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Structural Modeling of Luciferin 4-Monooxygenase from Human Respiratory Syncytial Virus Using Homology Techniques

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ABSTRACT

Background: Human Respiratory Syncytial Virus (HRSV) is a leading etiological agent of acute lower respiratory tract infections, particularly affecting infants, elderly populations, and immunocompromised individuals, thereby imposing a substantial global health burden. Despite its clinical significance, the structural biology of several HRSV-encoded enzymes remains insufficiently characterized. Luciferin 4-Monooxygenase(L4MO) represents one such poorly understood enzyme for which no experimentally resolved three-dimensional (3D) structure is currently available. This lack of structural information limits functional interpretation and hampers structure-based antiviral drug development.

Methods: The three-dimensional structure of HRSV-derived Luciferin 4-Monooxygenase was predicted using homology modeling approaches. Amino acid sequence alignment was performed against homologous luciferin monooxygenases. Model construction was carried out using the SWISS-MODEL server. Structural validation and quality assessment were conducted using established computational tools, including PROCHECK, ERRAT, and Verify3D.

Results: The predicted L4MO model exhibited a secondary structural composition of 30.91% alpha-helices, 20.55% extended strands, 7.64% beta-turns, and 40.91% random coils. Model validation demonstrated high structural reliability, as reflected by an ERRAT score of 96.6% and 94.8% of residues residing within the most favored regions of the Ramachandran plot. Furthermore, the Verify3D analysis yielded a score of 83.33%, supporting the overall consistency and accuracy of the predicted structure.

Conclusion: This study presents the first validated homology-based three-dimensional structural model of Luciferin 4-Monooxygenase from HRS. The generated model provides critical structural insights into this understudied viral enzyme and establishes a foundational framework for future investigations into its functional role in viral pathogenicity.

Keywords: Human Respiratory Syncytial Virus (HRSV); Luciferin 4-Monooxygenase (L4MO); Homology modeling; 3D structure; Bioluminescent enzymes; SWISS-MODEL

Introduction

Human Respiratory Syncytial Virus (HRSV) is a globally prevalent respiratory pathogen and a leading cause of acute lower respiratory tract infections (ALRTIs), particularly among neo-

nates, infants, elderly individuals, and immunocompromised populations. The virus is associated with substantial morbidity and mortality, accounting for over 100,000 deaths annually in children under five years of age and more than three million hospitalizations worldwide [1–2].

The high disease burden of HRSV is further exacerbated by its seasonal transmission patterns, antigenic variability, and the lack of long-lasting protective immunity, which collectively contribute to recurrent infections and sustained public health impact [3].

Despite extensive research efforts, therapeutic options for HRSV remain limited. Currently available interventions, including the monoclonal antibody palivizumab, are primarily prophylactic and exhibit only partial efficacy, particularly in high-risk populations [4]. Moreover, no widely effective antiviral treatment has yet been established, highlighting a critical need for the identification of novel viral targets and the development of innovative therapeutic strategies.

In this context, the exploration of less-characterized or putative viral proteins may provide new insights into HRSV biology and potential avenues for antiviral drug discovery. One such protein is Luciferin 4-Monooxygenase (L4MO), an enzyme classically associated with bioluminescent organisms such as the firefly *Photinus pyralis*, where it catalyzes the oxidative decarboxylation of luciferin to oxyluciferin, resulting in light emission [5]. The reported presence or annotation of L4MO-like sequences in HRSV is unusual and remains insufficiently characterized. Notably, HRSV is not a bioluminescent organism, and therefore, the functional role of any L4MO-like protein within its viral system is unclear and warrants critical investigation [6].

It is important to emphasize that the assignment of L4MO in HRSV is primarily based on sequence homology or computational annotation, rather than direct experimental validation. This raises important questions regarding the accuracy of such annotation and the possible existence of structurally related proteins with divergent biological functions. Therefore, a rigorous structural and bioinformatics-based investigation is necessary to evaluate whether the putative L4MO in HRSV shares conserved structural features with known monooxygenases and to explore its potential functional relevance. A major limitation in this area is the absence of an experimentally resolved three-dimensional (3D) structure for the putative L4MO protein in HRSV. Structural information is essential for understanding protein function, active site architecture, and ligand interactions, all of which are critical for rational drug design. The lack of such data represents a clear and significant research gap.

To address this gap, we aimed to employ homology modeling approaches to predict the 3D structure of the L4MO protein associated with HRSV. Homology modeling is a well-established computational technique that leverages sequence similarity with structurally characterized template proteins to generate reliable structural models in the absence of experimental data. Through this approach, the study sought to elucidate the secondary and tertiary structural features, identify potential active sites, and characterize ligand-binding regions of the protein. The significance of this work lies in providing the first predicted structural model of L4MO in the context of HRSV, while also critically evaluating its plausibility as a functional viral protein. Furthermore, the model will be validated using established structural assessment tools such as PROCHECK, ERRAT, and Verify3D, ensuring its reliability for downstream applications. Ultimately, this study not only contributes to the structural understanding of a poorly characterized protein but also lays the groundwork for future biochemical and antiviral investigations targeting HRSV [7].

Methods

Protein Sequence Retrieval

The amino acid sequence for Luciferin 4-Monooxygenase (L4MO) from HRSV (accession number V9VFW4) was retrieved from the UniProt database. The sequence served as the input for further computational analysis and structural Homology modeling [8].

Protein Sequence Analysis and primary Structure Prediction

To understand the protein's physicochemical properties and to predict the 1D structure, the ProtParam tool was employed. This tool was used to compute the molecular weight, theoretical isoelectric point (pI), atomic composition, amino acid composition, and hydrophobicity index of the L4MO protein. Additionally, the instability index and aliphatic index were calculated. These calculations help to infer the stability, solubility, and functional viability of the protein under different experimental conditions [9].

Sequence Alignment and Template Selection

The FASTA format sequence of L4MO (V9VFW4) was aligned with known homologous sequences from bioluminescent organisms to identify conserved regions and establish structural similarities. Using the BLAST tool and Protoparam tool, seven homologous sequences with varying degrees of similarity were selected [9]. These include sequences from Photinus pyralis (Common Eastern Firefly, H1AD96), Photinus pyralis (P08659), Aquatica lateralis (Heike Firefly, Q01158), Nipponoluciola cruciata (Genji Firefly, P13129), Luciola mingrelica (Southern Russian Firefly, Q26304), Photuris pensylvanica (Pennsylvania Firefly, Q27757), and Photinus pyralis (A0AA5N4A9N9), with sequence similarities ranging from 57% to 99.6%. Multiple sequence alignment (MSA) was performed using Clustal Omega to identify conserved regions across the selected species and facilitate the alignment with L4MO [10].

