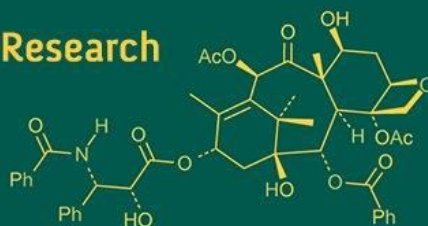


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Structural characterization of three important enzymes of lumpy skin disease virus: A comparative analysis

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Abstract

Lumpy skin disease (LSD) is a highly infectious and threatening disease among livestock mainly affecting cattle, caused by the Lumpy Skin Disease Virus (LSDV). The disease is associated with significant morbidity and mortality, leading to substantial economic losses for livestock owners and a marked decline in productivity within the dairy industry. Recent advances in genome sequencing have identified several hypothetical proteins of LSDV with unknown functions, which may play crucial roles in viral mode of action and could serve as potential targets for preparation of drugs. However, the lack of detailed structural information for these proteins poses a major challenge in the development of specific antiviral drugs and effective vaccines against LSD. In this context, the present study aimed to investigate three key viral proteins: A0A1W6S9T9_LSDV DNA ligase, A0A1C9HHI5_LSDV DNA-directed RNA polymerase, and A0A1C9HHQ2_LSDV DNA polymerase. Analysis of various physical and chemical properties of these proteins were executed using the ExPASyProtParam tool, including isoelectric point, molecular weight, instability index, half-life, amino acid composition and aliphatic index. Prediction of Secondary structure was extracted with the help of the GOR IV method to identify α -helices and β -pleated sheets. Furthermore, tertiary structures were predicted using the SWISS-MODEL server. Structural validation was conducted through Ramachandran plot analysis along with secondary (2D) structure assessments.

The interpretation and results of this study provide valuable informations to the structural and functional properties of LSDV proteins, which may facilitate rational drug design and vaccine development. However, further validation on the basis of experiment is more essential to confirm the structural predictions and elucidate the precise mechanisms of action of these viral proteins, thereby supporting the development of targeted therapeutic strategies for effective control and prevention of LSD.

Keywords: Lumpy skin disease virus, protein sequence, DNA ligase, DNA directed RNA polymerase, DNA polymerase

Introduction

Lumpy skin disease (LSD) is a highly threatening disease of livestock that significantly impacts animal health and welfare. Although LSD is not transmitted to human directly as zoonotic disease, hence poses no direct risk to humans, but it drastically affects the economic stability of livestock producers by causing reduced milk production, infertility, damage to hides and in severe cases, death of cattle.

Lumpy Skin Disease Virus (LSDV) is the causative agent of LSD which generally belongs to belongs to Capripoxvirus, a member of the family *Poxviridae* (Zhai *et al.*, 2023) [43]. The disease primarily affects cattle, although other species such as impala, springbok, water buffalo, giraffe and Arabian oryx may also be affected to varying degrees (Pieters, 2019; Hasib & Islam, 2025) [25, 14]. Exanthema nodularis bovis, pseudo-urticaria, Neethling virus disease, and knopvelsiekte are some other name of the same disease LSD in different regions and different situation. (Al-Salihi, 2014; Tuppurainen *et al.*, 2017) [2, 40]. LSDV is a double-stranded DNA virus transmitted mainly by arthropod vectors like mosquitoes and ticks. Clinical symptoms and manifestations of disease include high fever, lymphadenitis, arthritis, lacrimation, oedema, mastitis, and characteristic nodular skin lesions, leading to substantial economic losses (Chihota *et al.*, 2001; Haegeman *et al.*, 2023; Khalil *et al.*, 2021; Murcia *et al.*, 2009, Akther *et al.*, 2023) [6, 13, 17, 23, 1].

The World Organization for Animal Health recognizes Lumpy Skin Disease as a significant transboundary animal disease due to its economic impact and rapid spread, initially confined to Africa, the disease has progressively spread to Europe, the Middle East, and Asia (Mazloum, Sprygin *et al.*, 2023; An *et al.*, 2023) [36, 4]. In India, the first outbreak was reported in 2019, with widespread infections resembling the Neethling strain. In Odisha, approximately 5, 702 cases were reported in 2019, which increased dramatically to 51, 598 cases during 2022-2023. The rapid spread and high case numbers are largely attributed to the limited availability of specific antiviral drugs and vaccines.

