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Original Article

Characterization of the NS5 Methyltransferase Domain of Dengue Virus Serotype 4: Structural, Functional, and Post-Translational Insights

Afaf Salim Alwabli*

Biological Sciences Department, College of Science and Arts, Rabigh Campus, King Abdulaziz University, Jeddah, SAUDI ARABIA.

ABSTRACT

Background: The NS5 Methyltransferase (MTase) domain of Dengue Virus Serotype 4 (DENV-4) plays a central role in viral RNA capping, genome replication, and immune evasion. Understanding its structural and functional properties is essential for antiviral drug development. Hence, the present study was aimed to characterize the structural features, functional motifs, and potential post-translational regulation of the NS5 MTase domain using computational approaches. **Materials and Methods:** Bioinformatic analyses were conducted to examine the genomic organization and amino acid sequence of the NS5 MTase domain. Secondary structure prediction determined α-helix and β-strand composition. Sequence logo analysis identified conserved catalytic motifs. ProtScale analysis evaluated physicochemical property variations. Potential O-glycosylation and phosphorylation sites were predicted, along with nucleotide-binding residues. Distance constraint modeling was used to assess the Three-Dimensional (3D) structural organization. **Results:** The NS5 MTase domain consists of 263 amino acid residues, with approximately 30% forming α-helices and 16% forming β-strands. Flexible terminal regions may facilitate conformational adaptation during catalysis. Conserved catalytic motifs were identified, highlighting residues essential for methyl donor binding and enzymatic activity. ProtScale analysis revealed alternating hydrophobic and functional regions contributing to structural stability. Predicted O-glycosylation and phosphorylation sites, along with ATP-, AMP-, and GTP-binding residues, suggest possible post-translational regulatory mechanisms. Distance constraint modeling demonstrated a compact 3D structure with cooperative interactions among catalytic residues. **Conclusion:** This study provides a comprehensive bioinformatic characterization of the NS5 MTase domain of DENV-4, offering structural and functional insights that may support the rational design of antiviral agents targeting dengue virus replication.

Keywords: Amino acid, Contact map, Dengue virus structure, MTase, O-glycosylation, Phosphorylation Probability plot.

Correspondence:
Dr. Afaf Salim Alwabli
Biological Sciences Department, College of Science and Arts, Rabigh Campus, King Abdulaziz University, Jeddah-21589, SAUDI ARABIA.
Email: aalwabli@kau.edu.sa

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INTRODUCTION

Dengue Virus (DENV) is a mosquitoborne flavivirus. It has expanded rapidly in both geographic range and case burden over the last decade. In 2024 alone, the World Health Organization recorded more than 7.6 million cases by the end of April, with the Region of the Americas especially affected. These figures underscore the ongoing public health urgency of effective antiviral strategies (Who, 2024; eClinicalMedicine, 2024). Although multiple vaccine platforms are advancing, there are no broadly approved directacting antivirals for dengue. This keeps viral enzymes at the center of drugdiscovery efforts (Pal *et al*., 2025; Patra *et al*., 2025).

DENV possesses a positivesense, singlestranded RNA genome (~11 kb). This genome encodes a single polyprotein that is co and posttranslationally processed into three structural proteins (C, prM/M, and E) and seven Nonstructural proteins (NS1-NS5) (Li *et al*., 2022). Among these, NS5 is the largest and most conserved protein. It comprises an N-terminal Methyltransferase (MTase) and a C-terminal RNA-dependent RNA polymerase (RdRp). This dualenzyme architecture coordinates RNA capping and genome replication. It is conserved across flaviviruses (Bollati *et al*., 2009; Gladysheva *et al*., 2024).

Capping of the 5′ end of the viral RNA generates a type1 cap (mGpppAm) that mimics host mRNA. This process is required for efficient translation and stability. The capping pathway proceeds through a series of sequential reactions. First, the γphosphate is removed from the nascent RNA by an RNA triphosphatase

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(provided by the NTPase/RTPase activity of NS3). Then, GMP is transferred by a guanylyltransferase. Finally, two methylations are catalyzed by the NS5 MTase: these are guanine-N7 and ribose-2′-O methylation (Bollati *et al*., 2009; Egloff *et al*., 2007). Structural and biochemical studies demonstrate that the Nterminal MTase adopts a Rossmannlike fold and carries out both methyl transfers using SAdenosylLMethionine (SAM) as the methyl donor. A conserved LysAspLysGlu (KDKE) catalytic tetrad forms the core of the active site. This tetrad is essential for both N7 and 2′-O reactions (Zhou *et al*., 2007; Coutard *et al*., 2017; Lu *et al*., 2013).

