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Original Article

Integrated Structural and Physicochemical Profiling of Dengue Virus Type 2 NS1 Protein

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ABSTRACT

Background: Dengue virus Non-Structural Protein 1 (NS1) is a multifunctional glycoprotein and an important target for therapeutic and diagnostic research, as it participates in viral replication, immune evasion and pathogenesis. **Objectives:** The objective of this study was to characterize the structural and physicochemical properties of the Dengue Virus Serotype 2 (DENV-2) NS1 protein using an *in silico* approach. **Materials and Methods:** *In silico* analysis of the 380-residue NS1 sequence was performed to predict secondary structure, intrinsic disorder, membrane topology, signal peptide regions, solubility, charge distribution, fold propensity and surface physicochemical properties. **Results:** NS1 was predicted to adopt a β-sheet-rich architecture interspersed with α-helices and coil regions. A single N-terminal signal peptide and only limited intrinsically disordered regions were identified. Membrane topology analysis indicated a non-transmembrane protein. Solubility and electrostatic analyses identified a high predicted solubility together with discrete charged surface regions and localized hydrophobic patches that may support membrane interaction and oligomerization. **Conclusion:** DENV-2 NS1 is a largely ordered, membrane-associated protein whose structural features underpin its multiple biological functions. These findings provide molecular clues that may inform the future development of antiviral, vaccine and diagnostic products.

Keywords: DISOPRED3, *Flavivirus*, Intrinsic disorder, MEMSAT-SVM, PSIPRED, Solubility prediction.

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INTRODUCTION

Dengue is one of the fastest growing mosquito-borne viral diseases in the world and a major public health problem in tropical and subtropical countries (Lim *et al*., 2025; Pathak and Mohan, 2019; Umar *et al*., 2025). Dengue virus (DENV), a positive-sense single-stranded RNA virus of the genus *Flavivirus*, family *Flaviviridae*, is the causative agent. Four serotypes (DENV-1, DENV-2, DENV-3 and DENV-4) are recognized, and infection can produce a wide clinical spectrum ranging from asymptomatic infection to dengue fever and severe dengue (hemorrhagic fever and shock syndrome) (World Health Organization, 2024, 2025). Epidemiological reports have documented an unprecedented recent surge in the global spread of dengue, with more than 14 million cases reported worldwide in 2024 and a further increase in 2025; this reinforces the critical need for enhanced surveillance, diagnosis and therapeutic intervention (World Health Organization, 2024, 2025).

The DENV genome encodes three structural proteins – Capsid (C), Membrane (M) and Envelope (E) – and seven Non-Structural Proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5). Among these, Non-Structural Protein 1 (NS1) is a highly conserved glycoprotein with critical roles in viral replication, immune modulation, endothelial dysfunction and disease pathogenesis (Byk and Gamarnik, 2016; Sinha *et al*., 2024). NS1 is not a structural component of the virion but occurs in several forms, including membrane-bound monomers, intracellular monomers, secreted dimers and circulating hexamers during infection. Because NS1 is detectable in blood during the early phase of illness, it is a useful marker for the diagnosis and monitoring of dengue (Glasner *et al*., 2017; Muller and Young, 2013).

Recent studies have emphasized the multifunctional role of NS1 in host–virus interaction. Secreted NS1 compromises the integrity of the endothelial barrier, activates the complement pathway and induces vascular leakage, a hallmark of severe dengue. NS1 has also been proposed to possess immune-evasive properties that facilitate viral persistence and replication within the host (Glasner *et al*., 2017; Muller and Young, 2013; Zhang *et al*., 2023). These diverse biological activities have led to NS1 being considered a vaccine candidate, an antiviral drug target and an immunotherapeutic agent.