Due to the lack of effective therapeutic regimen, there is growing interest in identifying potential molecular targets using bioinformatics approaches. The proteomic analysis of LSDV will play important roles in identifying viral structure and development of vaccine (Schlosser, Perrin *et al.*, 2023) [28]. In addition to it, by using immunoinformatics approach, the based vaccine based on membrane protein for lumpy skin disease virus (LSDV) will be developed (Salaudin *et al.*, 2024) [27]. Several hypothetical protein sequences of LSDV with unknown functions have been identified and analyzed using computational tools. In this study, particular emphasis is placed on three important proteins: A0A1W6S9T9 (DNA ligase), A0A1C9HHI5 (DNA-directed RNA polymerase), and A0A1C9HHQ2 (DNA polymerase).

DNA ligase plays a pivot role in viral DNA repair and replication, facilitating the joining of DNA strands and contributing to nucleotide metabolism. It has also been explored as a potential marker for differentiating infected from vaccinated animals (DIVA), highlighting its diagnostic importance (Haegeman *et al.*, 2023; Suwankitwat *et al.*, 2025) [13, 38]. The DNA-directed RNA polymerase is a multi-subunit enzyme responsible for transcribing viral DNA into RNA within the host cytoplasm. It regulates early, intermediate, and late gene expression, interacting with transcription factors such as the early transcription factor (ETF) to initiate viral gene transcription (Yadav *et al.*, 2024) [42]. DNA polymerase is essential for viral genome replication and propagation within host cells. Due to its conserved nature and indispensable role in viral replication, DNA polymerase has emerged as a promising target for antiviral drug development. Targeting such essential enzymes may enable the design of specific inhibitors to control LSDV infection effectively (Zia *et al.*, 2024) [44]. Epidemiological studies indicate that the mortality rate of LSD in India ranges from 5% to 45% (Davies *et al.*, 1991; Mathivanan *et al.*, 2023) [10, 20]. States such as Maharashtra, Karnataka, West Bengal, Punjab, Haryana, Rajasthan, Gujarat, and Uttar Pradesh have reported significant morbidity and mortality (Sudhakar *et al.*, 2020) [37].

The detection of LSDV by polymerase amplification assay with the help of Real-time recombinase is widely applicable in clinical diagnosis (Zhai *et al.*, 2023) [43]. Currently, treatment is largely supportive, to alleviate fever and pain, non-steroidal anti-inflammatory drugs (NSAIDs) along with antihistamines, electrolyte therapy, and immune enhancers are used. Preventive measures rely primarily on vaccination, quarantine, and the isolation of infected animals.

Government of India has implemented several control strategies, including restrictions on animal movement, establishment of quarantine facilities, and large-scale vaccination campaigns using the goatpox vaccine to achieve

herd immunity. Despite these efforts, LSD continues to pose a major challenge due to limited awareness, difficulties in early diagnosis, and gaps in understanding disease transmission and management (Yadav *et al.*, 2024a) [42]. Therefore, this study was carried out to characterize these three proteins which can be targeted for drugs development against LSD virus.

Materials and Methods

In the present study, three key proteins of Lumpy Skin Disease Virus (LSDV), namely A0A1W6S9T9 (DNA ligase), A0A1C9HHI5 (DNA-directed RNA polymerase), and A0A1C9HHQ2 (DNA polymerase), were analyzed using a range of bioinformatics tools to predict their structural and functional characteristics. UniProt database software is used to extract the protein sequence (<https://www.uniprot.org>). Template identification and execution of tertiary structure were carried out using the SWISS-MODEL (<https://swissmodel.expasy.org>). Physicochemical properties were analyzed using the ExPASyProtParam tool, while secondary structure prediction was performed using the GOR IV method. Protein solubility and model quality were assessed using SOSUI, and structural validation was conducted using the ProSA server by evaluating Z-scores (1).

The selected proteins are known to play critical roles in viral replication, transcription, and host-virus interactions. Additionally, the presence of putative signal peptides and similarities with known immunomodulatory proteins suggest their involvement in modulating host immune responses (Tulman *et al.*, 2001) [39].

Retrieval of Protein Sequences

The sequences of amino acids of the selected LSDV proteins were extracted from the UniProt server using their respective accession numbers. These sequences were used as the basis for all subsequent computational analyses (2).

Prediction of Three-Dimensional Structure

The tertiary structures of the selected proteins were created using the SWISS-MODEL software, which employs homology modeling techniques (Sahoo *et al.*, 2023) [32]. The target sequences were aligned with suitable template structural form, available in the Protein Data Bank (PDB). Model selection was based on some physiochemical parameters such as Global Model Quality Estimation (GMQE) score, template coverage, and sequence identity. The best model, exhibiting the highest sequence identity and optimal GMQE score, was selected for further validation and analysis in this study(4).

