

ORIGINAL RESEARCH ARTICLE

An *in silico* approach to design a multi-epitope vaccine against small ruminant lentiviruses causing Maedi-Visna and caprine arthritis encephalitis in sheep and goats

Rumesa Ghazanfar^{1†}, Zainab Nagari^{1†}, Muhammad Atiq Ur Rehman^{2†},
Chandra Sourav³, Muhammad Zeeshan Shabbir^{1,4*}

¹Department of Biological Research, BioMind Research Institute, MindSet Development Foundation, Islamabad, Pakistan

²RI-Biomics, Advanced Radiation Technology Institute, Korean Atomic Energy Research Institute, Jeongeup, Jeollabuk, Republic of Korea

³Department of Electronics and Information Engineering, Jeonbuk National University, Jeonju, Jeollabuk, Republic of Korea

⁴Department of Animal Breeding and Genetics, Justus Liebig University Giessen, Gießen, Hesse, Germany

[†]These authors contributed equally to this work.

***Corresponding author:**

Muhammad Zeeshan Shabbir
(muhammad.zeeshan.shabbir@uni-giessen.de)

Citation: Ghazanfar R, Nagari Z, Rehman MAU, Sourav C, Shabbir MZ. An *in silico* approach to design a multi-epitope vaccine against small ruminant lentiviruses causing Maedi-Visna and caprine arthritis encephalitis in sheep and goats. *Microbes & Immunity*. 2026;3(4):025450121.
doi: 10.36922/MI025450121

Received: November 6, 2025

Revised: April 26, 2026

Accepted: May 6, 2026

Published online: June 9, 2026

Copyright: © 2026 Author(s). This is an Open-Access article distributed under the terms of the Creative Commons Attribution License, permitting distribution, and reproduction in any medium, provided the original work is properly cited.

Publisher's Note: AccScience Publishing remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abstract

Small ruminant lentiviruses are common viral pathogens affecting livestock, primarily sheep and goats, and are responsible for chronic and fatal diseases such as Maedi-Visna and caprine arthritis encephalitis. These infections compromise livestock productivity. Developing a safe and effective vaccine that provides broad protection across host species remains a major challenge. Consequently, we employed different bioinformatics tools to design a novel multi-epitope vaccine construct based on the envelope gene of the causative lentiviruses. The secondary and tertiary structures of the construct were predicted and subsequently refined. The stability and binding affinity of the vaccine construct were evaluated using advanced computational approaches, including molecular docking and molecular dynamics simulation. Also, the immunogenic potential was assessed through immune simulation studies, followed by codon optimization and *in silico* cloning. However, *in vivo* studies are required in the near future to confirm the vaccine construct's efficacy and immunogenicity.

Keywords: Reverse vaccinology; Small ruminant lentiviruses; Molecular docking; Molecular dynamics simulation

1. Introduction

Maedi-Visna (MV) is a common viral infection primarily affecting sheep and characterized by a slow progression throughout the animal's life cycle, ultimately leading to severe pneumonia along with neurological manifestations such as tremors, weakness, and paralysis.¹ Infected sheep also experience chronic indurative mastitis.² Another common disease is caprine arthritis encephalitis (CAE), which primarily affects goats. CAE-infected goats exhibit a wide range of clinical symptoms. In young goats,

incomplete muscle development may lead to encephalitis, characterized by paresis, dizziness, seizures, and impaired coordination, ultimately resulting in paralysis.³ In contrast, older goats develop arthritis, characterized by enlarged, painful joints. Both MV- and CAE-infected animals may exhibit weight loss, reduced milk production, and poor coat development.⁴ Two primary routes of transmission for these viruses include respiratory/oral (via inhalation of the infective agents) and ingestion via colostrum or milk.⁵

The etiological agents responsible for MV and CAE are Maedi-Visna virus (MVV) and caprine arthritis encephalitis virus (CAEV), respectively.⁶ Recent studies have demonstrated that both species form a viral continuum and can infect goats and sheep without species barriers. Hence, they are collectively referred to as small ruminant lentiviruses (SRLVs).⁷

The viral genome size is approximately 8.4–9.2 kb

in length^{8,9} and encodes both structural and regulatory proteins. The three structural genes, *gag*, *pol*, and *env*, encode the viral core, whereas the regulatory functions, such as viral replication modulation, are performed by *tat*, *vif*, and *rev* genes.^{10–13} Based on genome sequence analysis, particularly of *gag* and *pol* genes, SRLVs are classified into genotypes A–E. Genotype A includes MVV isolates, whereas CAEV isolates are included in Genotypes B. Genotypes C and E constitute the regional isolates.¹⁴

Timely diagnosis of SRLVs is crucial for effective disease control, as the virus often stays dormant in the host with minimal or no clinical symptoms.¹⁵ Among various control measures, vaccination remains the most promising approach. Although previous studies have explored vaccine candidates targeting Gag and Env proteins (Table 1), no commercially available vaccine currently provides cross-protection against the MVV–CAEV continuum.

Table 1. Major vaccine candidates against Maedi-Visna and caprine arthritis encephalitis

Type of vaccine	Targeted viral agent	Targeted species	Parameters of study	Outcomes	References
Attenuated molecular clone of Maedi-Visna virus (MVV) (2005)	MVV	Sheep	Four sheep were infected with an attenuated molecular clone of MVV (LV1-1KS1), while comparing them with a control group ($n = 4$)	Low viral titer was detected in the enzyme-linked immunosorbent assay, indicating partial protection	16
DNA-based vaccine based on the envelope gene (2005)	MVV	Sheep	pcDNA plasmid (pcDNA-env) encoding the env glycoproteins of MVV, boosted with combined pcDNA-env and pCR3.1-IFN- γ plasmid inoculations, was introduced in both the test and control groups	An early protective effect was observed in the vaccinated sheep that restricted the virus replication; however, it disappeared after two years	17
DNA vaccine containing the MVV gag gene (2007)	MVV	Sheep	Sheep were immunized eight times over a period of 2.5 years with the MVV <i>gag</i> gene, VR1012- <i>gag</i> -CTE, and pcDNA3.1- <i>gag</i> -CTE	No positive outcome was observed. In fact, the vaccine may have made them more susceptible to the virus	18
Use of B7 costimulatory molecules as adjuvants in a prime-boost vaccination against Visna/Maedi ovine lentivirus (2009)	Visna/Maedi ovine lentivirus	Sheep (Lleyen sheep breed)	Sheep were primed with particle-mediated epidermal bombardment using plasmids containing the MVV <i>gag</i> and <i>env</i> genes	Enhanced early cellular immune response	19
<i>In silico</i> discovery of epitopes of Gag and Env proteins for the development of a multi-epitope vaccine candidate against MVV virus (2023)	Maedi-Visna	Sheep	The DNA sequence for the gag and env genes of MVV KV1514 isolate was retrieved from the National Center for Biotechnology Information, and a multi-epitope vaccine was designed based on the 19 epitopes	Gag protein was detected to be more conserved, and a strong affinity was shown between the final multi-epitope vaccine and TLR-2/4	20
Recombinant vaccinia virus rWR63 expressing CAEV envelope glycoproteins	Caprine arthritis encephalitis virus (CAEV)	Goat	Goats ($n = 6$) were vaccinated thrice with rWR63, followed by different booster immunizations. The control group received rWRSC11	All vaccinated goats developed antibodies to CAEV glycoproteins, but no neutralizing antibodies were detected.	21

Notes: The table shows various vaccine candidates reported over the years against MVV and CAEV. The type of vaccine candidate is shown in the first column, while the targeted viral agent and species are listed in the second and third columns, respectively. Parameters chosen for the individual study and their specific outcomes are depicted in the fourth and fifth columns, respectively. Also, reference articles are mentioned in the sixth column.