Advances in computational biology and bioinformatics have made it possible to characterize viral proteins through sequence

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comparison and structure-based approaches. *In silico* methods provide insight into protein architecture, physicochemical properties, secondary structure organization, intrinsically disordered regions, membrane interactions and functional motifs (Si and Gong, 2026). Such analyses are particularly valuable for identifying structural determinants of stability, antigenicity and activity, and can reduce the time and cost of experimental investigation (Heo and Feig, 2020; Janes and Beltrao, 2024; Jumper *et al*., 2021). Building on this approach, our group has recently characterized the dengue NS5 methyltransferase across all four serotypes using integrated structural and physicochemical profiling (Alwabli, 2026a, 2026b; Bukhari, 2026a, 2026b), investigated its enzymatic activity, inhibitor discovery and ligand interactions (Alwabli, 2025; Alwabli *et al*., 2019b; Alwabli and Qadri, 2021), and profiled the gene-expression signature and molecular landscape of dengue infection (Alwabli, 2024; Alwabli *et al*., 2019a). Comparable computational structure-based profiling by our group has addressed the dengue HABD domain and related flaviviral and coronaviral protein targets (Alwabli, 2022, 2023; Patel *et al*., 2022); the present study extends this framework to the NS1 protein.

Given its biological significance and therapeutic potential, structural and functional characterization of NS1 is essential. The present study therefore examines the Dengue Virus Serotype 2 (DENV-2) NS1 protein using an *in silico* approach. To gain insight into its molecular properties and potential contribution to dengue pathogenesis, sequence features, secondary structural elements, membrane topology, intrinsically disordered regions, solubility, electrostatic properties and residue polarity distributions were analyzed.

MATERIALS AND METHODS

Genome and Domain Organization of Dengue Virus

The organization of the dengue virus proteome was analyzed by identifying and categorizing the viral proteins into the structural proteins – Capsid (C), Membrane (M) and Envelope (E) - and the Non-Structural Proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5). The DENV-2 NS1 protein sequence was retrieved from the NCBI Protein database (accession no. CAA78918). The complete 380-residue amino acid sequence was assembled and mapped onto the framework of the viral polyprotein.

Secondary Structure Prediction Using PSIPRED

Secondary structure was predicted using the PSIPRED server. The protein sequence was examined for α-helices, β-strands and coil regions, and a per-residue confidence score was obtained. The sequence plot was generated to display the structural assignment of each residue and to highlight potential functional features. The PSIPRED cartoon representation (Buchan *et al*., 2013) was then analyzed to establish the distribution of secondary structural elements along the protein sequence.

Sequence Feature Mapping, Disorder and Membrane Topology Prediction

A sequence feature map was generated to determine the distribution of the predicted α-helices and β-sheets along the protein sequence (He *et al*., 2021). Intrinsically disordered regions were predicted using the DISOPRED3 algorithm, with residues assigned as disordered at the default score threshold of 0.5 (http://bioinf.cs.ucl.ac.uk/psipred/). Membrane topology and membrane-associated regions were then screened for signal peptides, transmembrane helices, and extracellular and cytoplasmic segments using the MEMSAT-SVM tool (http://bioinf.cs.ucl.ac.uk/psipred/) (Nugent and Jones, 2009).

Physicochemical and Surface Property Analysis

Amino acid composition and sequence-derived parameters were used to predict protein solubility and to estimate the probability of soluble expression (Chen *et al*., 2023; Wang and Zou, 2023; Wu and Yu, 2021). Residue-based analyses were performed using a sliding-window approach to determine charge distribution and folding propensity along the protein sequence (https://protein-sol.manchester.ac.uk/). The three-dimensional protein model was subsequently analyzed to determine the electrostatic potential of the protein surface, presented as a potential field map indicating regions of positive and negative electrostatic potential. In addition, the surface distribution of non-polar and polar residues was calculated in order to define hydrophobic and hydrophilic regions (Hebditch and Warwicker, 2019). To characterize interaction sites and surface properties, the maximum non-polar/polar residue ratio was calculated and compared across the different structural regions.

RESULTS

Genome Organization of Dengue Virus

The structural organization of the Dengue Virus (DENV) genome and virion is presented in Figure 1. The mature virion comprises an Envelope (E) and a Membrane (M) protein surrounding a nucleocapsid that encloses the single-stranded viral RNA genome. The viral polyprotein is cleaved into three structural proteins - Capsid (C), Membrane (M) and Envelope (E) - and seven Non-Structural Proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) (Figure 1). The DENV-2 NS1 protein was 380 amino acids in length (accession no. CAA78918). The NS1 coding region follows the structural protein block and is implicated in viral replication and pathogenesis. The retrieved amino acid sequence contained no interruptions, consistent with the conserved nature of DENV-2 NS1. Overall, the genomic organization shows a clear separation between structural and non-structural proteins, with NS1 positioned as the first non-structural protein and a principal target for functional study.