Within this context, the NS5 MTase of Dengue Virus Serotype 4 (DENV4) merits close analysis. All four serotypes share conserved enzymology, but sequence differences can influence local structure, flexibility, and ligand recognition. These differences shape serotypespecific susceptibility to inhibitors (Cheng *et al*., 2025). Understanding this begins with sequencelevel and biophysical annotation. In this work, we employ a computational workflow to characterize the DENV-4 NS5 MTase domain. We annotate the genomic locus and the 263residue amino acid sequence. We predict secondary structure elements and disordered segments. We identify conserved catalytic motifs and sequence logos within the activesite corridor. We map physicochemical trends along the chain and forecast Post-Translational Modification (PTM) propensities (O-glycosylation, phosphorylation) that may influence activity or interactions. We estimate residuecontact probabilities and highlight likely nucleotidebinding residues in GTP/ATP/AMP pockets (Gille *et al*., 2014). Together, these in-silico annotations provide a high-resolution functional map that complements existing structural and biochemical information. They also nominate residues and subpockets that could be prioritized in structure-based inhibitor design against DENV replication (Zhou *et al*., 2007; Borujeni *et al*., 2024).

Finally, by integrating domain architecture, catalytic mechanism, innateimmunity interfaces, and druggability features, this study frames the DENV4 NS5 MTase as a tractable and timely antiviral target. The resulting annotations and residue-level hypotheses are intended to accelerate medicinal chemistry efforts around SAM-competitive ligands, GTP-site binders, and hybrid compounds. These compounds can engage multiple subpockets. The annotations also inform mutagenesis and enzymology to validate predicted regulatory PTMs and cooperative contact networks (Noble *et al*., 2014).

MATERIALS AND METHODS

NS5 Methyltransferase Domain in DENV-4

The sequence (amino acid) for the NS5 Methyltransferase (MTase) domain of dengue virus serotype 4 (DENV-4) was obtained from the NCBI GenBank database (Benson *et al*., 2013). Information on viral protein structure and genome organization was obtained from earlier DENV genome annotations (Lindenbach *et al*., 2003; Mukhopadhyay *et al*., 2005). The 263-amino acid MTase sequence was then analyzed to identify the catalytic region involved in RNA capping and replication (Zhou *et al*., 2007). Protein domains were mapped visually using Illustrator for Biological Sequences (IBS 2.0) (Liu *et al*., 2015), and a hand-drawn schematic of the dengue virion and genome showed both structural (C, prM, E) and Non-Structural (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5) proteins based on standard flavivirus references (Kuhn *et al*., 2002).

Structure of the DENV-4 NS5 methyltransferase

We predicted the secondary structure of the DENV-4 NS5-MTase domain using PSIPRED v4.0, which uses neural-network algorithms based on PSI-BLAST-derived profiles (Jones, 1999; Kaur *et al*., 2004). To identify intrinsically disordered regions, we employed DISOPRED3, a tool that integrates sequence profiles and structural templates (Ward *et al*., 2004). We visualized conserved residues in the catalytic core with sequence logos made by WebLogo 3 (Crooks *et al*., 2004). Next, we evaluated structural confidence and residue conservation to find functionally stable regions. Finally, we created a disorder probability plot with IUPred2A, which estimates flexibility and folding tendencies for each residue (Meszaros *et al*., 2018).

Physicochemical and structural properties

Analyzed using ProtScale from the ExPASy Bioinformatics Resource Portal (Gasteiger *et al*., 2003). Multiple amino acid scales including molecular weight, refractivity, β-strand tendencies, and residue accessibility were applied to evaluate positional variations along the 263-residue sequence. The profiles were plotted to identify hydrophobic hydrophilic transitions and structural periodicity that may influence folding and stability (Kyte *et al*., 1982). These analyses provided quantitative insights into domain organization and functional residue distribution.

The inter-residue distance constraints of the NS5-MTase domain

We used RaptorX Contact Map to predict residue-residue interactions and tertiary folding patterns (Wang *et al*., 2017). In parallel, NetPhos 3.1 identified phosphorylation sites on serine, threonine, and tyrosine residues (Blom *et al*., 1999), while GlycoEP further evaluated the potential for O-glycosylation (Chauhan *et al*., 2013). Additionally, ATPsite 2.0 predicted nucleotide-binding residues for ATP, ADP, AMP, GTP, and GDP using structure-based feature learning (Chauhan *et al*., 2013).

