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Original Article

Quantitative Physicochemistry Meets Epitope Prediction: A Blueprint for Peptide Vaccine Design against DENV-3 NS5 MTase

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ABSTRACT

Background: DENV NS5 is the most conserved viral protein; its N-terminal Methyltransferase (MTase) is essential for RNA capping and a prime drug target. The MTase domain mediates methylation of the viral RNA cap, ensuring genome stability, efficient translation, and evasion of host innate immune responses. **Objectives:** To computationally profile the DENV-3 NS5 Methyltransferase (MTase) domain (aa 1-262) in terms of key physicochemical properties, signal peptide/propeptide cleavage potential, and phosphorylation site distribution. To map and prioritize cross-validated linear B-cell epitopes on MTase as candidates to support structure-informed antiviral targeting and peptide-based vaccine/diagnostic development. **Materials and Methods:** We analyzed the Methyltransferase (MTase) domain of DENV-3 NS5 (amino acids 1-262) using physicochemical property analysis, SignalP software for predicting signal peptide or propeptide cleavage, NetPhos for potential phosphorylation site mapping, and multiple tools for linear B-cell epitope prediction, including the Emini surface accessibility scale, BepiPred 2.0, and the Kolaskar-Tongaonkar method. **Results:** The MTase domain (theoretical pI ~9.4) shows alternating charged/flexible segments with near-average predicted solubility and no detectable signal peptide or propeptide cleavage site. High-confidence phosphorylation hotspots cluster at ~3-45, 90-110, and 150-190 and extend toward the C-terminus. Cross-validated linear epitopes concentrate at ~10-59, 148-170, and 185-204/226-259. **Conclusion:** The DENV-3 NS5 MTase lacks secretory signals. It exhibits discrete phosphorylation and epitope clusters, yielding peptide candidates for diagnostic and vaccine applications. The study also defines conserved, accessible surfaces for structure-based inhibitor design. This workflow can be generalized to other flaviviral MTases.

Keywords: Bepipred, Dengue virus, Epitope mapping, MTase, Phosphorylation, Signal peptide.

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INTRODUCTION

Dengue Virus (DENV) is the most common arthropod-borne virus in humans, causing 100-400 million infections each year in more than 120 countries. Illness can range from mild fever to severe complications like plasma leakage, bleeding, and organ damage. The spread of *Aedes* mosquitoes, rapid urban growth, and climate change have led to frequent outbreaks that put pressure on public health systems in tropical and subtropical areas. Even with improved mosquito control and better diagnostics, prevention is not yet complete. Approved vaccines work differently depending on a person's previous exposure and age. There is still no widely used antiviral treatment made specifically for DENV. Due to these challenges, researchers are exploring new approaches, such as designing drugs that target crucial viral enzymes and identifying new components for future vaccines and diagnostic tests (Biswal *et al.*, 2019; Bhatt *et al.*, 2013; Guzman *et al.*, 2024; Sridhar *et al.*, 2018).

The DENV genome is a positive-sense RNA approximately 11 kilobases in length. Its codes for a polyprotein that is split into three structural proteins (core [C], precursor membrane/membrane [prM/M], and envelope [E]) and seven non-structural proteins (NS1 to NS5). NS5 is the most conserved and has several functions. It contains two main parts: the N-terminal Methyltransferase (MTase) domain, which adds methyl groups to RNA to help cap the viral RNA and avoid the immune system, and the C-terminal RNA-dependent RNA polymerase (RdRp) domain, which makes new RNA strands. The MTase creates a cap structure called m^7GpppAm, which is similar to the cap on host cell RNA. This helps the viral RNA stay stable, be translated, and escape immune detection, while the RdRp copies the viral genome. Inhibiting the MTase activity of NS5 weakens the virus,

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making it a key target for drug development (Egloff *et al*., 2002; Lindenbach *et al*., 2003).