Secondary Structure Prediction

The secondary structure of L4MO was predicted using PSIPRED and GOR IV sequence analysis tools. These tools provided predictions of alpha-helices, beta-sheets, and random coils. The percentage composition of each secondary structure element was calculated to provide a clearer understanding of the protein's folding and functional stability. This analysis helps to characterize the structural folding of the protein and provides valuable insights into its potential functional domains [11].

Tertiary Structure Prediction and Homology Modeling

Since no experimentally determined 3D structure exists for L4MO (V9VFW4), homology modeling was performed using the SWISS-MODEL server. The FASTA sequence of L4MO was aligned with the template sequences identified earlier, and a 3D model was generated. This model represents the entire amino acid sequence of L4MO, from Methionine (Met 1) to Valine (Val 550). The homology modeling process involves aligning the target sequence with structurally characterized templates and using the SWISS-MODEL server to generate the best possible model by refining the backbone and optimizing side-chain conformations [12].

Homology Model Validation

To validate the accuracy of the homology-modeled 3D structure of L4MO, several in silico validation tools were employed as follows:

1. The ERRAT tool was used to assess the quality and accuracy of the modeled structure. ERRAT evaluates the amino acid residue quality and identifies structural errors**.**
2. **PROCHECK** tool was used to generate the Ramachandran plot, which assesses the backbone dihedral angles and the spatial conformation of amino acids.

This plot helps determine whether the protein structure is within the allowed and most favored regions**,** indicating its stereochemical integrity**.**

3. The Verify3D tool (Institute for Genomics and Proteomics (IGP), 2021) was used to assess the compatibility of the modeled structure with its 1D amino acid sequence. A good match between the 3D model and the sequence confirms the accuracy of the model [13].

Structural Comparison

The three-dimensional (3D) structure of L4MO from HRSV was predicted using SWISS-MODEL, a homology modeling tool that aligns the target protein sequence with experimentally solved protein structures from the Protein Data Bank (PDB) [14]. The alignment of the modeled L4MO protein with the template was conducted to identify conserved regions and functional domains, particularly the active sites. The PDB structure (1lci.1.A) of the template was selected based on sequence similarity and functional relevance. The SWISS-MODEL output was used for further analysis and comparison of structural features [15].

Results

Primary structure Analysis

A homologous sequence was selected from the UniProt database, and its alignment results are presented in Figure 1, Supplementary Materials and Table 1,2,3. The 3D structure of L4MO from HRSV was successfully predicted and modeled using the SWISS-MODEL server based on available structural templates [15].

Table 1: Accession Number, E-value, and Similarity or Identity of Luciferin-4-monooxygenase from different strains of Human Respiratory Syncytial Virus

No	*Organism*	*Accession No.*	*Similarity (%)*
1	Photinus pyralis (Lampyris pyralis)	H1AD96	99.6
2	Photinus pyralis (Lampyris pyralis)	P08659	99.6
3	Aquatica lateralis (Luciola lateralis)	Q01158	68.9
4	Nipponoluciola cruciata (Luciola cruciata)	P13129	68.8
5	Luciola mingrelica	Q26304	63.3
6	Photuris pensylvanica	Q27757	59.7
7	Photinus pyralis (Lampyris pyralis)	A0AA5N4A9N9	57

The analysis of the primary structure was performed using the ProtParam tool [16], and the physicochemical characteristics of both the model and template proteins are presented in Table 2 and 3.

Table 2: Composition of the amino acids groups of model and template sequence

Amino acids	*The number of amino acids in the model*	*Amino acid percentage*	*The number of amino acids in the template*	*Percentage of amino acids*
Alanine – Ala (A)	43	7.8	42	7.6
Arginine – Arg (R)	20	3.6	20	3.6
Asparagine – Asn (N)	17	3.1	19	3.5%
Aspartic acid Asp (D)	32	5.8	31	5.6
Cysteine – Cys (C)	4	0.7	4	0.7
Glutamine Gln (Q)	16	2.9	16	2.9
Glutamic acid Glu (E)	33	6.0	33	6.0
Glycine – Gly (G)	46	8.4	45	8.2
Histidine – His (H)	14	2.5	14	2.5
Isoleucine – Ile (I)	39	7.1	38	6.9
Leucine – Leu (L)	51	9.3	52	9.5
Lysine – Lys (K)	39	7.1	40	7.3
Methionine – Met (M)	14	2.5	14	2.5
Phenylalanine – Phe (F)	30	2.5	30	5.3
Proline – Pro (P)	29	5.3	29	5.3
Serine – Ser (S)	28	5.1	29	5.3
Threonine – Thr (T)	29	5.3	29	5.3
Tryptophan – Trp (W)	2	0.4	2	0.4
Tyrosine – Tyr (Y)	19	3.5	19	3.5
Valine – Val (V)	45	8.2	44	8.0
Pyrrolysine – Pyl (O)	0	0.0	0	0.0
Selenocysteine – Sec (U	0	0.0	0	0.0

Table 3: Physicochemical properties of the model and template proteins of luciferin 4-monooxygenase from Human Respiratory Syncytial Virus (HRSV)

Properties	***Model***	***Template***
Number of amino acids	550	550
Molecular weight	60644	60745.17
Theoretical pI	6.19	6.42
Total number of negatively charged residues (Asp + Glu)	65	64
Total number of positively charged residues (Arg + Lys)	59	60
Total number of atoms	8605	8619
Chemical formula	C2755H4330N712O790S18	C2758H4337N715O791S18
Estimated half-life	30 Hours	30 Hours
Aliphatic index	95.3	94.65
Hydropathicity value (GRAVY score)	-0.14	-0.040

Secondary Structure Analysis

The secondary structure of the protein was predicted using the PSIPRED protein sequence analysis software [17]. The results for the model and template proteins, including alpha helices, coils, beta sheets, standard, extended strands, polar, aromatic plus, and small nonpolar residues, are illustrated in Figures 2-5. Additionally, analysis using the GOR IV software indicated that the total number of amino acids is 550 [18].

