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Namitha

1) Manasa Gangotri College,
 University of Mysore,
 Karnataka, India
 2) CSB-Central Sericultural
 Research & Training Institute,
 Mysuru, Karnataka, India

Pradeep C

CSB-Central Sericultural
 Research & Training Institute,
 Mysuru, Karnataka, India

Comprehensive *in silico* structural and evolutionary characterization of *Bombyx mori* hemolin reveals its potential interaction with baculoviral GP64

Namitha and Pradeep C

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Abstract

Bombyx mori nucleopolyhedrovirus (BmNPV) is a major viral pathogen causing significant economic losses in sericulture. Hemolin, an insect-specific immunoglobulin superfamily protein, plays an important role in innate immunity; however, its structural characteristics and interaction with the viral envelope glycoprotein GP64 remain poorly understood. This study employed an integrated *in silico* approach to characterize *Bombyx mori* hemolin. Phylogenetic analysis was performed using the Maximum Likelihood method. Three-dimensional structures of hemolin and GP64 were predicted by homology modeling and validated using Ramachandran plot, MolProbity, ERRAT, and ProSA analyses. Conserved domains and motifs were identified using SMART, NCBI Conserved Domain Database, and MEME Suite. Protein-protein docking and interaction analyses were conducted using HDOCK and PLIP. Phylogenetic analysis revealed that *B. mori* hemolin clustered closely with *Bombyx mandarina*, indicating strong evolutionary conservation. Structural modeling generated reliable three-dimensional models with favorable validation statistics. Domain analysis identified four conserved immunoglobulin-like domains, while motif analysis revealed three highly conserved motifs across lepidopteran hemolins. Molecular docking predicted a stable hemolin-GP64 complex (Docking score: -241.36) stabilized by extensive hydrogen bonds, hydrophobic interactions, salt bridges, and π -cation interactions, suggesting a favorable binding interface. The findings demonstrate that hemolin is a structurally conserved immune recognition protein with the potential to interact with the baculoviral GP64 glycoprotein. This study provides structural insights into host-virus recognition in *B. mori* and establishes a foundation for future experimental validation and the development of disease-resistant silkworm lines.

Keywords: *Bombyx mori*, Hemolin, GP64, BmNPV, molecular docking, phylogenetic analysis

1. Introduction

Sericulture is an economically important agro-based industry that supports the livelihoods of millions of rural households worldwide, particularly in Asian countries such as China and India, which together account for the majority of global silk production (Sharma *et al.*, 2020) [1]. The domesticated silkworm, *Bombyx mori* (Lepidoptera: Bombycidae), serves as the principal producer of commercial silk and has also emerged as an important model organism for studies on insect genetics, physiology, immunity, and host-pathogen interactions (Ayaz *et al.*, 2025) [2]. However, the productivity and profitability of sericulture are severely constrained by infectious diseases caused by viruses, bacteria, fungi, and microsporidia. Among these, grasserie disease, caused by *Bombyx mori* nucleopolyhedrovirus (BmNPV), is regarded as one of the most destructive viral diseases of silkworms, frequently causing substantial reductions in larval survival, cocoon yield, and silk quality (Chauhan *et al.*, 2025) [3]. Outbreaks of BmNPV can spread rapidly under rearing conditions and result in considerable economic losses to the sericulture industry, highlighting the need for a better understanding of the molecular mechanisms governing host resistance and viral infection (Ayaz *et al.*, 2025) [2].

BmNPV belongs to the genus *Alphabaculovirus* within the family *Baculoviridae* and possesses a circular double-stranded DNA genome (Yang *et al.*, 2025) [4]. During infection, the virus produces two morphologically and functionally distinct virion phenotypes: the Occlusion-Derived Virus (ODV), responsible for initiating primary infection in the larval midgut following oral ingestion, and the Budded Virus (BV), which mediates systemic

Corresponding Author:**Pradeep C**

CSB-Central Sericultural
 Research & Training Institute,
 Mysuru, Karnataka, India

dissemination of the infection throughout the host (Blissard *et al.*, 2018) [5]. Among the viral structural proteins, GP64 is the major envelope fusion glycoprotein present on BV particles and is indispensable for viral attachment, membrane fusion, endosomal escape, and cell-to-cell transmission. Owing to its essential role in viral infectivity, GP64 has become one of the best-characterized baculoviral proteins and represents an attractive target for investigating host-virus interactions and developing antiviral strategies (Monsma *et al.*, 1996) [6].

Unlike vertebrates, insects rely exclusively on innate immunity to defend themselves against invading pathogens (Eleftherianos *et al.*, 2022) [7]. In *B. mori*, the hemolymph functions as the principal circulatory fluid and serves as a dynamic reservoir of immune molecules, transport proteins, storage proteins, enzymes, antimicrobial peptides, and signaling factors that collectively contribute to physiological homeostasis and pathogen defense (Merzendorfer *et al.*, 2026) [8]. Proteomic investigations have demonstrated that the composition of hemolymph changes substantially following microbial or viral infection, reflecting activation of immune signaling pathways, oxidative stress responses, apoptosis, and metabolic reprogramming (Wang *et al.*, 2021) [9]. Characterization of these protein alterations provides valuable insights into the molecular events associated with pathogen recognition and antiviral defense.

Among the immune-associated proteins present in lepidopteran hemolymph, hemolin has attracted considerable attention because of its multifunctional role in innate immunity. Hemolin is a member of the immunoglobulin superfamily and functions as a pattern-recognition molecule capable of binding pathogen-associated molecular patterns and facilitating immune activation (Bian *et al.*, 2026) [10]. Previous studies have reported significant induction of hemolin expression following bacterial, fungal, and viral infections, suggesting its involvement in pathogen recognition and host defense (He *et al.*, 2025) [11]. Structural analyses have revealed that hemolin contains conserved immunoglobulin-like domains that have been maintained throughout lepidopteran evolution, emphasizing its biological importance (Aathmanathan *et al.*, 2018) [12]. Despite accumulating evidence supporting the immunological significance of hemolin, its structural characteristics and potential interaction with essential viral proteins during BmNPV infection remain insufficiently understood (Bian *et al.*, 2026) [10].

Recent advances in proteomics and computational biology have greatly enhanced our ability to investigate host-pathogen interactions at both molecular and structural levels (Jean *et al.*, 2017) [13]. Biochemical approaches, including SDS-PAGE and quantitative protein estimation, enable the identification of differentially expressed proteins associated with infection, whereas bioinformatics tools facilitate the prediction of physicochemical properties, conserved domains, evolutionary relationships, three-dimensional structures, protein-protein interactions, and molecular docking (Sen *et al.*, 2016) [14]. Integrating experimental observations with computational analyses provides a comprehensive framework for understanding protein function and identifying molecular determinants involved in antiviral defense (Dix *et al.*, 2016) [15]. Such multidisciplinary approaches have become increasingly valuable for elucidating the mechanisms underlying insect

immunity and for identifying candidate proteins that could be exploited in breeding or molecular improvement programs aimed at enhancing disease resistance.

Although several studies have documented changes in gene expression and protein abundance during BmNPV infection, relatively few have done structural and interaction analyses of key host and viral proteins. In particular, the molecular basis of the interaction between the host immune protein hemolin and the viral envelope glycoprotein GP64 remains poorly characterized. Therefore, the present study focuses on comprehensive in silico characterization of hemolin and GP64, including analyses of their physicochemical properties, conserved domains, structural features, evolutionary relationships, and protein-protein interactions. This integrated approach provides new insights into the potential role of hemolin in antiviral defense and advances our understanding of the molecular mechanisms underlying host-BmNPV interactions.