Secondary Structure Prediction of the DENV-2 NS1 Protein

PSIPRED analysis of the 380-residue DENV-2 NS1 protein indicated a typical α/β architecture composed of α-helices, β-strands and coil regions. Helical segments were located predominantly at residues 65-100 and 200-230, whereas β-strands were distributed across the N-terminal, central and C-terminal portions of the sequence (Figure 2A). The PSIPRED cartoon representation confirmed the alternating arrangement of helices and β-sheets, with high confidence scores across most residues (Figure 2B). Extended β-sheet regions were identified at residues 110-150 and 235-350 and near the C-terminus, and may contribute to structural stability. The predominance of ordered secondary structure connected by flexible coil regions indicates that NS1 is a structurally stable protein, consistent with roles in dimerization, viral replication, immune modulation and host–virus interaction.

Sequence Feature Map, DISOPRED3 and MEMSAT-SVM Analyses

The sequence feature map of DENV-2 NS1 confirmed a typical α/β architecture in which the predicted α-helices clustered within the N-terminal and central regions (approximately residues 1-120 and 210-280), while β-sheets were distributed throughout the protein and were particularly prominent in the central and C-terminal regions (Figure 3A). This organization provides a stable framework that is important for protein folding and functional interaction. DISOPRED3 analysis identified only limited intrinsically disordered regions, confined mainly to the N- and C-termini (residues 1-25 and 365-380, respectively), with the remainder of the protein predicted to be ordered; this indicates a relatively stable structure in which the flexible terminal regions may contribute to protein-protein interaction and functional adaptability (Figure 3B). MEMSAT-SVM analysis predicted an N-terminal signal peptide and no transmembrane helices, indicating that NS1 is a secreted, membrane-associated