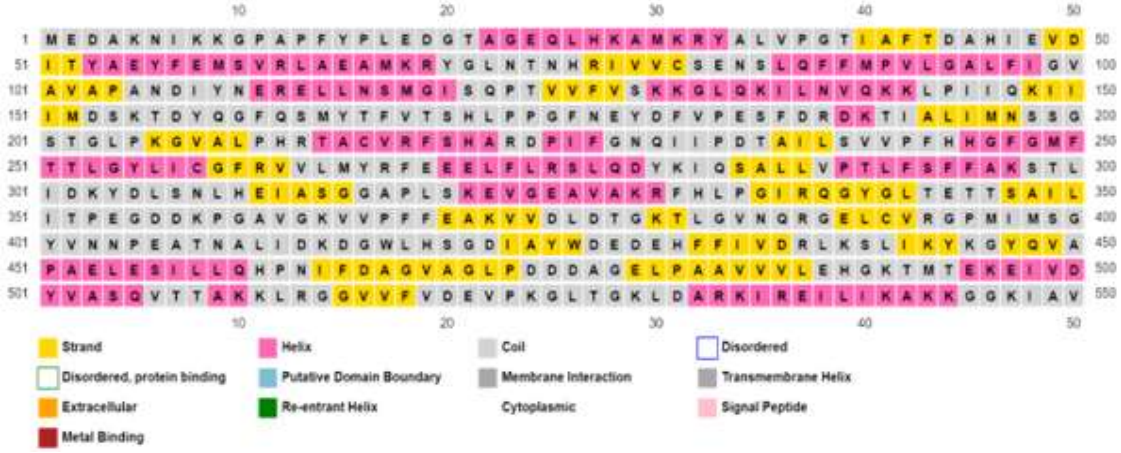

Figure 2: The model displays the presence of helices, strands, coils, turns, and loops

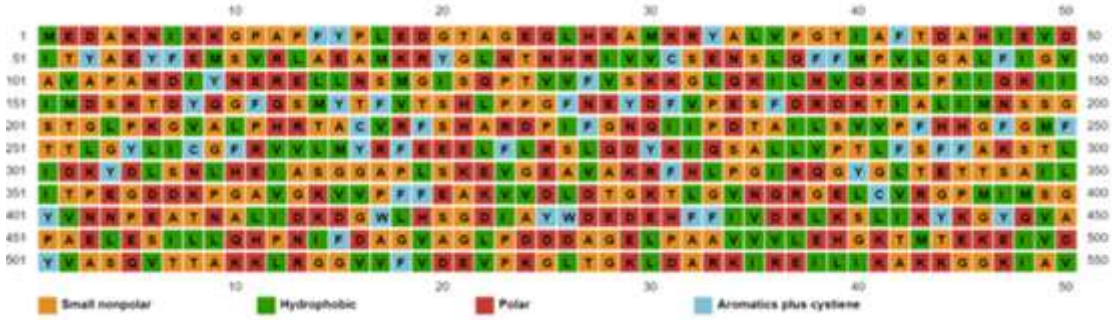

Figure 3: The model shows the presence of polar, small nonpolar, hydrophobic, aromatic plus, and cysteine residues

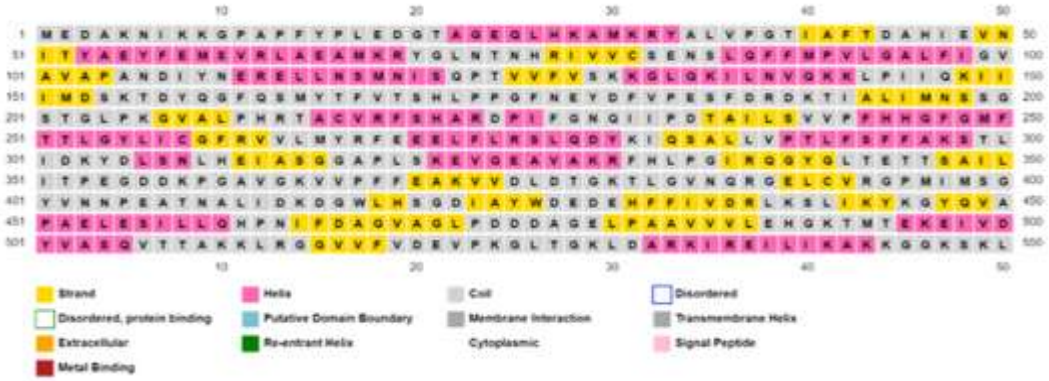

Figure 4: The template displays the presence of helices, strands, coils, turns, and loops

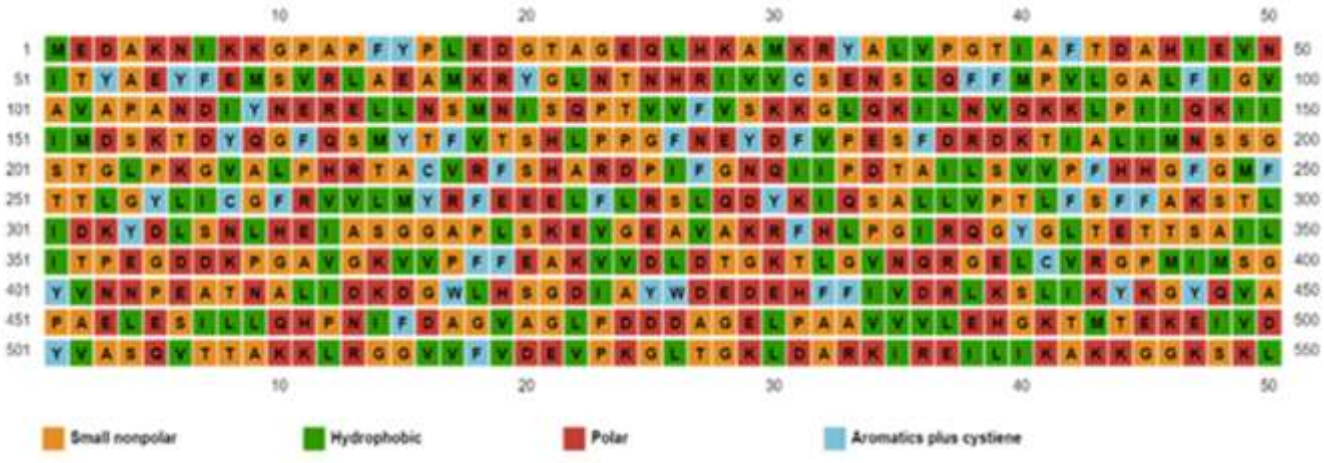

Figure 5: The template shows the presence of polar, small nonpolar, hydrophobic, aromatic plus, and cysteine residues

The percentage of the constituants structure

Each protein unit consists of helices, strands, coils, turns, and loops. The secondary structure of the model and template proteins was predicted using the GOR IV and SOPMA software tools. The results are presented in Tables 4 and 5, and illustrated in Figures 6, 7, and 8 [19].