2. Materials and Methods

2.1 Phylogenetic analysis

The CDS sequences of hemolin from *Bombyx mori* and other related insects were assembled using Clustal Omega (<https://www.ebi.ac.uk/Tools/msa/clustalo/>). The hemolin sequences from other insect species were retrieved from the NCBI database. Sequences were aligned using ClustalW with default settings in BioEdit (Version 7.2.5) (Hall, 1999) [16]. The molecular phylogenetic tree was generated in MEGA 11 (Tamura *et al.*, 2021) [17] using the Maximum Likelihood (ML) method with complete gap deletion and resampled with 100 bootstrap replications.

2.2 Three-dimensional structure prediction and visualization of hemolin and GP64

The nucleotide sequences of *Bombyx mori* hemolin and nucleopolyhedroviral receptor GP64 were translated into amino acid sequences using the ExPASy Translate tool (<https://web.expasy.org/translate/>). The resulting protein sequences were used for homology-based three-dimensional (3D) structure prediction using the SWISS-MODEL server (<https://swissmodel.expasy.org/>). Suitable template structures were automatically identified based on sequence similarity, and multiple models were generated. The best-quality models were selected based on structural validation parameters including GMQE, QMEAN scores, and Ramachandran plot analysis, with preference given to models showing a higher proportion of residues in favored regions. The selected models were downloaded in Protein Data Bank (.pdb) format and visualized using UCSF Chimera (<https://www.cgl.ucsf.edu/chimera/>) to generate cartoon representations and PyMOL (<https://pymol.org/>) to produce surface representations of the predicted 3D structures. The quality of the predicted three-dimensional structures of hemolin and GP64 was further evaluated using ERRAT and ProSA-web servers. ERRAT (<https://servicesn.mbi.ucla.edu/ERRAT/>) was used to analyze the statistics of non-bonded atom-atom interactions and to assess the overall quality factor of the models. ProSA-web (<https://prosa.services.came.sbg.ac.at/prosa.php>) was employed to determine the overall model quality by calculating the Z-score, which reflects the degree of nativeness of the predicted structures relative to experimentally determined protein structures. The ERRAT overall quality factor and ProSA Z-scores were used as

additional validation parameters to assess the reliability of the homology-modeled structures.

2.3 Protein domain prediction of hemolin from *Bombyx mori* and related species

The Simple Modular Architecture Research Tool (SMART) (<https://smart.embl-heidelberg.de/>) and NCBI conserved domain search (<https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi>) was used to analyse the functional and conserved domains of hemolin protein. The obtained domains were compared with the related insect species domains. The amino acid sequence of target genes was obtained using the exPasy translation tool (<https://web.expasy.org/translate/>).

2.4 Conserved motif analysis of hemolin proteins

Conserved motif analysis of Hemolin proteins from *Bombyx mori* and related lepidopteran species was performed using the Multiple Em for Motif Elicitation (MEME) Suite (<https://meme-suite.org/meme/>). The analysis was conducted with default parameters, including the identification of statistically significant conserved motifs within the input protein sequences. Motif distribution, sequence conservation, and positional patterns among Hemolin proteins were examined to assess evolutionary conservation and potential functional domains. The identified motifs were subsequently visualized and compared across species to determine similarities and variations in motif architecture.

2.5 Molecular docking analysis of hemolin and GP64

Protein-protein docking between hemolin and the GP64 was performed using the HDock server (<http://hdock.phys.hust.edu.cn/>) with default parameters. The homology-modeled three-dimensional structures of hemolin and the GP64 were uploaded in PDB format, and docking was carried out without defining any binding site residues. The docking model exhibiting the lowest docking energy score was selected as the most favorable complex. The selected docked complex was visualized using PyMOL, and three-dimensional surface representations were generated to examine the interaction interface between the two proteins. Identification of interacting residues was performed using Protein Ligand Interaction Profiler (<https://plip-tool.biotec.tu-dresden.de/plip-web/plip/index>) which determines hydrogen bonds and hydrophobic interactions. UCSF Chimera (<https://www.rbvi.ucsf.edu/chimera/>) and PyMOL (<https://pymol.org/2/>) software tools were used to visualize the docking confirmations and the cavity site of the protein interacting with the ligand.

3. Results

3.1 Phylogenetic analysis

Multiple sequence alignment and phylogenetic reconstruction using the Maximum Likelihood method grouped the *B. mori* hemolin with hemolin proteins from other lepidopteran species, clustering most closely with *Bombyx mandarina* (Fig 1). This close association reflects the well-established evolutionary relationship between the domesticated silkworm and its wild progenitor. Hemolin proteins from *Vanessa cardui*, *Maniola jurtina*, *Maniola hyperantus*, and *Aricia agestis* formed a neighboring clade, indicating a high degree of sequence conservation among

these species. In contrast, hemolin sequences from *Samia ricini*, *Hyalophora cecropia*, *Antheraea pernyi*, *Antheraea mylitta*, *Manduca sexta*, *Lonomia obliqua*, *Mythimna separata*, *Heliothis virescens*, *Trichoplusia ni*, *Ectropis obliqua*, and *Dendrolimus kikuchii* were grouped into more divergent branches, suggesting moderate evolutionary divergence while retaining conserved hemolin characteristics. The most distantly related hemolin proteins were observed in *Amyelois transitella* and *Ostrinia furnacalis*, which formed separate branches away from the *Bombyx* cluster. Overall, the phylogenetic analysis revealed a clear pattern of evolutionary divergence among lepidopteran hemolin proteins while maintaining their conserved functional identity, supporting the annotation of the cloned *B. mori* sequence as a member of the hemolin family.

3.2 Three-dimensional structure prediction of hemolin

The three-dimensional structure of *Bombyx mori* hemolin was predicted using homology-based modelling in the SWISS-MODEL server. Among the available templates, hemolin from *Bombyx mandarina* (Wild silk moth) (AlphaFold ID: A0A6J2KGT4.1. A) was identified as the most suitable template based on sequence similarity and structural quality parameters. The selected model exhibited a Global Model Quality Estimation (GMQE) score of 0.90, indicating a reliable and well-supported structural model (Fig 2). Structural validation further confirmed the quality of the predicted model, with a MolProbity score of 0.97, reflecting favourable stereochemical properties. Ramachandran plot analysis showed that 94.85% of residues were located in the favoured regions, while 0.74% were classified as outliers, suggesting acceptable backbone geometry and overall structural integrity.

The predicted three-dimensional structure of *Bombyx mori* hemolin was further evaluated using ERRAT and ProSA-web servers to assess the reliability of the homology-modeled structure. ERRAT analysis yielded an overall quality factor of 91.008%, indicating acceptable non-bonded atomic interactions within the model (Fig 3A). ProSA-web analysis resulted in a Z-score of -8.06, which falls within the range reported for experimentally determined protein structures of comparable size (Fig 3B). These validation results, together with Ramachandran plot and MolProbity analyses, support the overall stereochemical quality and structural reliability of the predicted hemolin model.