RESULTS

Structure, Motifs, and Genome of DENV-4

We describe the structure and genome of DENV-4, with a focus on the NS5 Methyltransferase (MTase) domain Figure 1A. The virion diagram illustrates the E and M envelope proteins within

a lipid membrane that surrounds the nucleocapsid and the positive-sense RNA genome, highlighting the role of NS5 in replication Figure 1A. The genome map shows a single ORF that is translated into a polyprotein, which is then cleaved into C, prM, E, and NS1–NS5. NS5, located at the 3′ end, has an N-terminal MTase and a C-terminal RdRp that work with NS3 for RNA capping and synthesis Figure 1B. The NS5 MTase sequence (residues 1-263) contains the conserved K-D-K-E catalytic tetrad, SAM/SAH and cap-binding motifs, as well as flexible loops that act as substrate gates, confirming the analysis of the full catalytic domain Figure 1C.

Secondary structure and intrinsic disorder profile

Figure 2A shows the predicted secondary structure and intrinsic disorder profile, indicating that 30% of residues are in α-helices, 16% in β-strands, and 17% in intrinsically disordered regions. The α-helices, marked in Figure 2A, are found in conserved regions that help stabilize the SAM-binding core. Short β-strands, also in Figure 2A, form sheet-like areas around the catalytic cavity. The disorder profile in Figure 2A highlights flexible regions at the termini and in inter-helical loops, which may help the protein adapt its shape during substrate binding or product release. Figure 2B presents a sequence logo analysis of conserved motifs