Table 4: In the V9VFW4 enzyme model, the number and percentage of alpha helices, extended strands, beta turns, and random coils were calculated

Components of the secondary structure	*Number*	*Percentage*
Alpha helix	170	30.91
Extended strand	113	20.55
Beta turn	42	7.64
Random coil	225	40.91
Length of sequence	550	

Table 5 : The proportions of helices, strands, and loops in the model and template proteins were analyzed

Composition of secondary structure	***Model(%)***	***Template(%)***
Helices	25.73	23.36
Strands	16.24	20.07
Coils, turns loops,	58.03	56.75

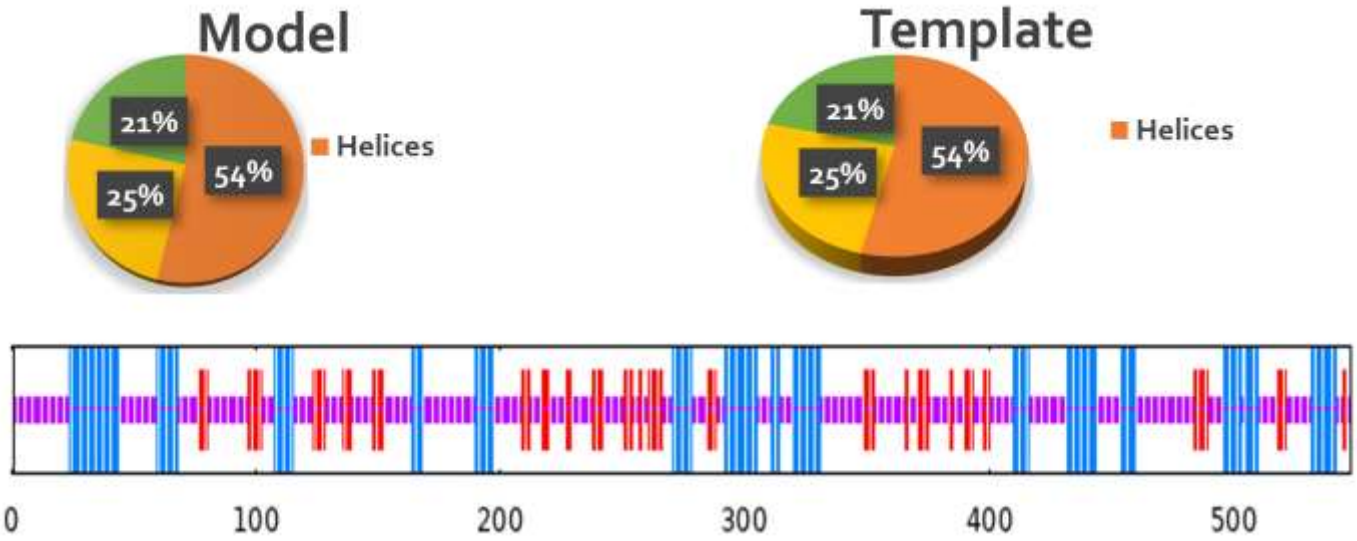

Figure 6: The secondary structure was predicted using SOPMA, which illustrates the presence of alpha helices, beta turns, extended strands, and random coils

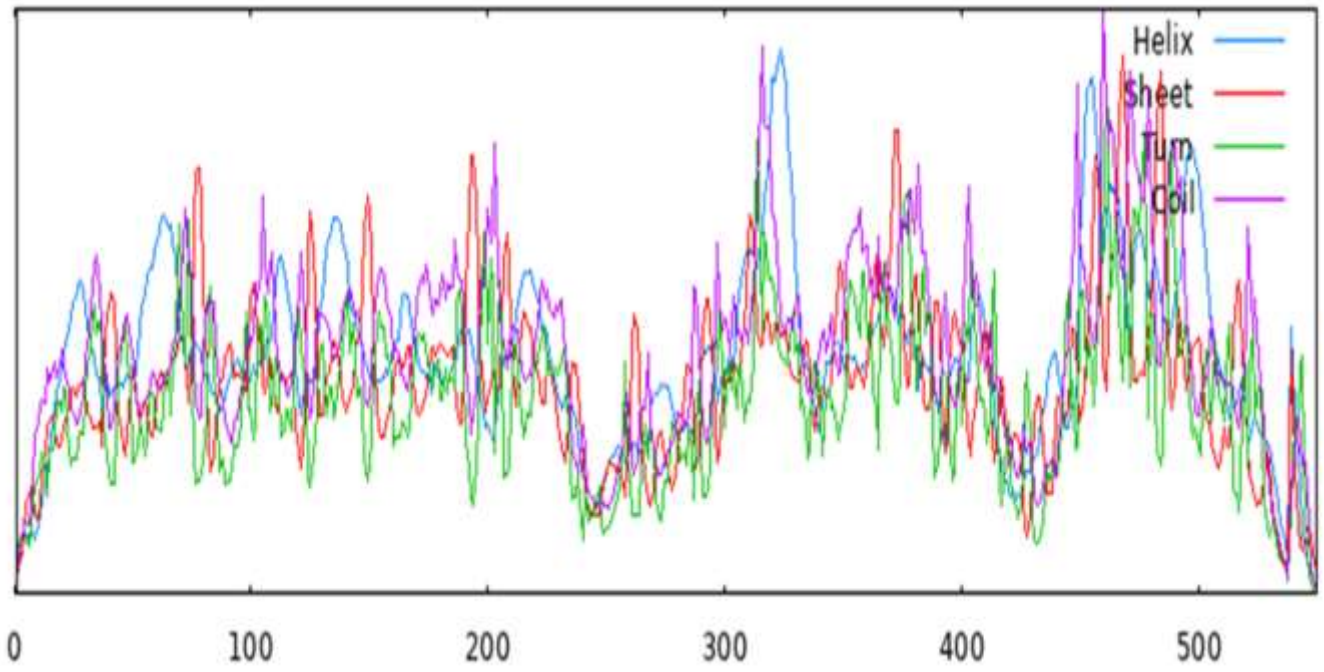

Figure 7: The secondary structure predicted by SOPMA, including alpha helices, beta turns, extended strands, and random coils, is presented in graphical form

