



A Comparative Analysis of the Gene Sequences, Structural Models and Identification of Candidate Epitope of Dengue Envelope Protein Domain III

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Abstract

Dengue is a major public health concern in tropical and subtropical regions worldwide. The World Health Organization (WHO) classifies dengue as an endemic disease in more than 125 countries, with approximately 75% of the affected population residing in the Asia-Pacific region, including the Philippines. This study aimed to compare and analyze the four-dengue virus (DENV) serotypes in terms of gene and protein sequence alignment, structural modeling, and identification of candidate epitopes within envelope protein domain III (E-DIII). The DENV E-DIII region plays a critical role in host-cell interaction and is considered a promising immunogenic target for the development of dengue subunit vaccines and diagnostic tools. Bioinformatics tools were employed for gene and protein sequence alignment, protein structure prediction, structural annotation, and peptide analysis. Conserved and variable regions among the four DENV serotypes were identified and analyzed using multiple epitope prediction methods. The

study computationally predicted two candidate linear epitope regions: discriminatory residues from K16–T25 and non-discriminatory residues from N72–E81 or N72–D81. These peptide regions were predicted to be non-toxic, non-glycosylated, and exhibited average to high scores across multiple epitope prediction algorithms. The findings provide insights into the structural and sequence characteristics of DENV E-DIII and contribute to the identification of potential peptide targets for future dengue antigen and vaccine development.

Keywords: dengue virus, serotypes, envelope protein domain III, epitope prediction, conserved sequences.

1 Introduction

Dengue is a significant global public health concern and remains one of the most prevalent mosquito-borne viral diseases in the Philippines. Understanding the molecular characteristics of the dengue virus (DENV), including its genetic variation and serotype diversity, is essential for improving disease surveillance, diagnosis, and vaccine development.

Citation

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The World Health Organization (WHO) classifies dengue as an endemic disease in more than 125 countries, with approximately 75% of affected individuals residing in the Asia-Pacific region [1]. The global burden of dengue remains difficult to quantify because of limitations in disease surveillance, misdiagnosis, and underreporting [2–4]. It is estimated that approximately 3.6 billion people live in tropical and subtropical regions where DENV transmission occurs. Brady et al. [5] (2012) conducted evidence-based mapping of global dengue incidence to improve estimates of populations at risk.

The Philippines, a tropical archipelago consisting of more than 7,000 islands, remains highly susceptible to dengue outbreaks. Historical records indicate that dengue was previously referred to as hemorrhagic fever or infectious acute thrombocytopenic purpura during the 1950s [6, 7]. Epidemiological data reported in 2019 indicate that children aged 5–9 years accounted for approximately 42% of the country's reported 170,000 dengue cases, highlighting the disproportionate burden of dengue among younger age groups in the Philippines [8].

2 Related Work

Dengue fever is caused by the dengue virus (DENV), which exists as four genetically related but antigenically distinct serotypes: DENV-1, DENV-2, DENV-3, and DENV-4. All four serotypes have been reported in the Philippines [9]. Although infections caused by different serotypes generally produce similar clinical manifestations, several serotype-specific characteristics have been documented. DENV-1 infections are commonly associated with red eyes and retro-orbital discomfort, whereas DENV-2 infections are frequently associated with joint pain and thrombocytopenia. In contrast, DENV-3 and DENV-4 infections have been associated with lower lymphocyte counts compared with other serotypes. Infection with one serotype does not confer lifelong immunity against the remaining serotypes; therefore, secondary and sequential infections may occur [10, 11]. Effective surveillance systems are consequently essential to track multi-serotype transmission and identify populations at risk of severe disease [12].

2.1 Gene Sequence Alignment

Gene sequence alignment plays a crucial role in identifying genetic variations among viral strains and determining evolutionary relationships between

related viruses. Comparative analysis of viral genomes enables the detection of mutations, identification of specific genotypes, and characterization of sequence diversity. Furthermore, sequence alignment provides valuable information regarding viral genetic structure and the mechanisms underlying viral evolution and mutation [13].

In DENV research, sequence alignment is commonly used to evaluate genetic diversity and identify conserved and variable regions within the viral genome. Comparative analyses of DENV protein sequences provide insights into conserved and variable amino acid residues among DENV serotypes, contributing to the understanding of viral evolution, adaptation, and antigenic diversity [9].

2.2 Structural Modeling and Binding Interaction

Structural modeling of viral proteins enables visualization of molecular architecture and facilitates the identification of potential epitope candidates for vaccine and diagnostic development. Understanding the interaction between viral proteins and antibodies is essential because strong and specific antibody binding can inhibit viral infection and neutralize viral activity.