Therefore, the present study aims to leverage advances in bioinformatics, reverse vaccinology, and protein structure prediction to design a multi-epitope vaccine capable of providing broad protection against both MV and CAE across diverse goat and sheep breeds. Notably, the epitopes identified in this study are novel, and the adjuvant Pam3CSK4 has not been used for SRLV vaccine development previously.²⁰

2. Materials and methods

2.1. Protein sequence retrieval

Protein (amino acid) sequences of the envelope gene from prevalent SRLV genotypes namely A1 (National Center of Biotechnology Information [NCBI] accession no. M60609), A1 (NCBI accession no. OL436272), A2 (NCBI accession no. AY101611), B1 (NCBI accession no. OL436260), and B2 (NCBI accession no. OL436262) were retrieved in FASTA format from the NCBI database (Table 2). The sequences were subjected to multiple sequence alignment using Clustal Omega.²² Conserved regions were further analyzed using JalView, and conservation scores were used to generate a consensus sequence representing the SRLV envelope gene.²³ The analysis was also validated using BioEdit software.²⁴ A flow chart describing the methodology used in this study is provided in Figure S1.

2.2. B-cell epitope prediction

Since antibodies play a crucial role in adaptive immunity, identifying B-cell epitopes is essential for vaccine design. B-cell epitopes are broadly classified into linear and conformational epitopes. Several publicly accessible tools were used for prediction, including ABCPred (for antigenicity),²⁵ BCEPred (predicts the flexibility of predicted B-cell epitopes),²⁶ and BepiPred (for linear B-cell epitope prediction).²⁷ Epitopes with the highest antigenicity scores were selected. Furthermore, the antigenicity was further validated using VaxiJen 2.0 (threshold > 0.4).²⁸ Selected epitopes were evaluated for allergenicity using AllerTOP,²⁹ and for toxicity using ToxinPred.³⁰ Only epitopes that were antigenic, non-allergenic, and non-toxic were selected.

2.3. Cytotoxic T lymphocyte and helper T lymphocyte epitope prediction

2.3.1. CD8⁺ T cell epitopes

Prediction was performed using ProPred-I,³¹ EpiJen,³² and the IEDB major histocompatibility complex (MHC)-I binding prediction tool (NetMHCpan EL 4.1).³³ Due to the lack of sheep/goats MHC datasets, bovine (BoLA) alleles were used as surrogates.³⁴ This surrogate strategy has also been reported previously based on evidence of similar responses in ruminants.^{35,36} Selected epitopes were

filtered based on AllerTOP (allergenicity), VaxiJen 2.0 (antigenicity), and ToxinPred (toxicity).

2.3.2. CD4⁺ T cell epitopes

Helper T lymphocyte epitopes were predicted using the NetMHCIIpan 2.1 tool, which is efficiently trained by making use of artificial neural networks and includes the bovine leukocyte alleles of the MHC-II.³⁷ The predicted epitopes were selected based on stringent criteria, including binding affinity ($IC_{50} < 50$ nM), percentile rank (<0.5), and prediction score (>0.9). Further validation was performed using VaxiJen 2.0, EpiJen, ToxinPred, and AllerTOP.

2.4. Prediction of discontinuous epitopes

Discontinuous epitopes are of significant importance because they help identify antigenic residues that may strongly interact with host antibodies. Hence, the ElliPro web server was used to predict discontinuous epitopes using default parameters.³⁸

2.5. Design of the small ruminant lentiviruses-based vaccine

To ensure an optimal balance between size (stability) and immunogenicity, only the top-ranked B-cell (10), cytotoxic T lymphocyte (CTL; four), and helper T lymphocyte (HTL; four) epitopes were selected based on antigenicity, toxicity, and allergenicity, and subsequently fused to construct the multi-epitope vaccine. These selected epitopes were highly conserved across diverse SRLV genotypes and were antigenic, non-toxic, and non-allergenic.

At the N-terminal of the construct, a TLR2 agonist adjuvant, Pam3CSK4, was incorporated to enhance the immune response. The adjuvant was linked to the first CTL epitope using the "EAAAK" linker, which provides flexibility between the adjuvant and the multiepitopic regions. In addition, the CTL epitopes were joined using "AAY" linkers, while "EAAAK" linkers were used to connect CTL and HTL epitopes. The HTL epitopes were linked using "GPGPG" linkers, which were also used to link HTL epitopes to B-cell epitopes. Finally, an "EAAAK" linker was used to attach a 6 × His tag at the C-terminal end of the construct to facilitate purification/recovery following expression.³⁹

2.6. Physicochemical properties

The physicochemical properties of the vaccine candidate were analyzed using the ExPASy ProtParam online server. Parameters, including molecular weight, molecular formula, isoelectric point, instability index (II), estimated half-life, number of atoms, and grand average of hydropathicity (GRAVY), were calculated.⁴⁰ Additionally, the construct's solubility was predicted using the SolPro server to assess its expression in living cells.⁴¹

Table 2. Reference isolates for the generation of an *in silico*-based vaccine candidate against Maedi-Visna and caprine arthritis encephalitis

Serotype	National Center of Biotechnology Information accession number	Region	Gene type	Accession links
A1	M60609	Iceland	Envelope	https://www.ncbi.nlm.nih.gov/nucleotide/M60609
A1	OL436272	Poland	Envelope	https://www.ncbi.nlm.nih.gov/nucleotide/OL436272
A2	AY101611	United States of America	Envelope	https://www.ncbi.nlm.nih.gov/nucleotide/AY101611
B1	OL436260	Poland	Envelope	https://www.ncbi.nlm.nih.gov/nucleotide/OL436260
B2	OL436262	Poland	Envelope	https://www.ncbi.nlm.nih.gov/nucleotide/OL436262

Notes: The first column lists the genotypes of selected small ruminant lentiviruses isolates, and the second column provides their accession numbers. The third column contains information about the region, followed by its targeted gene type in the fourth column. All the information is retrieved from NCBI, and the link is provided in the last column.

2.7. Secondary structure prediction

The secondary structure of the vaccine construct was predicted using the self-optimized prediction method (SOPMA) with online alignment server with default parameters. The proportions of alpha helix, beta sheets, random coils, and beta-turns were estimated.⁴² Furthermore, PSIPRED was used to provide insights into the amino acid composition and structural elements.⁴³

2.8. Tertiary structure prediction

Tertiary structure of the vaccine construct was predicted using the I-TASSER server,⁴⁴ which employs a multiple-threading approach combined with iterative structural assembly. The linear amino acid sequence of the vaccine construct was submitted to the I-TASSER server, and the resulting models were evaluated using the PROCHECK server.⁴⁵ The best model was selected based on Ramachandran plot analysis and subsequently refined using the Galaxy Refine server.⁴⁶ The refined models were re-evaluated using the PROCHECK server, and the final model was selected based on the percentage of residues in the favored region, clash scores, MolProbity scores, and root mean square deviation (RMSD). The overall quality of the final model was further evaluated using the ProSA webserver.⁴⁷

2.9. Molecular docking analysis with TLR-2 and TLR-4

Protein-protein interactions between the vaccine construct and the most common transmembrane pattern recognition receptors (TLR-2 and TLR-4) were predicted using the ClusPro webserver.⁴⁸ ClusPro integrates rigid-body docking, energy-based scoring, and clustering algorithms. It utilizes a Fast Fourier Transform (FFT)-based PIPER algorithm to evaluate billions of potential docking

conformations based on van der Waals, electrostatic, and desolvation energies.

