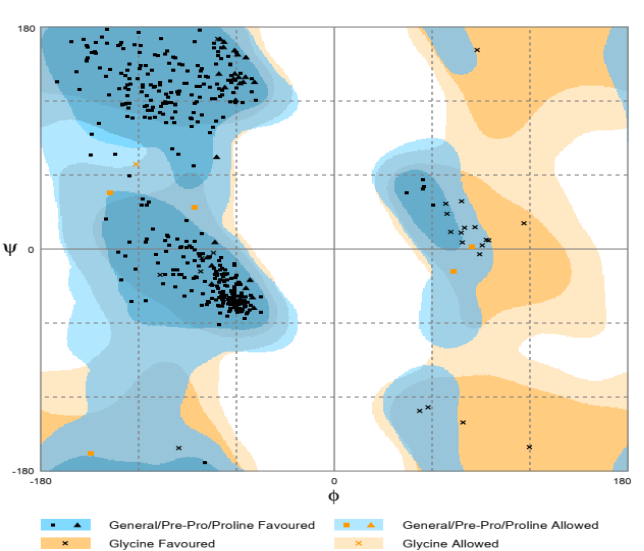
**Figure 3:** PROCHECK analysis for best model of Dengue 4 Ns3 Full-length Protein (PDB: 2VBC\_A)

**Table 4:** Structural validation studies by various online tools

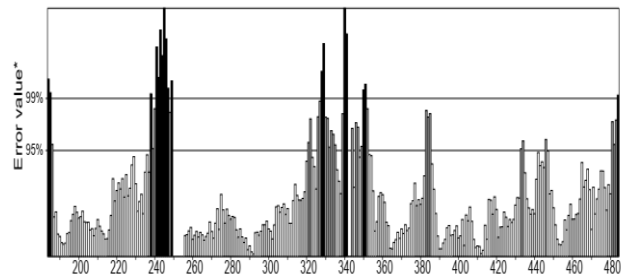
| Model number | PROCHECK*<br>(Regions of Ramachandran Plot, %) | RAMPAGE** (%) | ERRAT*** (%) | Verify_3D**** (%) | RMSD***** (Å) |
|--------------|--|---------------|--------------|-------------------|---------------|
| Model 01     | 86.5   | 94.6          | 65.961       | 85.71             | 1.420         |
| Model 02     | 82.9   | 84.9          | 43.902       | 49.57             | 22.728        |
| Model 03     | 84.1   | 87.8          | 46.358       | 64.64             | 21.117        |
| Model 04     | 90.0   | 98.6          | 87.915       | 93.85             | 0.616         |
| Model 05     | 87.6   | 94.0          | 83.333       | 83.33             | 1.222         |

\*Residues in most favoured regions. a good quality model would be expected to have equal or over 90% in the most favoured regions; \*\*Amino acid residues in most favoured region. Expected or standard of good quality model around 98% or higher; \*\*\*Good high resolution structures generally produces values around 95% or higher. Low resolution structures produces values around 91%;\*\*\*\*Evaluates the compatibility of 3D molecular model with its own (1D) amino acid sequence where the score ranges from -1(not acceptable) to +1(acceptable); \*\*\*\*\*as lower the RMSD value near 0 means it perfectly aligned comparatively than others.

atomic interactions. For non-bonded atomic interactions, ERRAT is a renowned as "overall quality factor," with higher scores implying higher quality. For a high quality product, the generally accepted range is > 50 [29]. Table 3 shows the predicted ERRAT score ranging from 46.358 to 87.915 percent. In accordance with that best model of ERRAT showed in Fig. 5.