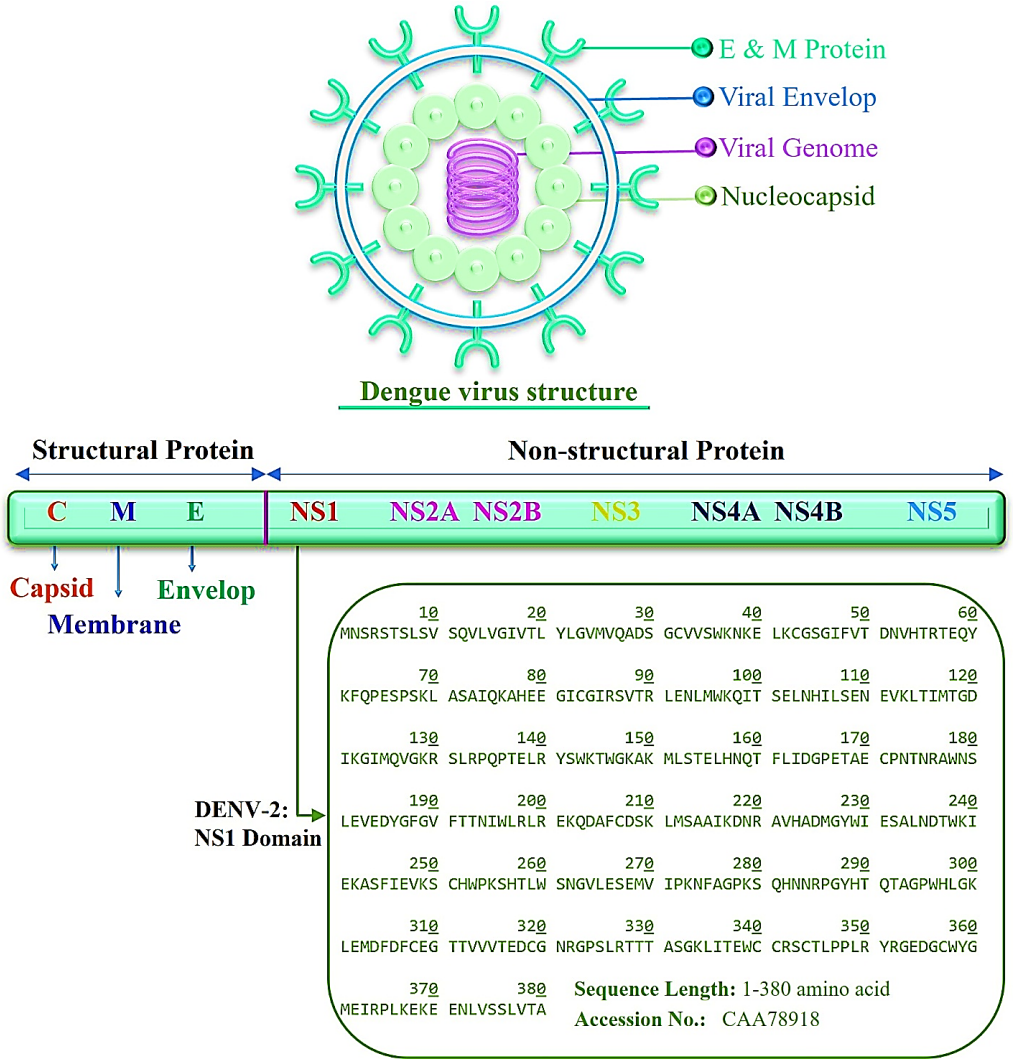


Figure 1: Structural organization of the dengue virus and genomic protein map, showing the structural (C, M, E) and non-structural (NS1-NS5) proteins together with the amino acid sequence of the DENV-2 NS1 domain (accession no. CAA78918).

glycoprotein rather than an integral membrane protein. The hydrophobicity profile showed predominantly moderately hydrophilic segments interspersed with relatively hydrophobic stretches, consistent with membrane association through hydrophobic interaction rather than membrane insertion (Figure 3C). Taken together, these results indicate that NS1 is a structurally stable protein with multiple functions in viral replication, immune evasion and host–pathogen interaction.

Physicochemical and Surface Property Analysis

DENV-2 NS1 was predicted to be a highly soluble protein, with a favorable amino acid composition characterized by a low content of hydrophobic residues, a balanced charge distribution, and ordered and flexible regions that would support correct folding and intermolecular interaction (Figure 4A). Electrostatic surface mapping revealed several potential electrostatic interaction interfaces with membranes that may serve as sites for host recognition, immune modulation and viral replication (Figure 4B). Hydrophobicity analysis showed an uneven distribution of polar and non-polar residues: predominantly polar surface regions, consistent with the high predicted solubility, together with hydrophobic patches that may mediate membrane association, oligomerization and stability (Figure 4C). Leu346 was identified as the most hydrophobic residue in the local hydrophobicity profile