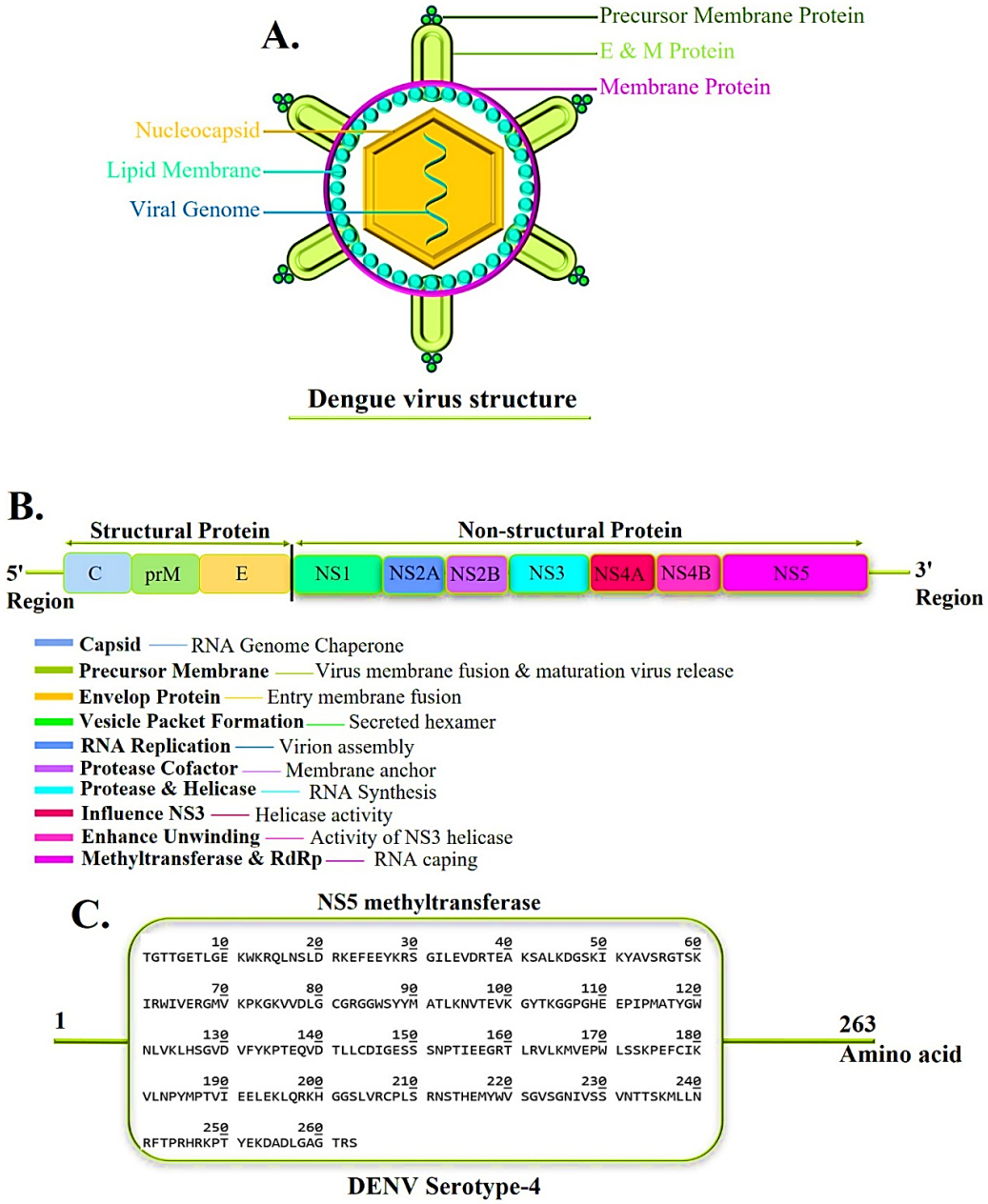

Figure 1: (A) Schematic illustration of the dengue virus structure, indicating the nucleocapsid, lipid membrane, and surface proteins: E, M, and prM. (B) Schematic layout of the viral genome, depicting structural proteins and non-structural proteins. (C) Amino acid sequence of the NS5 Methyltransferase (MTase) domain from dengue virus serotype 4.

between residues 120 and 200, which make up the catalytic core of the MTase domain. In Figure 2B, residues forming the Lys-Asp-Lys-Glu (K-D-K-E) catalytic tetrad and nearby glycine- and histidine-rich segments are highly conserved, showing their importance in methyl transfer and in the interaction between SAM and SAH. The variable flanking residues in Figure 2B may allow for serotype-specific cofactor or RNA recognition. Figure 2C shows the predicted probability of disorder for each residue. Most residues have a low disorder chance (less than 0.5), as seen in Figure 2C, which supports a stable and compact structure. The C-terminal segment (residues 240 to 260) in Figure 2C has a higher disorder potential, suggesting this flexible tail may help with dynamic movements involved in RNA capping or communication between domains within NS5.

ProtScale- mical Profiling

Profiling of the DENV-4 NS5 Methyltransferase (MTase) domain shows that different biophysical indices fluctuate across the 263-residue sequence. Plots of codon number and molecular weight indicate rhythmic variations corresponding to alternating hydrophobic and polar segments, supporting structural partitioning within the MTase core Figures 3A-B. Antiparallel and parallel β-strand propensities peak moderately between residues 80 and 180, suggesting β-sheet-rich subregions that may