This study focuses on the comparative structural analysis of envelope protein domain III (E-DIII) among the four DENV serotypes. The E-DIII region forms part of the viral surface and is believed to contain receptor-recognition sites involved in host-cell attachment and viral entry. Because of its surface accessibility and immunogenic potential, E-DIII is considered a promising target for antibody recognition. Structural prediction and epitope mapping of this domain may therefore contribute to the identification of candidate epitopes and improve understanding of antibody–antigen interactions involved in dengue immunity.

3 Methodology

This study employed several bioinformatics tools to analyze DENV E-DIII sequences obtained from the NCBI GenBank database. MEGA X software was used to perform multiple sequence alignment and identify conserved regions among the four DENV serotypes [14]. The aligned nucleotide sequences were subsequently translated into protein sequences for further structural and epitope analyses. The methodological procedures, analytical approaches, and criteria used for data interpretation are described in the following sections.

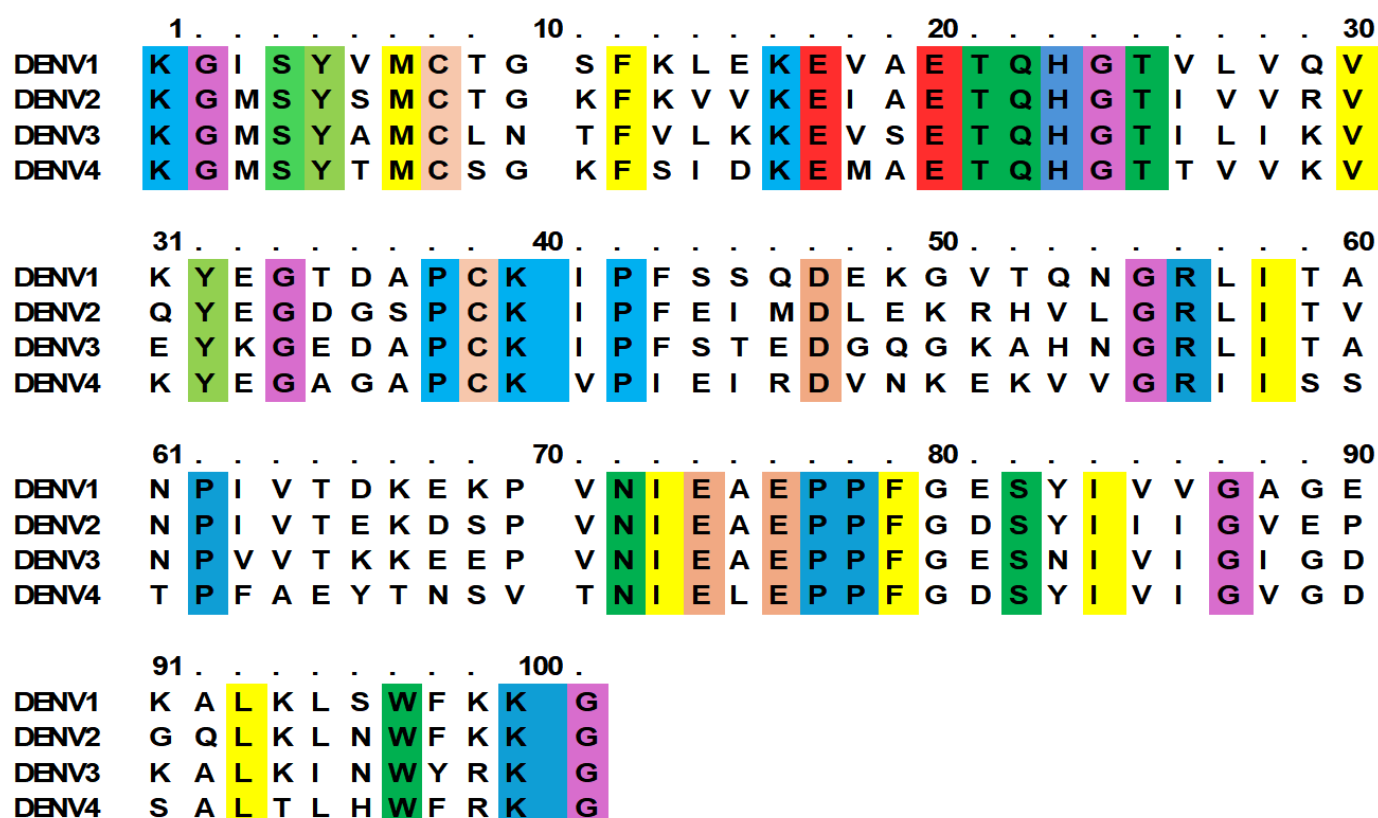


Figure 1. Multiple sequence alignment of dengue virus envelope protein domain III (E-DIII) across the four DENV serotypes. Conserved amino acid residues are highlighted according to residue type, whereas non-conserved residues remain unshaded. Sequences were aligned using MEGA X software.