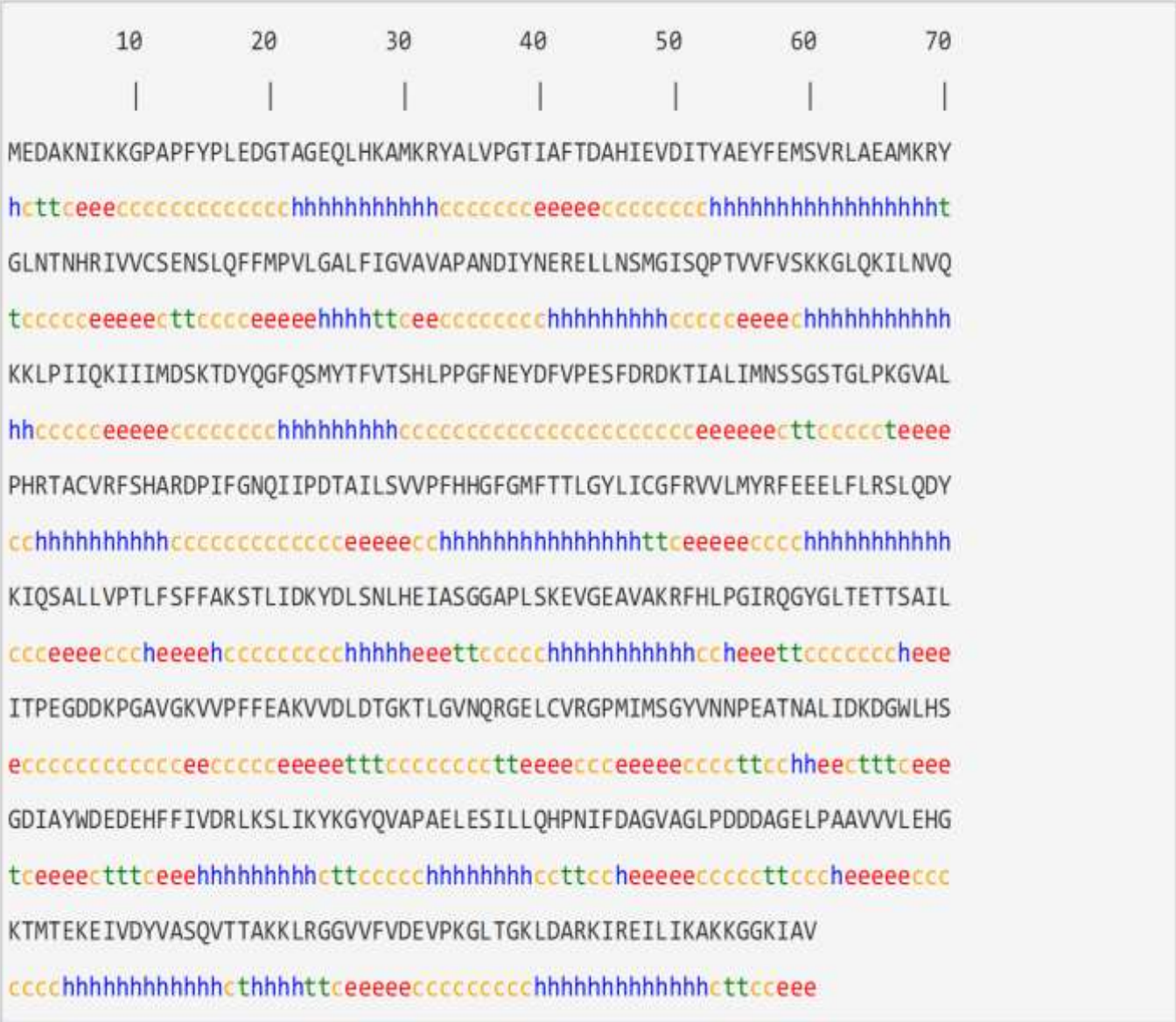

Figure 8: The secondary structure sequence predicted by SOPMA is represented using letters, where alpha helix (H), beta turn (T), random coil (C), and extended strand (E) are indicated based on these symbols

Tertiary Structure predication

The 3D model of the V9VFW4 enzyme was constructed using the Swiss-Model software. To select an appropriate template, multiple protein alignments were submitted to various alignment search tools. The results from the Swiss-Model servers were evaluated, as shown in Figures 9, and 10 [20].