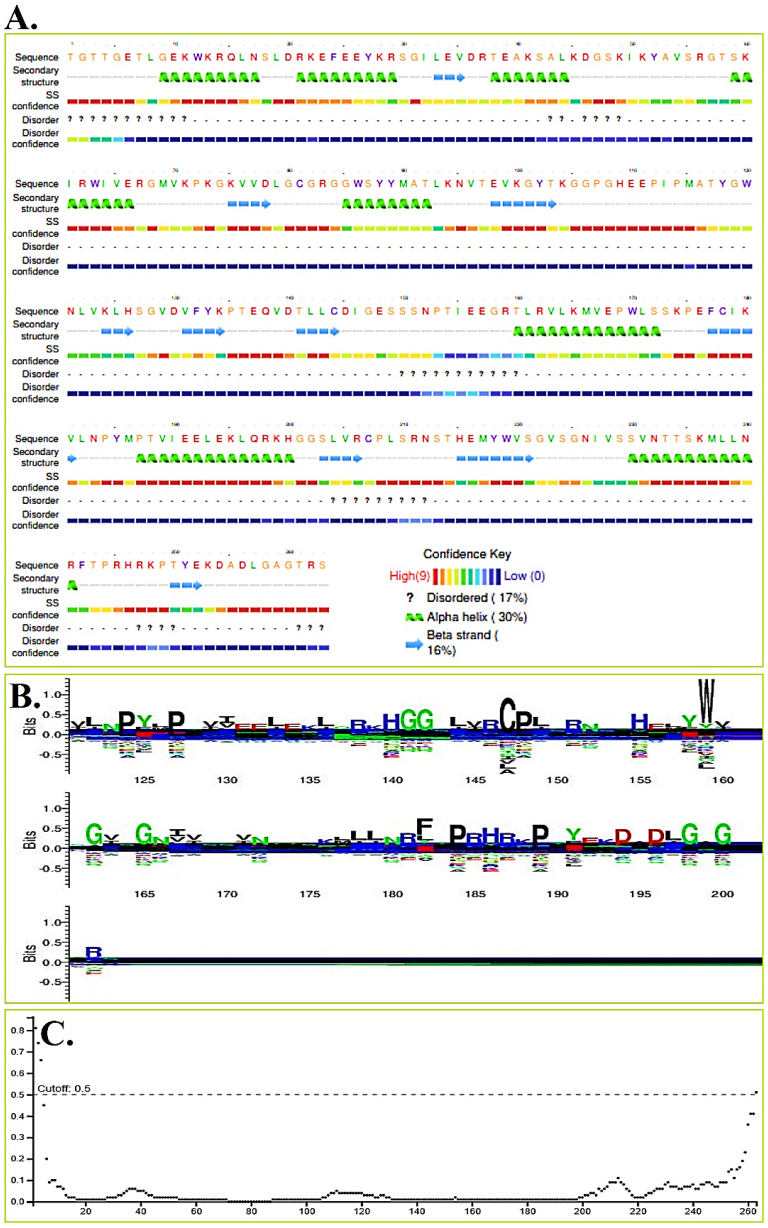

Figure 2: (A) Shows the predicted secondary structure and disorder profile of the NS5-MTase domain, relating α-helices, β-strands, and disordered regions to protein function. (B) Presents a sequence logo indicating conserved residues critical for the catalytic core enzymatic activity. (C) Displays a disorder probability plot marking stable core regions and flexible termini in the 263-amino-acid NS5-MTase.

stabilize the Rossmann-like α/β fold characteristic of flaviviral MTases Figures 3C-D. Refractivity and recognition factor plots reveal dynamic oscillations, reflecting shifts in side-chain volume and binding affinity that likely mark motifs involved in SAM/SAH and guanine cap binding or cofactor interaction Figures 3E-F. Alternating peaks in amino acid composition and accessible residue percentage indicate regions of surface exposure interspersed with buried hydrophobic cores, consistent with solvent-accessible catalytic loops flanking a compact enzymatic core Figures 3G-H.

Functional Site Predictions and Distance Constraint Analysis of MTase

Figure 4 shows the predicted structural constraints and possible functional modification sites in the DENV-4 NS5 Methyltransferase (MTase) domain. The contact map in Figure 4A presents the predicted distances between residues. Strong