3.1 Protein Structure Modeling and Analysis

The DENV E-DIII protein sequences representing the four serotypes were submitted to the Zhang Laboratory I-TASSER server for protein structure prediction and structure-based functional annotation [15]. Following model generation, the predicted structures were retrieved as Protein Data Bank (PDB) files. The resulting PDB files were visualized and analyzed using PyMOL software to examine structural features and identify candidate epitope regions [16]. Protein structures were annotated, highlighted, and compared to evaluate structural similarities and differences among serotypes.

3.2 Mapping of Potential Epitope and Analysis of Residues

Multiple sequence alignment of the DENV E gene and protein sequences facilitated the identification of candidate epitope regions for further analysis. Predicted epitope sequences were evaluated using the Immune Epitope Database Analysis Resource (IEDB-AR) B-cell epitope prediction tools.

Several IEDB-AR methods were employed to

identify potential linear B-cell epitopes suitable for antigen design. Candidate peptide sequences were subsequently analyzed using ToxinPred [17, 18] to evaluate peptide toxicity and determine relevant physicochemical properties. These analyses included the assessment of hydrophobicity, hydrophilicity, charge, steric properties, and other peptide characteristics that may influence antigenicity and immunogenicity.

The combined results from epitope prediction and physicochemical analyses were used to identify and characterize potential epitope candidates for future dengue antigen development.

4 Results and Discussion

4.1 Multiple Sequence Alignment (MSA) Analysis

The genome sequences representing the four DENV serotypes were retrieved from the NCBI GenBank database and verified using nucleotide BLAST analysis. The study focused on sequences corresponding to Philippine isolates whenever available. BLAST analysis was performed to confirm sequence identity and ensure that the selected accession numbers corresponded to dengue virus strains reported in the