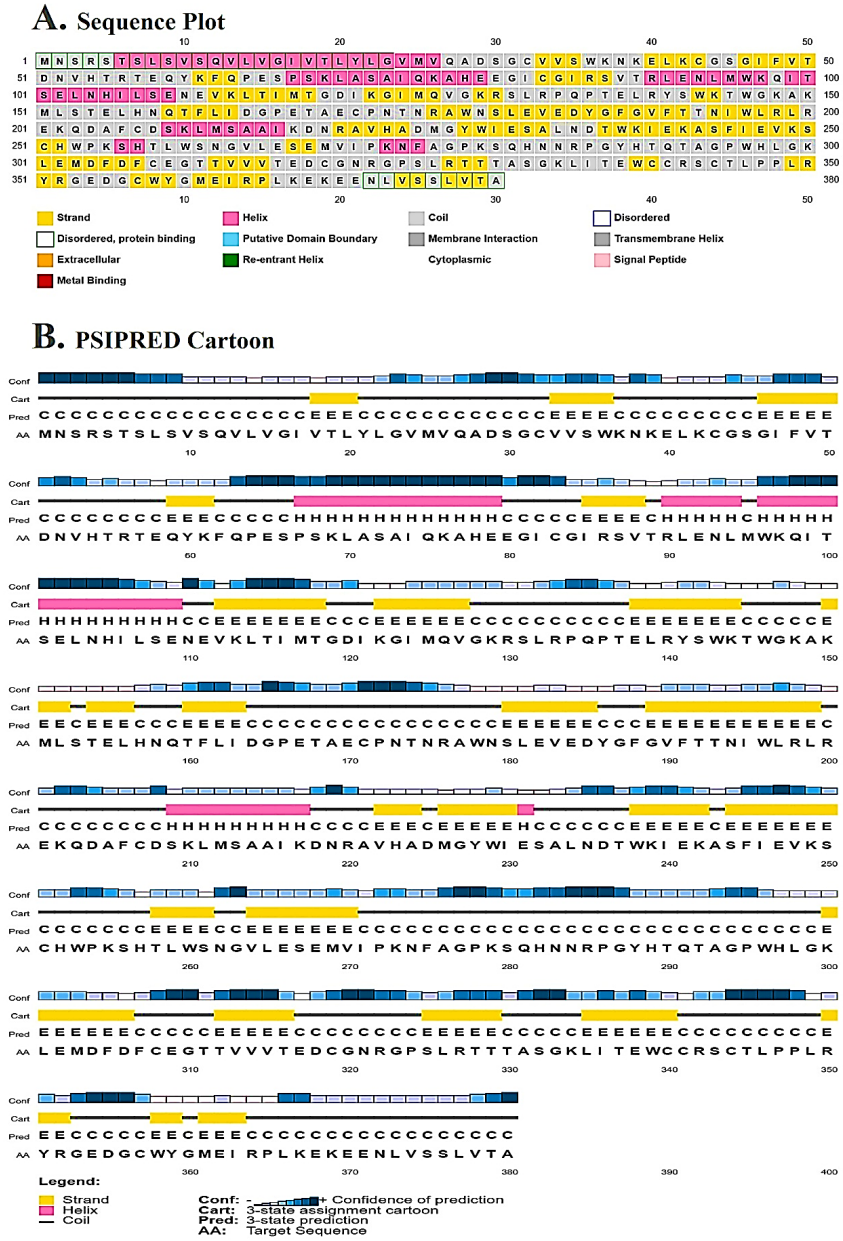


Figure 2: Secondary structure prediction of the DENV-2 NS1 protein by PSIPRED. (A) Per-residue secondary structure assignment along the 380-residue sequence, showing α-helices, β-strands and coil regions. (B) PSIPRED cartoon representation showing the alternating arrangement of helices and β-sheets with per-residue confidence scores.

(non-polar/polar ratio ≈ 3.16), suggesting a hydrophobic hotspot that may contribute to structural stabilization, protein assembly and intermolecular interaction (Figure 4D). Collectively, these results indicate that the solubility, electrostatic character and hydrophobic surface distribution of NS1 are consistent with its multifunctional role in the dengue virus life cycle.

DISCUSSION

In the present study, a detailed *in silico* characterization of the DENV-2 NS1 protein was performed, encompassing sequence organization, membrane-associated properties, structural features and physicochemical parameters. NS1 has critical roles in viral replication, immune evasion, pathogenesis and host-cell interaction, making it one of the most conserved and functionally important non-structural proteins of dengue virus. Recent studies have also highlighted NS1 as a diagnostic, therapeutic and vaccine development target (Morgan *et al*., 2024; Nasar *et al*., 2024; Tan *et al*., 2023). Analysis of the dengue viral genome confirmed that NS1 is the first non-structural protein encoded downstream of the structural proteins (C, M and E). The retrieved DENV-2 NS1 sequence comprised 380 amino acids, consistent with previously reported *Flavivirus* NS1 proteins when the N-terminal signal sequence is included. Conservation of NS1 across dengue serotypes indicates an essential role in the viral life