3.3 Three-dimensional structure prediction of viral GP64

The three-dimensional structure of Nucleopolyhedroviral GP64 was predicted using homology-based modelling through the SWISS-MODEL server. The major envelope glycoprotein from baculovirus (PDB ID: 8YG6.1. A) was identified as the most suitable template based on sequence similarity and overall model quality parameters. The selected model exhibited a Global Model Quality Estimation (GMQE) score of 0.77 and a QMEANDisCo global score of 0.75 ± 0.05 , indicating moderate to good reliability of the predicted structure (Fig 4). Structural validation using MolProbity analysis yielded a MolProbity score of 1.03, reflecting acceptable stereochemical quality for a large receptor protein. Ramachandran plot analysis showed that 93.52% of residues were located in favored regions,

supporting the overall conformational validity of the predicted model.

The homology-modeled three-dimensional structure of Nucleopolyhedroviral GP64 was further validated using ERRAT and ProSA-web analyses. ERRAT evaluation showed an overall quality factor of 78.025%, reflecting average quality of non-bonded interactions in the receptor model, but acceptable (Fig 5A). ProSA-web analysis produced a Z-score of -5.29, which lies within the expected range for proteins of similar size and fold (Fig 5B). Along with Ramachandran plot statistics and MolProbity scores, these results confirm the reasonable stereochemical quality and reliability of the predicted GP64 structure.

3.4 Protein domain architecture

In silico protein domain prediction revealed four conserved domains in *B. mori* hemolin: Ig1_hemolin_like (28-117 aa), Ig2_hemolin_like (119-217 aa), Ig3_hemolin_like (232-323), and Ig4_hemolin_like (327-409 aa). All four domains were identified as members of the immunoglobulin (Ig)-like superfamily and belong to the I-set of immunoglobulin superfamily (IgSF) domains. The presence of these tandemly arranged Ig-like domains is a characteristic structural feature of hemolin proteins and supports their role in immune recognition and cell adhesion processes. The first Ig-like domain (Ig1_hemolin_like) exhibited the highest level of conservation, with a highly significant E-value of 9.21×10^{-51} , while the second, third, and fourth domains also showed strong statistical support with E-values of 1.02×10^{-26} , 2.42×10^{-24} , and 1.37×10^{-26} , respectively (Table 1).

Comparative domain architecture analysis of hemolin proteins from *Bombyx mori* and related lepidopteran species revealed a highly conserved organization of immunoglobulin (Ig)-like domains (Fig 6). Most hemolin proteins contained four tandemly arranged Ig-like domains corresponding to Ig1, Ig2, Ig3, and Ig4, indicating strong structural conservation across species. The domain arrangement observed in *B. mori* was nearly identical to that of its closest homolog, *Bombyx mandarina*, further supporting their close evolutionary relationship. Several species, including *Samia ricini*, *Hyalophora cecropia*, and *Antheraea mylitta*, exhibited a conserved four-domain architecture with clearly identifiable Ig1-, Ig3-, and Ig4-like domains. Likewise, *Lonomia obliqua*, *Heliothis virescens*, *Mythimna separata*, and *Dendrolimus kikuchii* retained the characteristic terminal Ig4-like domain, suggesting functional conservation of the C-terminal region. In contrast, *Ostrinia furnacalis* and *Manduca sexta* displayed a prominent Ig2-like domain, indicating lineage-specific variations in domain composition and organization. Species such as *Maniola hyperantus*, *Maniola jurtina*, *Vanessa cardui*, *Amyelois transitella*, and *Aricia agestis* showed domains primarily annotated as members of the Ig superfamily, reflecting high sequence divergence that may have limited the assignment of specific hemolin-like domain subclasses.

Despite these variations, the overall conservation of the four-domain immunoglobulin superfamily framework across all examined species suggests that hemolin proteins have maintained a common structural scaffold throughout lepidopteran evolution. The preservation of this domain architecture highlights the functional importance of Ig-like domains in mediating protein-protein interactions, pathogen

recognition, and innate immune responses. Furthermore, the high similarity in domain organization between *B. mori* and other lepidopteran hemolins supports the evolutionary conservation of hemolin as an insect-specific immune molecule.

3.5 Conserved motif analysis of hemolin proteins

Conserved motif analysis of hemolin proteins from *Bombyx mori* and related lepidopteran species was performed using the MEME suite to identify shared sequence signatures and assess evolutionary conservation. Three highly conserved motifs were detected across the majority of the analyzed hemolin proteins (Fig 7). The motifs were consistently distributed in a similar order and relative position along the protein sequences, indicating strong conservation of structural and functional regions among lepidopteran hemolins. The motif architecture of *B. mori* hemolin was nearly identical to that observed in *Bombyx mandarina*, reflecting their close evolutionary relationship. Similar motif arrangements were also observed in *Samia ricini*, *Manduca sexta*, *Antheraea pernyi*, *Hyalophora cecropia*, *Ectropis obliqua*, *Lonomia obliqua*, *Mythimna separata*, *Heliothis virescens*, *Vanessa cardui*, *Trichoplusia ni*, and other examined species, suggesting that these sequence elements have been maintained throughout lepidopteran evolution. Motif 1 was located predominantly in the central region of the proteins, whereas motifs 2 and 3 were distributed toward the C-terminal region. The high statistical significance of motif occurrence, reflected by low p-values ranging from 10^{-102} to 10^{-128} , indicates that these motifs are unlikely to occur by chance and are therefore likely associated with important biological functions. Minor variations in motif position and spacing were observed in some species, particularly in the more distantly related taxa, suggesting lineage-specific sequence divergence while preserving the overall motif framework.

3.6 Protein-protein docking results of hemolin-gp64 complex

Protein-protein docking analysis between hemolin and the hemolin receptor was performed using the HDock server. Among the generated docking solutions, the top-ranked model exhibiting the lowest docking score (-241.36) and a high confidence score (0.8614) was selected as the most stable complex. The docked complex was visualized using PyMOL, and surface representation analysis revealed a well-defined interaction interface with complementary binding surfaces between hemolin and the GP64, indicating favorable molecular recognition and stable complex formation (Fig 8). Detailed interaction analysis using PLIP identified several key residues involved in stabilizing the complex through hydrogen bonding, Vander Waals interactions and hydrophobic interactions. (Table 2). A total of 55 hydrophobic interactions, 43 hydrogen bonds, 14 salt bridges, and 3 π -cation interactions were identified within the complex. The large number of hydrophobic contacts suggests that hydrophobic forces play a major role in stabilizing ligand binding within the hemolin binding pocket. Hydrogen bonding interactions were distributed throughout the binding interface and are likely to contribute significantly to binding specificity and complex stabilization. In addition, fourteen salt bridges were detected between charged residues and ligand atoms, indicating strong electrostatic complementarity and further enhancing

the stability of the docked complex. Three π -cation interactions were also observed, providing additional non-

covalent stabilization through interactions between aromatic residues and positively charged groups.