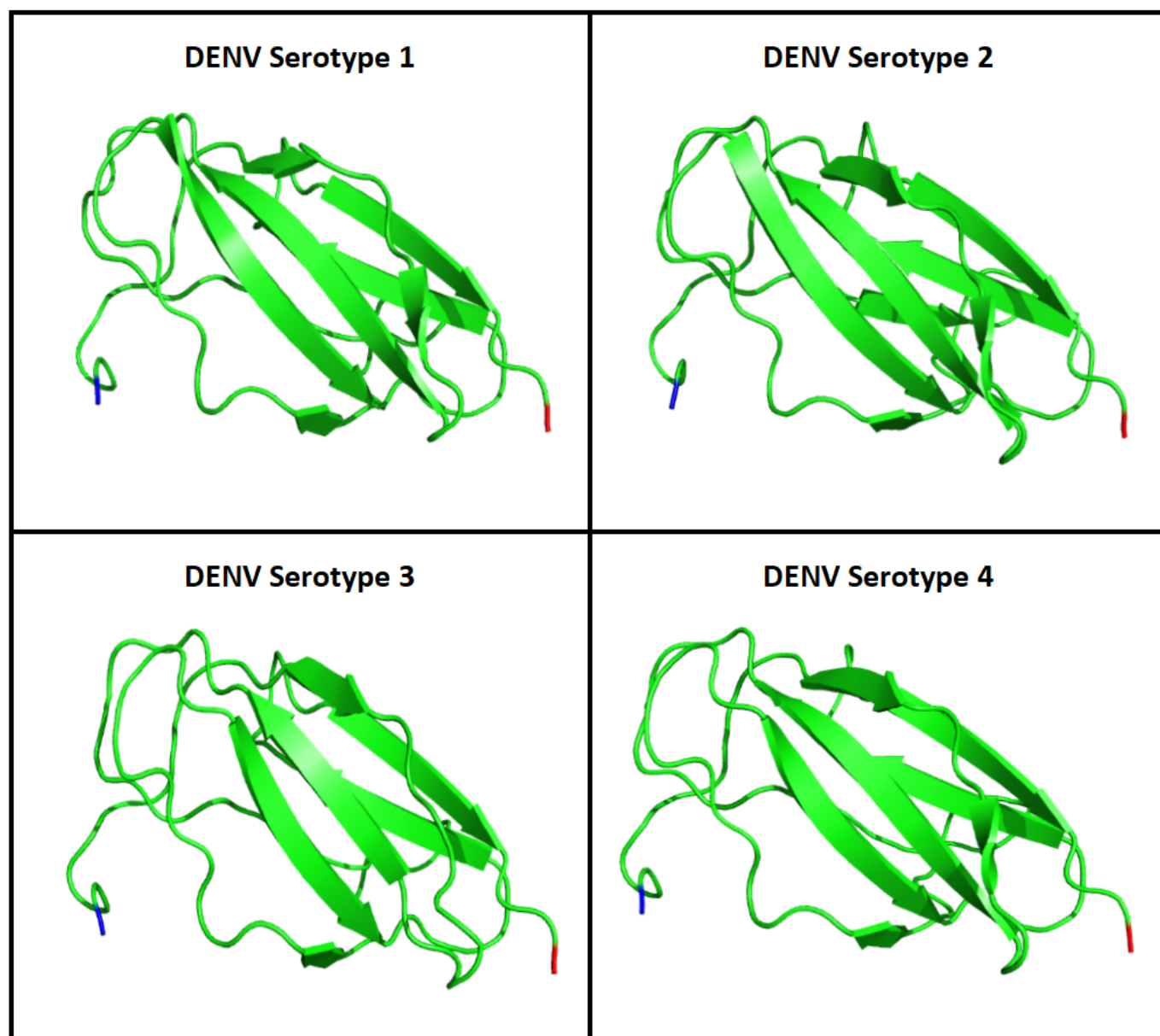


Figure 2. Predicted three-dimensional structures of DENV envelope protein domain III (E-DIII) generated using the I-TASSER server. Models correspond to DENV serotypes 1–4. The N-terminus is shown in blue, whereas the C-terminus is shown in red. All predicted structures predominantly consist of antiparallel β -sheet arrangements characteristic of flavivirus envelope protein domain III.

Philippines. The selected accession numbers were AF425627.1, KU509276.1, P27915, and Q58HT7 for DENV serotypes 1–4, respectively.

The identified DENV E-DIII gene regions consisted of 303 nucleotides. Multiple sequence alignment revealed that 169 nucleotide positions were conserved across all four serotypes, representing 55.78% sequence conservation. In contrast, 134 nucleotide positions were variable, corresponding to 44.22% sequence variation. These findings indicate moderate conservation within the E-DIII coding region while highlighting regions of genetic diversity among the

DENV serotypes.

The nucleotide sequences were translated into amino acid sequences using the DNA-to-Protein Translation function of MEGA X. The resulting protein sequences were validated using corresponding entries from the NCBI protein database. The validated DENV E-DIII protein sequences consisted of 101 amino acid residues for each serotype.

The aligned DENV E-DIII protein sequences are presented in Figure 1. Conserved amino acid residues are highlighted, whereas variable residues remain unshaded. Among the 101 amino acid positions

