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Original Article

# Analysis of DENV-2 NS5 Methyltransferase Reveals Structural Features and Potential Epitope Regions

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## ABSTRACT

**Background:** The NS5 Nterminal Methyltransferase (MTase) of Dengue Virus Serotype2 (DENV2) catalyzes RNA cap methylation. MTase is indispensable for replication and is an attractive target for antivirals and epitopebased vaccines. Purpose of this study is to characterize the DENV-2 NS5 N-terminal Methyltransferase (MTase) domain using integrated computational structural and immunoinformatic analyses. To identify surface-exposed linear B-cell epitope candidates and potential phosphorylation sites that could support antiviral, diagnostic, or vaccine development. **Materials and Methods:** We profiled the MTase domain (residues 1-263) using an integrated computational workflow that included physicochemical characterization, secondarystructure and disorder prediction, residue contact mapping, and 3D modeling. Immunoinformatic screens mapped linear Bcell accessible regions and predicted serine/threonine/tyrosine phosphorylation sites. **Results:** The protein comprises 263 amino acids (~29.37 kDa) with a basic theoretical pI (~9.33). Predicted secondary structure and contact topology support a Rossmann-like MTase fold, characterized by alternating β-strands and α-helices. There is low intrinsic disorder outside the termini, which is consistent with a wellpacked catalytic domain. Antigenicity profiling highlights surfaceexposed stretches near the Nterminus, a central loop region, and the Cterminus. Seven short linear peptides (6-13 residues) were consistently prioritized between positions 10-254. The highestscoring epitope is located in the Cterminal region (~242-254). Multiple putative phosphorylation sites were detected, forming serine/threoninerich clusters around ~20-40, ~120-140, and ~200-240. These may regulate enzyme function or host interactions. **Conclusion:** This study delineates the structural organization and immunogenic landscape of the DENV2 NS5 MTase domain. It proposes discrete peptide candidates for experimental validation in the context of diagnostics or vaccine design. The modeled fold and residuelevel features provide a framework for structureguided inhibitor discovery.

**Keywords:** Antigenicity, DENV, Epitope, MTase, Phosphorylation, Secondarystructure.

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## INTRODUCTION

DENV is a positive-sense, single-stranded RNA virus of the genus *Flavivirus* (Family *Flaviviridae*) (Umar *et al*., 2025). Its 10.7-kb genome carries a single open reading frame flanked by structured 5′ and 3′ untranslated regions. These regions regulate translation and replication (Sinha *et al*., 2024; Clyde *et al*., 2006; Marianneau *et al*., 1988; Chambers *et al*., 1990). The polyprotein is co- and post-translationally processed into three structural proteins: Capsid (C), precursor Membrane/Membrane (prM/M), and Envelope (E). It also produces seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) (Norazharuddin *et al*., 2018; Osawa *et al*., 2023). These nonstructural proteins orchestrate RNA synthesis and antagonize host immunity (Chambers *et al*., 1990; Apte-Sengupta *et al*., 2014; Brett *et al*., 2007). NS5 is one of the numerous conserved proteins of the flavivirus family. It consists of an N-terminal Methyltransferase (MTase) domain and C-terminal RNA-dependent RNA polymerase (RdRp) domain (Goh *et al*., 2024; Brett *et al*., 2007). This modular architecture supports a coupled capping and replication program. Both processes are essential for the viral life cycle.

The NS5 MTase catalyzes two sequential methylation reactions that generate a type-1 cap on the 5′ end of viral RNA (Liu *et al*., 2010). The steps include N7-methylation of the cap guanine and 2′-O-methylation of the first transcribed nucleotide ribose (Egloff *et al*., 2002; Ahola *et al*., 1995; Lim *et al*., 2005; Dong *et al*., 2014). S-Adenosyl-L-Methionine (SAM) acts as a methyl donor. This process produces S-Adenosyl-L-Homocysteine (SAH). Cap maturation increases RNA stability and translation efficiency. It also masks the RNA from innate sensors. Notably, 2′-O-methylation helps the virus evade IFIT-mediated restriction (Dong *et al*., 2014). Biochemical and structural studies have established a Rossmann-like fold for the MTase. This fold features a conserved catalytic K-D-K-E tetrad and binding

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pockets for SAM/SAH and cap analogs (Ahola *et al*., 1995; Lim *et al*., 2015). Mutations in the active site or SAM-binding region attenuate replication. These results underline the essentiality of MTase activity (Dong *et al*., 2014). Because the domain is close, conserved, and has well-defined small-molecule pockets, it is widely regarded as an attractive antiviral target. The NS5 polymerase is also considered an attractive target (Yap *et al*., 2007; Tarantino *et al*., 2016).