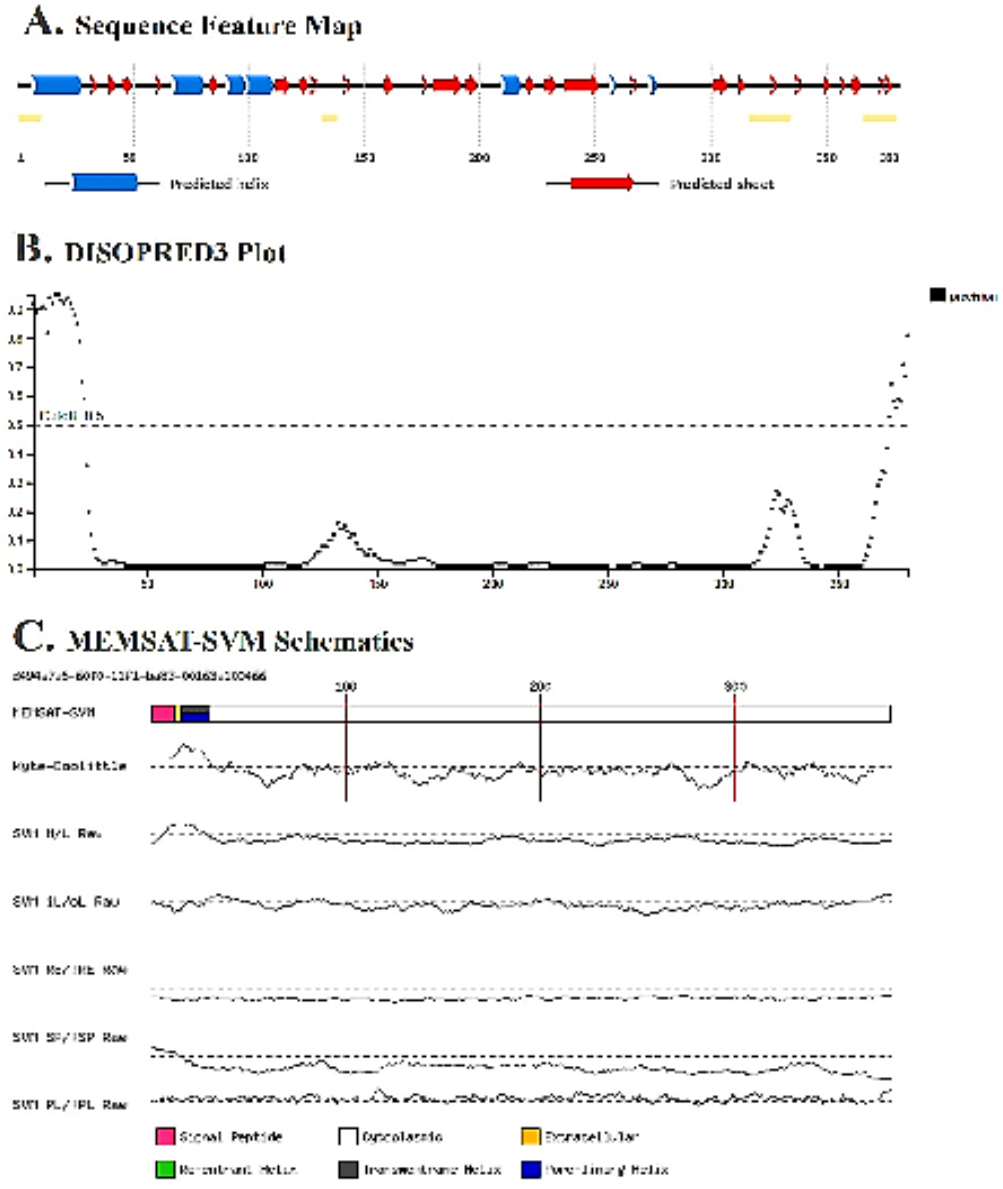


Figure 3: Sequence feature mapping, intrinsic disorder prediction and membrane topology analysis of the DENV-2 NS1 protein. (A) Sequence feature map showing the distribution of predicted α-helices and β-sheets. (B) DISOPRED3 disorder profile identifying intrinsically disordered regions at the N- and C-termini. (C) MEMSAT-SVM topology prediction and hydrophobicity profile, indicating an N-terminal signal peptide and the absence of transmembrane helices.

cycle and supports its potential as an antiviral target (Fan *et al*., 2014; Rastogi *et al*., 2016; Scaturro *et al*., 2015).

Secondary structure prediction revealed a mixed α/β architecture comprising several α-helices, β-sheets and coil regions, which is characteristic of flaviviral NS1 proteins and is required for structural stability and oligomerization. The abundance of β-sheet regions in the central and C-terminal domains is consistent with experimentally determined NS1 structures, in which dimer and hexamer formation involves β-sheet-rich domains. The predicted helices, located mainly in the N-terminal and central portions of the protein, may be important for folding and intermolecular interaction (Minami *et al*., 2017; Sakuma *et al*., 2024).