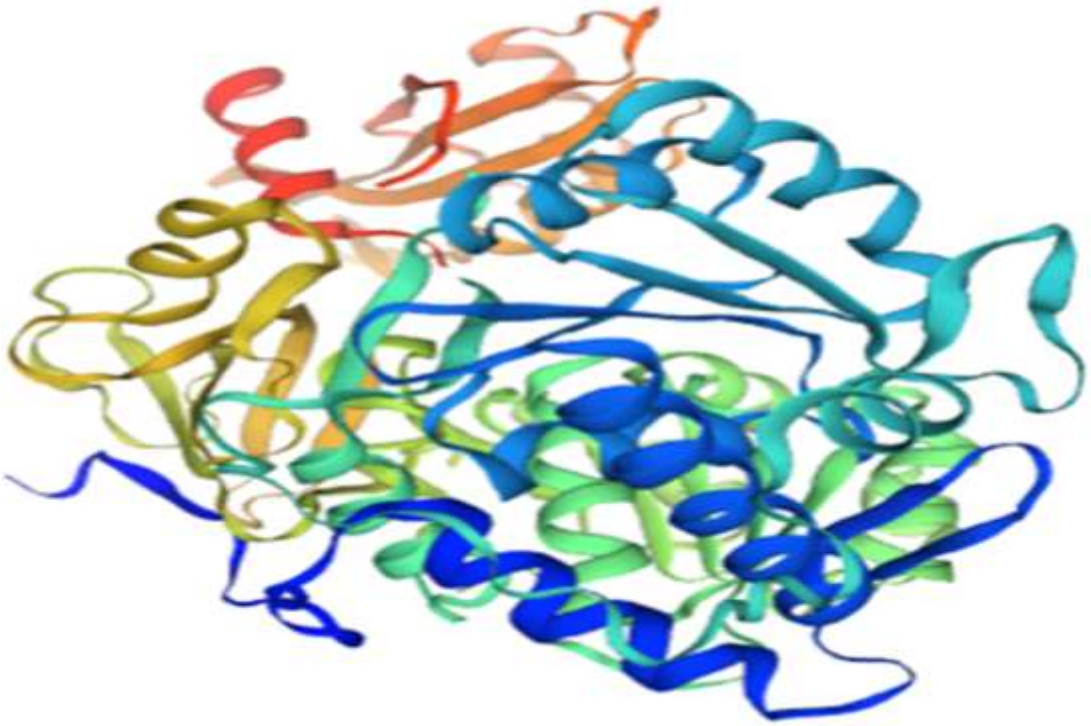

Figure 9: The tertiary structure of the template predicted by Swiss-Model

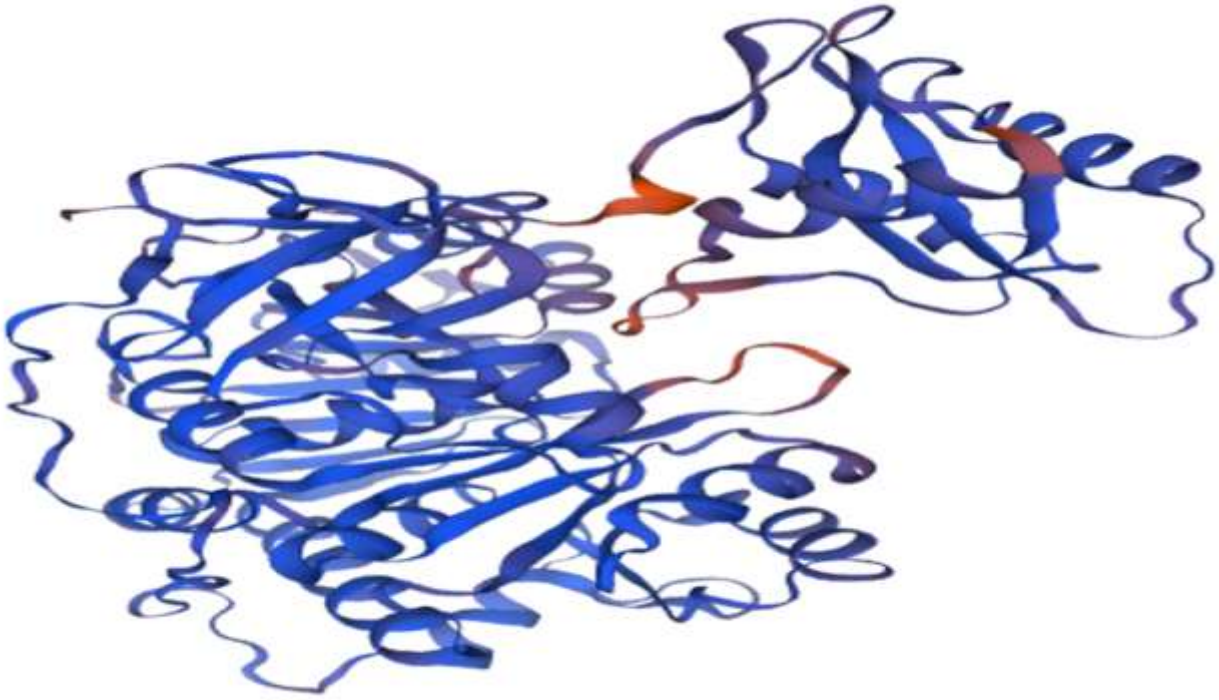

Figure 10: The tertiary structure of luciferin-4 monooxygenase was predicted entirely using Swiss-Model

Homology Modelling Validation

The predicted three-dimensional model (V9VFW4), in PDB file format, was validated using the ERRAT tool. The PROCHECK tool was used to generate the Ramachandran plot, and the Verify 3D tool [21] . was employed to further assess the model's quality. The results are illustrated in Figures 11, 12, and 13 [22].