Validation of Protein structure by Ramachandran Plot

The Ramachandran plot generated through Discovery Studio software and the analysis of stereochemical quality of the predicted protein models was evaluated(5). The plots classified amino acid residues into four regions such as disallowed, favored, generously allowed, and additionally allowed regions based on backbone dihedral angles (ϕ and ψ). A higher proportion of residues in favored regions indicates better structural reliability (Sahoo *et al.*, 2018) [29].

Model Validation and Quality Assessment

Protein solubility and stability were evaluated using SOSUI software (Hirokawa *et al.*, 1998) [15]. This analysis helped

determine whether the predicted proteins are likely to be soluble or membrane-associated, providing insight into their functional behavior.

Estimation of Physicochemical Parameters

The computation of Physicochemical properties of the proteins were done by using the ExPASyProtParam tool (Gasteiger *et al.*, 2005) [11]. Parameters analyzed included aliphatic index, molecular weight, theoretical instability index, isoelectric point (pI), grand average of hydropathicity (GRAVY), estimated half-life, and the number of charged residues like positive and negative. These data and informations are essential for understanding protein stability, solubility, and potential functional behavior.

Secondary Structure Prediction

Secondary structure elements, including α -helices, β -sheets, and random coils, were predicted using the GOR IV method

via the NPS @ (Network Protein Sequence Analysis) server (Combet *et al.*, 2005) [8]. The results were expressed as percentage compositions and visualized using color-coded structural representations.

Results

The analysis of amino acid sequence revealed, the DNA ligase, DNA-directed RNA polymerase, and DNA polymerase proteins consisted of 559, 1156, and 1010 amino acid residues, respectively. Their corresponding molecular weights were calculated as 64, 619.67 Da for DNA ligase, 132, 669.03 Da for DNA-directed RNA polymerase, and 117, 557.00 Da for DNA polymerase. Among these, the DNA-directed RNA polymerase exhibited the highest number of amino acid residues and molecular weight, indicating greater structural complexity and potential functional significance.

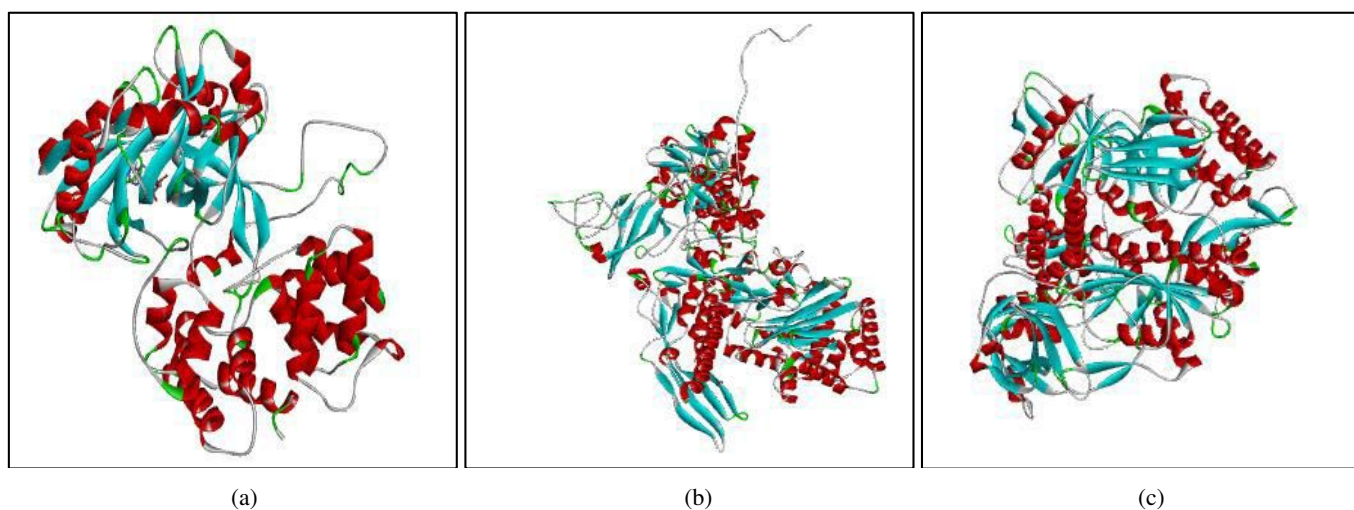


Fig 1: Showing the quaternary structure of a. DNA ligase b. DNA-directed RNA polymerase c. DNA Polymerase

The analysis of Ramachandran plot described the different stereochemical quality of the predicted protein models. A majority of the residues of amino acid for all three proteins were present in the favored place of interest, with only a minimal number of residues falling in disallowed regions.