Fig 1: Phylogenetic tree of Hemolin proteins from *Bombyx mori* and other lepidopteran species, illustrating their evolutionary relationships and sequence conservation

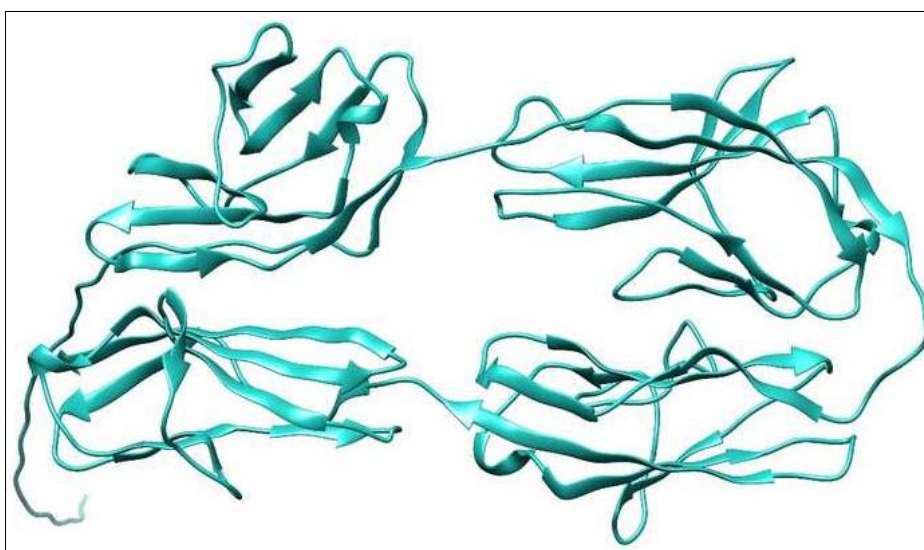


Fig 2: Three-dimensional structure of hemolin protein from *Bombyx mori*

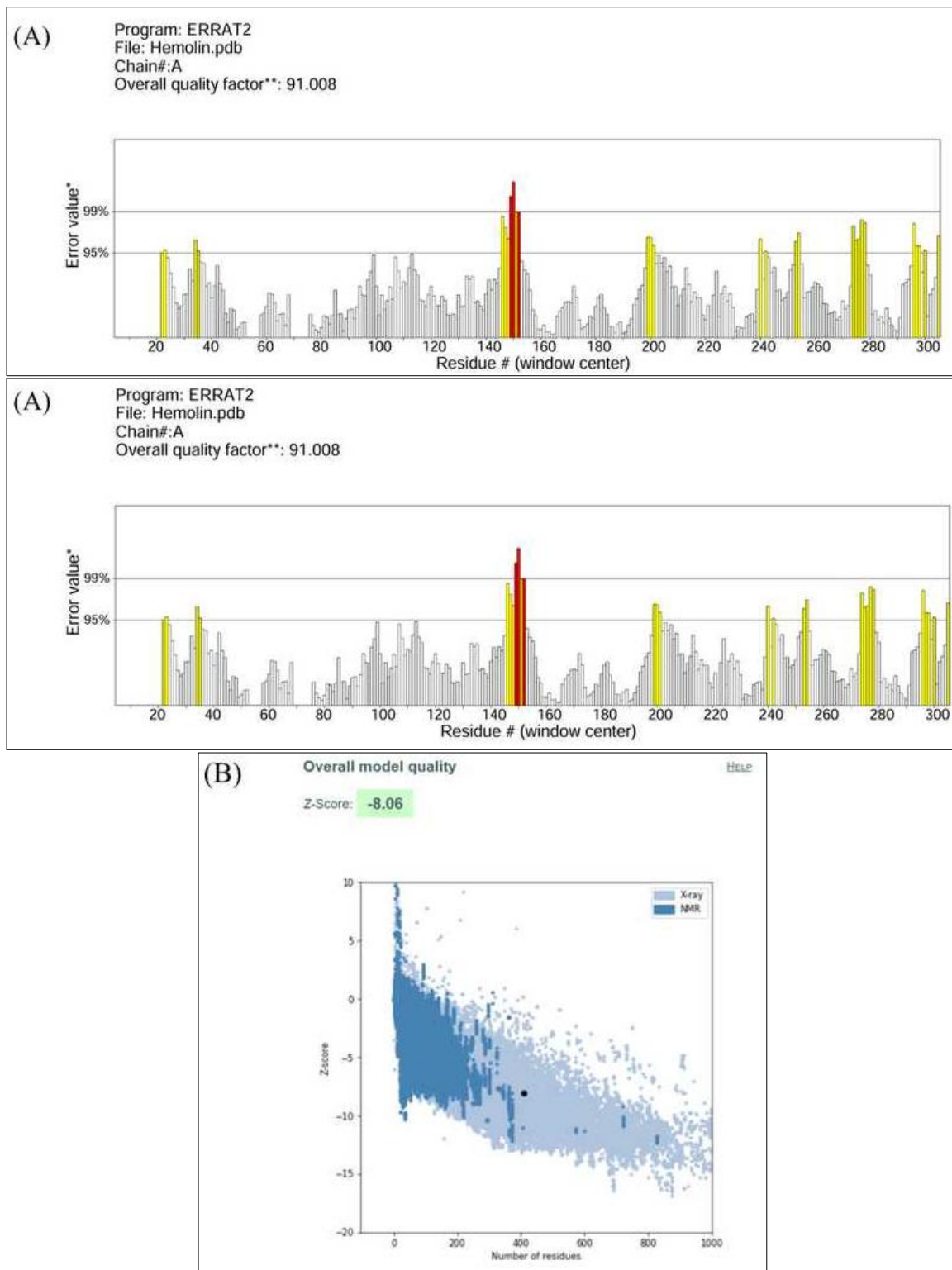


Fig 3: (A) ERRAT quality assesment of the predicted hemolin protein model; (B) ProSA analysis of predicted hemolin protein model

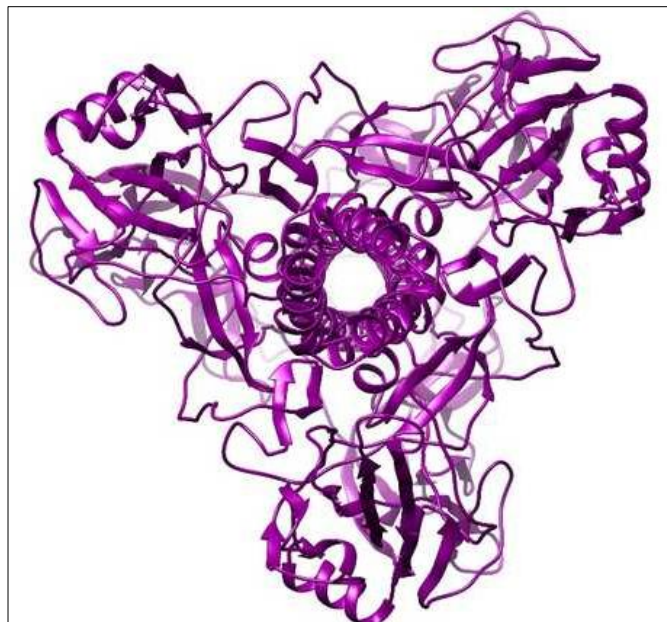


Fig 4: Three-dimensional structure of GP64 protein from *Bombyx mori* nucleopolyhedrovirus (BmNPV)