Docked complexes were ranked using multiple scoring schemes (balanced, electrostatically favored, and hydrophobically favored) and clustered based on interface RMSD. The largest cluster size was considered indicative of the most probable binding mode.

The structures of the human TLR-2 (PDB ID: 6NIG) and TLR-4 (PDB ID: 4G8A) were retrieved from the Protein Data Bank, and their extracellular domains were used for docking. The best docking poses were selected based on low energy scores and large cluster sizes using the ClusPro web server.

Furthermore, the docked complexes were further analyzed using the PRODIGY server to estimate binding affinity, dissociation constant, and interfacial properties.⁴⁹ PDBePISA was used to assess the biological relevance of the predicted complexes and to analyze the interaction interfaces.⁵⁰ Structural visualization was performed using Chimera⁵¹ and PyMol.⁵² Furthermore, additional interaction details, such as the number of chains, bonds, and interacting residues, were identified using PDBSum.⁵³

2.10. Molecular dynamics simulation

The stability of the docked complexes was evaluated using molecular dynamics simulations in Schrödinger Maestro.⁵⁴ The system was prepared using the Protein Preparation Wizard, and simulations were conducted using the OPLS4 force field and TIP3P water model under periodic boundary conditions. Equilibration was performed at 300 K and 1 bar pressure under number-volume-temperature and number-pressure-temperature (NPT) ensembles. A 100 ns production run under NPT conditions was conducted to analyze RMSD, root mean square fluctuation

(RMSF), radius of gyration (Rg), and solvent-accessible surface area (SASA).

2.11. Immune simulation studies

The immunogenic potential of the vaccine construct was tested using the C-ImmSim server, a machine learning-based platform that simulates the host immune response.⁵⁵ Simulation parameters include a lipopolysaccharide-free injection, a random seed of 12.643, a simulation volume of 50, and 1,000 simulation steps with time points at 1, 84, and 168.

2.12. Codon optimization and *in silico* cloning

To ensure efficient expression in *Escherichia coli*, codon optimization was performed using the JCat Server,⁵⁶ which provided optimized codon usage, guanine–cytosine (GC)% content, and codon adaptation index (CAI). Furthermore, the proper insertion of the linear construct into the cloning vector was facilitated by introducing *XhoI* and *BamHI* restriction sites at the N- and C-termini, respectively, to enable cloning into the pET28a (+) vector. The optimized gene sequence was inserted into the vector using SnapGene.

3. Results

3.1. Sequence retrieval

The amino acid sequences coding for the envelope protein

of the selected SRLV isolates were successfully retrieved from the NCBI database and aligned. Conserved residues were analyzed using Jalview and BioEdit. Based on this analysis, a consensus envelope gene sequence for the most prevalent SRLV isolates was generated.

3.2. Linear B-cell epitope prediction

Linear B-cell epitopes were predicted and validated using highly interactive bioinformatics tools. Initially, several candidate B-cell epitopes were predicted. However, after stringent filtering, only the top 10 B-cell epitopes (15-mer) were selected based on antigenicity, allergenicity, and toxicity scores. The antigenicity scores of the selected epitopes ranged from 0.51 to 1.26, while the toxicity-predicting support vector machine scores ranged from −0.57 to −1.40. Detailed results of the linear B-cell epitope prediction are provided in Table 3.

3.3. Cytotoxic T lymphocyte epitope prediction

Cytotoxic T lymphocyte epitopes play a vital role in eliciting cell-mediated immunity. At first, a total of 21 CTL epitopes were predicted using the ProPred-I server. After filtering, to maintain a suitable vaccine size, only four 9-mer epitopes were selected for inclusion in the final vaccine construct. The above selection was based on antigenicity score, allergenicity, and toxicity. Minimal percentile rank of the selected epitopes ranged from 0.02

Table 3. List of selected B-cell epitopes for the generation of an *in silico*-based vaccine candidate against Maedi-Visna and caprine arthritis encephalitis

Sequence	Sequence length	Score	AllerTop	VexiJen	ToxinPred	Support vector machine score	Molecular weight
YMEAQGENRRSRRLNQ	16	0.80	Non-allergen	Antigen (0.6151)	Non-toxin	−1.04	1,880.28
RVKAKYSCESNLGGLD	16	0.77	Non-allergen	Antigen (1.1465)	Non-toxin	−0.59	1,625.09
DLGTERGEEKQKVVV	16	0.89	Non-allergen	Antigen (0.7215)	Non-toxin	−1.40	1,716.11
TWKSMEPLRETWGQV	16	0.80	Non-allergen	Antigen (0.5101)	Non-toxin	−0.80	1,846.31
QQEQEKLLTEGKQKSW	16	0.79	Non-allergen	Antigen (0.5243)	Non-toxin	−1.29	1,744.20
SKNRPSRVTWKSMEPP	16	0.70	Non-allergen	Antigen (0.8433)	Non-toxin	−0.99	1,803.27
EGKQKSWVSIDLLGTE	16	0.60	Non-allergen	Antigen (1.0230)	Non-toxin	−1.01	1,661.12
NESSKWTCAPRGKKGK	16	0.89	Non-allergen	Antigen (0.9891)	Non-toxin	−0.57	1,649.06
NCSLPHINESSKWTC	16	0.70	Non-allergen	Antigen (0.5145)	Non-toxin	−0.57	1,719.13
DRTGNALGNVKNKNC	16	0.82	Non-allergen	Antigen (1.2684)	Non-toxin	0.73	1,557.91

Notes: The table shows the selected B-cell lymphocyte epitope (BCL) for vaccine development. The first column shows the sequence of selected BCL sequences, whereas the sequence length and scores are listed in the second and third columns, respectively. Furthermore, the results of the allergenicity, antigenicity, and toxicity analyses are depicted in columns four to six, respectively. Support vector machine scores and molecular weight information are listed in the seventh and eighth columns, respectively.

to 0.07, antigenicity scores ranged from 0.76 and 0.94, and strong binding affinity ($IC_{50} < 50$ nM) across all four BoLA alleles (BoLA-T2c, BoLA-HD6, BoLA-1:02301, and BoLA-2:00801). The results of the CTL epitope predictions are summarized in Table 4.

3.4. Helper T lymphocyte epitope prediction

Helper T lymphocyte epitopes are crucial to both cellular and humoral immunity, and they were predicted using a combination of tools. Four HTL epitopes with the highest binding affinity ($IC_{50} < 50$ nM), percentile rank (<0.5), and prediction scores (>0.9) were selected. Net Chop analysis indicated a C-terminal cleavage score of 3.0 at a threshold of 0.5. HTL epitopes prediction results are provided in Table 5.

3.5. Prediction of discontinuous epitopes

Discontinuous epitopes were predicted using the ElliPro web server. Interacting amino acid residues and their antigenicity scores were identified. The results are presented in Table S1 and Figure S2.

3.6. Epitope selection criteria

All selected epitopes possessed three desirable attributes: non-toxic, non-allergenic, and highly antigenic/immunogenic, as validated using ToxinPred, AllerTop, and VaxiJen. The tools utilized in this study are summarized in Table 6.

3.7. Design of the small ruminant lentivirus vaccine construct

The multi-epitope vaccine was designed by assembling ten B-cell epitopes linked via “GPGPG” linkers. Likewise, four CTL and four HTL epitopes were connected using the “AAY” and “GPGPG” linkers, respectively. The adjuvant (Pam3CSK4) was kept at the N-terminal of the construct, and linked to CTL epitopes (MHC-I) molecules using the “EAAAK linkers.” CTL epitopes were further

connected with the HTL (MHC-II) epitopes using the “EAAAK” linkers, and the HTL epitopes were linked to the B-cell epitopes employing the “GPGPG” linkers. The C-terminal end of the vaccine construct contained the $6 \times$ His tag molecule, attached via “EAAAK” linkers to facilitate purification. The final vaccine construct consisted of 361 amino acids. The amino acid sequence and overall geometry of the final vaccine construct are shown in Figure 1A.