This distribution confirms the reliability and acceptable quality of the predicted three-dimensional models. The Ramachandran plots along with the corresponding tertiary structures are presented in Figures 2

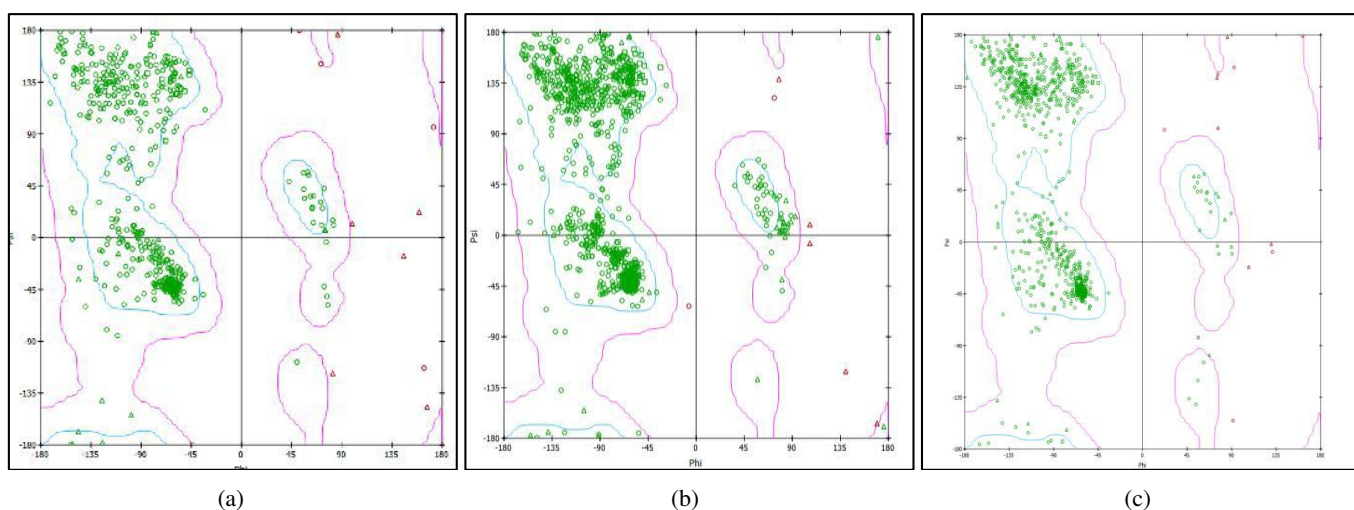


Fig 2: Showing the Ramachandran Plot a. DNA ligase b. DNA-directed RNA polymerase c. DNA Polymerase

Protein solubility analysis using SOSUI indicated that all three proteins are soluble in nature, suggesting their suitability for further experimental and functional studies.

Hydrophobicity profiles of the proteins are illustrated in Figures 3.