**Figure 4:** RAMPAGE output of best models for Dengue 4 Ns3 Full-length Protein (PDB: 2VBC\_A)

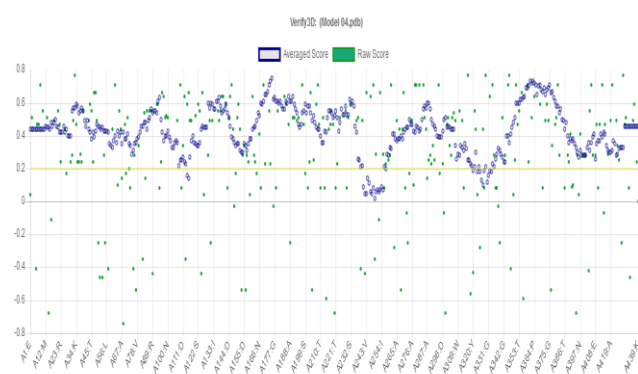


**Figure 5.** ERRAT score of best models for Dengue 4 Ns3 Full-length Protein (PDB: 2VBC\_A)

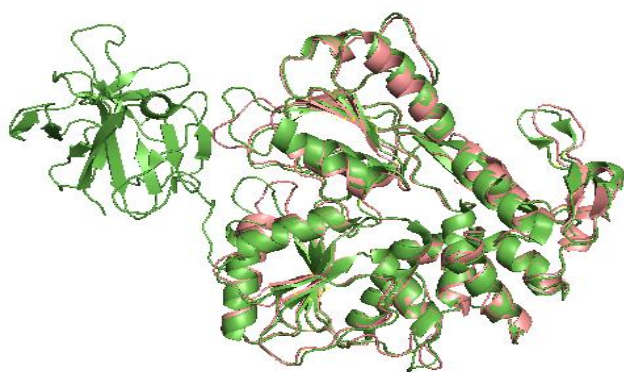
Verify3D; analyzes atomic design (3D) conformity of one's own amino acid sequence (1D) where the score ranges from -1 (not acceptable) to +1 (acceptable). VERIFY3D process by assigning a structural class based on the location and environment of each residue position and by comparing the results to good structures. According to Table 3, 49.57 to 93.85% of the residues had an averaged 3D–1D score >0.2. Graphical view of the residues of 93.85% is presented in Fig. 6.

The resemblance within these two, three-dimensional protein structures is typically measured by the root-mean-square-distance (RMSD) between sets of identical Ca atoms, estimated after the two structures have optimally superimposed themselves. In that case, we used the PyMOL software to measure the aligned based similarity between grouped 3D protein structures. And as lower the RMSD value near 0 means it perfectly aligned comparatively than others.

The best superimposed structure containing estimated lower RMSD value of 0.616 is given in Fig. 7.



**Figure 6:** Verify\_3D output of best models for Dengue 4 Ns3 Full-length Protein (PDB: 2VBC\_A).



**Figure 7.** Superimposed structure of best models with target Dengue 4 Ns3 Full-length Protein (PDB: 2VBC\_A) visualized in PyMol

Evaluating the results of various validation tools, the best model for Dengue 4 NS3 full-length protein was established. From Table 3 it was studied that model 4 of respective protein is outperformed by other models based on the validation scores obtained from various tools.

#### 4. Conclusions

The NS3-protease from Dengue virus type 4 is involved in the replication and assembly of dengue virus through the cleavage of viral polyprotein. In this particular study, physicochemical properties of Dengue 4 NS3 full length protein was analyzed by using Protparam tool from Expasy server which enabled us to know different properties of the protein. Secondary structural properties were predicted by SOPMA tool to know structural conformation.

Conserved region in motifs analyzed by MEME suit give us insight about functional domain and help us to design specific degenerate primers for identification and isolation. 3D structure of the protein was predicted using Phyre2 server and the models were validated by various validation tools, such as- PROCHECK, RAMPAGE, ERRAT, Verify 3D and finally RMSD value from PyMol. Validation and evaluation result of 3D structure of this particular protein shows that predicted model is a stable structural model and of good quality. This study provides information on structural properties of the Dengue 4 NS3 protease which can help us in further docking studies to use it as target and design a specific identification tool and type specific antiviral drug for dengue virus.

#### Data Availability

Data will be made available on request

#### Author Contributions:

Conceptualization, T.D.; Methodology, M.H.U.C.; Software, M.H.U.C.; Validation, M.H.U.C and T.D.; Formal Analysis, M.H.U.C.; Investigation, M.H.U.C. and S.S.; Resources, M.H.U.C.; Data Curation, M.H.U.C. and S.S.; Writing – Original Draft Preparation, M.H.U.C. and S.S.; Writing – Review & Editing, T.D.; Visualization, M.H.U.C. and T.D.; Supervision, T.D.

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#### Conflicts of interest

All authors declare that there were no competing interests

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