3.8. Physicochemical properties

ProtParam analysis revealed that the vaccine construct contains 5,426 atoms with a molecular formula of $C_{1723}H_{2679}N_{505}O_{500}S_{19}$. The extinction coefficient was calculated as $108,440 \text{ M}^{-1} \text{ cm}^{-1}$. The estimated half-life was 30 h in mammalian reticulocytes (*in vitro*). The instability index (II) of 35.15 indicated that the protein is stable, supported by an aliphatic index (53.02) and GRAVY (-0.840). SolPro predicted that the construct is soluble with a score of 0.978 upon overexpression. Detailed physicochemical properties are listed in Table 7.

3.9. Secondary structure prediction

A SOPMA revealed that the vaccine consists of alpha-helices (26.04%), turns, extended strands (18.28%), and loops. This predicted composition suggests that the construct may have sufficient structural flexibility for epitope exposure and potential receptor interactions. The details of secondary structure prediction are provided in Table S2. PSIPRED analysis supported these findings and further highlighted the distribution of hydrophobic and hydrophilic residues (Figures 1A and S3).

3.10. Tertiary structure prediction

Tertiary structure was predicted using I-TASSER, and the best model was selected based on structural evaluation. Ramachandran plot analysis indicated that $> 90\%$ of

Table 4. List of selected cytotoxic T lymphocyte epitopes for the generation of an *in silico*-based vaccine candidate against Maedi-Visna and caprine arthritis encephalitis

Allele	Epitope sequence	Score	Percentile rank	AllerTOP	VaxiJen	ToxinPred
BoLA-T2c	SMMHQQMLL	0.933478	0.04	Non-allergen	Non-immunogen (100%)	Non-toxin
BoLA-HD6	RQQEKEKLL	0.945614	0.03	Non-allergen	Immunogen (100%)	Non-toxin
BoLA-1:02301	RQQEKEKLL	0.768478	0.07	Non-allergen	Immunogen (100%)	Non-toxin
BoLA-2:00801	YIAGGKQFW	0.859677	0.02	Non-allergen	Immunogen (66%)	Non-toxin

Notes: This first column shows the respective allele used to predict the cytotoxic T lymphocyte epitope sequence for the vaccine construct, whereas the epitope sequence length, score, and percentile length are listed in the second, third, and fourth columns, respectively. Furthermore, the results of the allergenicity, antigenicity, and toxicity analyses are depicted in columns four to seven, respectively.

Table 5. List of selected helper T lymphocyte epitopes for the generation of an *in silico*-based vaccine candidate against Maedi-Visna and caprine arthritis encephalitis

Sequence	Length	Allele	Affinity (nM)	Percentile rank	Binding level	AllerTOP	VaxiJen	Toxin Pred (support vector machine, molecular weight)
VTWKSMEPLRETWG	16	BoLA-DRB3*0301	0.644	30.63	Strong binder	Non-allergen	Immunogen (100%)	Non-toxin (−0.89, 1,817.31)
LWVIQMVWLGCLIWE	16	DRB1*0102	4.41	7.00	Strong binder	Non-allergen	Immunogen (66%)	Non-toxin (−0.14, 1,889.64)
VLWVIQMVWLGCLIW	16	DRB1*0104	0.761	10.00	Strong binder	Non-allergen	Immunogen (66%)	Non-toxin (−0.30, 1,859.62)
TWKSMEPLRETWGQ	16	DRB1*0120	0.648	29.46	Strong binder	Non-allergen	Immunogen (66%)	Non-toxin (−0.80, 1,846.31)

Notes: The predicted helper T lymphocyte epitope sequence for the vaccine construct is shown in the first column, whereas the epitope sequence length, respective allele, binding affinity, and percentile rank are depicted in the second, third, and fourth columns, respectively. Furthermore, the results of the allergenicity, antigenicity, and toxicity analyses are depicted in columns 4–7, respectively.

residues were in the favored region. Refinement using Galaxy Refine improved model quality, with > 95% of residues falling within the allowed region. The refined model was validated using ProSa, yielding a Z-score of −2.0. Structural validation with ProCHECK confirmed the model's geometric quality. The quality and validation plots for the three-dimensional structure are shown in [Figure 1B](#) and [1C](#), with additional details in Table S3 and Figure S4.

3.11. Molecular docking analysis of the small ruminant lentivirus vaccine construct with TLR-2 and TLR-4 receptors

Molecular docking between the vaccine construct and TLR-2/TLR-4 receptors was performed using ClusPro. The best docking models were selected based on cluster size, energy scores, and interaction profiles (Tables S4 and S5). PDBSum analysis revealed strong interactions. The TLR-2 complex exhibited 16 hydrogen bonds, three salt bridges, and 205 non-bonded contacts. In contrast, the TLR-4 complex demonstrated strong interactions, including 33 hydrogen bonds, 10 salt bridges, and 340 non-bonded contacts ([Figure 2](#)). PRODIGY analysis confirmed higher binding affinity for TLR-4 (−22.5 kcal/mol) compared to TLR-2 (−17.4 kcal/mol) ([Table 8](#)). Also, the TLR-4 complex had more interfacial residues and stronger binding ([Table 8](#)). These results indicate favorable interaction potential; however, these computational estimates do not directly represent biological efficacy and require experimental validation.

3.12. Molecular dynamics simulation

A 100 ns molecular dynamics simulation was performed to evaluate the stability of the docked complexes. RMSD analysis showed initial fluctuations followed by stabilization after approximately 40 ns, indicating attainment of structural equilibrium. The TLR-2 complex exhibited RMSD variations in the range of ~5–6 Å, while the TLR-4 complex showed higher deviations within ~13–14 Å, suggesting greater conformational rearrangement while maintaining overall stability. RMSF analysis demonstrated localized flexibility confined to loop regions, while core residues remained relatively stable. The TLR-2 complex exhibited values ranging from ~2.0 to 13.7 Å, whereas the TLR-4 complex showed reduced fluctuations, ranging from ~1.0 to 4.0 Å, indicating greater residue-level rigidity. The Rg analysis further indicated the compactness of the complexes over time. The average Rg value was ~4.6–4.7 nm for the TLR-2 complex and ~3.1–3.2 nm for the TLR-4 complex, indicating that it adopts a more compact conformation. Additionally, SASA analysis revealed that the TLR-2 complex exhibited higher solvent accessibility, with average values ranging from ~80–160 Å², while the TLR-4 complex showed lower values ranging from 20–60 Å², indicating tighter packing and reduced solvent accessibility. Overall, both complexes demonstrated stable dynamic behavior over the simulation period; however, the relatively higher RMSD and RMSF values for the TLR-4 complex are consistent with the large size and multi-domain nature of the docked complex ([Figure 3](#)).