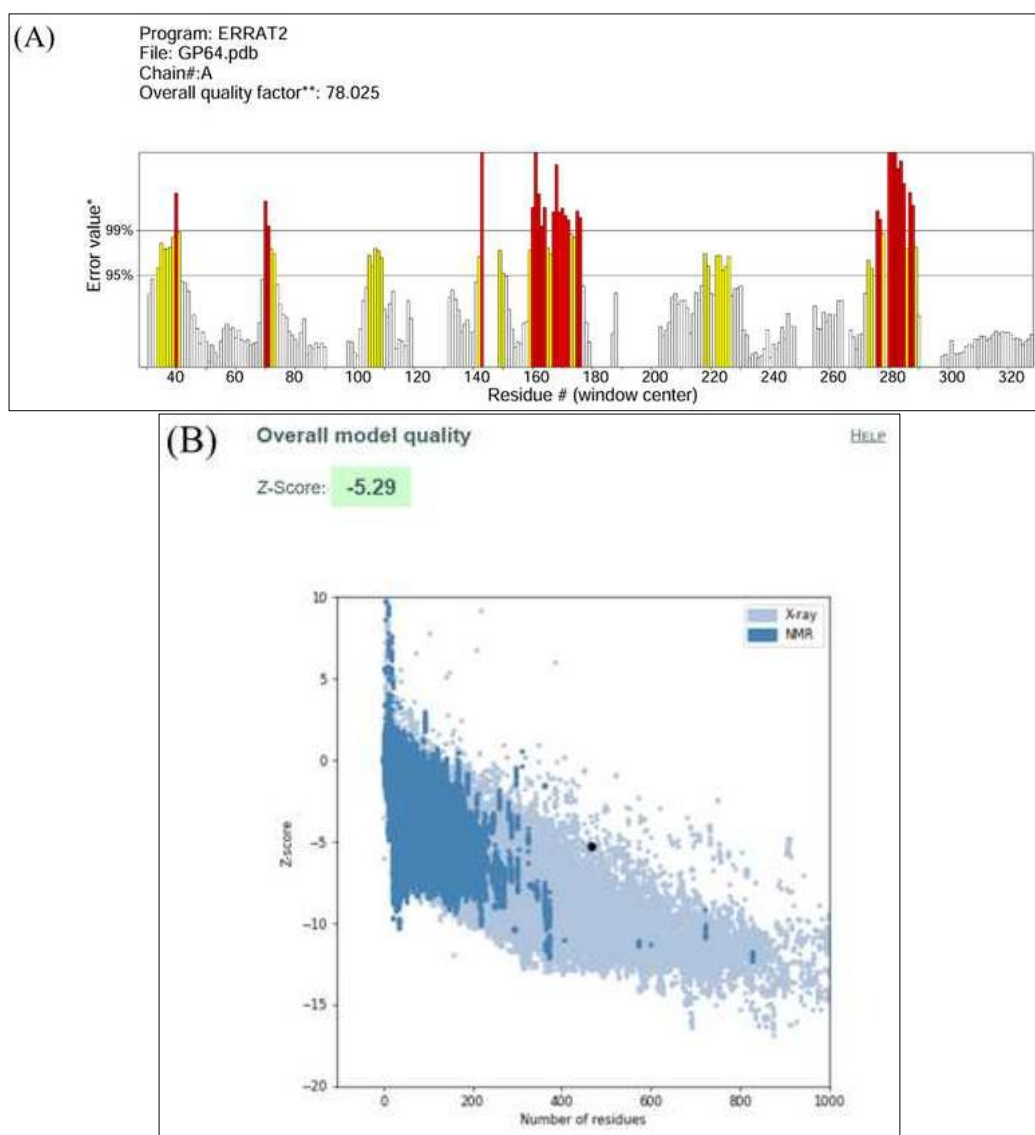


Fig 5: (A) ERRAT quality assesment of the predicted GP64 protein model; (B) ProSA analysis of predicted GP64 protein model

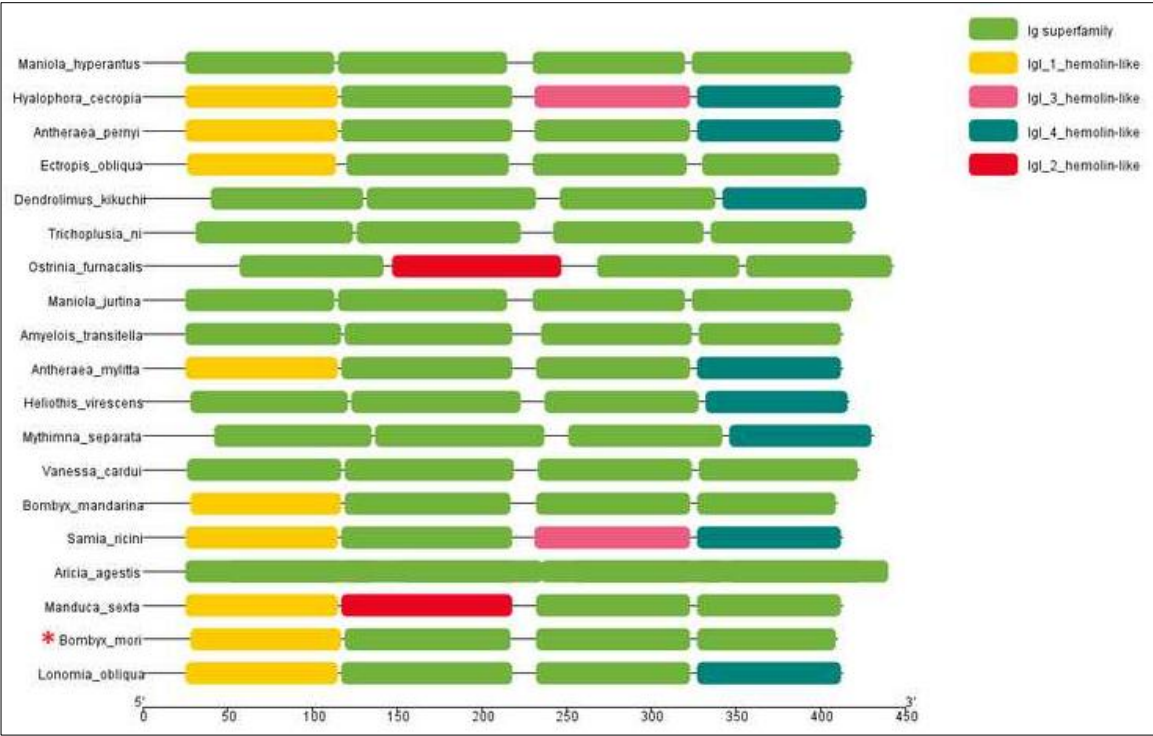


Fig 6: Comparative domain architecture of hemolin proteins among selected lepidopteran species

Name	p-value	Motif Locations
Bombyx_mori	4.82e-105	
mandarina_1	3.69e-105	
Samia_ricini	1.37e-126	
Manduca_sexta_1	5.36e-120	
Lonomia_obliqua_1	5.26e-115	
Antheraea_pernyi	5.23e-109	
Hyalophora_cecropia	8.64e-124	
Ectropis_obliqua_2	1.08e-119	
mylitta_1	7.71e-103	
Maniola_jurtina	9.96e-127	
Aricia_agestis	2.98e-111	

Maniola_jurtina	9.96e-127	
Aricia_agestis	2.98e-111	
Amyelois_transitella	4.77e-112	
Dendrolimus_kikuchii	1.80e-115	
Mythimna_separata_123	1.11e-118	
Heliothis_virescens	2.26e-123	
Maniola_hyperantus_98	1.22e-128	
Aricia_agestis_67	7.95e-117	
Vanessa_cardui	1.61e-111	
Trichoplusia_ni	4.58e-111	
Ostrinia_furnacalis	4.27e-102	

Motif

Symbol

Motif Consensus

1.

MMAKAGDVTMIYCIYGGTPLAYPDWFKDGKBVNGK

2.