Disorder prediction indicated that the majority of NS1 is structurally ordered, with intrinsic disorder confined to the N- and C-termini. Terminal disordered regions are frequently involved in molecular recognition and protein-protein interaction, suggesting that these segments may mediate interaction with the viral replication complex or with host cell factors. The predominance of ordered regions is consistent with the requirement for NS1 to oligomerize into dimers and higher-order assemblies during viral replication and secretion (Holehouse and Kragelund, 2024; Pajkos *et al*., 2023; Zhang *et al*., 2023).

MEMSAT-SVM analysis predicted no transmembrane helices in the mature NS1 sequence but identified an N-terminal signal peptide. This is consistent with NS1 biology, since the protein is synthesized in the endoplasmic reticulum and secreted as a soluble glycoprotein rather than functioning as an integral membrane protein. Membrane association of NS1 has previously been attributed to hydrophobic interaction and oligomeric assembly rather than to permanent membrane insertion (Muller and Young, 2013; Zhang *et al*., 2023).

NS1 was also predicted to possess high solubility, supported by positive solubility scores from the prediction models applied. The heterogeneous distribution of charged residues and the electrostatic surface potential indicate discrete interaction interfaces that may participate in host-cell binding, immune modulation and viral replication. Surface electrostatic mapping identified patches of both positive and negative charge, a feature