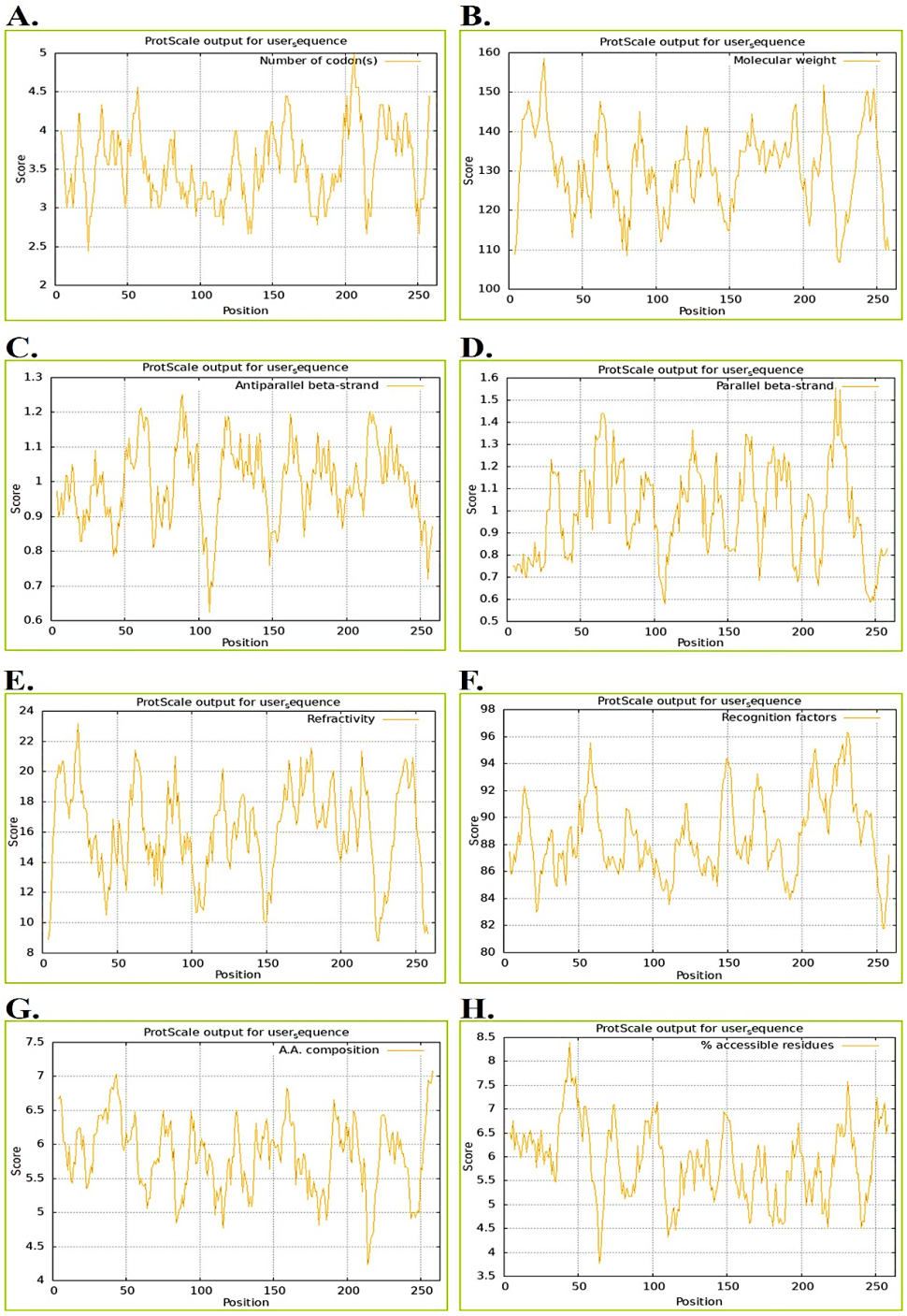

Figure 3: (A-H) ProtScale analysis of NS5 Methyltransferase (DENV-4) shows variation along the 263-residue sequence for several physicochemical indices. Plots represent codon number, molecular weight, β-strand tendencies, refractivity, recognition factors, amino acid composition, and residue accessibility.

contacts, shown in blue with scores above 0.7, suggest the domain is compact and tightly folded. Average to weak interactions, characterized by colors ranging from green to red, signify surface loops or flexible linkers that may facilitate the structure adaptation during catalysis. This practice supports the structural integrity of the 263-residue MTase. Panel B shows that O-glycosylation analysis predicts a few low-potential sites, primarily located in surface-exposed regions. Although the general possibility is low, these sites may be involved in Post-Translational Modifications (PTMs) under specific cellular conditions, which could potentially impact protein stability or localization. The phosphorylation plot in Figure 4C highlights several serine, threonine, and tyrosine residues above the prediction threshold, indicating possible regulatory phosphorylation sites. These modifications may help control enzyme activity, subcellular localization, or interactions with other viral and host proteins, which aligns with