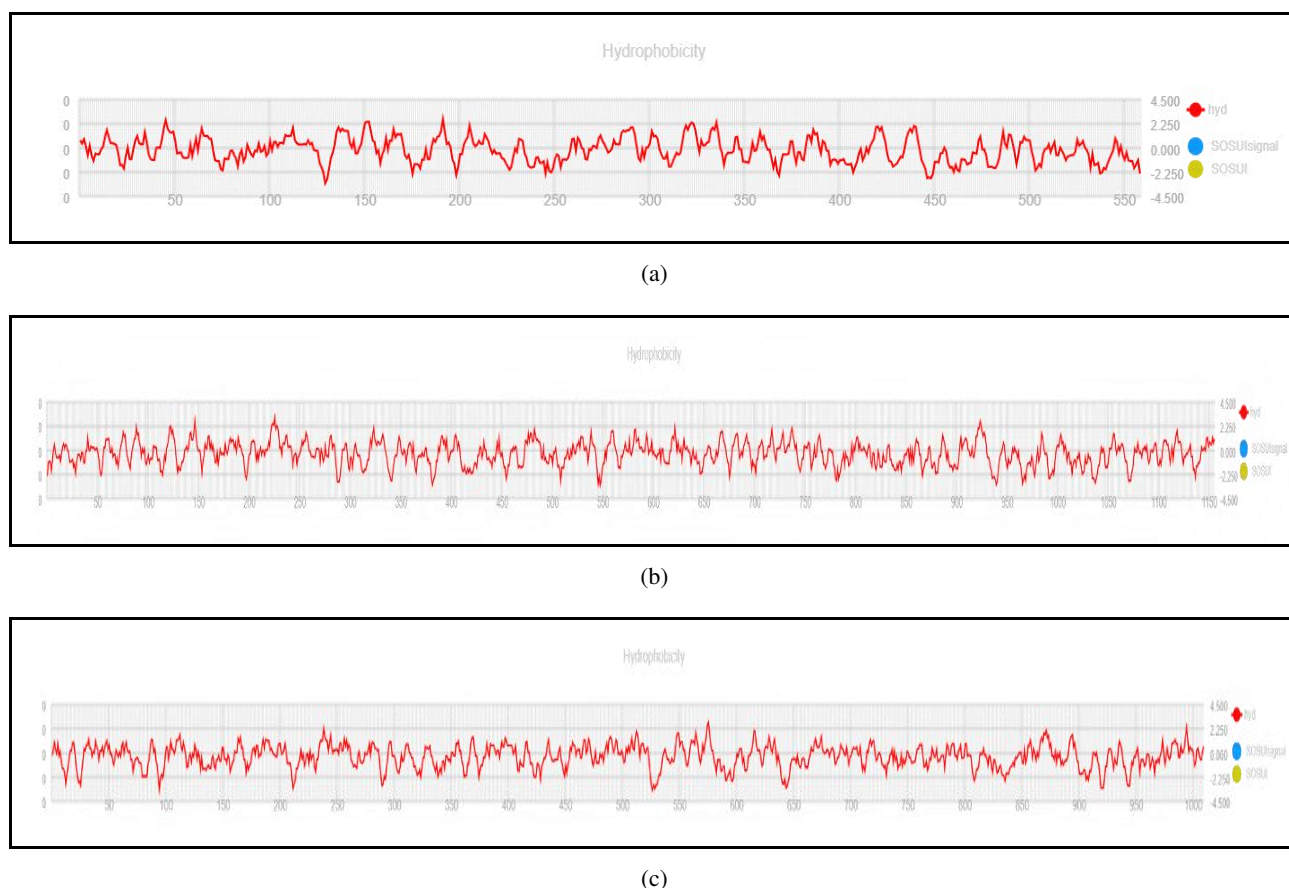


Fig 3: Showing hydrophobicity a. DNA ligase b. DNA-directed RNA polymerase c. DNA polymerase

Further structural validation was carried out using the ProSA server, which evaluates model quality based on Z-score analysis. The Z-scores obtained for all three proteins were within the acceptable range typically observed for

native protein structures, supporting the accuracy and reliability of the predicted models. Additionally, energy profile plots generated by ProSA are shown in Figures 4.