However, NS5 plays a key role in dengue immunology, particularly in relation to E and NS1. Strong T-cell responses to nonstructural proteins (NS3 and NS5) are well-documented, and anti-NS5 antibodies play a crucial role in the diagnosis of acute infections (Weiskopf *et al*., 2014; Jung *et al*., 2001). Mapping linear, surface-exposed NS5 MTase regions is beneficial: short peptides facilitate the development of serodiagnostic assays and guide the design of multi-epitope vaccines. They can identify regions where immune pressure may limit resistance to inhibitors. Understanding possible post-translational regulation of NS5, such as host kinase phosphorylation, can reveal interactions with cellular pathways that may affect the assembly, localization, and function of the replication complex (Zhao *et al*., 2015; Kroschewski *et al*., 2008).

Modern in-silico pipelines enable the fast and integrative characterization of such features with minimal experimental input (Jung *et al*., 2001). Structure (Secondary) and disorder predictions rely on amino acid sequence arrangement to identify helices, strands, and loops. These predictions usually emphasize lax segments that harbor antigenic determinants (Wilkins *et al*., 1999; Buchan *et al*., 2019). Template-based and ab initio fold prediction approaches enable the construction of plausible 3D models. These models are useful when experimental structures are unavailable. They are also helpful when serotype-specific differences must be considered. Widely used platforms include Phyre2 and I-TASSER (Kelley *et al*., 2015; Yang *et al*., 2015). Contact-map inference further validates topology by leveraging co-evolutionary signals in sequence families. In immunoinformatics, the B-cell epitope predictors tool BepiPred-2.0 combines amino acid propensities with structural context to rank linear epitopes (Jespersen *et al*., 2017). Semi-empirical antigenicity rankings remain valuable for accentuating solvent-exposed, flexible areas (Kolaskar *et al*., 1990; Hofmann *et al*., 1990). Epitope prospects can be cross-referenced against the Immune Epitope Database for prior evidence and probable cross-reactivity (Vita *et al*., 2018; Ponomarenko *et al*., 2011). In parallel, NetPhos and related tools estimate serine/threonine/tyrosine phosphorylation potential based on local sequence context. These tools identify candidate regulatory hotspots for downstream analysis. Three main reasons drive the analysis of the DENV-2 NS5 MTase domain (residues 1-263). First, DENV-2 dominates many outbreaks and displays broad spread and genetic variation (Bhatt *et al*., 2013; Guzman *et al*., 2015).

We created a detailed computer-based profile of the DENV-2 NS5 MTase (1-263 aa) using physicochemical analysis, secondary structure prediction, residue contact mapping, and homology modeling. We also screened for B-cell epitopes and phosphorylation sites to highlight short, manageable peptide segments and regulatory motifs for further testing. Our structural model and epitope map can help develop peptide-based diagnostic tests and multi-epitope vaccines, as well as guide the search for small molecules that target SAM/cap pockets or regulatory loops. By focusing on this key dengue enzyme, our work provides new insights for antiviral research and monitoring in areas where dengue is prevalent.

## MATERIALS AND METHODS

### Structural Characterization of NS5-MTase Domain of DENV-2

The genomic organization and structural layout of Dengue virus serotype 2 (DENV-2) were constructed using an integrative bioinformatics approach. This approach illustrates the structural and non-structural protein regions of the viral genome. The full-length DENV-2 genome sequence was retrieved from the National Center for Biotechnology Information (NCBI) database (Zhou *et al*.,2007; Egloff *et al*., 2002). Sequence data were analyzed to identify coding regions for structural proteins (Capsid, Membrane, and Envelope) and non-structural proteins (NS1-NS5). These form the polyprotein organization typical of flaviviruses. The Conserved Domain Database (CDD) and InterProScan v5.62 were used to annotate and confirm the positions of the non-structural proteins (Jones *et al*., 2014). This includes the NS5 Methyltransferase (MTase) domain at the N-terminus of the NS5 gene. Functional domain mapping was cross-validated with the Pfam database.

### Secondary structure analysis

We analyzed the secondary structure and sequence properties of the *DENV-2 NS5 methyltransferase* (residues 1-263) using several bioinformatics tools. We used PSIPRED v4.0 to predict α-helices and β-strands based on residue-specific confidence scores (Buchan *et al*., 2019). ExPASy ProtParam (https://web.expasy.org/cgi-bin/protparam/protparam) helped classify amino acids as hydrophobic, polar, or aromatic (Gasteiger *et al*., 2003). We mapped structural motifs and visualized secondary structures with SOPMA and RaptorX to confirm the α/β pattern typical of SAM-dependent methyltransferases. PyMOL v2.5 was used to create graphical representations of the predicted helices and sheets.

### Contact Map and *in silico* Analysis

We performed structure prediction using the Phyre2 *in silico* tool, which delivered homology-based protein folding models that we verified for accuracy and residue confidence (Kelley *et al*.,

2015). Then, RaptorX Contact analyzed the interactions between residues and created a contact map for visualization. We used PyMOL v2.5 to examine the 3D structure, focusing on features such as alpha helices and beta sheets. Finally, we confirmed sequence alignment and secondary structure stability using ProSA-web and PSIPRED, which supports the strength of the mixed alpha/beta fold (Buchan *et al*., 2019; Alwabli *et al*., 2019).