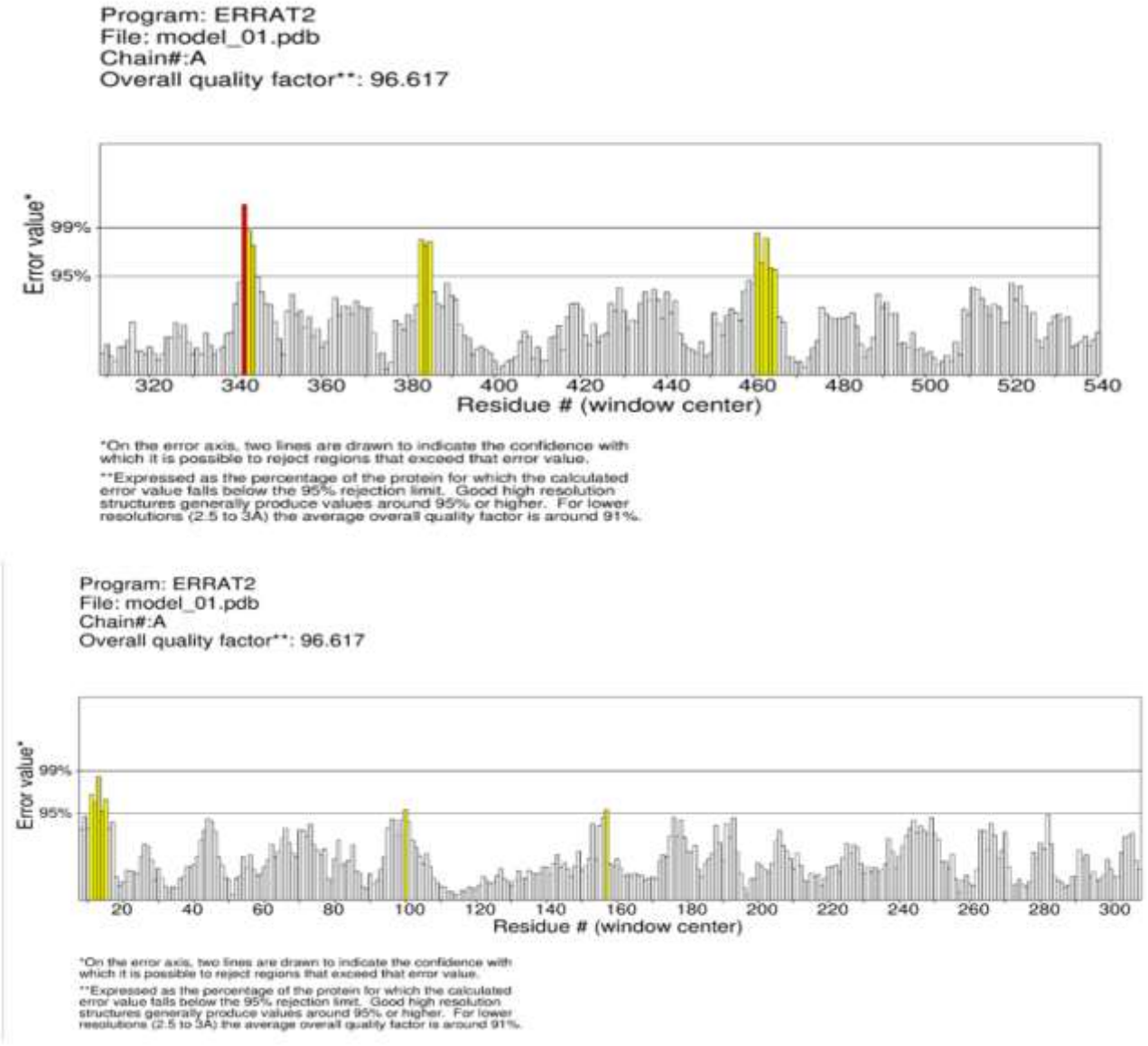

Figure 11: (A,B)Structural validation was performed using ERRAT. Two reference lines were drawn to highlight regions that are likely to be rejected due to having error values exceeding the acceptable threshold

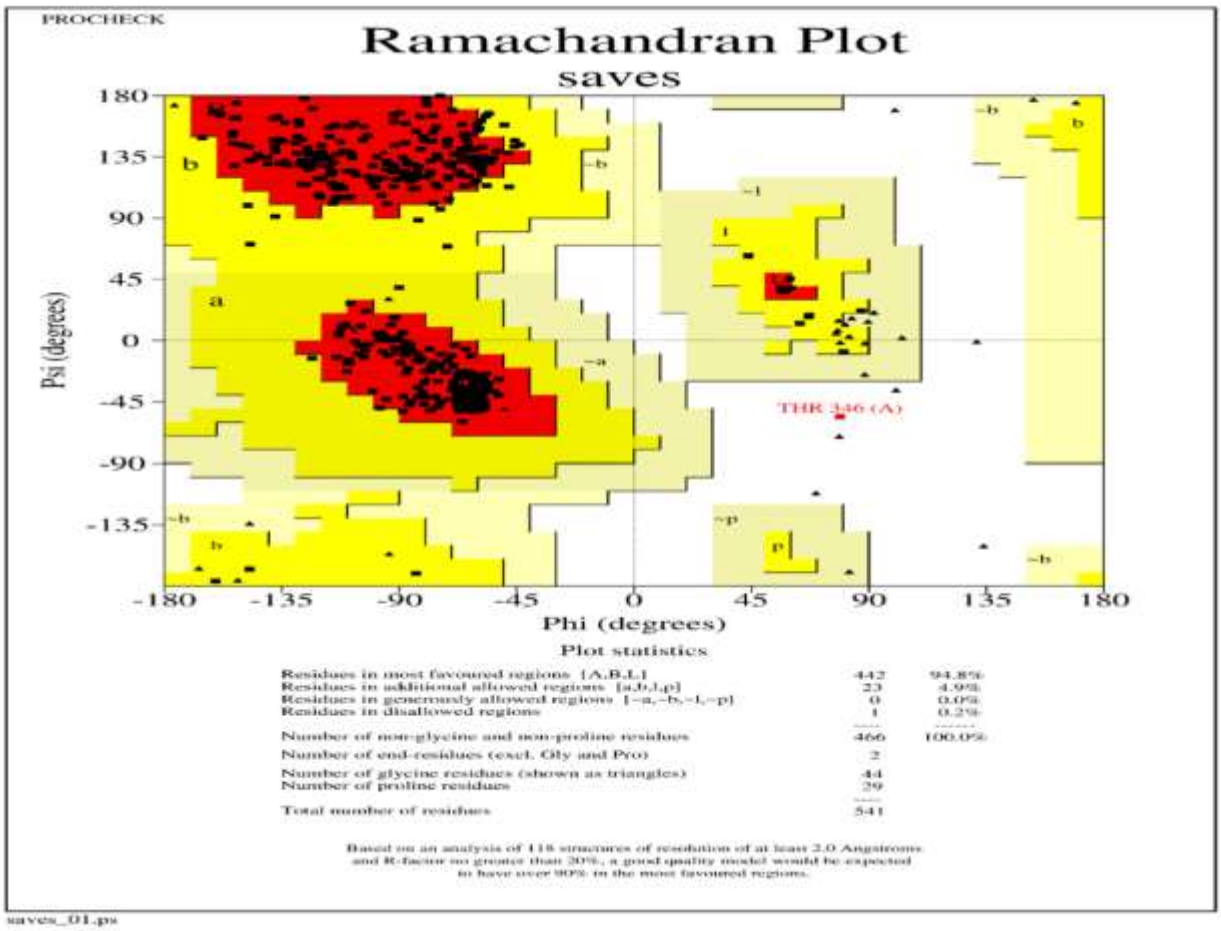

Figure 12: The structural validity of the L4MO enzyme model was assessed using a Ramachandran plot, which displays the distribution of psi–phi angle probabilities