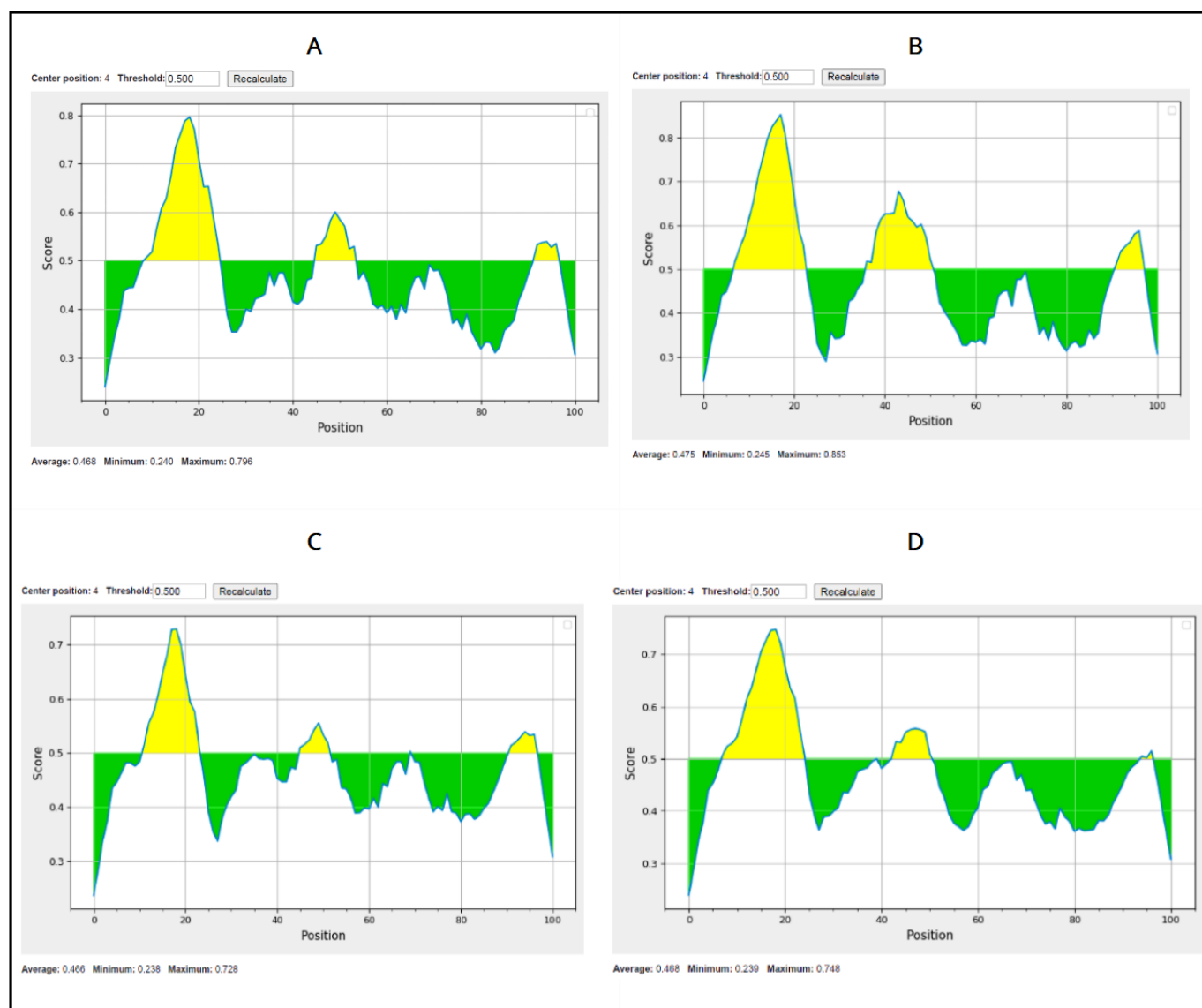


Figure 3. BepiPred linear B-cell epitope prediction scores for DENV envelope protein domain III (E-DIII) across all four serotypes. (A) DENV-1, (B) DENV-2, (C) DENV-3, and (D) DENV-4. The x-axis represents amino acid position, whereas the y-axis represents the prediction score. Regions highlighted in yellow exceed the threshold value (0.500) and indicate potential linear B-cell epitope candidates.

analyzed, 42 residues were conserved across all four serotypes, corresponding to 41.58% sequence conservation. Conversely, 59 residues were variable, representing 58.42% sequence variation. These results indicate that the E-DIII protein contains both conserved and serotype-specific regions that may influence antigenic characteristics and epitope recognition.

Identification of conserved protein regions facilitated the selection of candidate linear epitopes for further analysis. Conserved amino acid sequences are frequently associated with structurally and functionally important regions of proteins. Analysis of these conserved regions provides insight into

protein stability, evolutionary conservation, and their potential suitability as targets for antigen design and vaccine development [19, 20].

4.2 Structural Prediction of DENV E-DIII Using I-TASSER

The DENV E-DIII amino acid sequences were submitted to the I-TASSER server [15] for three-dimensional structure prediction. Predicted structural models were evaluated using the confidence score (C-score) and cluster density values provided by I-TASSER. The model with the highest confidence score for each serotype was selected for subsequent structural analysis and epitope mapping.