### Amino Acid Profiling and B-Cell Epitope Prediction

The sequence of amino acids was studied for its composition and epitope characteristics using tools such as ProtParam and BepiPred 2.0 (http://tools.immuneepitope.org/bcell/result/). Hydrophilicity and surface accessibility profiles identified loop-rich and solvent-exposed residues. Seven high-scoring linear B-cell peptide predictions were purified through IEDB's BepiPred server, (Alwabli *et al*., 2019; Jespersen *et al*., 2017) and graphical representations were created with GraphPad Prism v9 to illustrate amino acid frequency and epitope mapping.

### Atomic composition and post-translational

The study found that the DENV-2 NS5 methyltransferase contains specific elemental components that suggest a stable molecular structure. Bioinformatics analysis also revealed predicted phosphorylation (netphos-3.1b) sites at serine, threonine, and tyrosine residues, indicating possible regulatory roles for these modifications (Blom *et al*., 1999).

## RESULTS

### Genome organization

Dengue virus architecture and genome organization. The virion contains a positive-sense RNA genome capped at the 5′ end and flanked by 5 ′ and 3 ′ UTRs, enclosed by nucleocapsid, membrane, and envelope proteins (Figure 1A). Translation produces a single polyprotein that is cleaved into three structural proteins (capsid, membrane/prM, and envelope) and seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5). NS2B functions as the essential cofactor for the NS3 protease/helicase. NS5 is bifunctional, comprising an Nterminal methyltransferase and a Cterminal RNAdependent RNA polymerase; the displayed sequence corresponds to the DENV2 NS5 methyltransferase domain (residues 1-263), Figure 1B.

### Secondary structure prediction

The secondary structure of the NS5 methyltransferase domain (DENV-2, residues 1-263) is important for its catalytic activity. The model displays both α-helices (coiled segments shown in blue) and β-sheets (flat segments shown in red), arranged in an α/β fold, a common structural motif where helices and sheets alternate, found in SAM-dependent methyltransferases (enzymes that use S-adenosyl methionine as a methyl donor). Short helices appear between β-strands, and a glycosylation site (a region where sugar molecules may attach) near the C-terminus may help folding or stability Figure 2A. An amino-acid composition diagram uses colors to emphasize small nonpolar, hydrophobic, polar, and aromatic residues (types of amino acids grouped by their chemical properties), revealing a hydrophobic (water-repelling) core with polar residues outside. This arrangement aids solubility and stability Figure 2B. Secondary structure prediction extremely supports the α-helix and β-strand regions, ensuring the organization needed for NS5 methyltransferase function, RNA cap recognition, and efficient catalysis Figure 2C.

### Predicted structure, residue interactions, and conformational profile

The residuewise precision curve indicates low confidence for most longrange contacts (values below the 0.5 cutoff), with a noticeable rise toward the Cterminus. This pattern suggests flexible segments along much of the sequence and relatively better-defined structure near the Cend Figure 3A.

The contact map reveals different off-diagonal clusters, consistent with a compact α/β core, where β-strands pack together and interact with neighboring helices. These interaction blocks keep the existence of a folded catalytic core despite flexible termini Figure 3B. The 3D model displays a Rossmann-like α/β architecture: a central β-sheet (yellow) flanked by α-helices (magenta) and connected by loops. This topology matches that of SAdenosylLMethionine (SAM)dependent methyltransferases and is compatible with RNAcap binding and methyl transfer Figure 3C. The alignedtermini profile highlights strong structural definition over the central region (~80–200), while the N and Cterminal segments are enriched in coil. Together, the metrics indicate a stable catalytic core with flexible ends that may facilitate conformational adjustments during catalysis Figure 3D.

### Predicted peptide, surface accessibility, and amino acid composition

The NS5 methyltransferase domain of DENV2 comprises 263 amino acids, with a molecular weight of ~29.37 kDa and a theoretical pI of 9.33. Panel A shows the residue composition: Glu (E) is most abundant (~10.65%), followed by Leu (~8.3%) and Val (~8.0%), with Gly/Ser/Asn each at approximately 7.2%. Basic residues are well represented (Lysine and Arginine ~6.8% each), whereas Cystine and Histidine are scarce (~1.9% each), Figure 4A. This profile reveals a soluble, basic protein that relies on Lysine/Arginine for RNA and cap-substrate binding. The lower cysteine content implies that disulfide bond stabilization is unlikely, consistent with a cytosolic enzyme. Figure 4B shows the sequence propensity profile across positions 1-263; the glimmer features multiple surface-exposed peaks (e.g., around 20-60, 110-130, 190-230, and 240-260) separated by troughs typical of buried core segments. The absence of long hydrophobic stretches suggests no transmembrane helices, supporting overall hydrophilicity in line with experimental knowledge

that the NS5 Nterminus forms a soluble α/β SAMdependent methyltransferase fold. Figure 4C lists seven predicted surface peptides aligned with the highpropensity regions: EKWKSR (10-15, length 6), QIYKSG (25-31, 7), EGIKRGFD (42-50, 9), SSPNPT (149-154, 6), MEALQRKY (193-200, 8), PLSRNSIHE (208-216, 9), and FTMRHKKATVEPD (242-254, 13). The basic/aromatic composition, as well as the location of these peptides in loop segments, supports their surface exposure and compatibility with RNA, SAM/SAH, or partner-protein interactions. They also provide tractable motifs for epitope mapping, peptide inhibitor design, or diagnostic assay development.