RVTRHNRTSGKRLLIKETLLEDEGTYTCEVDNEVGKPKHSMKLTTVVSAP

3.

ILDRIITLSPDGTLYFSNVTKEVDSDKYKYVCVAKTPASDEDVLAHHI I

Fig 7: Conserved motif analysis of hemolin proteins

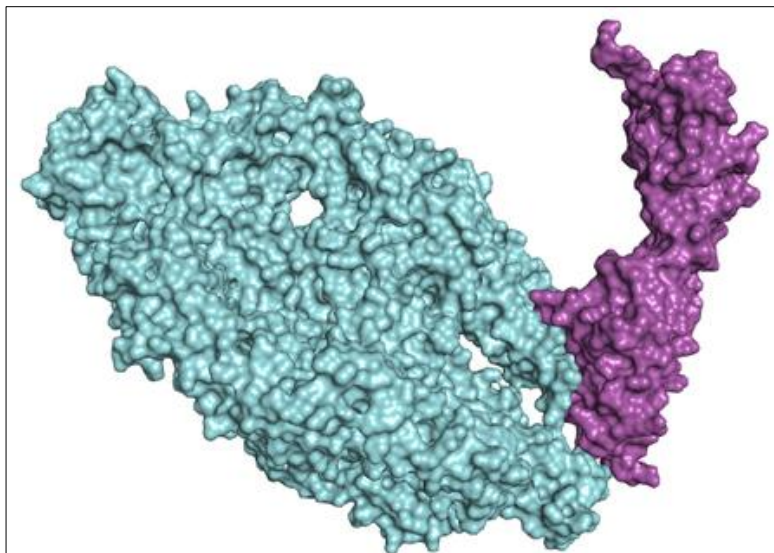


Fig 8: Protein-protein docking complex showing the predicted interaction interface between hemolin and GP64

Table 1: Domain architecture of hemolin protein

Sl. No.	Name	Accession	Interval	E-value
1.	Igl_1_hemolin like	Cd20979	28-117	9.21×10^{-51}
2.	Igl_2_hemolin like	Cd20965	119-217	1.02×10^{-26}
3.	Igl_3_hemolin like	Cd20978	327-409	1.37×10^{-26}
4.	Igl_4_hemolin like	Cd20977	232-323	2.42×10^{-24}

Table 2: Molecular interaction profile of the hemolin-GP64 docked complex

A. Hydrophobic interactions

Index	Residue	AA	Distance (Å)	Ligand Atom	Protein Atom
1	34B	TYR	3.59	6160	6827
2	35B	LYS	3.57	6132	6841
3	35B	LYS	3.75	6130	6839
4	76B	TYR	3.67	1987	7177
5	76B	TYR	3.87	1986	7179
6	85B	PRO	3.86	1874	7242
7	90B	GLU	3.92	1987	7282
8	130B	PHE	2.80	1988	7607
9	143B	LYS	3.94	5935	7710
10	156B	TYR	2.47	1174	7826
11	284B	TRP	3.58	5286	8866
12	300B	LYS	3.83	5235	8992
13	302B	ASP	3.72	5135	9004
14	303B	LEU	3.96	5275	9015
15	303B	LEU	3.81	5285	9014
16	303B	LEU	3.91	5310	9015
17	306B	ILE	3.47	5128	9041
18	308B	GLU	3.57	5108	9056
19	310B	LEU	3.56	5346	9075
20	312B	TYR	3.78	5097	9094
21	312B	TYR	3.75	4677	9093
22	316B	LEU	3.83	4676	9129
23	316B	LEU	3.42	4693	9129
24	317B	LEU	3.86	5407	9136
25	318B	LYS	3.83	5408	9143
26	319B	MET	3.97	3216	9151
27	321B	ILE	3.49	5441	9170
28	321B	ILE	3.82	3182	9169
29	323B	LEU	3.98	4863	9187
30	323B	LEU	3.86	4822	9186

31	328B	ILE	3.57	5524	9227
32	335B	LEU	3.84	5555	9284
33	338B	LEU	3.88	5582	9311
34	339B	ILE	3.49	5582	9318
35	340B	VAL	3.77	3122	9326
36	349B	LEU	3.66	5581	9395
37	353B	LEU	3.90	5571	9424
38	368B	PHE	3.69	3120	9537
39	368B	PHE	3.93	3098	9538
40	370B	LEU	3.69	3101	9554
41	372B	PRO	3.97	3072	9569
42	420B	PHE	3.56	5825	9951
43	420B	PHE	3.45	5731	9950
44	420B	PHE	3.91	5839	9947
45	421B	TRP	3.86	5854	9965
46	444B	ALA	3.88	6000	10149
47	445B	GLN	3.96	6025	10155
48	450B	LEU	3.88	6468	10197
49	451B	ILE	3.43	6407	10204
50	459B	PHE	3.92	6458	10272
51	466B	LEU	3.65	6529	10311
52	473B	ALA	3.70	6569	10362
53	480B	LYS	3.91	6678	10409
54	484B	PHE	3.77	6733	10441
55	485B	MET	3.96	1622	10448

B. Hydrogen bonds

Index	Residue	AA	Distance H-A (Å)	Distance D-A (Å)	Donor angle (°)	Protein donor?	Side chain?	Donor atom	Acceptor atom
1	24B	HIS	2.86	3.40	118.42	X	✓	3389 [O3]	6759 [Nar]
2	24B	HIS	3.35	4.00	125.16	X	✓	3977 [Nam]	6759 [Nar]
3	28B	GLN	1.98	2.90	154.94	✓	✓	6787 [Nam]	4507 [O2]
4	33B	PRO	2.97	3.92	161.05	X	X	6135 [Nam]	6819 [O2]
5	35B	LYS	2.08	3.03	159.79	X	X	6153 [Nam]	6838 [O2]
6	35B	LYS	2.43	2.89	108.05	✓	X	6835 [Nam]	6138 [O2]
7	37B	LYS	2.01	2.92	153.00	✓	X	6852 [Nam]	6156 [O2]
8	45B	LYS	2.65	3.05	104.47	X	✓	4806 [Nar]	6922 [N3]
9	46B	GLU	3.05	4.00	163.50	X	✓	5101 [Ng+]	6931 [O2]
10	46B	GLU	2.30	3.26	164.98	X	✓	4694	6931 [O2]
11	76B	TYR	3.00	3.64	123.42	X	✓	2073 [Ng+]	7181 [O3]
12	80B	GLY	3.05	3.97	156.30	X	X	1195 [Ng+]	7210 [O2]
13	86B	ASN	3.16	3.82	125.71	✓	✓	7251 [Nam]	2051 [N2]
14	90B	GLU	3.12	4.02	161.18	X	✓	1990 [O3]	7284 [O2]
15	141B	LYS	2.18	3.16	161.00	✓	✓	7701 [N3]	5880 [O2]
16	141B	LYS	1.89	2.85	164.82	X	✓	5888 [Nar]	7701 [N3]
17	144B	GLU	2.95	3.82	148.48	✓	X	7715 [Nam]	5934 [O2]
18	156B	TYR	3.00	3.73	132.24	X	✓	1170 [Nam]	7829 [O3]
19	156B	TYR	2.42	3.22	137.73	X	✓	1078 [Ng+]	7829 [O3]
20	156B	TYR	2.62	3.37	133.30	X	✓	1079 [Ng+]	7829 [O3]
21	157B	HIS	3.32	4.02	129.58	X	✓	2070 [Ng+]	7836 [N2]
22	284B	TRP	2.82	3.76	160.46	X	X	5321 [Nam]	8856 [O2]
23	298B	ALA	2.26	3.20	159.17	X	X	5246 [Nam]	8978 [O2]
24	300B	LYS	3.11	3.90	137.74	✓	X	8987 [Nam]	5221 [O2]
25	307B	GLN	2.59	3.32	131.53	✓	✓	9050 [Nam]	5338 [O.co2]
26	309B	GLU	2.64	3.60	162.65	X	✓	5124 [Nam]	9067 [O2]
27	312B	TYR	2.05	2.91	145.36	X	✓	4678 [Nam]	9096 [O3]