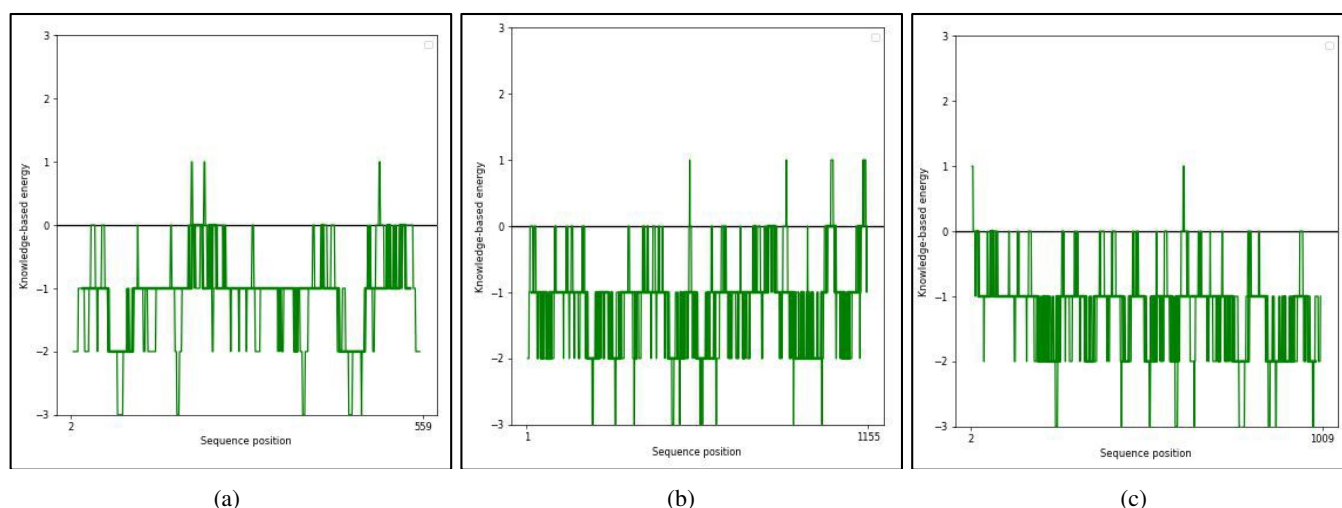


Fig 5: Showing energy sequence plot a. DNA ligase b. DNA-directed RNA polymerase c. DNA polymerase

The physical and chemical properties of the proteins, including aliphatic index, molecular weight, instability index, theoretical isoelectric point (pI), GRAVY, half-life, and charged residues, were computed and compared. These

parameters provide important insights into protein stability, solubility, and thermostability. A detailed comparative summary is presented in Table 1

Table 1: Showing Physiochemical Parameters of three proteins

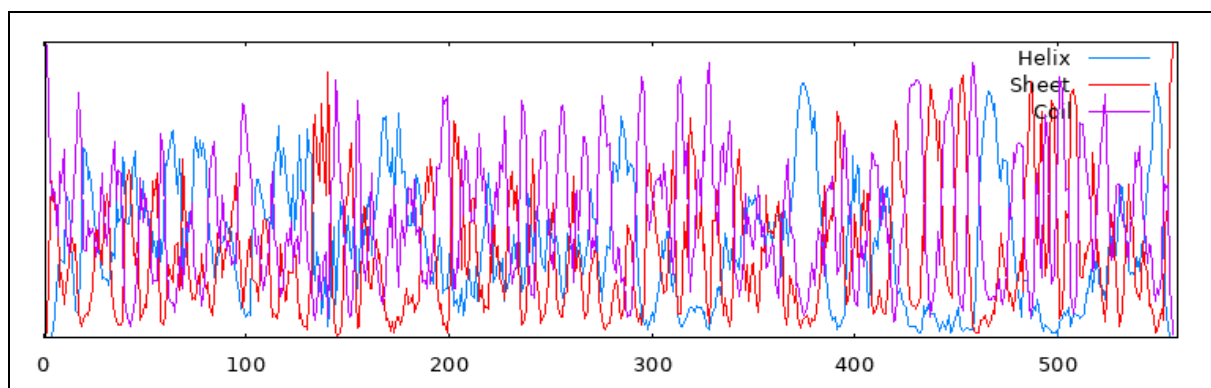
Sl. No	Physio-chemical parameters	Name of the Protein		
		DNA ligase	DNA-directed RNA polymerase	DNA polymerase
1	Number of amino acids	559	1156	1010
2	Theoretical pI	9.22	8.47	8.55
3	Molecular weight	64619.67	132669.03	117557.00
4	Total number of negatively charged residues (Asp + Glu)	64	135	125
5	Total number of positively charged residues (Arg + Lys)	87	145	137
6	Total number of atoms	9215	18708	16576
7	Primary accession	A0A1W6S9T9	A0A1C9HHI5	A0A1C9HHQ2
8	Ext. coefficient	65500	104630	118150
9	Estimated half-life:	30 hours (mammalian reticulocytes, <i>in vitro</i>).	30 hours (mammalian reticulocytes, <i>in vitro</i>).	30 hours (mammalian reticulocytes, <i>in vitro</i>).
10	Instability index:	27.67	37.27	39.10
11	Aliphatic index	96.39	90.85	87.88
12	Grand average of hydropathicity (GRAVY)	-0.200	-0.311	-0.338
13	Z Score	-9.49	-13.13	-13.18
14	Model quality	Soluble protein	Soluble protein	Soluble protein
15	GMQE Value	0.76	0.83	0.78

Secondary structure prediction revealed varying proportions of random coils, α -helices, and β -sheets among the three proteins. The distribution of these structural elements suggests differences in protein folding patterns and