Table 6. List of tools used in the study

Tool used	Link
Clustal Omega	https://www.ebi.ac.uk/jdispatcher/msa/clustalo
JalView	https://www.jalview.org/
BioEdit	https://bioedit.software.informer.com/7.2/
ABCPRED	https://webs.iitd.edu.in/raghava/abcpred/
BCEPRED	https://webs.iitd.edu.in/raghava/bcepred/bcepred_submission.html
BEPIPRED	https://services.healthtech.dtu.dk/services/BepiPred-2.0/
VaxiJen 2.0	https://www.ddgpharmfac.net/vaxijen/VaxiJen/VaxiJen.html
AllerTOP	https://www.ddg-0pharmfac.net/allertop_test/
ToxinPred	https://webs.iitd.edu.in/raghava/toxinpred/
ProPred-I	http://crdd.osdd.net/raghava/propred1/
EpiJen	https://www.ddg-pharmfac.net/epijen/
Ellipro	http://tools.iedb.org/ellipro/
IEDB MHC-I binding prediction tools	http://tools.iedb.org/mhci/
NetMHCIIpan 2.1 server	https://services.healthtech.dtu.dk/services/NetMHCIIpan-2.1/
ExPASy ProtParam	https://web.expasy.org/cgi-bin/protparam/protparam
SolPro	https://scratch.proteomics.ics.uci.edu/
SOPMA	https://npsa.lyon.inserm.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_sopma.html
PSIPRED	https://bioinf.cs.ucl.ac.uk/psipred/
I-TASSER	https://zhanggroup.org/I-TASSER/
ProCheck server	https://www.ebi.ac.uk/thornton-srv/software/PROCHECK/
Galaxy Refine server	https://bio.tools/galaxyrefine
ProSa webserver	https://prosa.services.came.sbg.ac.at/prosa.php
ClusPro	https://cluspro.bu.edu/
Protein Data Bank	https://www.rcsb.org/
PRODIGY	https://rascar.science.uu.nl/prodigy/
PDBePISA	https://www.ebi.ac.uk/pdbe/pisa/
PDBSum	https://www.ebi.ac.uk/thornton-srv/databases/pdbsum/
Schrodinger Maestro software package	https://www.schrodinger.com/platform/products/maestro/
C-ImmSim server	https://wwwold.iac.rm.cnr.it/
SnapGene	https://www.snapgene.com/
Chimera	https://www.cgl.ucsf.edu/chimera/
Pymol	https://www.pymol.org/

Notes: The table describes the tools utilized in the current study. The first column lists the tool names, and the corresponding links are given in the second column.

3.13. Immune simulation studies

Immune simulation demonstrated strong immune responses at both primary and secondary levels. IgM peaked early (20–30 days), followed by IgG1 and IgG2 responses, indicating effective class switching along with immune memory formation. Elevated levels of B cells,

cytotoxic and helper T cells, dendritic cells, and natural killer cells were observed, suggesting robust and long-term immunological memory. The results of the immune simulations are illustrated in Figure 4.

3.14. Codon optimization and in silico cloning

Codon optimization was performed using the JCat codon

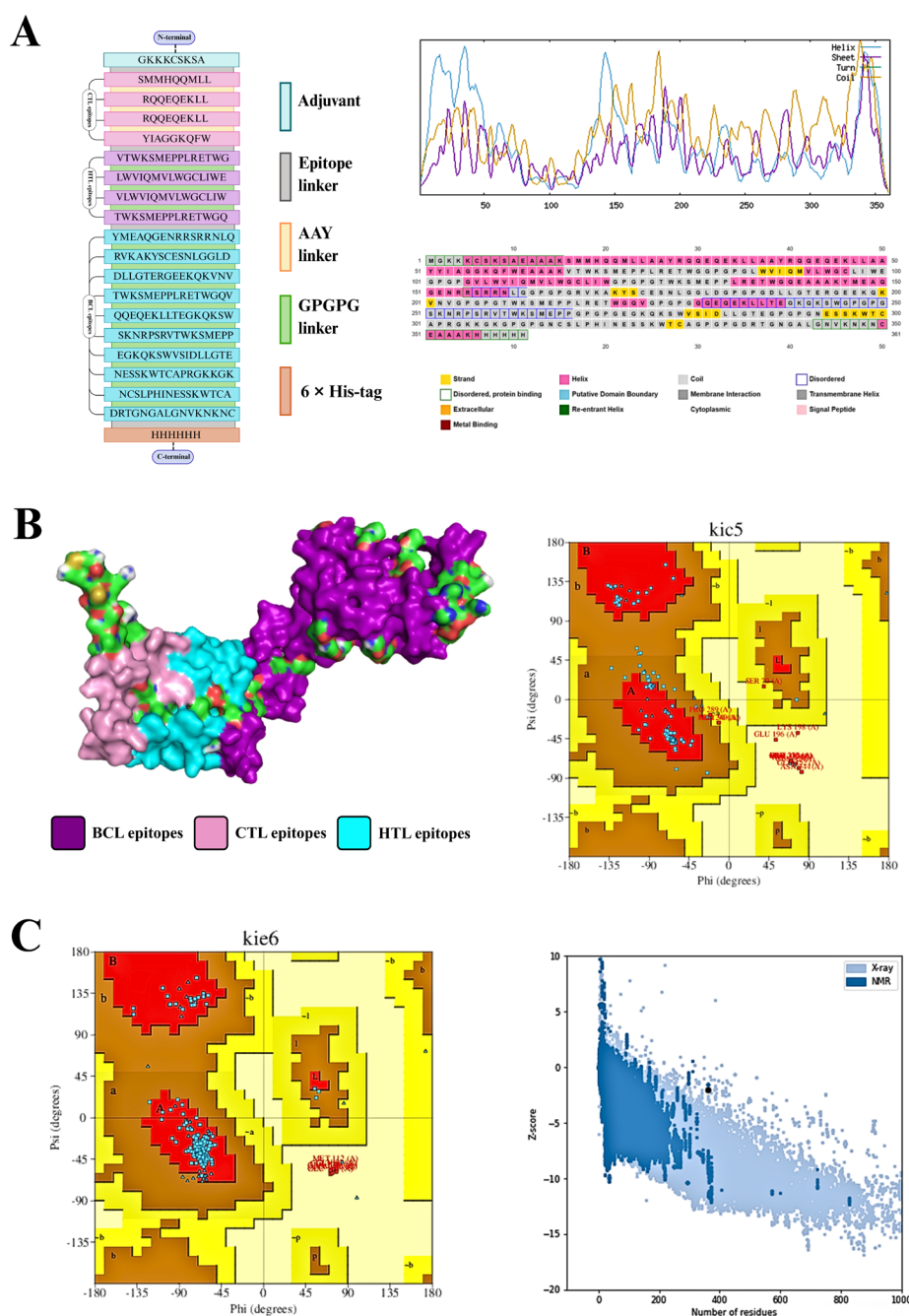


Figure 1. Schematic diagram of the small ruminant lentivirus vaccine construct and predicted structural features. (A) The geometry of the generated vaccine construct by joining adjuvant, B-cell, CTL, and HTL together with the help of linker molecules. All the components of the construct are represented in different colors. Furthermore, the results of the SOPMA secondary structure analysis indicate the presence of various elements, including helices, turns, and coils, in the generated vaccine construct. Whereas the other elements of the structure are highlighted in different-colored boxes. (B) The tertiary structure of the protein construct is shown in different colors (highlighting different epitopes) when generated using the I-TASSER protein prediction server. The visualization of the prediction structure was aided by Chimera and PyMol. Furthermore, the quality of the predicted model was assessed using a Ramachandran plot generated with the ProCheck web server. (C) Ramachandran plot generated via the ProCheck server for the selected protein model (refined with the Galaxy Refine tool), showing >90% of the residues in the allowed regions. The quality of the predicted model was also analyzed using the ProSa webserver by examining the predicted Z-scores for each amino acid residue in the vaccine construct. Abbreviations: BCL: B cell lymphocyte; CTL: Cytotoxic T lymphocyte; HTL: Helper T lymphocyte; kie6: Keratinocyte immortalized cell line 6; SOPMA: Self-optimized prediction method with alignment.