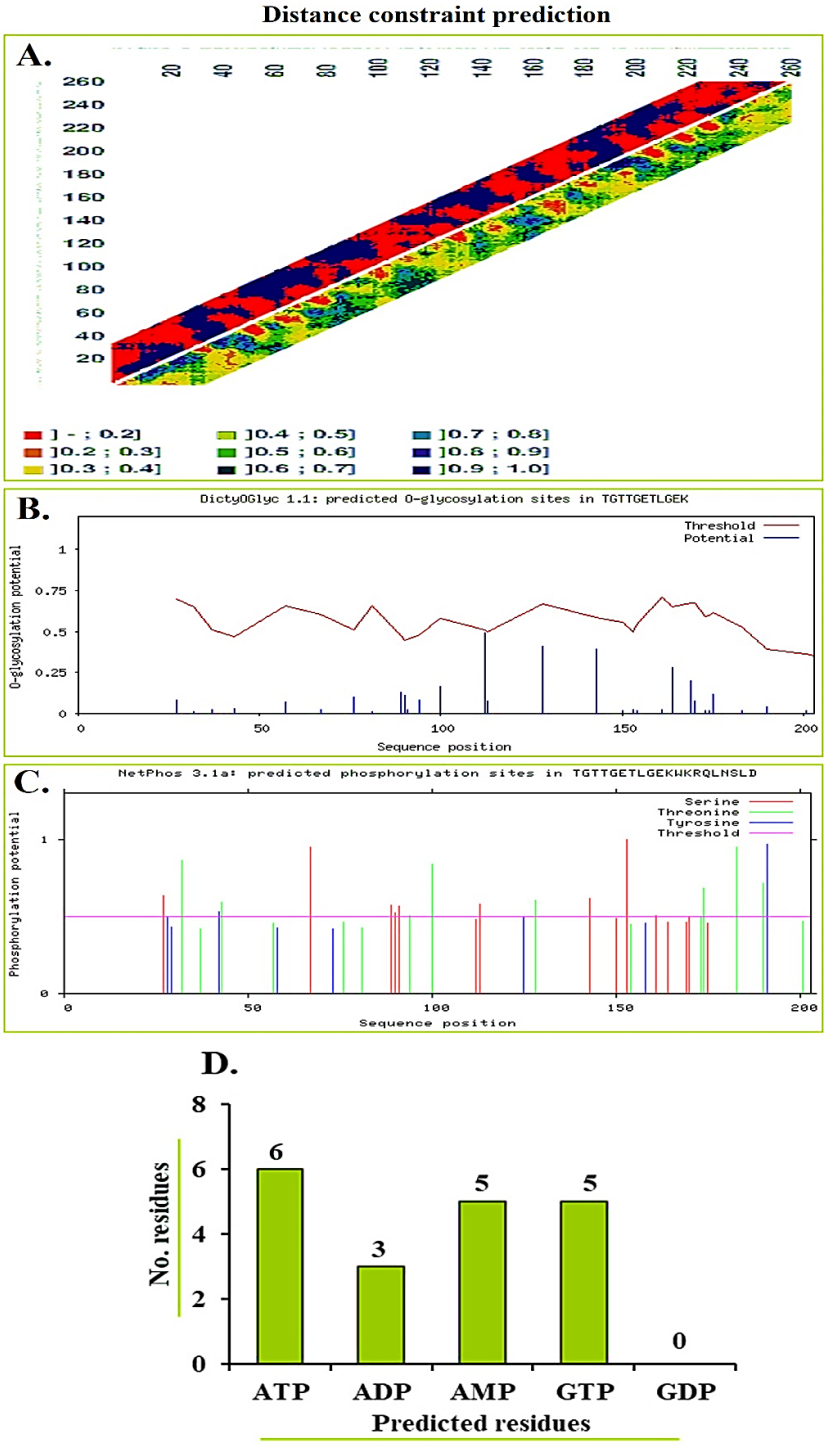

Figure 4: (A-D) Functional predictions for the NS5 methyltransferase domain of DENV-4. (A) Residue contact map; (B) O-glycosylation; (C) Phosphorylation on Ser/Thr/Tyr; (D) Nucleotide-binding residues. Predicted modification and binding sites suggest key regulatory and catalytic roles.

previous studies on NS5 regulation in flaviviruses. Finally, the nucleotide-binding residue prediction in Figure 4D shows strong binding potential for ATP (six residues), GTP (five residues), and AMP (five residues). ADP has three binding residues, and GDP has none. This pattern suggests a strong ability to interact with nucleotides, which fits with the MTase role in RNA capping, where GTP is a key substrate.

DISCUSSION

Our *in silico* analysis of DENV-4 NS5 methyltransferase draws on structural, biophysical, and functional evidence. This aligns with established flaviviral capping biology and identifies residue-level features unique to serotype 4. Figure 1 illustrates the position of NS5-MTase within the genome and virion, as well as its coordination with NS3 and the NS5 polymerase during RNA capping and synthesis (Lindenbach *et al*., 2003; Zhou *et al*., 2007; Alwabli, 2024). The 263 residues studied are located within the canonical N-terminal MTase module, which encompasses the SAM/SAH pocket and the guanine-cap binding site (Alwabli, 2025; Noble *et al*., 2014; Lim *et al*., 2011). These sites act sequentially to methylate cap N7 and 2′-O, producing a cap-1 structure essential for translation and immune evasion (Daffis *et al*., 2010; Zhou *et al*., 2007).

Figure depicts a Rossmann-like α/β core with short β-strands and helices. The flexible termini probably allow 'breathing' near the SAM pocket and cap-binding channel, consistent with structural data showing alternate rotamers and loop placements in related MTases (Alwabli, 2019; Jia *et al*., 2022; Noble *et al*., 2014). Sequence logo enrichment across residues 120-200 highlights the conserved catalytic corridor, where the K-D-K-E tetrad and neighboring residues stabilize the substrate and transition states (Zhou *et al*., 2007). Motifs retained in DENV-4 indicate that serotype differences affect local dynamics or electrostatics, rather than altering the core viral mechanism.

ProtScale profiles exhibit recurring hydropathy and refractivity waves, indicating alternating surface ridges and grooves that are well-suited for transient ligand or cofactor contacts. Accessibility peaks then mark likely cap or nucleotide entry paths. Consistency across scales supports functional segmentation. The hydrophobic-hydrophilic patchwork defines cavity B and side pockets at the SAM site in Wesselsbron and West Nile MTases, features promising for medicinal chemistry (Alwabli, 2019; Bollati *et al*., 2009; Bollati *et al*., 2009; Chauhan *et al*., 2013).