## Atomic composition and phosphorylation

The DENV2 NS5 methyltransferase domain contains C1288H2051N375O385S13 atoms (4,112 total). Elemental percentages are: H ~49.9%, C ~31.3%, O ~ 9.4%, N, ~9.1%, and S ~0.32%. High hydrogen and carbon content is typical of proteins with aliphatic side chains and peptide backbones Figure 5A. The oxygen and nitrogen reflect backbone carbonyls, amides, and polar side chains that support hydrogen bonding and solubility. The low sulfur content, along with the limited cysteine and methionine residues, argues against disulfide-bond stabilization, which is typical of insoluble, cytosolic enzymes.

NetPhos predictions along residues show many serine (green) and threonine (red) candidates scoring above the default threshold (horizontal line), but tyrosine (blue) sites are fewer in number. High-scoring peaks cluster at the N-terminus (<70), mid-region (~100–150), and later in the 1–200 window (~160-190). These hotspots are located in loop/coil segments, rather than the packed α/β core, and are therefore accessible to host kinases.

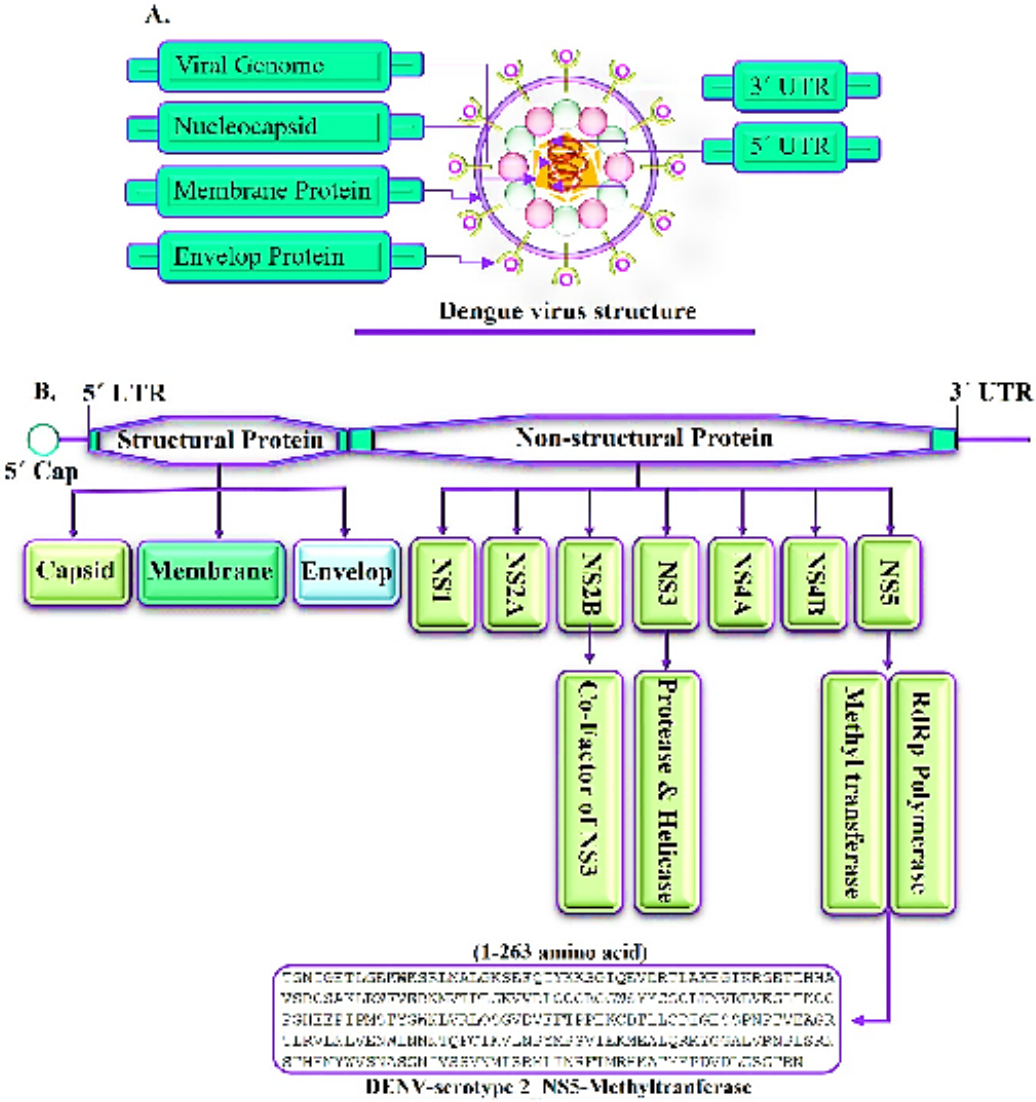


**Figure 1:** Structural and genomic organization of Dengue virus and NS5-MTase domain (DENV-2). (A) The genome is enclosed within the nucleocapsid, surrounded by a lipid envelope with membrane and envelope proteins. (B) Polyprotein schematic showing three structural proteins (Capsid, Membrane, Envelope) and seven non-structural proteins (NS1-NS5); NS3 functions as a protease/helicase, and NS5 contains N-terminal MTase and C-terminal RdRp domains.

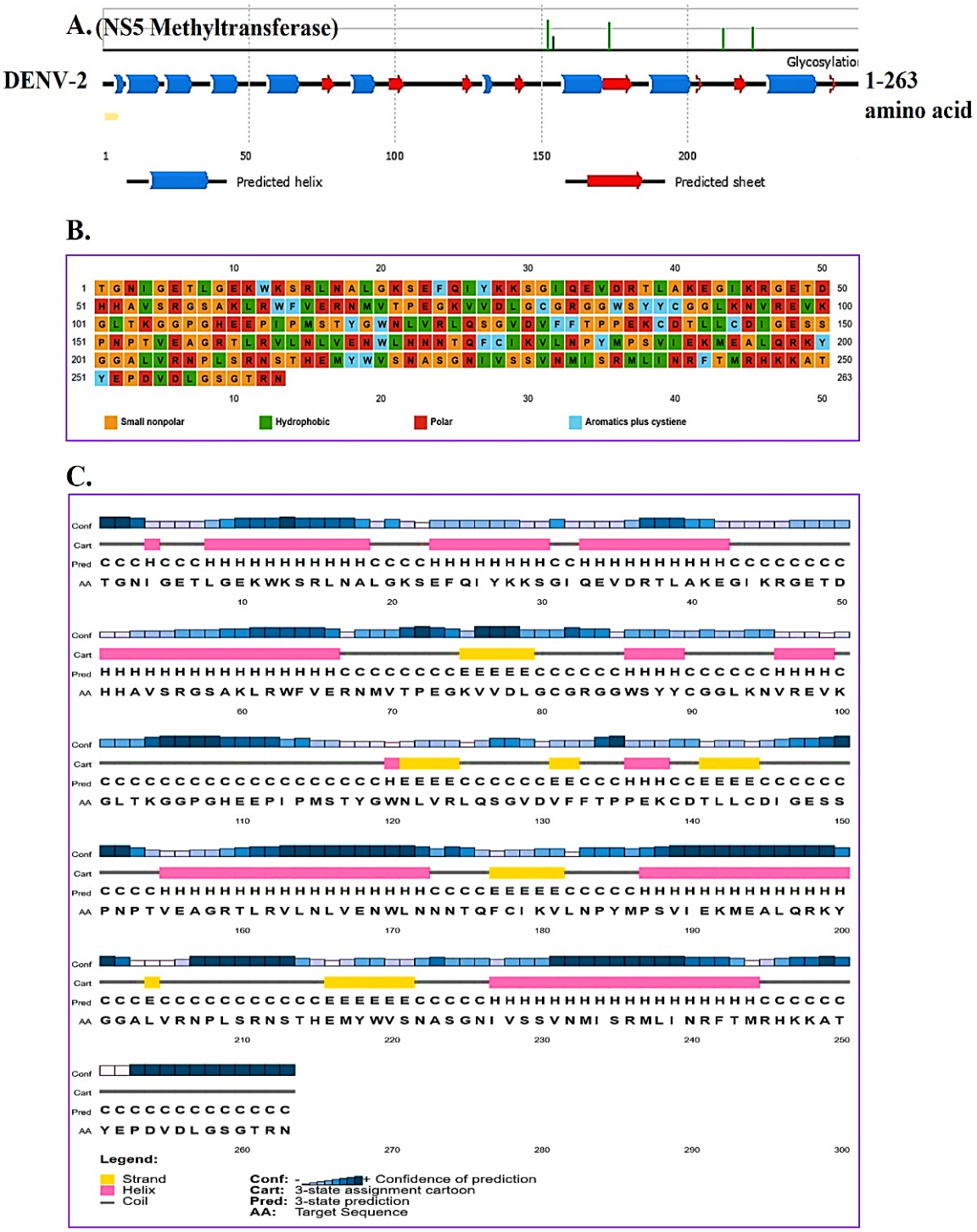


**Figure 2:** Structural and physicochemical features of DENV-2 NS5-MTase. (A) Predicted secondary-structure map of the NS5-MTase, highlighting α-helices (blue) and β-sheets (red) along the sequence. (B) Amino acids classified by physicochemical properties: small nonpolar, hydrophobic, polar, and aromatic residues. (C) Predicted elements show helices (H), strands (E), and coils (C) with confidence levels, supporting a compact α/β-fold.