The N-terminal MTase part of NS5 has a Rossmann-like fold, a shape often found in enzymes that bind nucleotides. It possesses a catalytic tetrad, which consists of four essential amino acids crucial for the reaction, and a pocket that accommodates the cap structure. This setup allows the transfer of methyl groups from the cofactor S-Adenosylmethionine (SAM) to two spots on the viral RNA: the guanosine cap and the 2'-hydroxyl group of the first nucleotide's ribose. Studies of the structure show conserved grooves where RNA binds and connections between SAM binding and product release (Medvedev *et al*., 2021; Yap *et al*., 2010; Zhao *et al*., 2015). Researchers have tested inhibitors that copy SAM, mimic the cap, or block these binding sites. However, it remains challenging to identify inhibitors that are effective, can enter cells, and do not affect similar enzymes in the host. Detailed physical and sequence analysis of DENV-3 NS5 MTase can help identify regions that are easily accessible, conserved among virus strains, and less likely to change, which aids in guiding the design of new inhibitors (Ahmed-Belkacem *et al*., 2024; Alwabli *et al*., 2021; Alwabli *et al*., 2019; Dong *et al*., 2014).

Accurately predicting linear B-cell epitopes, which are short segments of viral proteins that antibodies bind to, is critical for developing vaccines and diagnostic tests that target specific antigens. Good epitopes should be easily accessible to antibodies, capable of triggering an immune response, and located on the surface of the protein. Since NS5 is found inside infected cells, its B-cell epitopes are likely to be seen by the immune system during virus replication or when infected cells die and release their contents. To select the most appropriate epitopes for diagnostics, it is beneficial to combine data on surface exposure, immune response, and other key characteristics, which can also mitigate issues with cross-reactivity from related viruses (Liu *et al*., 2024; Israeli *et al*., 2024; Ras-Carmona *et al*., 2022).

In this investigation, we provide a detailed examination of DENV-3 NS5 MTase, analyzing its physical and chemical properties, signaling pathways, and potential to elicit an immune response. We highlight important features, such as molecular weight, isoelectric point, and solubility, to aid in laboratory work. Our analysis reveals that there is no typical N-terminal signal peptide, which aligns with its role in the cell's cytosol. We also identified phosphorylation motifs that may have regulatory functions and warrant further investigation. Using predictive models, we selected B-cell epitopes that are both likely to trigger an immune response and possess favorable properties for peptide and antibody production. These results support the use of medicinal chemistry to target conserved regions of the MTase.

MATERIALS AND METHODS

Schematic Representation of Dengue Virion

This schematic of the dengue virus emphasizes the NS5 region, a pivotal protein (non-structural) with MTase activity that caps the viral RNA, improving stability and immune evasion. The sequence (a.a) of the NS5-MTase domain, comprising residues 1-262, was sourced from GenBank for molecular accuracy. Other features include the lipid membrane, envelope (E), membrane (M), nucleocapsid (C), the single-stranded RNA genome, the 5′ UTR cap, and 3′ UTR poly(A) tail. The genome contains one Open Reading Frame (ORF) that encodes a polyprotein, which is subsequently cleaved into structural (C, prM, E) and Non-Structural (NS1-NS5) proteins (El Sahili *et al*., 2017; Li *et al*., 2022).

Sequence Analysis

The protein FASTA was studied using ExPASy ProtParam to determine its molecular weight, theoretical pI, amino acid percentages/counts, and proportions of carbon, hydrogen, nitrogen, oxygen, and sulfur. Protein-Sol was used to predict solubility, providing a solubility score and highlighting feature differences (Hoogland *et al*., 1999; Hebditch *et al*., 2017; Wilkins *et al*., 1999). Default sliding-window plots for net charge and fold tendency were created to highlight regions that enhance or hinder solubility. Results were summarized in bar charts for composition and Protein-Sol panels. All steps can be repeated with the same FASTA file and default settings; no structure templates or modeling were used.

Signal peptide cleavage and phosphorylation sites

Sequence-only analysis of the FASTA sequence was performed. Putative signal-peptidase and proprotein-convertase cleavage sites were scored with ProP 1.0; positions above threshold were exported and plotted as vertical bars. N-terminal signal peptides were assessed with SignalP-NN; C/S/Y traces over the first 70 residues, and the server (SignalP 6.0 - DTU Health Tech - Bioinformatic Services) D-score classification was exported directly. Ser/Thr/Tyr phosphorylation sites were predicted with NetPhos 3.1, and residues scoring above the cut-off were plotted by position with color coding. Outputs from all servers were collated in spreadsheets and visualized without smoothing or normalization (Bonne Køhler *et al*., 2020; Blom *et al*., 2004; Duckert *et al*., 2004; Van de Ven *et al*., 1993).