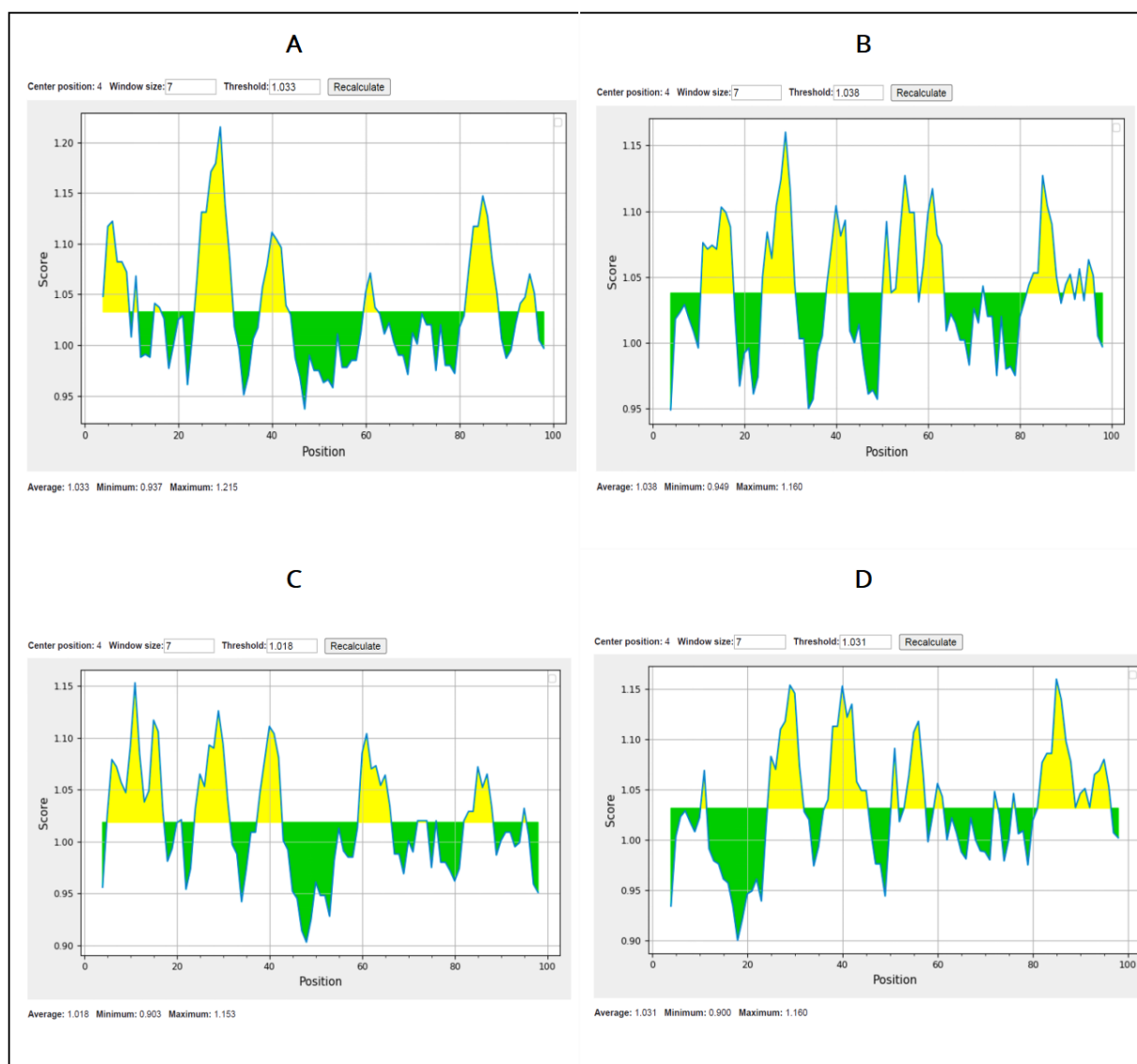


Figure 4. Kolaskar and Tongaonkar antigenicity prediction scores for DENV E-DIII across all four serotypes. (A) DENV-1, (B) DENV-2, (C) DENV-3, and (D) DENV-4. The x-axis represents amino acid position, whereas the y-axis represents antigenic propensity. Yellow regions correspond to residues with antigenicity scores above the threshold values and therefore represent potential antigenic determinants.

Figure 2 illustrates the predicted three-dimensional structures of the DENV E-DIII proteins for all four serotypes. Each model consists of 101 amino acid residues organized primarily into antiparallel β -sheet structures. Structural comparison revealed minor differences in β -strand arrangement among serotypes. DENV-1 contained seven β -strands, DENV-2 contained eight β -strands, DENV-3 contained six β -strands, and DENV-4 contained seven β -strands. Despite these variations, the overall structural fold remained highly conserved across the four serotypes.

4.3 Epitope Prediction Analysis Using IEDB

The DENV E-DIII protein sequences from all four serotypes were analyzed using multiple prediction

tools available in the Immune Epitope Database Analysis Resource (IEDB-AR). The resulting prediction profiles are presented in Figures 3 and 4. In the graphical outputs, regions highlighted in yellow represent residues with prediction scores above the defined threshold values, indicating potential epitope candidates. In contrast, green regions represent residues with lower prediction scores. The epitope prediction analyses focused on identifying peptide regions that consistently exhibited high prediction scores across multiple algorithms.

4.3.1 BepiPred Linear Epitope Prediction

The BepiPred algorithm predicts linear B-cell epitopes based on amino acid sequence characteristics.

Identification of linear B-cell epitopes is important because these regions may be recognized by antibodies and contribute to humoral immune responses. The incorporation of linear B-cell and T-cell epitopes into synthetic peptide-based vaccine formulations has been demonstrated as a viable immunization strategy across diverse pathogens; for example, a fully synthetic polyoxime vaccine incorporating both B-cell and universal T-cell epitopes successfully elicited immune responses across diverse HLA types in volunteers [21], illustrating the translational potential of computationally identified linear epitopes.

Although linear epitopes represent only one component of antigen recognition, their identification serves as an important first step in the characterization of antigenic determinants and the development of peptide-based vaccine candidates. Structural flexibility of peptide regions has also been recognized as a key determinant of epitope accessibility, as flexible loops and turns are more likely to be surface-exposed and recognized by antibodies [22].

4.3.2 Kolaskar & Tongaonkar Antigenicity

The Kolaskar and Tongaonkar method is a semi-empirical approach used to predict antigenic determinants based on the physicochemical properties and occurrence frequencies of amino acid residues within known epitopes [23]. Emini et al. [24] (1985) demonstrated that surface accessibility of peptide regions is a key determinant for antibody recognition, providing a quantitative basis for identifying candidate antigenic peptides on protein surfaces. The method provides approximately 75% prediction accuracy and remains one of the most widely used antigenicity prediction tools.

Regions exhibiting scores above the threshold value are considered potential antigenic determinants and may contribute to antibody recognition.

4.3.3 ToxinPred Analysis and Physicochemical Characterization

The combined IEDB analyses identified two candidate linear epitope regions within the DENV E-DIII proteins. These regions corresponded to residues K16–T25 and N72–E81/D81 (Table 1). Both peptide regions consistently exhibited moderate to high prediction scores across multiple epitope prediction algorithms, suggesting their potential suitability as antigenic targets.