Phosphorylation would add a negative charge to a protein with a basic pI (~9.33), potentially regulating RNA cap binding, cofactor (SAM/SAH) interactions, and partner docking. Overall, the enzyme is soluble, basic, and features a stable catalytic core with exposed regulatory patches well-suited for mutagenesis (S/T/Y→A/E) and phosphoproteomics Figure 5B.

## DISCUSSION

Our studies integrate genome organization, sequence features, and structure prediction to contextualize the N-terminal Methyltransferase (MTase) domain of dengue virus NS5 (DENV-2, residues 1-263) as a catalytic hub for RNA capping and an attractive

target for antiviral development. Figure 1 summarizes the virion and polyprotein architecture; NS5 bifurcates into an Nterminal MTase that installs N7 and 2′Omethyl groups on the 5′ cap and a Cterminal RNAdependent RNA polymerase (RdRp) that replicates the genome. Crystallographic and cryoEM work across flaviviruses establishes that the MTase adopts a Rossmannlike α/β fold built around a central βsheet with surrounding helices and harbors adjacent binding sites for SAdenosylLMethionine (SAM/SAH) and the capped RNA. These properties rationalize why the domain's active site can catalyze both N7 and 2′-O reactions, and why the SAM pocket has become the dominant locus for small-molecule design (Alwabli *et al.*, 2019; Zhou *et al.*, 2007; Ackermann *et al.*, 2001; Benarroch *et al.*, 2004).

Figure 2 indicates an αhelix-rich, βstrandinterleaved architecture that matches the canonical SAMdependent MTase scaffold. The alternating hydrophobic/polar runs and the predicted short β-α-β motifs are consistent with a buried hydrophobic core and solventexposed loops that often cooperate in nucleotide positioning. These aspects align with experimental examinations that reveal the MTase stimulates RdRp initiation/elongation by enhancing RNA engagement, probably through composite RNA-binding surfaces that bridge the two domains. Notably, sequence scans can detect glycosylationlike sequons in this region, but NS5 resides in the cytoplasm/nucleus rather than the ER lumen; glycosylation is a hallmark of structural E protein, not NS5, so enzymatic glycosylation of NS5 is not expected in cells