Linear B-cell epitope mapping

The study screened the NS5 methyltransferase fragment of DENV-3 using sequence-only predictors on the IEDB server. Three methods were used: Emini surface accessibility, BepiPred-2.0, and the Kolaskar and Tongaonkar antigenicity index. Analyses used server defaults. Peptide segments were generated by applying threshold scores. Segments shorter than

seven residues were discarded to minimize false positives. Segments identified by two or more methods were considered higher-confidence candidates for peptide ELISA or immunogen design. All computations were scripted for reproducibility. The predictions will be experimentally validated (Emini *et al.*, 1985; Jespersen *et al.*, 2017; Zhou *et al.*, 2007).

RESULTS

Architecture and genome organization

The figures depict the canonical architecture and genome organization of dengue virus: an enveloped virion with a lipid membrane bearing the Envelope (E) and Membrane (M) proteins surrounding a nucleocapsid (C) that packages a positive-sense RNA genome capped at the 5′ end (m^7GpppA) and terminating with a 3′UTR poly(A) tail (Figure 1A). The genomic map displays a single open reading frame that is translated into a polyprotein and subsequently cleaved into structural proteins (C-prM-E) and non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5). The accentuated region corresponds to DENV serotype-3 NS5 N-terminal MTase (residues 1-262), for which the complete amino-acid sequence is provided to enable subsequent structural/functional analyses (Figure 1B).

Physicochemical profile of DENV-3 NS5 methyltransferase

The NS5 methyltransferase (1-262 aa) has an elemental composition dominated by hydrogen (2095 atoms) and carbon (1309). Nitrogen (379), oxygen (382), and sulfur (14) contribute much smaller amounts (Figure 2A). The polypeptide is 262 residues (29,703.18 Da) with a theoretical pI of 9.44. It is enriched in basic/helix-friendly residues, notably Lys, Leu, Ser, and Glu. Rare residues such as Cys, Met, Trp, and Tyr are underrepresented (Figure 2B). The scaled solubility profile is generally close to the population average.

There is modest N-terminal depression, followed by alternating soluble and less-soluble segments (Figure 2C-a). The windowed charge plot reveals central acidic stretches, shown as negative scores around the mid-region. These are flanked by basic clusters near the termini (Figure 2C-b). The fold-propensity track indicates mixed structural behavior: a lower propensity (more disorder) at the extreme N-terminus and midsection, near ~150-190, and a higher fold propensity (more ordered) across ~70-110 and ~200-240. This is consistent with a soluble, basic N-terminal enzyme domain punctuated by flexible loops (Figure 2C-c).