The first candidate epitope region (K16–T25) exhibited sequence variation among serotypes and

may therefore serve as a discriminatory marker for serotype differentiation. Several amino acid substitutions were observed within this region, particularly between residues K16 and E20, indicating potential usefulness for serotype-specific diagnostic applications. The second candidate epitope region (N72–E81/D81) exhibited a higher degree of sequence conservation across serotypes and may therefore represent a broad-spectrum antigenic target capable of recognizing multiple DENV serotypes.

Table 1. Candidate linear epitopes identified within DENV E-DIII proteins.

Serotype	Epitope	
	A (K16-T25)	B (N72-E81/D81)
DENV-1	KEVAETQHGT	NIEAEPPFGE
DENV-2	KEIAETQT	NIEAEPPFGD
DENV-3	KEVSETHT	NIEAEPPE
DENV-4	KEMAETQT	NIELEPPFD

ToxinPred analysis was performed to evaluate peptide toxicity and characterize the physicochemical properties of the identified epitope candidates. The analysis included measurements of hydrophobicity, hydrophilicity, hydropathicity, steric hindrance, isoelectric point (pI), and net charge. These parameters provide valuable information for peptide design because they influence peptide stability, antigenicity, and biological activity.

The identified epitope candidates were predicted to be non-toxic and therefore represent promising targets for future antigen and vaccine development studies [17, 18]. The study provided a comprehensive evaluation of conserved epitopes across dengue virus serotypes, and illustrated an integrative assessment of potential diagnostic targets relevant to the genetic diversity of dengue viruses in the Philippines.

4.4 Structural Annotation of Predicted Epitopes

The DENV E-DIII sequences were sent to I-TASSER for model prediction. The predicted model was used for visualization, analysis of secondary structure, and annotated through the use of PyMOL software (Figure 5).

The identified conserved sequence that revealed high score values in all methods is from residue K16 to T25, highlighted in yellow, and N72 to E81/D81, depicted in cyan. Residues K16 to T25 represent the first candidate epitope region exhibiting consistently