Table 7. Physicochemical properties of the small ruminant lentivirus vaccine construct

Physicochemical property	Result
No. of amino acids	361
Theoretical isoelectric point	9.38
Molecular weight	39,077.45
Total number of negatively charged residues (Asp + Glu)	33
Total number of positively charged residues (Arg + Lys)	47
Total no. of atoms	5,426
Formula	$C_{1723}H_{2679}N_{505}O_{500}S_{19}$
Extinction coefficients	Extinction coefficient 108440 M ⁻¹ cm ⁻¹ ; Absorbance 0.1% (=1 g/L) 2.775, assuming all pairs of Cys residues form cystines
Estimated half-life	30 hours (mammalian reticulocytes, <i>in vitro</i>); >20 h (yeast, <i>in vivo</i>); >10 h (<i>Escherichia coli</i> , <i>in vivo</i>)
Instability index	35.15 (stable)
Aliphatic index	53.02
Grand average of hydropathicity	-0.840

Notes: This table provides the outcome of the physicochemical properties predicted for the vaccine construct using the ExPASy ProtParam online server. The first column describes the respective physicochemical property whereas the results of each analysis are listed in the second column.

Table 8. PRODIGY analysis for the docked vaccine construct with 6NIG and 4G8A ligand

Protein-Protein Complex	ΔG (kcal mol ⁻¹)	K_d (M) at °C	ICs charged-charged	ICs charged-polar	ICs charged-apolar	ICs polar-polar	ICs polar-apolar	ICs apolar-apolar	NIS charged	NIS apolar
6NIG-construct	-17.4	1.7×10^{-13}	22	21	50	16	33	39	27.14	33.27
4G8A-construct	-22.5	3×10^{-17}	30	38	96	13	31	20	23.69	37.85

Abbreviations: IC: Inter-chain contacts; ΔG : Binding affinity; K_d : Dissociation constant; NIS: Non-interacting surface.

optimization server. The optimized codon had 361 amino acids, a CAI value of 1.0, and a GC content of 54.85%, indicating high expression potential in *E. coli*. The gene was successfully cloned into the pET28a (+) vector using SnapGene. The length of the generated recombinant construct was 6,430 base pairs. The final clone is shown in Figure 5.

4. Discussion

Maedi-Visna and CAE, caused by SRLVs, are among the most prevalent viral infections affecting livestock, particularly goats and sheep. These viruses have longer incubation periods, which often lead to ineffective or delayed diagnosis. Furthermore, the RNA genome of SRLVs undergoes frequent mutations, thereby limiting the efficacy of the existing strategies.

Vaccination remains the most effective approach for controlling viral diseases, as it stimulates the host's immune system to recognize and eliminate invading pathogens. However, conventional vaccine development is often complex, tedious, and expensive. In contrast, reverse vaccinology offers an efficient alternative by using cutting-edge bioinformatics and computational biology tools to screen proteomic, genomic, and transcriptomic datasets for potential antigenic targets. This approach enables the rapid identification of the vaccine candidates that are immunogenic, non-allergenic, and non-toxic. Nevertheless, wet-lab experimentation remains essential to confirm the biological efficacy of computationally designed vaccine constructs.

In this study, a reverse vaccinology approach was employed to design a multi-epitope vaccine construct

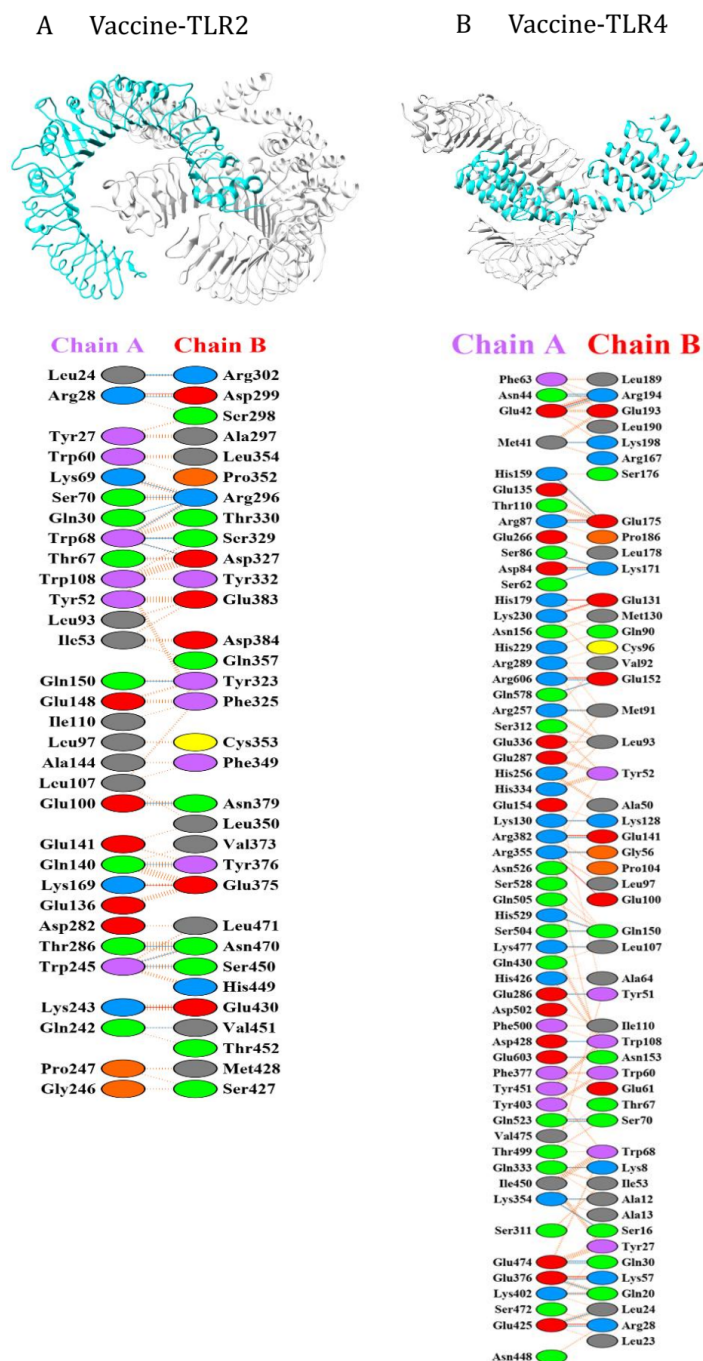


Figure 2. Molecular docking analysis for the small ruminant lentivirus (SRLV) vaccine construct. (A) Results of the molecular interaction/docking analysis between the SRLV vaccine construct and the TLR-2 receptor molecule (PDB ID:6NIG). The results were analyzed with the PDBSum web server. Here, chain A represents the vaccine construct, chain B the receptor molecule, and the different-colored boxes show interacting amino acid residues in the respective chains. The differently colored lines represent various intermolecular forces, such as hydrogen bonds and salt bridges. (B) Results of the molecular interaction/docking analysis between the SRLV vaccine construct and the TLR-4 receptor molecule (PDB ID:4G8A). The results were analyzed with PDBSum web server. Here, chain A corresponds to the receptor molecule, whereas chain B corresponds to the vaccine construct sequence. Different-colored boxes show interacting amino acid residues in the respective chains, whereas different-colored lines indicate intermolecular forces such as hydrogen bonds and salt bridges. The results collectively reveal a stronger molecular interaction between the generated SRLV vaccine construct and the TLR-4 receptor than with the TLR-2 receptor.