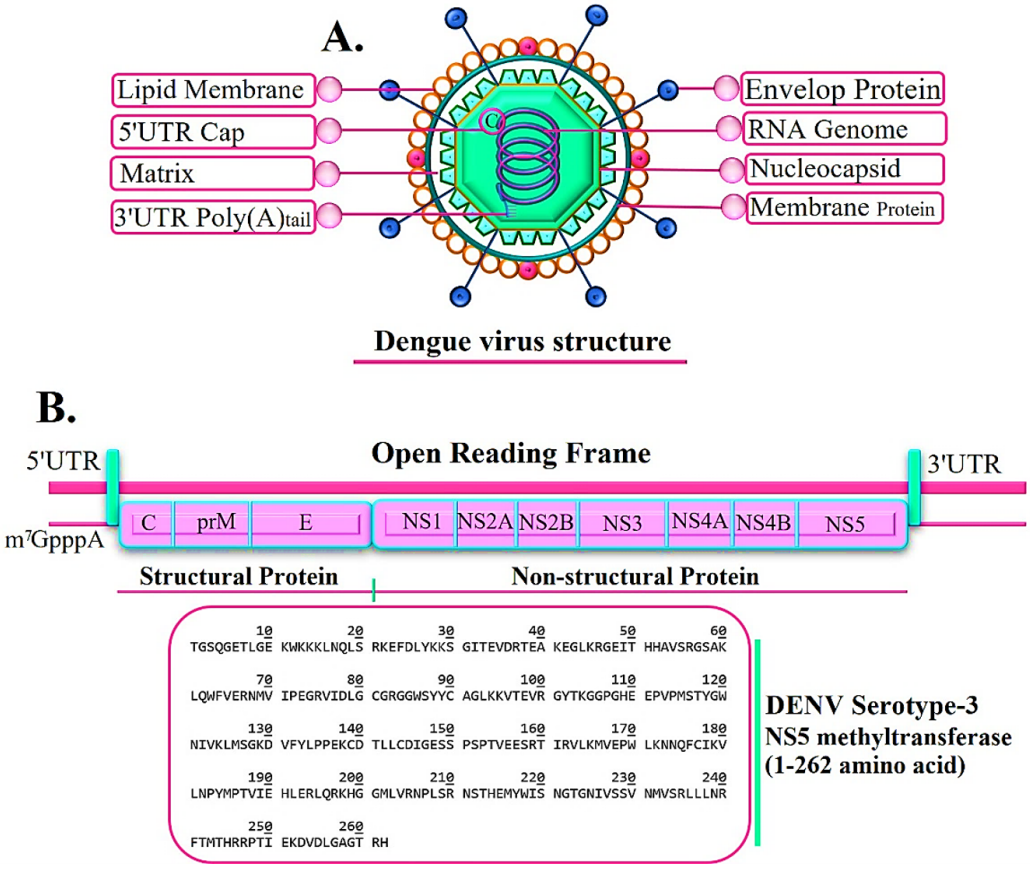

Figure 1: (A) Schematic of a dengue virion showing the lipid envelope with E and M proteins, and the nucleocapsid (C), which are encoded by the structural protein region of the genome. (B) Genome organization reveals that a single open reading frame encodes structural proteins and non-structural proteins. Specifically, the NS5-MTase from DENV serotype 3, highlighted by its sequence, is encoded in the non-structural region.