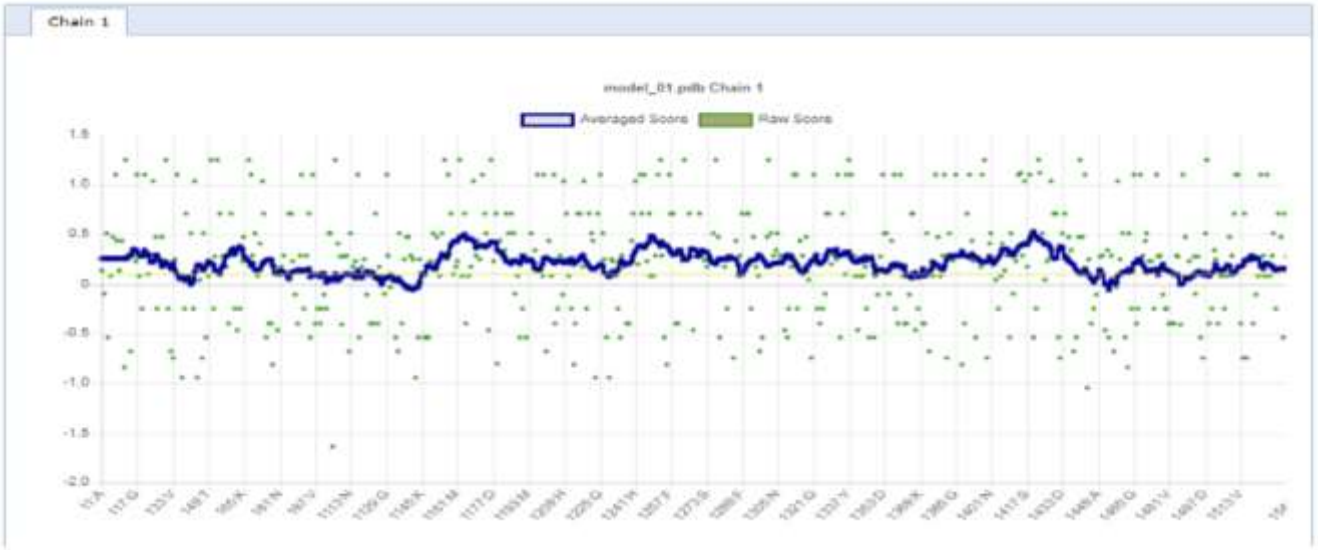

Figure 13: The atomic 3D model was validated against the complete amino acid (1D) sequence using structural assessment based on Verify 3D, to ensure model reliability

Discussion

The primary objective of this study was to predict the 3D structure of L4MO from HRSV using homology modeling approaches in the absence of experimentally resolved structural data. Since no experimentally validated structure is available in major databases such as AlphaFold, this study provides a preliminary structural framework for a protein that remains poorly characterized at both structural and functional levels [23]. However, the biological interpretation of this protein must be approached with caution, as its annotation is primarily derived from sequence-based predictions rather than direct experimental evidence.

From a biological perspective, the presence of L4MO-like annotation in a non-bioluminescent virus such as HRSV raises important questions regarding evolutionary origin and functional relevance. In well-characterized bioluminescent organisms such as Photinus pyralis, luciferin 4-monooxygenases are involved in highly specialized oxidative reactions that produce bioluminescence. In contrast, viruses generally lack metabolic enzymes of this type, suggesting that any L4MO-like sequence in HRSV may either represent a distant homolog with divergent function or a misannotated protein fragment [24]. This highlights a fundamental limitation in purely computational annotation systems, where functional predictions may not always reflect true biological roles. Comparative analysis with previously reported structural modeling studies of luciferase and monooxygenase enzymes provides further insight into this discrepancy. In classical luciferase systems, such as those from Photinus pyralis and related firefly species, structural studies have consistently demonstrated a highly conserved catalytic core with a well-defined substrate-binding pocket essential for luciferin oxidation. These enzymes also show strict conservation of active-site residues that directly participate in the bioluminescent reaction [25]. In contrast, although the predicted L4MO structure shares some conserved motifs with these enzymes, the overall sequence similarity and catalytic residue conservation are comparatively lower, suggesting possible structural resemblance without guaranteed functional equivalence. This pattern is consistent with previous homology-based studies in which proteins adopt similar folds yet perform entirely different biological roles due to divergence in active-site architecture [26].

Furthermore, study on monooxygenase-like proteins in non-bioluminescent systems have shown that structural conservation often reflects evolutionary stability of protein scaffolds rather than preservation of enzymatic function [27]. This is particularly relevant in viral proteins, where structural mimicry can occur without direct enzymatic activity. Therefore, while the predicted L4MO model exhibits a luciferase-like fold, its biological role in HRSV remains speculative and may not involve catalytic activity at all.

The physicochemical characteristics of the model protein are broadly consistent with those observed in related monooxygenase templates, suggesting that the protein can maintain a stable folded conformation under physiological conditions [28]. However, in comparative studies of homologous enzymes, such physicochemical similarity has not been sufficient to infer functional conservation, particularly when active-site residues are not strictly preserved. This further supports the interpretation that structural similarity alone cannot be used as definitive evidence of enzymatic function. Secondary structure comparison with previously characterized monooxygenases reveals a similar distribution of helices, strands, and coils, which is typical for oxidoreductase enzyme families [29]. However, such secondary structural patterns are also widely observed across non-enzymatic proteins, limiting their diagnostic value. In established luciferases, secondary structure or-

ganization is tightly linked to catalytic efficiency and substrate specificity, whereas in the predicted L4MO model, such functional linkage cannot yet be confirmed. At the tertiary structural level, the SWISS-MODEL prediction suggests a stable globular architecture with a putative active-site region [30]. When compared to experimentally resolved luciferase structures, which typically display highly optimized and well-defined substrate-binding cavities, the predicted L4MO binding region appears less structurally constrained. This difference further supports the possibility that, even if structurally related, the protein may not function as a true monooxygenase in HRSV. Model validation results indicate that the predicted structure is stereochemically reliable, with favorable quality scores across multiple assessment tools [31]. However, as observed in previous protein modeling studies, high-quality validation scores primarily confirm structural plausibility rather than biological activity [32]. Therefore, functional inference based solely on computational validation remains limited [33].

While the L4MO protein model shares certain structural features with known monooxygenases, significant uncertainties remain regarding its biological identity and function. The comparative analysis with established luciferase systems suggests that structural similarity does not necessarily translate into functional equivalence, particularly in viral contexts where protein annotation is often uncertain. This underscores the importance of integrating computational modeling with experimental validation, such as gene expression profiling, protein purification, and enzymatic assays, to confirm the true biological role of this protein [34].

Conclusion

We successfully used homology modeling to predict the 3D structure of L4MO from HRSV. Our model provides insights into the structural characteristics of L4MO, which can be crucial for its role in HRSV pathogenesis. Future experimental validation, as well as the development of antiviral drugs and vaccines, could be guided by this structural data. Further investigations will be required to explore the therapeutic potential of L4MO inhibitors and to refine the 3D model to address the areas identified as potentially problematic.

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Data Availability

The datasets generated and analyzed during the current study, including sequence alignments, homology models, structural validation results for Luciferin 4-Monooxygenase (L4MO) from Human Respiratory Syncytial Virus (HRSV), and supplementary materials are available from the corresponding author upon reasonable request.

Conflict of Interests

The authors declare no conflict of interest.

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