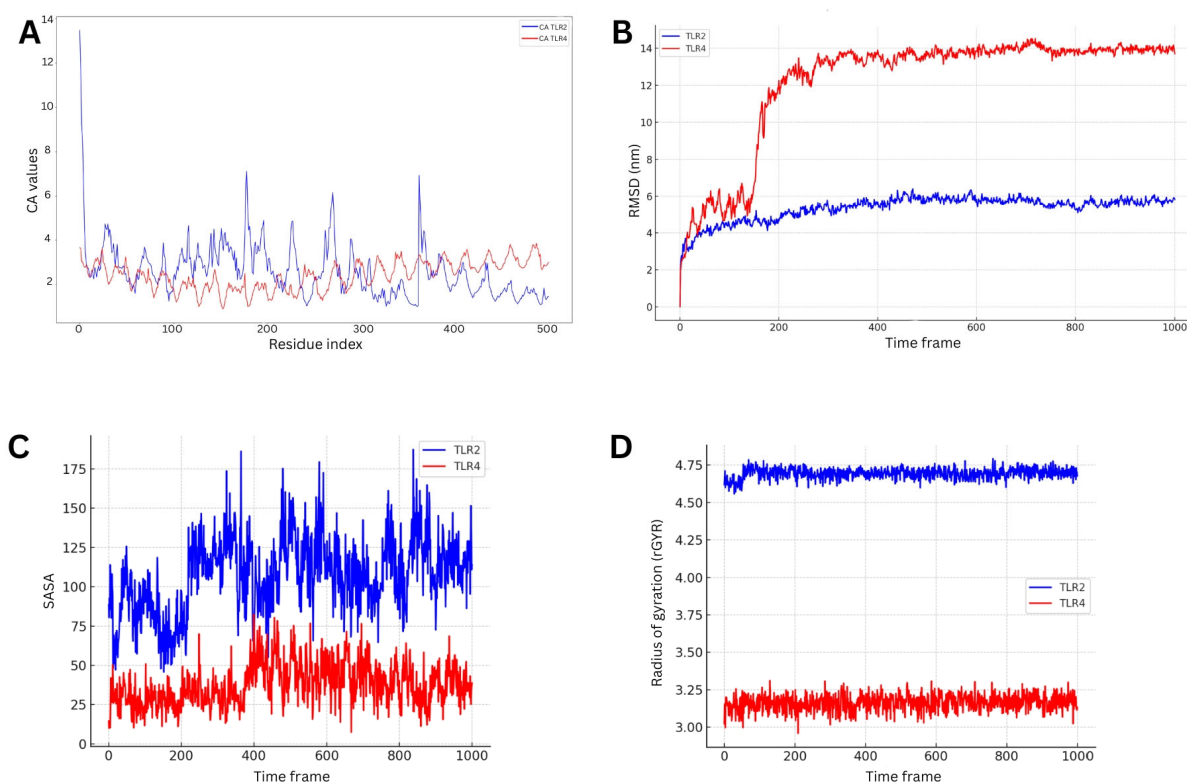


Figure 3. Molecular dynamics simulations for the small ruminant lentivirus vaccine construct. The outcomes of molecular dynamics simulations were performed with the generated vaccine construct and two receptor molecules, i.e., TLR-2 (6NIG) and TLR-4 (4G8A), using the Schrodinger Maestro software package. Different parameters, including (A) CA, (B) RMSD, (C) SASA, and (D) Rg, were calculated and compared for both ligands relative to the construct molecule.

Abbreviations: CA: C-alpha atom; Rg: Radius of gyration; RMSD: Root mean square deviation; SASA: Solvent accessible surface area.

capable of providing broad species protection against SRLV infections in both goats and sheep. Previous studies have primarily focused on vaccine development targeting MVV in sheep. However, the current construct is based on both MVV and CAEV reference isolates, thereby enhancing the potential applicability across diverse host species.

The selected epitopes were derived from the conserved region of the viral envelope protein, which plays a crucial role in host attachment and viral entry. Both linear and discontinuous B-cell epitopes were predicted, along with CTL and HTL epitopes, using relevant MHC alleles. All selected epitopes were filtered to ensure high antigenicity and the absence of toxicity and allergenicity.

The final vaccine construct comprised ten B-cell epitopes, four CTL epitopes, and four HTL epitopes, linked by appropriate linker sequences. An adjuvant (Pam3CSK4) was added at the N-terminus to enhance immunogenicity, while a 6 × His tag was added at the C-terminus to facilitate purification. The final construct consisted of 361 amino

acids and demonstrated favorable properties, including high solubility, high antigenicity, non-toxicity, and non-allergenicity.

Secondary structure revealed a composition of alpha helix (26.04%), extended beta-strands (18.28%), and random coils (55.68%). Tertiary structure was predicted with I-TASSER and refined with Galaxy Refine, and validated with PROCHECK and ProSA, confirming the model's structural reliability.

Molecular docking analysis demonstrated strong interactions between the vaccine construct and toll-like receptors, particularly TLR-4, which showed a higher binding affinity (−22.5 kcal/mol) than TLR-2 (−17.4 kcal/mol). Interaction analysis also supported these findings, demonstrating a greater number of interacting residues and hydrogen bonds in the TLR-4 complex (Table 8).

Molecular dynamics simulations indicated that both TLR-2 and TLR-4 complexes remained structurally stable throughout the simulation. Notably, the TLR-4 complex