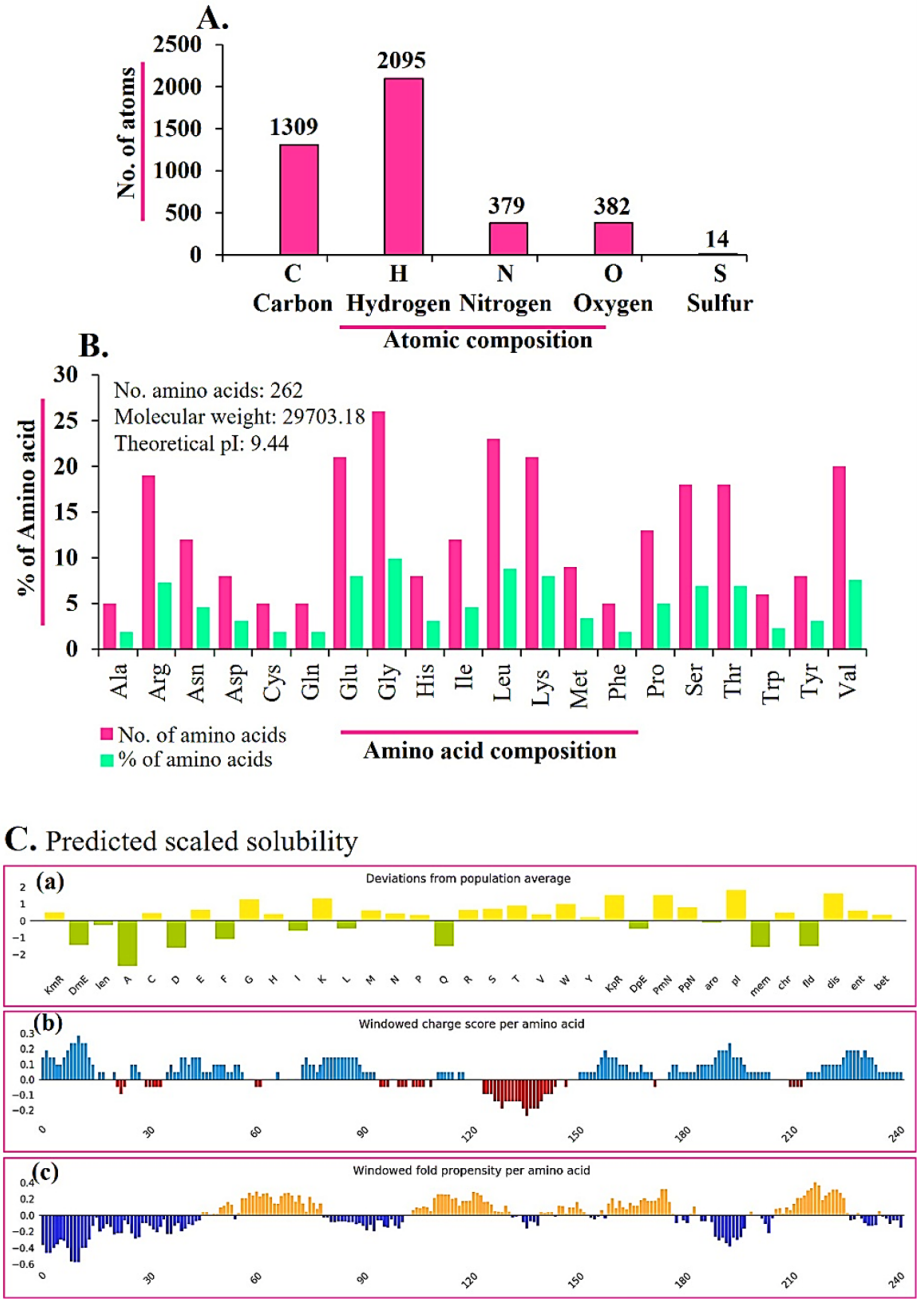

Figure 2: (A) Atomic counts for C, H, N, O, S; H and C predominate; S is scarce. (B) Amino-acid composition: counts (pink) and percentages (green); inset indicates length, molecular weight, and theoretical pI. (C) Scaled-solubility tracks: residue-wise deviation from average, windowed charge (blue >0 basic, red <0 acidic), and fold-propensity (orange >0 ordered, purple <0 disordered) along the N→C axis.

Sequence-based features of DENV-3 NS5 MTase

Across the NS5 methyltransferase N-terminal domain (1-262 aa), the signal/propeptide cleavage scan shows no peak surpassing the decision threshold at any position (Figure 3A), indicating the absence of a classical signal peptide or propeptide processing site. Consistently, signal peptide neural-network output for the first ~70 residues keeps the C, S, and Y scores well below their cutoffs (Figure 3B), arguing against an N-terminal signal sequence and supporting a non-secretory, intracellular enzyme. In contrast to the negative signal-peptide prediction, the NetPhos analysis identifies numerous phosphorylation candidates on serine and threonine and fewer on tyrosine with several residues exceeding the significance threshold (Figure 3C). These sites are not uniformly spread; instead, they form clusters in the N-terminal third (~35-45), the central portion (~90-110), and a broader span in the C-proximal half (~150-190), with additional isolated high-scoring residues toward the extreme C-terminus. The coexistence of multiple Ser/Thr hotspots suggests that this domain is amenable to multisite regulation. Taken together, the figures indicate that the DENV-3 NS5 methyltransferase lacks secretory targeting signals but contains

multiple high-confidence phosphorylation motifs, supporting a model in which post-translational modification, rather than membrane trafficking, predominates in modulating its activity and interactions.

Epitopic mapping

Epitopic profiling of the DENV-3 NS5 methyltransferase (1-262 aa) converges on several surface-exposed, antigenic stretches that recur across three predictors. Emini surface accessibility (Figures 4A-4B) highlights discrete peaks above threshold, led by a long N-terminal segment at residues ~10-30 (e.g., EKWKKKLNQLSRKEFDLYKKS, 21 aa) and additional accessible peptides at ~35-45 (KDRTEAKEGKI, 11 aa), ~153-159 (PTVIESR, 7 aa), ~192-199 (LERLQRKH, 8 aa), ~207-215 (PLSSPSTHE, 9 aa), and ~244-250 (IHRPPTI, 7 aa). BepiPred 2.0 (Figures 4C-4D) yields the broadest epitope at the extreme N-terminus (~5-59; 55 aa; sequence shown in the panel), followed by shorter positive windows at ~63-71, ~97-101, ~130-137, a prominent block at ~148-169, scattered sites across ~171-192, and C-terminal clusters around ~210-225 and ~242-259.

The Kolaskar and Tongaonkar antigenicity model (Figures 4E-4F) independently selects several overlapping peptides with above-average antigenic propensity, including regions near ~51-59 (e.g., IHHAVSRG, 9 aa), ~87-99 (SYYCAGLKKVTEV, 13 aa), ~120-126 (NVNKLAM, 7 aa), ~139-147 (DYVLPDPE, 8-9 aa), ~160-169 (CDILQDIG, 8-9 aa), ~174-183 (IRVLMKPEPW, ~10 aa), ~185-194 (LKNQFCIKIV, 10 aa), ~195-204 (MPVMEHLER, ~10 aa), and a C-terminal stretch at ~226-239 (IYSSVNMVSRLLNR, 14 aa). Collectively, the most emphatic consensus emerges at the N-terminus (residues ~10-59), in a