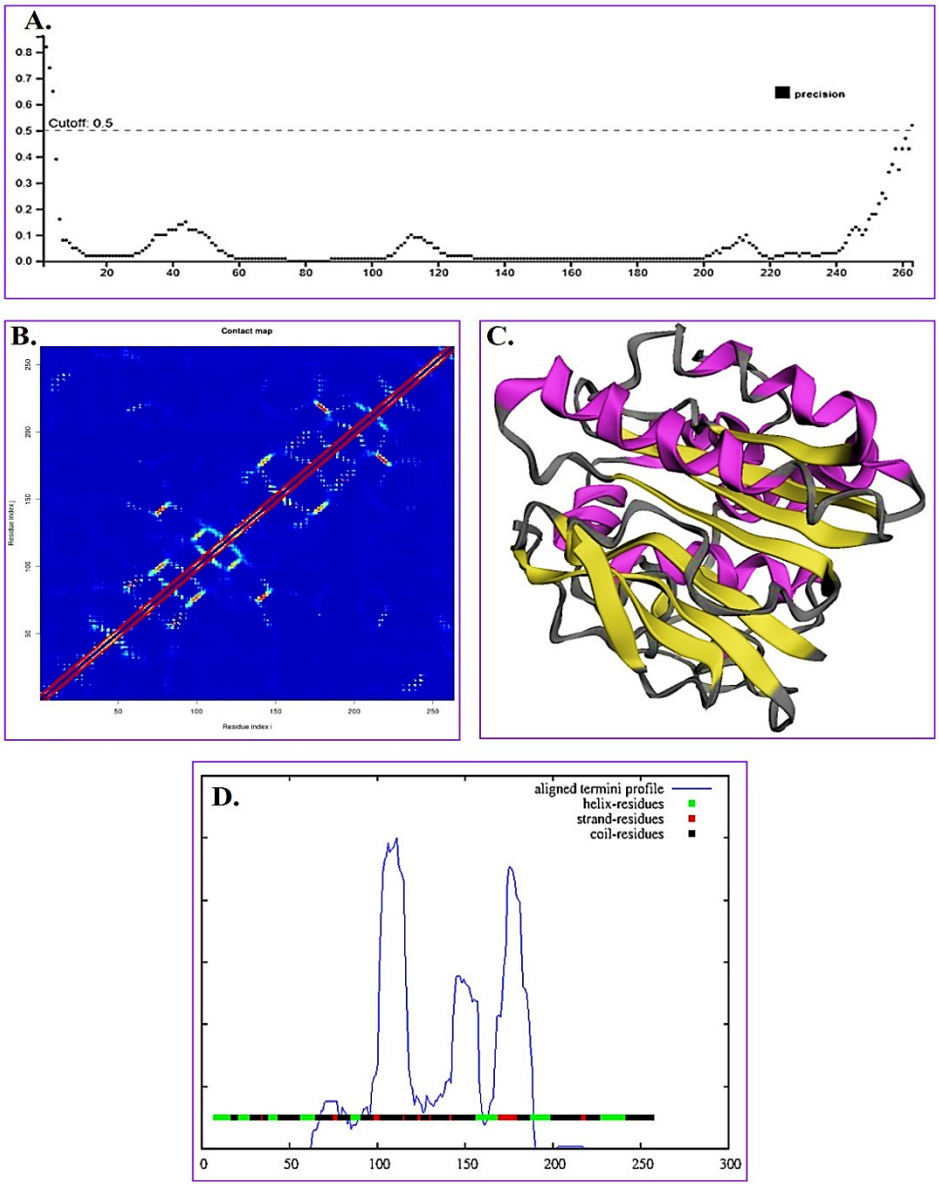


**Figure 3:** Structural prediction and 3D modeling of DENV-2 NS5 MTase. (A) Confidence plot illustrating residue-level prediction precision, with most regions above the 0.5 threshold indicating reliable modeling. (B) Predicted contact map emphasizing strong intra-domain interactions, keeping a compact fold. (C) 3D model displaying α-helices (magenta) and β-strands (yellow). (D) Alignment profile showing the distribution of helices (green), strands (red), and coils (black) along the sequence.

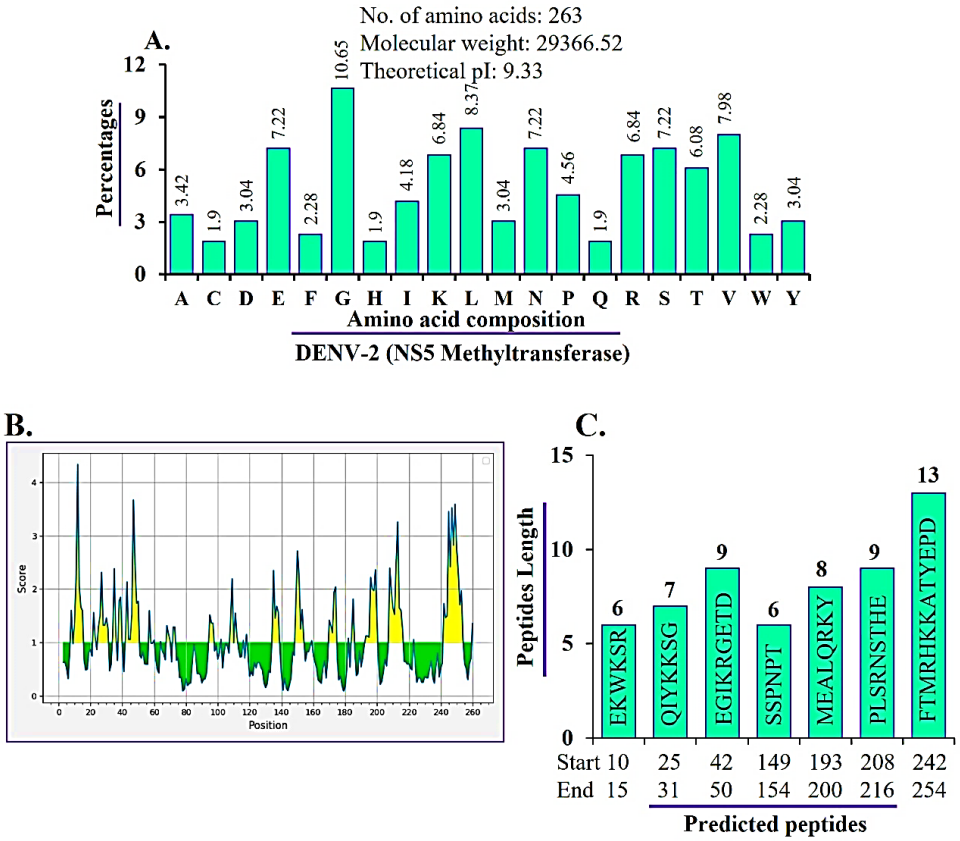


**Figure 4:** Amino acid composition and predicted antigenic peptides of DENV-2 NS5-MTase. (A) Amino acid makeup shows main residues (Phe, Lys, Leu, Val), sequence length (263 aa), MW (29.37 kDa), and pI (9.33). (B) Antigenicity along the sequence highlights surface areas. (C) Predicted B-cell epitopes with their positions; FTMHRKKATVEPD (242-254) has the highest antigenic score.