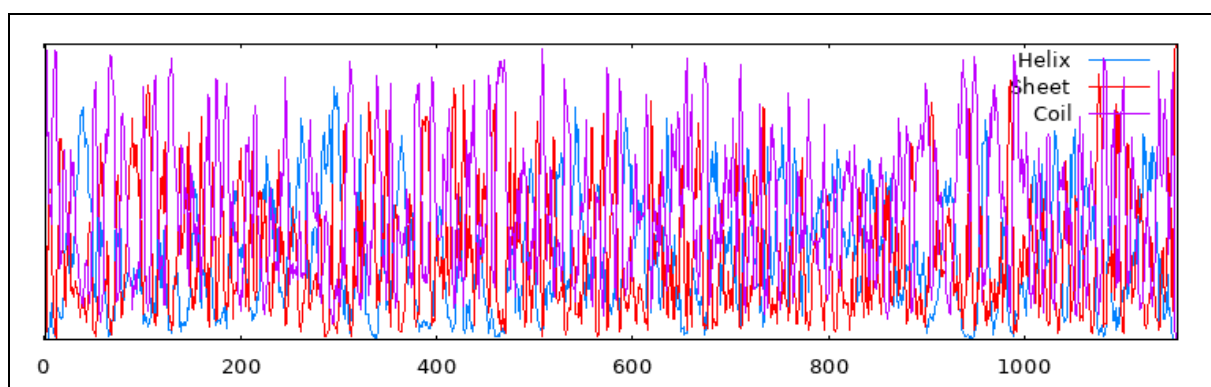
functional diversity. The percentage composition of secondary structure elements is summarized in Table 2, and their graphical representations are provided in Figures 6.

Table 2: Showing Secondary structure of the aforesaid proteins

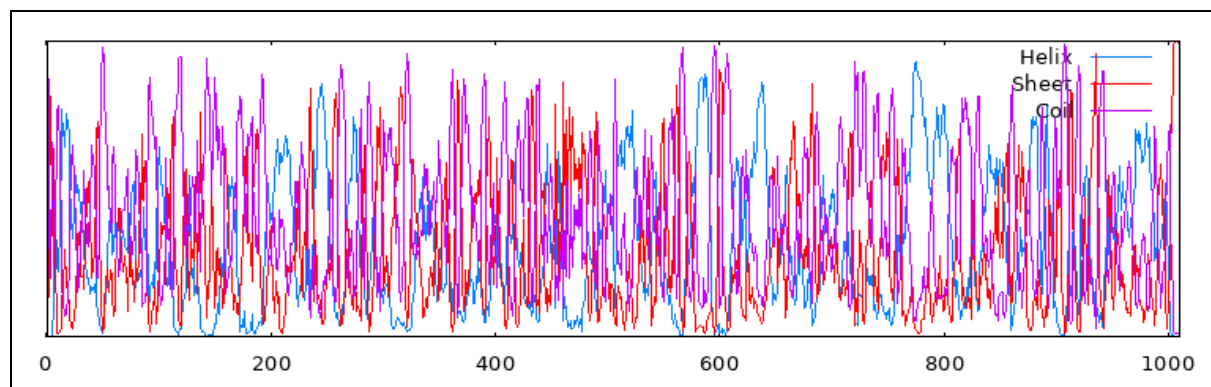
	DNA ligase	DNA-directed RNA polymerase	DNA polymerase
Alpha helix (Hh)	168 is 30.05%	318 is 27.51%	286 is 28.32%
Beta bridge (Bb)	0 is 0.00%	0 is 0.00%	0 is 0.00%
Extended strand (Ee)	116 is 20.75%	256 is 22.15%	255 is 25.25%
Beta turn (Tt)	0 is 0.00%	0 is 0.00%	0 is 0.00%
Bend region (Ss)	0 is 0.00%	0 is 0.00%	0 is 0.00%
Random coil (Cc)	275 is 49.19%	582 is 50.35%	469 is 46.44%



(a)



(b)



(c)

Fig 6: Showing Secondary structure a. DNA ligase b. DNA-directed RNA polymerase c. DNA polymerase

Discussion

In the present study, comprehensive *in silico* structural and physicochemical characterization of three important enzymes, namely DNA ligase, DNA-directed RNA polymerase, and DNA polymerase, was carried out to evaluate their structural stability, folding characteristics, and functional significance. These proteins are essential components of nucleic acid metabolism and play critical roles in DNA replication, transcription, and repair processes. Their structural features are closely associated with catalytic activity, substrate specificity, and molecular interactions (Alberts *et al.*, 2015) [3].

The amino acid sequence analysis showed significant variation in protein length and molecular weight among the three proteins. DNA-directed RNA polymerase exhibited the highest number of amino acid residues (1156) and molecular weight (132, 669.03 Da), followed by DNA polymerase (1010 residues; 117, 557.00 Da) and DNA ligase (559 residues; 64, 619.67 Da). The larger size of DNA-directed RNA polymerase indicates a more complex structural organization, which is consistent with its multifunctional role in RNA synthesis, promoter binding, and interaction with regulatory molecules (Cramer, 2019) [9]. Variations in molecular weight and amino acid composition often reflect differences in catalytic domains and protein architecture (Nelson and Cox, 2017) [24].