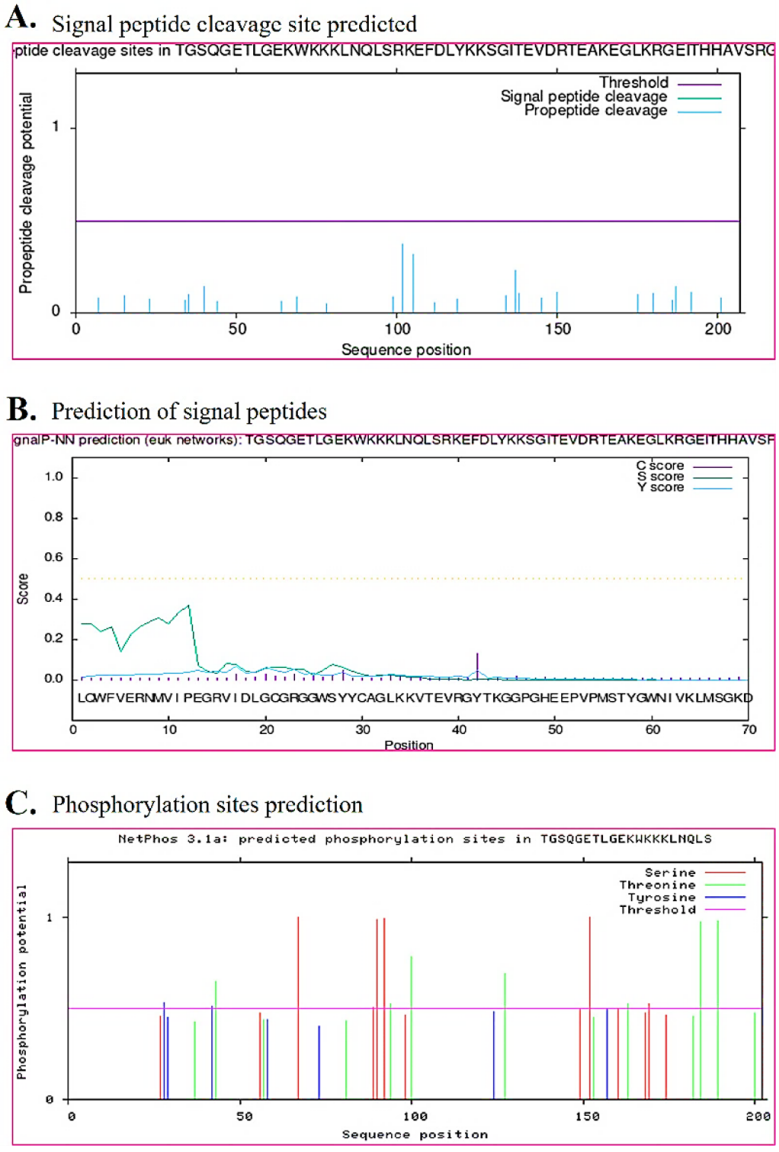

Figure 3: (A) Cleavage prospect is plotted across the sequence, with a horizontal threshold line marking the cutoff; as no peaks cross this limit, there is no classical signal or propeptide cleavage site. (B) C, S, and Y scores for the N-terminal region all remain below the dotted cutoff, further confirming the lack of an N-terminal signal peptide. (C) Per-residue phosphorylation potential is shown for Ser (red), Thr (green), and Tyr (blue), while the magenta line indicates the decision threshold.