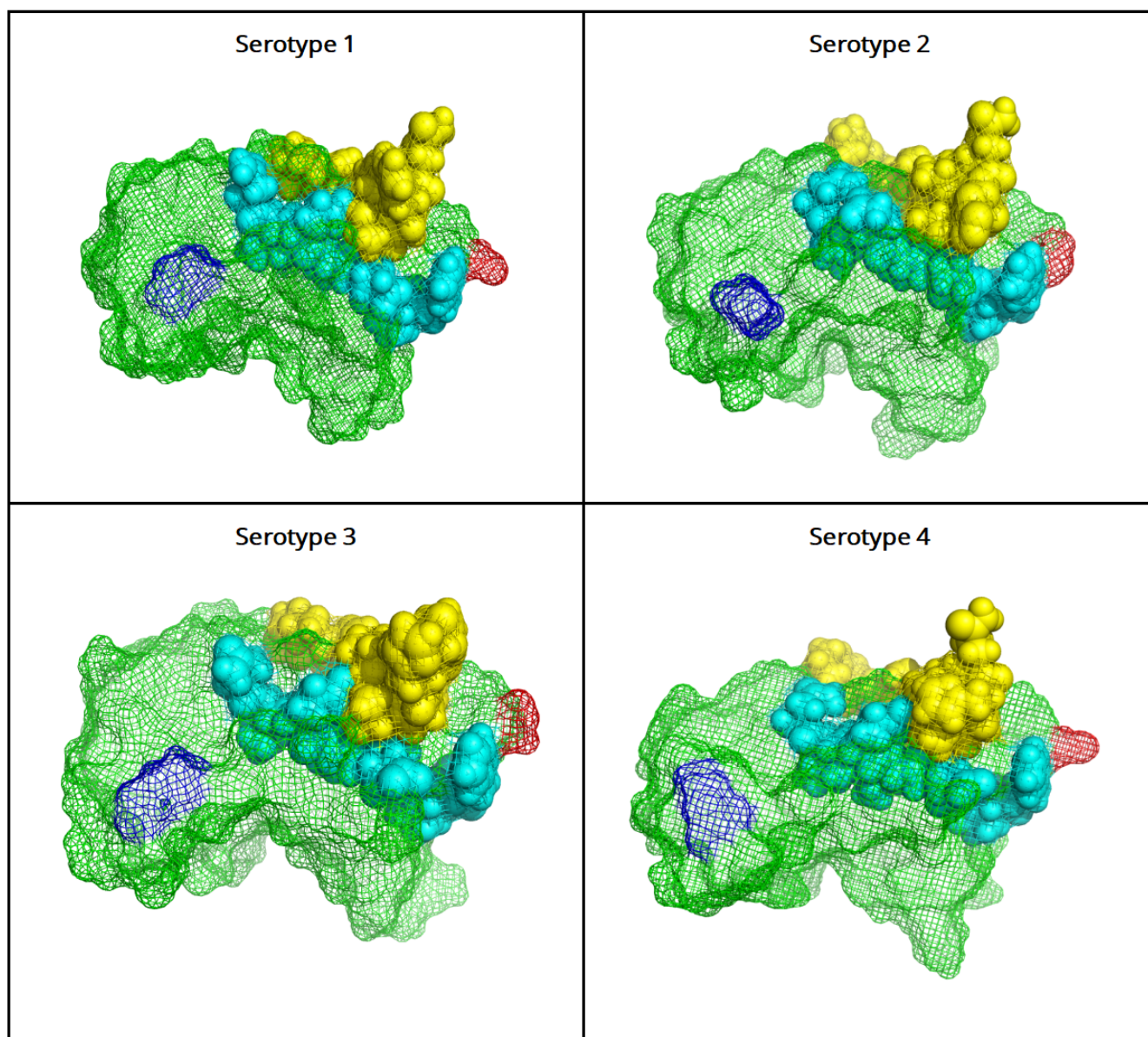


Figure 5. Structural annotation of candidate epitopes within predicted DENV E-DIII models. Candidate epitope regions are highlighted in cyan and yellow, whereas the remaining protein surface is displayed as a green mesh representation. The N-terminus is shown in blue and the C-terminus in red.

high scores across all prediction methods applied to the DENV E-DIII sequences. Additionally, residues N72–E81/D81 represent the second candidate epitope region showing high scores across all prediction methods.

The secondary structures predicted through the use of I-TASSER for all the DENV E protein sequences are anti-parallel beta-pleated sheets. The model is presented in mesh to depict the surface accessibility of the regions. The result illustrates that the regions of the selected epitope are accessible as displayed in the data. The selected residue position K16 to T25 for DENV E DIII serotype 1 protein sequences fall in the beta-strand and loops of the protein structure, while the position

N72 to E81 is positioned in the beta-strand of the protein structure. Secondly, the residue position K16 to T25 for DENV E DIII serotype 2 protein sequences landed in the beta-strand as well as in loops and turns of the protein structure, accordingly, the position N72 to D81 is depicted in the beta-strand extending to loops of the protein structure. Furthermore, the residue position K16 to T25 for DENV E-DIII serotype 3 protein sequences can be found in the loop and turns of the protein structure, and the residue position N72 to G81 is exhibited in the beta-strands extending up to the loops of the protein structure. Lastly, the residue position K16 to T25 for DENV E DIII serotype 4 protein sequences is depicted in loops, and beta-strands of the

protein sequence, while the residue position from N72 to D81 is displayed in beta-strands extending up to the loop of the protein structure.

5 Conclusion

This study performed a comparative bioinformatic analysis of DENV envelope protein domain III (E-DIII) across the four DENV serotypes through sequence alignment, structural modeling, and epitope prediction approaches.

Multiple sequence alignment revealed that 42 of the 101 amino acid residues were conserved among all serotypes, representing 41.58% sequence conservation. These conserved regions provided important targets for epitope identification and comparative structural analysis.

Protein structure prediction using I-TASSER demonstrated that all DENV E-DIII proteins retained a predominantly antiparallel β -sheet architecture despite minor structural differences among serotypes. The overall structural similarity supports the conserved functional role of E-DIII in dengue virus biology.

Epitope prediction analyses identified two promising candidate linear epitope regions located at residues K16–T25 and N72–E81/D81. These regions consistently exhibited favorable scores across multiple prediction algorithms and were predicted to be non-toxic and potentially antigenic. The K16–T25 region demonstrated serotype-specific sequence variation and may be useful for serotype discrimination, whereas the N72–E81/D81 region exhibited greater conservation and may serve as a broad-spectrum antigenic target.

The predicted epitopes exhibited favorable characteristics based on computational analyses, and these findings represent promising preliminary predictions. Experimental validation remains necessary to further confirm the biological relevance and immunogenic potential of the study. Such validation is to determine the suitability as candidate antigens for diagnostic applications or as components for dengue vaccine formulations.

Overall, the findings provide valuable insights into the sequence conservation, structural characteristics, and antigenic potential of DENV E-DIII proteins. The identified candidate epitopes may contribute to future studies focused on dengue diagnostics, antigen design, and vaccine development.

Data Availability Statement

All sequence data analyzed in this study were obtained from publicly accessible databases, including the NCBI GenBank database. Accession numbers used in the analyses are reported within the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

AI Use Statement

The authors declare that no generative AI was used in the preparation of this manuscript.

Ethical Approval and Consent to Participate

Ethical approval was not required because the study utilized publicly available sequence data and did not involve human participants, animals, or clinical specimens.

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