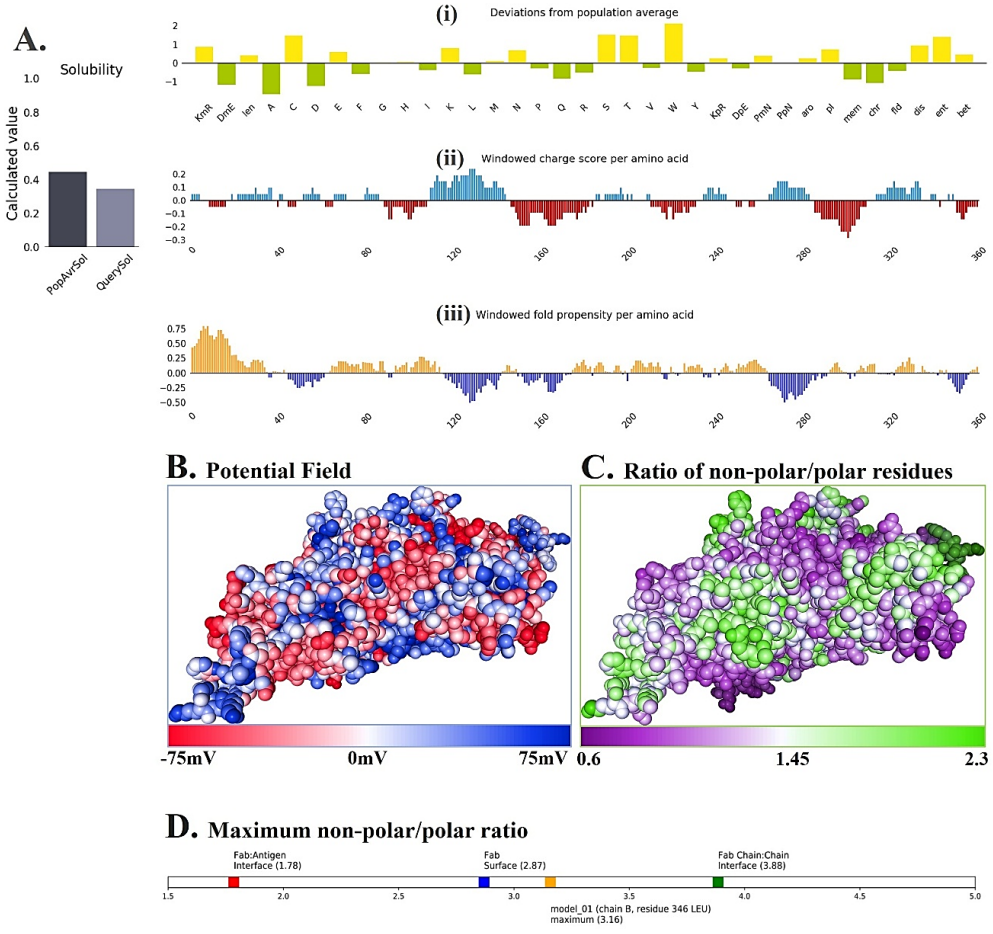


Figure 4: Physicochemical and surface property analysis of the DENV-2 NS1 protein. (A) Solubility prediction together with charge distribution and folding propensity along the sequence. (B) Electrostatic potential mapped onto the protein surface, showing regions of positive and negative potential. (C) Surface distribution of non-polar and polar residues. (D) Local hydrophobicity profile identifying Leu346 as the principal hydrophobic hotspot (non-polar/polar ratio ≈ 3.16).

typical of proteins engaged in diverse molecular interactions; flaviviral NS1 has been reported to interact with complement proteins, endothelial cells and host immune factors (Glasner *et al*., 2017; Zhang *et al*., 2023).

Hydrophobicity analysis revealed a balanced distribution of polar and non-polar residues, with localized hydrophobic hotspots such as the region surrounding Leu346. This region may contribute to structural stabilization, oligomerization and membrane association. The prevalence of polar surface residues accounts for the soluble character of NS1, whereas the hydrophobic patches may underlie its dimeric and secreted oligomeric forms. Recent structural studies have demonstrated that NS1 adopts several oligomeric states, including dimers, tetramers and hexamers, in which hydrophobic interactions are important for stabilization (Almeida *et al*., 2021; Cedano *et al*., 2025).

Taken together, the computational analyses indicate that DENV-2 NS1 is structurally stable and highly soluble, with low intrinsic disorder and defined electrostatic and hydrophobic interaction surfaces. These features are consistent with its multifunctional roles in viral replication, immune evasion, endothelial dysfunction and pathogenesis, and support the growing interest in NS1 as a promising vaccine target, therapeutic target and biomarker of dengue virus infection.

CONCLUSION

This study presents a comprehensive *in silico* characterization of the DENV-2 NS1 protein, a non-structural glycoprotein of 380 amino acids with a high proportion of α/β secondary structure and a low proportion of intrinsically disordered regions. Structural analysis indicates that NS1 is a stable, soluble protein composed of organized helices, β-sheets and flexible loop segments capable of supporting its essential viral functions. Membrane topology prediction identified an N-terminal signal peptide but no transmembrane helices, supporting the classification of NS1 as a secreted, membrane-associated protein rather than an integral membrane protein. Physicochemical and surface property analyses revealed a balanced charge distribution, favorable solubility, and distinct electrostatic and hydrophobic regions that may permit oligomerization, membrane interaction and host–pathogen communication. Collectively, the structural stability, surface heterogeneity and interaction-prone character of DENV-2 NS1 are consistent with multifunctional roles in viral replication, immune modulation and disease progression. These findings support the value of NS1 as a target for antiviral therapy, vaccine design and diagnostics, and provide a framework for further experimental and structure-based investigation (Alwabli *et al*., 2019c).

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ABBREVIATIONS

DENV-2: Dengue Virus Serotype 2; **NS1:** Non-Structural Protein 1; **C:** Capsid; **M:** Membrane; **E:** Envelope; **IDR:** Intrinsically disordered region; **SVM:** Support Vector Machine; **MEMSAT:** MEMbrane protein Structure and Topology; **DISOPRED3:** DISOrdered Protein PREDiction version 3; **PSIPRED:** PSI-blast based secondary structure PREDiction.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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AUTHOR CONTRIBUTIONS

Ghadeer Bukhari: Conceptualization; Methodology; Data curation; Formal analysis; Investigation; Writing - original draft. **Afaf Salim Alwabli:** Methodology; Formal analysis; Validation; Investigation; Writing - review and editing.

SUMMARY

DENV-2 NS1 is a highly conserved 380-amino-acid glycoprotein with low intrinsic disorder, a stable α/β-rich architecture and high predicted solubility. It is secreted and membrane-associated, possessing an N-terminal signal peptide but no transmembrane helices. Distinct electrostatic and hydrophobic surface properties indicate roles in oligomerization and host interaction. Together, these properties underline the contribution of NS1 to dengue virus replication and pathogenesis and its potential as an antiviral, vaccine and diagnostic target.

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