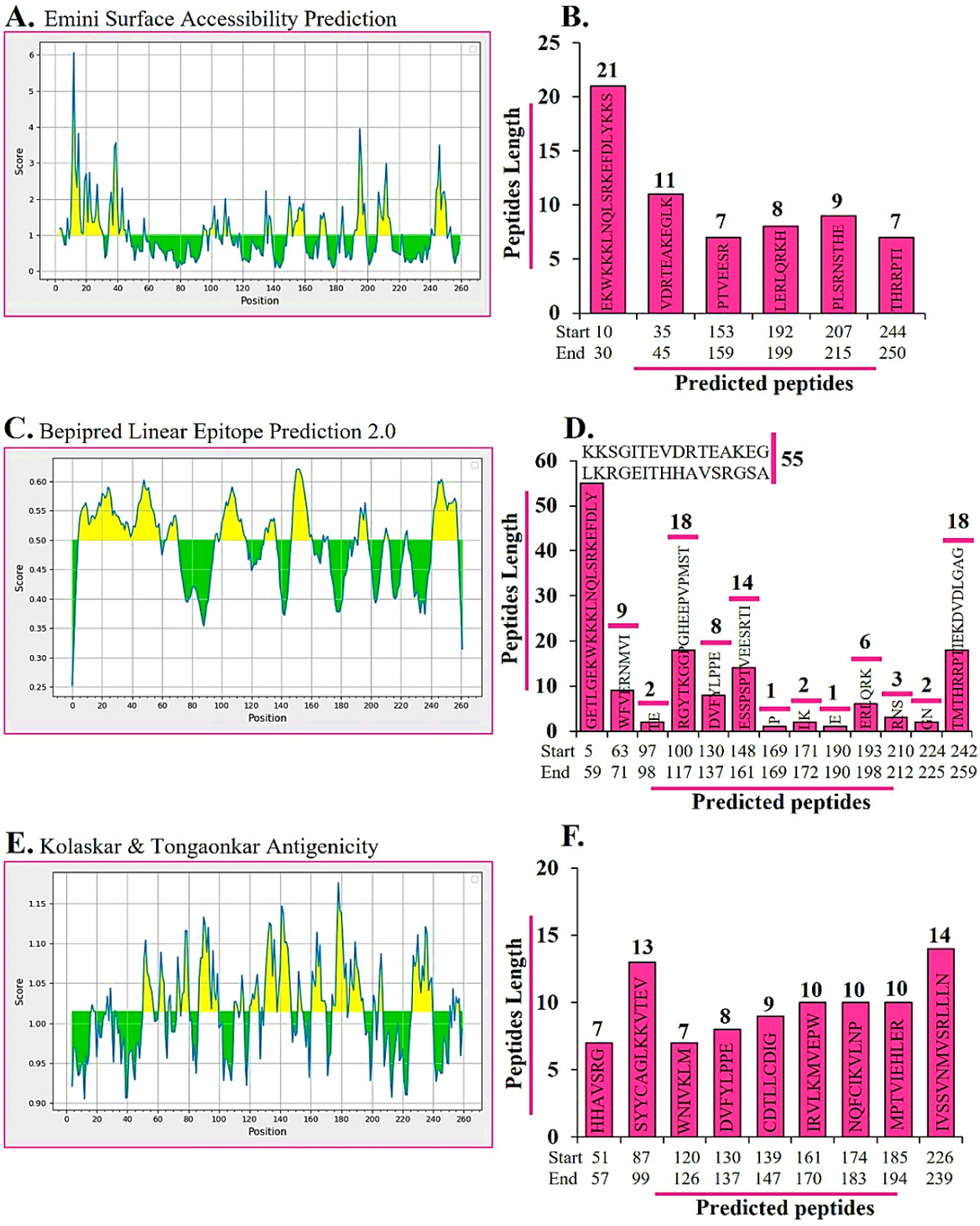

Figure 4: (A) Emini surface accessibility scores along the sequence (threshold line shown). (B) Surface-accessible peptides selected from A with start/end positions and lengths. (C) BepiPred 2.0 linear-epitope scores across the sequence (cutoff indicated). (D) BepiPred-derived epitope peptides with coordinates and lengths. (E) Kolaskar-Tongaonkar antigenicity profile (average-propensity threshold shown). (F) Antigenic peptides chosen from E with start/end positions and lengths.

mid-region spanning ~148-170, and toward the C-terminus (~185-204 and ~226-259), where accessibility (Emini) and antigenicity (Kolaskar) overlap with BepiPred predictions. These clusters represent high-confidence linear B-cell epitope candidates suitable for downstream peptide synthesis, immunoreactivity testing, and vaccine/diagnostic design.

DISCUSSION

The Dengue Virus (DENV) makes a single polyprotein that is split into three structural proteins (C, prM/M, and E) and seven non-structural proteins (NS1-NS5). NS5 is the largest and most conserved of these proteins. It has an N-terminal Methyltransferase (MTase) and a C-terminal RNA-dependent

RNA polymerase (RdRp). This study looks at the MTase domain (residues 1-262). The MTase domain alone can catalyze RNA cap methylation. It is often produced as a soluble domain for biochemical studies (Alwabli, 2025; Alwabli, 2024; Klema *et al*., 2016).