The functional predictions in Figure 4 provide testable hypotheses. Contact-map banding presents a compact fold with medium-range couplings between the catalytic corridor and nearby loops, which are compatible with allosteric communication observed in other flaviviruses. Further, changes in peripheral loops can alter methylation efficiency without affecting the active site (Noble *et al*., 2014). Predicted phosphorylation and O-glycosylation sites are mostly outside the catalytic core. Although the glycosylation of NS5 in cells remains uncertain, the phosphorylation of NS5 in similar flaviviruses is linked to nuclear localization and replication fitness, suggesting that some Ser/Thr sites in DENV-4 MTase may influence dynamics or protein interactions (Alwabli, 2021; Best, 2017; Fajardo *et al*., 2020). In addition, predicted ATP, AMP, and GTP-binding residues partly overlap with solvent-exposed basic patches that may guide capped RNA, making these residues good targets for studying RNA engagement or discovering cryptic allosteric sites for bisite ligands. Drug-design priorities are as follows: (i) Target the validated SAM/SAH pocket. SAM-competitive scaffolds, such as sinefungin analogs, bind deeply but require selectivity to avoid host MTases. Our residue-level electrostatics and accessibility maps can guide the placement of substituents to utilize DENV-4-specific side pockets (Noble *et al*., 2014; Chauhan *et al*., 2013). (ii) The cap and GTP channel present opportunities for non-SAM scaffolds. Sequence changes near this corridor could tune affinity and avoid targeting human capping enzymes (Alwabli, 2025; Bollati *et al*., 2009; Bollati *et al*., 2009; Jia *et al*., 2022). (iii) Loops connecting the catalytic core to solvent, which are only moderately conserved, may be allosteric levers.

Finally, by bringing these findings together, it is evident that NS5 MTase-installed cap-1 dampens IFIT sensing. Viruses lacking 2′-O methylation are attenuated. Thus, serotype-4 residues affecting 2′-O versus N7 activity may allow rational vaccine attenuation. Predicted PTMs imply host-kinase regulation and can be probed with phosphomimetic mutants to link structure to replication (Daffis *et al*., 2010). Overall, evidence from motifs, zoning, contact networks, and PTMs positions NS5 MTase as a compact, druggable target and guides the development of selective inhibitors and targeted mutagenesis.

CONCLUSION

This study provides an integrated computational characterization of the NS5 methyltransferase domain from Dengue Virus Serotype 4 (DENV-4). It combines structural, biophysical, and functional analyses. The findings confirm that the MTase domain (residues 1-263) is highly conserved, compact, and catalytically competent. It contains the Lys-Asp-Lys-Glu (K-D-K-E) catalytic tetrad and the SAM/SAH binding motifs, both of which are typical of flaviviral enzymes. Secondary-structure and disorder predictions revealed a Rossmann-like α/β fold with flexible termini. These features support dynamic conformational changes during RNA capping. Physicochemical profiling with ProtScale showed alternating hydrophobic and hydrophilic regions. These likely define substrate-binding surfaces and internal structural stability.

Functional predictions highlighted several potential phosphorylation and O-glycosylation sites. This suggests regulation by host-dependent post-translational modifications. The potent binding propensities of ATP, GTP, and AMP further

support their critical role in RNA cap formation and nucleotide interaction. Distance-constraint mapping indicated a compact, well-ordered domain with peripheral flexibility. This is consistent with the multifunctional nature of NS5.

Overall, this complete *in silico* assessment confirms that the DENV-4 NS5 MTase is structurally stable and dynamically adaptable. It enables efficient methyl transfer and interaction with viral and host factors. These insights establish the MTase as a promising target for antiviral therapy. They also provide a theoretical foundation for structure-guided drug design and mutational studies. Such efforts may disrupt viral replication or enhance rational vaccine development.

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ABBREVIATIONS

MTase: Methyltransferase; **DENV:** Dengue virus; **SAM:** S-adenosylmethionine; **E:** Envelope; **M:** Membrane; **C:** Nucleocapsid; **PTMs:** Post-translational modifications.

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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.

SUMMARY

Computational analysis shows the DENV-4 NS5 MTase (1–263) is a conserved, compact, catalytically active domain with the K-D-K-E tetrad and SAM/SAH-binding motifs. It likely adopts a Rossmann-like α/β fold with flexible termini for RNA-capping dynamics. ProtScale suggests alternating hydrophobic/hydrophilic regions supporting binding and stability, while predicted phosphorylation/O-glycosylation sites and ATP/GTP/AMP-binding residues imply post-translational and nucleotide-linked regulation. Overall, the ordered core with peripheral flexibility supports MTase as a prime antiviral target.

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