28	322B	GLU	2.40	3.30	151.35	X	✓	3210 [Nam]	9179 [O2]
29	332B	ASN	2.48	3.26	136.49	✓	✓	9261 [Nam]	3143 [O2]
30	336B	HIS	2.29	3.24	162.15	X	✓	3140 [Nam]	9295 [N2]
31	352B	ASN	1.80	2.78	170.41	✓	✓	9416 [Nam]	5603 [O3]
32	368B	PHE	2.22	3.05	140.68	X	X	3094 [Nam]	9532 [O2]
33	368B	PHE	2.34	3.28	159.64	✓	X	9529 [Nam]	3097 [O2]
34	370B	LEU	3.12	3.84	130.56	✓	X	9548 [Nam]	3086 [O2]
35	374B	THR	3.61	4.01	107.30	X	✓	3037 [Nam]	9582 [O3]
36	389B	TYR	3.38	3.84	112.91	✓	✓	9701 [O3]	5624 [N3]
37	389B	TYR	3.18	3.84	123.45	X	✓	5624 [N3]	9701 [O3]
38	419B	GLU	2.20	3.03	140.96	X	✓	5719 [Nam]	9941 [O2]
39	438B	SER	2.88	3.67	139.82	✓	✓	10101 [O3]	5742 [O2]
40	447B	LYS	2.01	2.97	155.25	✓	✓	10176 [N3]	6348 [O2]
41	448B	SER	2.84	3.29	107.48	X	✓	5981 [N3]	10182 [O3]
42	460B	GLY	2.23	2.90	123.87	X	X	6516 [Nam]	10276 [O2]
43	480B	LYS	3.24	3.75	112.41	✓	✓	10411 [N3]	6672 [O2]

C. π -cation interactions

Index	Residue	AA	Distance (Å)	Offset (Å)	Protein Charged?	Ligand Group	Ligand Atoms
1	88B	ARG	3.88	0.81	✓	Aromatic	2050, 2051, 2052, 2053, 2054
2	305B	HIS	5.51	0.38	X	LYS291a	5198
3	447B	LYS	3.58	1.34	✓	Aromatic	6402, 6403, 6404, 6405, 6406, 6407

D. Salt Bridges

Index	Residue	AA	Distance (Å)	Protein positive?	Ligand group	Ligand atoms
1	24B	HIS	5.06	✓	Glu144a	3993, 3994
2	30B	LYS	4.98	✓	Asp208a	4510, 4511
3	37B	LYS	5.31	✓	Glu411a	6151, 6152
4	37B	LYS	5.17	✓	Asp416a	6188, 6189
5	84B	ASP	4.84	X	His261a	1876, 1879
6	90B	GLU	4.62	X	Lys276a	1999
7	127B	GLU	3.77	X	Arg284a	2070, 2072, 2073
8	294B	GLU	3.61	X	His286a	5155, 5158
9	300B	LYS	3.44	✓	Asp302a	5277, 5278
10	308B	GLU	2.68	X	Lys291a	5198
11	313B	GLU	5.14	X	Arg280a	5099, 5101, 5102
12	330B	LYS	3.10	✓	Asp415a	6180, 6181
13	366B	ASP	4.26	X	Lys35a	3114
14	455B	GLU	2.66	X	Lys435a	6353

The combined presence of hydrophobic interactions, hydrogen bonds, salt bridges, and π -cation contacts indicates a highly favorable binding environment and suggests strong affinity between the ligand and hemolin. These interactions collectively contribute to the structural stability of the protein-ligand complex and may be important for the biological activity of the bound ligand. Detailed information regarding the interacting residues, interaction types, and bond distances is provided in Table 2.

4. Discussion

Hemolin is an insect-specific member of the immunoglobulin superfamily that plays an essential role in innate immunity by mediating pathogen recognition and immune responses (Jiang *et al.*, 2010) [28]. Although its involvement in antibacterial, antifungal, and antiviral

defense has been reported in several lepidopteran insects, the structural basis of its interaction with baculoviral proteins has remained largely unexplored. In this study, an integrated computational approach was employed to characterize *Bombyx mori* hemolin and evaluate its potential interaction with the major baculoviral envelope glycoprotein GP64.

Phylogenetic analysis revealed that *B. mori* hemolin is closely related to *Bombyx mandarina*, consistent with their evolutionary relationship and previous reports describing the high conservation of hemolin among Lepidoptera (Bian *et al.*, 2026; Pradeep *et al.*, 2015; Jiang *et al.*, 2010) [10, 27, 28]. The conservation of four immunoglobulin-like domains and three conserved motifs across diverse lepidopteran species further indicates that the structural framework of hemolin has been maintained during evolution, highlighting its

functional importance in immune recognition. Such conservation is characteristic of proteins involved in host defense, where preservation of structural integrity is critical for recognizing pathogen-associated molecular patterns (Ledvina *et al.*, 2024) [29].

The predicted three-dimensional models of hemolin and GP64 exhibited favorable stereochemical quality based on multiple validation parameters, supporting their suitability for structural analyses. The characteristic immunoglobulin fold of hemolin and the conserved architecture of GP64 provide a reliable framework for investigating host-virus interactions. Protein-protein docking predicted a stable hemolin-GP64 complex stabilized by hydrophobic interactions, hydrogen bonds, salt bridges, and π -cation interactions. These non-covalent interactions collectively suggest a structurally favorable binding interface and support the possibility that hemolin may participate in the early recognition of BmNPV during infection. Given the essential role of GP64 in viral attachment and membrane fusion, its interaction with hemolin may contribute to the activation of antiviral immune responses in *B. mori* (Sun *et al.*, 2025) [31].

Although the findings provide important structural insights, they are based on computational predictions and require experimental validation using approaches such as co-immunoprecipitation, pull-down assays, site-directed mutagenesis, or molecular dynamics simulations. Nevertheless, the agreement among phylogenetic, structural, domain, motif, and docking analyses provides strong evidence supporting the proposed interaction between hemolin and GP64. Overall, this study improves our understanding of the structural biology of hemolin and identifies potential interaction interfaces that may be exploited in future functional studies and molecular breeding strategies to enhance resistance against BmNPV in silkworms.