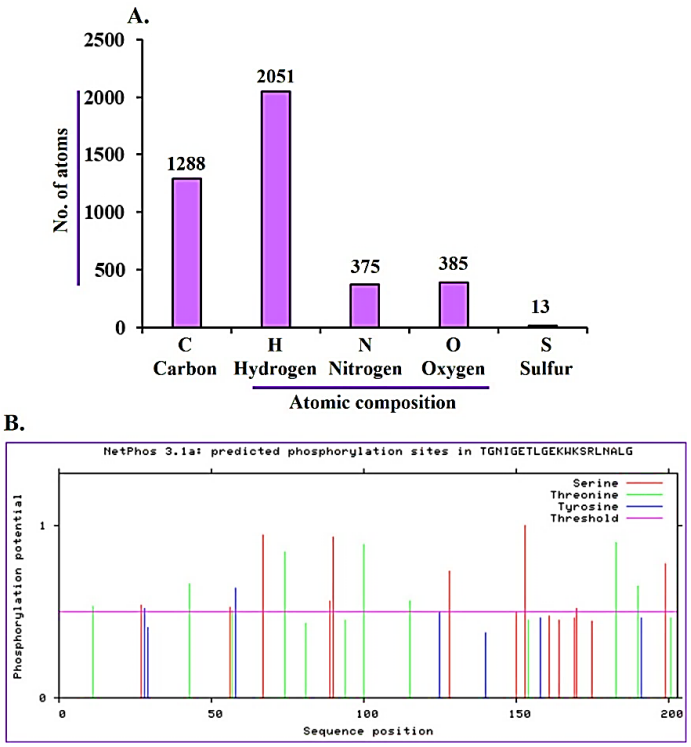


**Figure 5:** Atomic composition and predicted phosphorylation sites of DENV-2 NS5-MTase. (A) Atomic composition showing the relative abundance of carbon, hydrogen, nitrogen, oxygen, and sulfur atoms within the NS5-MTase structure. (B) Predicted phosphorylation sites on serine, threonine, and tyrosine residues generated by NetPhos 3.1, highlighting potential regulatory regions that exceed the threshold value.

(Alwabli *et al*., 2024; Alwabli *et al*., 2017; Supanee *et al*., 2014; Ruigrok *et al*., 2011).

The homology-based 3D model Figure 3C recapitulates the MTase α/β core. The contact map clusters Figure 3B and termini profile Figure 3D highlight a compact catalytic center bracketed by flexible ends. Emerging cryoEM work reveals that NS5 samples multiple interdomain arrangements and binds the 5′UTR StemLoop A (SLA) to initiate replication; SLA binding and interactions with host factors (e.g., STAT2) reshape the MTase–RdRp relative orientation. These data support a "conformational selection" model in which ligand/partner binding stabilizes productive NS5 states consistent with our prediction that loops near the active site are dynamic and poised for remodeling (Obi *et al*., 2025).

The domain's basic pI (~9.3) and Lys/Arg enrichment Figure 4A fit its role in binding negatively charged RNA and cap phosphates. Surfaceaccessibility profiling Figure 4B and Bcell epitope prediction Figure 4C highlight loop segments enriched in basic/aromatic residues chemistries associated with cap and SAM/SAH interactions. While NS5 is a nonstructural protein and not displayed on virions, such exposed peptides are useful for reagent development (e.g., antibodies for mechanistic studies) and for mapping protein-protein interfaces, provided conservation and structural context are validated experimentally. Phosphorylation predictions Figure 5B cluster at coil/loop regions and may modulate localization and partner binding. Prior studies show that NS5 toggles between the cytoplasm and nucleus and engages host transcriptional and STAT2 pathways, suggesting room for regulatory phosphorylation to tune these functions (Goh *et al*., 2024; Pierson *et al*., 2020).

## CONCLUSION

Our combined analyses support a clear structural and functional model for the DENV-2 NS5 Methyltransferase (MTase) domain, covering residues 1 to 263. Genome mapping reveals that this MTase is situated at the N-terminus of NS5, where it performs N7 and 2′-O methylation of the 5′ RNA cap in sequence. Predictions of secondary structure and homology modeling, combined with contact mapping, indicate a Rossmann-like alpha/beta fold with a compact beta-sheet core surrounded by helices and loops; this structure helps explain how the active site can mediate both reactions while nearby loops remain free to interact with RNA and cofactors. The protein primary and atomic composition is basic, rich in lysine and arginine, and low in sulfur, which matches that of a soluble cytosolic enzyme that binds the negatively charged cap. Surface accessibility and epitope scans reveal exposed, basic, or aromatic peptides that likely facilitate RNA binding, offering useful targets for antibody development or inhibitor design. Additionally, predicted phosphorylation sites in flexible coil regions may enable the enzyme activity and interactions to be adaptable. Overall, these results show a stable catalytic core with flexible surface loops, making the MTase a strong candidate for antiviral targeting.

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## ABBREVIATIONS

**MTase:** Methyltransferase; **DENV-2:** Dengue Virus Serotype-2; **SAM:** S-adenosyl-L-methionine; **SAH:** S-adenosyl-L-homocysteine; **NS:** Nonstructural Proteins; **SLA:** Stem-loop.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## GENERATIVE AI STATEMENT

The author states that generative AI tools, with 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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