The MTase has a basic theoretical pI of about 9.4. It shows a balanced mix of polar and charged residues. Protein-Sol analysis (Hebditch *et al*., 2017) found that its solubility is close to the average for *E. coli* expression. The analysis also found alternating charge clusters. These clusters help the protein fold correctly and stay soluble in the cytosol. These features are similar to the Rossmann-like fold seen in flaviviral MTases that bind S-Adenosyl-L-Methionine (SAM) and capped RNA. SignalP and ProP showed there are no signal peptides or propeptide cleavage sites. This fits with NS5 being located inside cells at replication membranes (Duckert *et al*., 2004; Almagro Armenteros *et al*., 2019; Liu *et al*., 2010; Nielsen, 2017).

Mapping showed the conserved KDKE catalytic tetrad (K61-D146-K182-E218). This group of residues is the main site for the N7 and 2′-O methylations that create the viral type I RNA cap (Bhatnagar *et al*., 2023; Zhou *et al*., 2007). Each residue is needed for the reaction. D146 is especially important for N7 methylation. NetPhos 3.1 (Blom *et al*., 2004) found several possible Ser/Thr/Tyr phosphorylation sites. These findings align with earlier reports that NS5 phosphorylation is linked to nuclear movement and immune evasion (Cheng *et al*., 2024; Kumar *et al*., 2013; Zou *et al*., 2015).

B-cell epitope mapping used Emini surface accessibility (Emini *et al*., 1985), BepiPred 2.0 (Jespersen *et al*., 2017), and the Kolaskar-Tongaonkar antigenicity index. This study found overlapping linear antigenic segments in the N-terminal region (about residues 10-30), a central loop, and the C-terminal region (Kolaskar and Tongaonkar, 1990). These peptides could be used as diagnostic antigens for ELISA tests. NS5 is not found on the surface of virions (Wahala and Silva, 2011). Sequence-based methods are not always precise, but consensus predictions still provide useful leads for serological testing. They also help check for cross-reactivity with other flaviviruses, like Zika or West Nile (Alwabli *et al*., 2019; Alwabli *et al*., 2025; Narayan *et al*., 2016; Patel *et al*., 2022).

In summary, the DENV-3 NS5 MTase (aa 1-262) is likely a soluble enzyme with a conserved KDKE catalytic core, predicted phosphorylation sites, and defined antigenic regions. These features support its suitability for recombinant cytosolic expression and structural research. Targeting the conserved SAM/cap-binding pocket may facilitate the design of inhibitors and the development of antiviral drugs.

CONCLUSION

The DENV-3 NS5 methyltransferase is a cytosolic enzyme well-suited for studies of recombinant proteins and the design of inhibitors. The Methyltransferase (MTase) domain, located in Nonstructural Protein 5 (NS5), has been analyzed for its amino acid sequence. Analytical data show a molecular weight of about 29.7 Kilodaltons (kDa) and a theoretical isoelectric point (pI) of 9.44. Protein-Sol predicts that its average solubility is identical to that of characteristic proteins. The structure features a Rossmann-like core fold and a nucleotide-binding domain. Surface loops accommodate affinity tags or truncations without concerning catalytic motifs. Sequence analysis identifies consensus linear B-cell epitope regions recognized by antibodies in the N-terminus, central loop, and C-terminus. Since NS5 is not exposed on the viral surface, these epitopes are suitable for laboratory diagnostic peptide assays. Data support expressing the MTase as a soluble, cytosolic protein, preferably with an N-terminal solubility-enhancing tag placed away from the catalytic KDKE tetrad. Next steps include assays for binding cofactors and capped RNA, phosphoproteomic analysis of phosphorylation patterns, development of serological diagnostics, and structure-guided discovery of MTase inhibitors.

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ABBREVIATIONS

MTase: Methyltransferase; **DENV:** Dengue virus; **SAM:** S-adenosylmethionine; **E:** Envelope; **M:** Membrane; **C:** Nucleocapsid; **kDa:** Kilodaltons; **pI:** Isoelectric point.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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ETHICAL APPROVAL

This research did not relate to humans or animals.

AUTHOR CONTRIBUTION

Afaf Salim Alwabli: Conceptualization; Methodology; Data curation; Formal analysis; Investigation; Writing original draft.

GENERATIVE AI STATEMENT

The author declares that generative AI tools, including Grammarly and QuillBot, were used solely to enhance the language and clarity of this work, and takes full responsibility for the accuracy and integrity of the content.

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