5. Conclusion

The present study provides a comprehensive in silico characterization of the hemolin protein from *Bombyx mori* and its homologs across diverse lepidopteran species. Sequence analysis, phylogenetic reconstruction, domain architecture comparison, and motif analysis demonstrated that hemolin is a highly conserved member of the immunoglobulin superfamily, possessing four characteristic Ig-like domains and conserved sequence motifs that have been maintained throughout lepidopteran evolution. The close evolutionary relationship between *B. mori* hemolin and its homologs, particularly *Bombyx mandarina*, further highlights the functional importance of this immune-related protein. Molecular docking and interaction analyses revealed extensive interactions between hemolin and the baculoviral envelope glycoprotein GP64, including numerous hydrophobic contacts, hydrogen bonds, salt bridges, and π -cation interactions. These findings suggest that hemolin may play an important role in host-pathogen recognition and could contribute to the immune response against nucleopolyhedrovirus (NPV) infection in silkworms. The identification of key interacting residues involved in hemolin-GP64 binding provides valuable targets for future functional studies. Advances in modern biotechnological approaches, including CRISPR/Cas-mediated genome editing and transgenic technologies, offer opportunities to modify or enhance critical interaction sites within the

hemolin protein. Such targeted alterations may strengthen antiviral defense mechanisms and contribute to the development of silkworm lines with improved resistance to nucleopolyhedrovirus infection. Therefore, the findings of this study establish a foundation for future experimental validation and the application of precision breeding strategies aimed at enhancing disease resistance in sericulture.

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Data availability statement

All data generated or analysed during this study are included in this article.

Competing interest

Authors have no conflict of interest

Ethics approval statement

This study does not include any animals which require any kind of ethical clearance. We confirm that all experiments were performed in accordance with relevant guidelines and regulations of the Institute.

References

- Sharma K, Kapoor B. Sericulture as a profit-based industry - a review. *Indian J Pure Appl Biosci.* 2020;8(4):550-562.
- Ayaz S, Kong WW, Wang J, Liu SH, Xu JP. Host immunity mechanisms against bacterial and viral infections in *Bombyx mori*. *Insects.* 2025;16(11):1167.
- Chauhan P, Bashir M, Pandey A, Kaur A. Silkworm disease management. In: *Sericulture*. Boca Raton: CRC Press; 2025. p.128-148.
- Yang X, Lei C, Peng X, Min Y, Hu J, Sun X. Genotypic diversity and biological characterization of *Alphabaculovirus mabrassicae* isolates from a wide host range. *Pest Manag Sci.* 2025;81(4):2215-2224.
- Blissard GW, Theilmann DA. Baculovirus entry and egress from insect cells. *Annu Rev Virol.* 2018;5(1):113-139.
- Monsma SA, Oomens AG, Blissard GW. The GP64 envelope fusion protein is an essential baculovirus protein required for cell-to-cell transmission of infection. *J Virol.* 1996;70(7):4607-4616.
- Eleftherianos I, Tafesh-Edwards G, Mohamed A. Pathogen infection routes and host innate immunity: lessons from insects. *Immunol Lett.* 2022;247:46-51.
- Merzendorfer H. *Hemolymph: a fluid tissue with versatile functions*. In: *Insect anatomy*. Amsterdam: Academic Press; 2026. p.1-29.
- Wang G, Xu D, Guo D, Zhang Y, Mai X, Zhang B, *et al.* Unraveling the innate immune responses of *Bombyx mori* hemolymph, fat body, and midgut to *Bombyx mori* nucleopolyhedrovirus oral infection by metabolomic analysis. *Arch Insect Biochem Physiol.* 2021;108(4).
- Bian DD, Mei XY, Zhang G, Liu QN, Tang BP, Zhu B. Hemolin: a molecular integrator of insect immunity and

- developmental signaling. *J Agric Food Chem.* 2026;74(11):9044-9063.
11. He L, Liu L, Liu H, Tang X, Meng Y, Xie H, *et al.* Functional analysis of BmHemolin in the immune defense of silkworms. *Insects.* 2025;16(8):778.
 12. Aathmanathan VS, Jothi N, Prajapati VK, Krishnan M. Investigation of immunogenic properties of Hemolin from silkworm, *Bombyx mori* as carrier protein: an immunoinformatic approach. *Sci Rep.* 2018;8(1):6957.
 13. Jean Beltran PM, Federspiel JD, Sheng X, Cristea IM. Proteomics and integrative omic approaches for understanding host-pathogen interactions and infectious diseases. *Mol Syst Biol.* 2017;13(3).
 14. Sen R, Nayak L, De RK. A review on host-pathogen interactions: classification and prediction. *Eur J Clin Microbiol Infect Dis.* 2016;35(10):1581-1599.
 15. Dix A, Vlaic S, Guthke R, Linde J. Use of systems biology to decipher host-pathogen interaction networks and predict biomarkers. *Clin Microbiol Infect.* 2016;22(7):600-606.
 16. Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser.* 1999;41:95-98.
 17. Tamura K, Stecher G, Kumar S. MEGA11: molecular evolutionary genetics analysis version 11. *Mol Biol Evol.* 2021;38(7):3022-3027.
 18. Waterhouse A, Bertoni M, Bienert S, Studer G, Tauriello G, Gumienny R, *et al.* SWISS-MODEL: homology modelling of protein structures and complexes. *Nucleic Acids Res.* 2018;46(W1).
 19. Guex N, Peitsch MC, Schwede T. Automated comparative protein structure modeling with SWISS-MODEL and Swiss-PdbViewer: A historical perspective. *Electrophoresis.* 2009;30(S1).
 20. Schwede T. QMEANDisCo-distance constraints applied on model quality estimation. 2019.
 21. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, *et al.* UCSF Chimera - a visualization system for exploratory research and analysis. *J Comput Chem.* 2004;25(13):1605-1612.
 22. DeLano WL. PyMOL: an open-source molecular graphics tool. *CCP4 Newsl Protein Crystallogr.* 2002;40(1):82-92.
 23. Wiederstein M, Sippl MJ. ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins. *Nucleic Acids Res.* 2007;35(Suppl 2).
 24. Yan Y, Tao H, He J, Huang SY. The HDock server for integrated protein-protein docking. *Nat Protoc.* 2020;15(5):1829-1852.
 25. Yan Y, Zhang D, Zhou P, Li B, Huang SY. HDock: a web server for protein-protein and protein-DNA/RNA docking based on a hybrid strategy. *Nucleic Acids Res.* 2017;45(W1).
 26. Bailey TL, Johnson J, Grant CE, Noble WS. The MEME suite. *Nucleic Acids Res.* 2015;43(W1).
 27. Pradeep AR, Anitha J, Panda A, Pooja M, Awasthi AK. Phylogeny of host response proteins activated in silkworm *Bombyx mori* in response to infestation by dipteran endoparasitoid revealed functional divergence and temporal molecular adaptive evolution. *J Clin Cell Immunol.* 2015;6:370. DOI:10.4172/2155-9899.1000370.
 28. Jiang H, Vilcinskas A, Kanost MR. Immunity in lepidopteran insects. In: *Invertebrate immunity.* 2010. p.181-204.
 29. Ledvina HE, Whiteley AT. Conservation and similarity of bacterial and eukaryotic innate immunity. *Nat Rev Microbiol.* 2024;22(7):420-434.
 30. Pradeep C, Ashok K, VinayKumar TM, Babu SS, Subhaharan K, Nagesha SN, *et al.* Integrative functional validation of pheromone binding proteins in *Spodoptera frugiperda* via in silico and RNAi approaches. *Int J Biol Macromol.* 2025:148440.
 31. Sun L, Xu Y, Chen K, Nan W, Wang M, Zhang Y, *et al.* Unraveling dual fusion mechanisms in BmNPV GP64: critical roles of CARC motifs and signal peptide retention. *J Virol.* 2025;99(1).