Ramachandran plot analysis demonstrated that the majority of amino acid sequences of all these three numbers of predicted protein models were situated within favourable regions, with very few residues in disallowed regions. This confirms good stereochemical quality and structural reliability of the predicted models. Ramachandran plots are widely used for validating backbone conformations of proteins and assessing the quality of predicted tertiary structures (Ramachandran *et al.*, 1963; Lovell *et al.*, 2003) [26, 19].

Solubility prediction using the SOSUI server indicated that all three proteins are soluble in nature. Protein solubility is an important parameter for recombinant protein expression and purification, particularly in bacterial expression systems such as *Escherichia coli*. Soluble proteins generally possess balanced hydrophobic and hydrophilic residues, which contribute to proper folding and structural stability (Hirokawa *et al.*, 1998) [15]. These hydrophobicity and protein length are widely used in protein classification computation (Mitaku and Hirokawa, 1999) [21].

The ProSA Z-score values obtained for the predicted protein models were placed in between the acceptable range as

observed for desire proteins, confirming structural accuracy and reliability. The ProSA tool evaluates model quality based on statistical analysis of experimentally determined protein structures and provides insight into possible structural errors (Wiederstein and Sippl, 2007) [41]. The negative Z-score values observed in this study indicate energetically favorable conformations and stable protein folding.

Physicochemical characterization revealed that the isoelectric point (pI) values of the proteins present in between 8.47 to 9.22, suggesting slightly basic proteins such as arginine and lysine with a higher proportion of positive charged amino acids. These residues facilitate interaction with DNA and RNA the negative charged nucleic acids. (Bjellqvist *et al.*, 1993) [5]. The instability index values indicated that DNA ligase is comparatively more stable than DNA-directed RNA polymerase and DNA polymerase, although all three proteins fall within the acceptable stability range (Guruprasad *et al.*, 1990) [12]. The aliphatic index values were relatively high (87.88-96.39), suggesting good thermostability of the proteins (Ikai, 1980) [16]. This aliphatic index parameter determines protein interaction and helps in determining the membrane water interface regions (Mitaku *et al.*, 2002) [22]. GRAVY values indicated slight variation in hydrophobicity, which influences protein folding and structural compactness (Kyte and Doolittle, 1982) [18].

Secondary structure prediction revealed that random coil regions constitute a major portion of the protein structure (46-50%), followed by α -helices (27-30%) and β -sheets (20-25%). The presence of higher random coil regions suggests structural flexibility, which is essential for enzymatic function and substrate binding. α -helices and β -sheets contribute significantly to protein stability and domain formation (Chou and Fasman, 1974) [7]. The variation in secondary structural elements among the three proteins reflects their functional diversity and molecular interactions. The GMQE values ranging from 0.76 to 0.83 indicate good reliability of the predicted tertiary models, supporting their suitability for further molecular docking and functional studies. Collectively, stereochemical validation, physicochemical characterization, and structural prediction confirmed that the modeled proteins possess stable conformations and functional relevance.

Overall, the present study provides valuable structural insight into three essential enzymes involved in DNA replication and transcription processes. The findings indicate that DNA-directed RNA polymerase possesses

higher structural complexity, while DNA ligase exhibits comparatively greater stability. These predicted models may serve as a foundation for future studies including molecular docking, protein engineering, inhibitor design, and recombinant protein expression. Such structural information is particularly useful in biotechnology, drug discovery, and molecular diagnostic research.

Conclusion

The present *in silico* analysis demonstrated that DNA ligase, DNA-directed RNA polymerase, and DNA polymerase possess structurally stable and reliable three-dimensional models supported by favorable Ramachandran plot distribution, acceptable ProSA Z-scores, and good GMQE values. Physicochemical characterization indicated suitable acceptable instability index, alkaline pI, molecular weight, balanced GRAVY values, and high aliphatic index suggesting good stability, solubility, and thermo stability of the proteins. Secondary structure analysis revealed predominance of random coils along with α -helices and β -sheets, indicating appropriate folding and functional flexibility. Among the three proteins, DNA-directed RNA polymerase exhibited greater structural complexity, whereas DNA ligase showed comparatively higher stability. Overall, the predicted models provide a reliable structural basis for future studies such as molecular docking, protein engineering, and functional characterization.

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