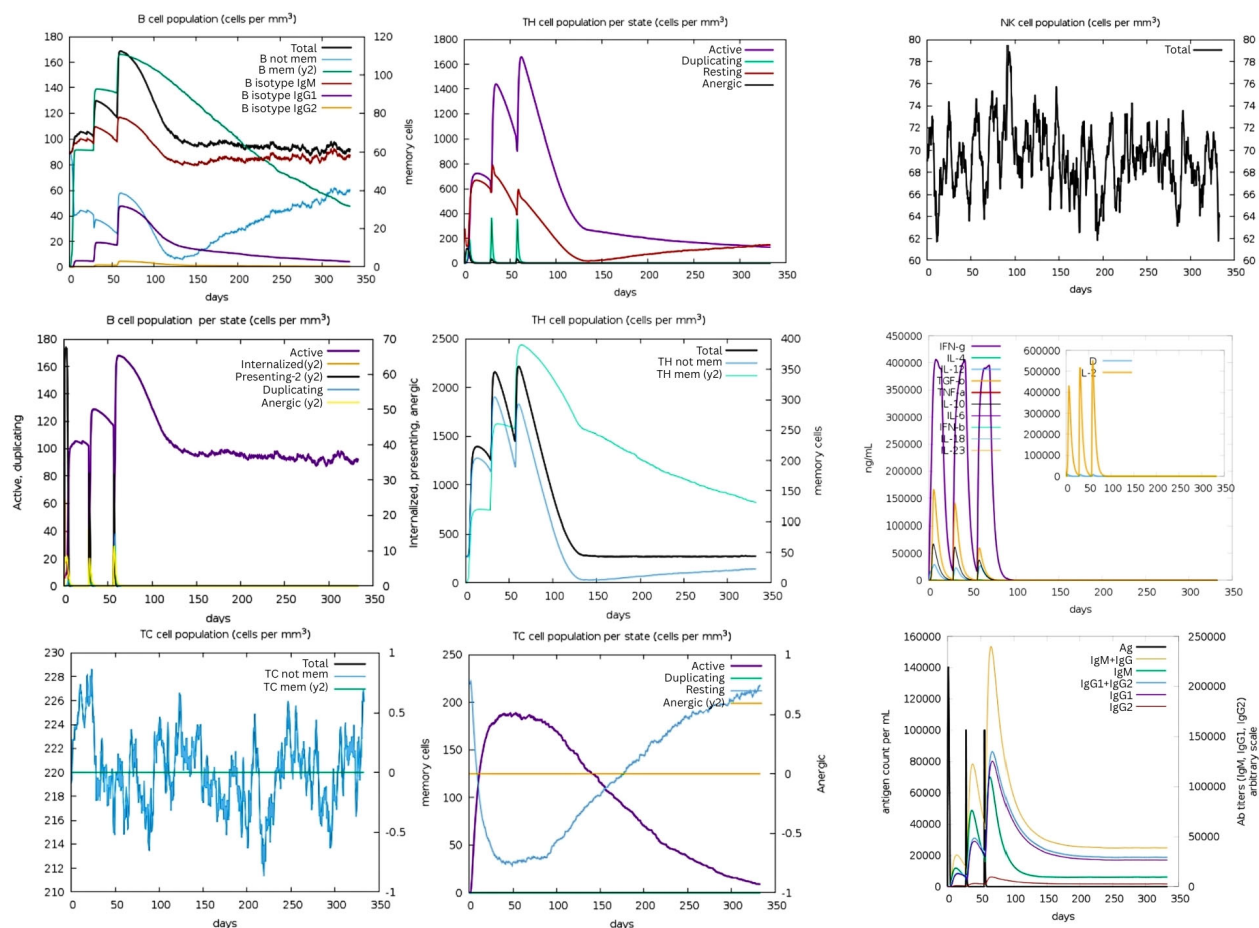


Figure 4. Immune simulations for the small ruminant lentivirus vaccine construct. The outcomes of the immune simulations were generated using the vaccine construct via the online C-Imm Sim server. Different parameters, including B cell, helper and cytotoxic T cell populations, were determined, along with the concentration of memory cells.

exhibited greater stability and stronger interactions than the TLR-2 complex. However, it is important to emphasize that molecular docking and simulation studies provide predictive insights and should not be interpreted as direct measures of immunogenicity or protective efficacy. Experimental validation, including receptor activation assays, cytokine profiling, and animal challenge studies, is required to confirm the biological relevance of the proposed vaccine construct. Additionally, the immune simulation studies further supported the construct's immunogenic potential, demonstrating robust activation of both humoral and cellular immunity. Elevated levels of B cells, cytotoxic T cells, helper T cells, and natural killer cells were observed along with the formation of immunological memory, indicating the potential for long-term protection.

Additionally, codon optimization and *in silico* cloning were performed to facilitate large-scale protein expression

in bacterial systems. The optimized sequence (361 amino acids) exhibited a high CAI along with a suitable GC content. It was successfully cloned into the pET28a (+) expression vector, resulting in a construct of 6,430 base pairs. Despite showing potential as an effective vaccine, the proposed vaccine candidate requires comprehensive *in vitro* and *in vivo* validation to confirm its safety, immunogenicity, and protective efficacy.

The present study is subject to several limitations. Although highly conserved epitopes were selected, bioinformatics tools primarily focus on predicting epitopes based on expression and binding properties, potentially overlooking other immunological factors involved in host-pathogen interactions. Also, the CTL epitopes were predicted using the surrogate strategy because ovine/caprine MHC datasets were unavailable. Hence, epitope-binding predictions may differ between sheep/goats, and

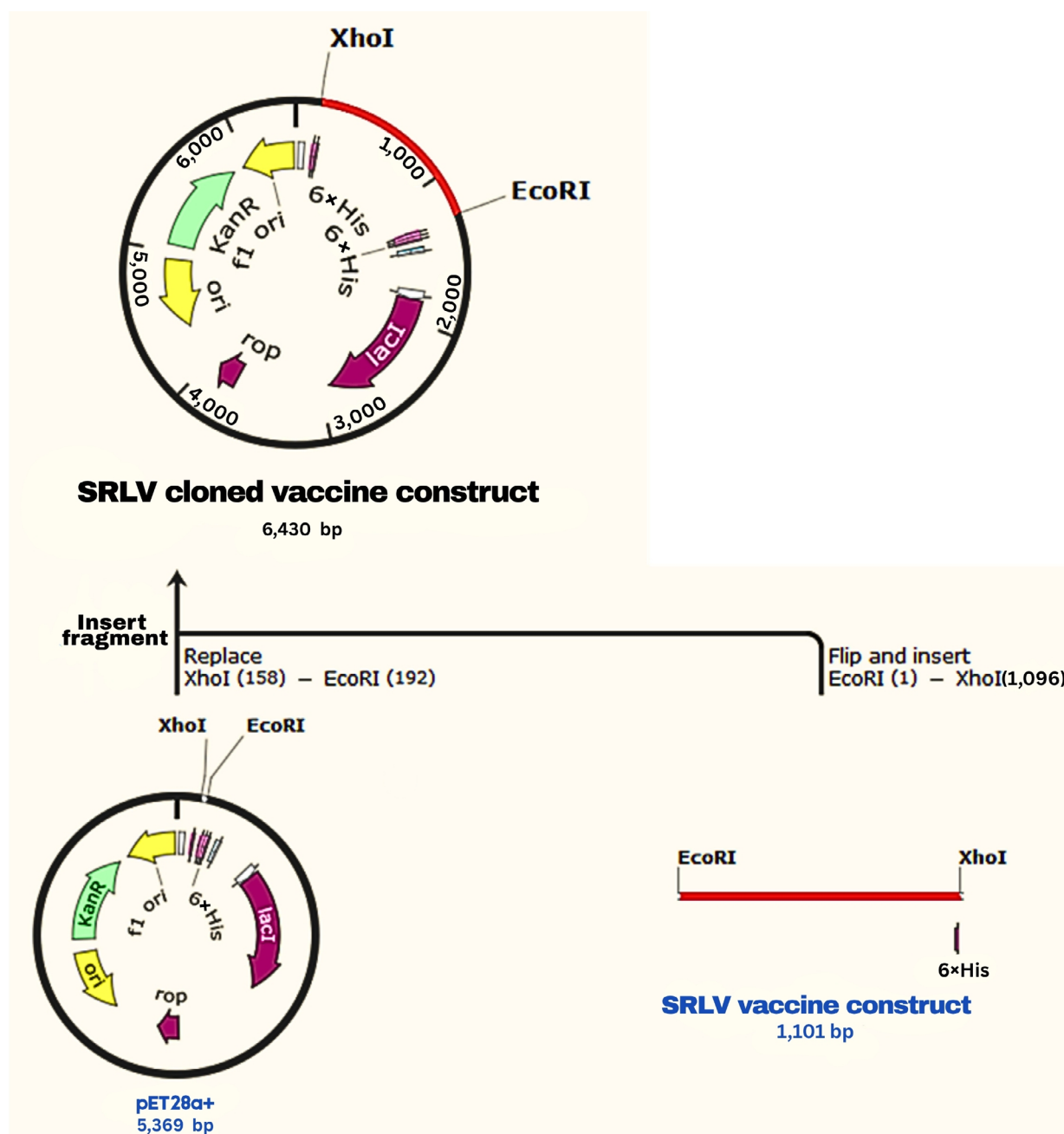


Figure 5. *In silico* cloning and codon optimization for the small ruminant lentivirus (SRLV) vaccine construct and the results of *in silico* cloning. First, the coding sequence was optimized using the JCat Server; later, the vaccine construct was cloned into the commercially available *E. coli* (pET28a [+]) vector using the SNAPGene tool (the clone is highlighted in red). The generated clone was 6,430 base pairs long.

future validation using ovine/caprine MHC datasets will be necessary.

Additionally, linker sequences used in the vaccine construct may destabilize the construct and raise safety concerns. Also, the above vaccine construct development is entirely computational, and its safety/efficacy validation in

the practical world would require animal experimentation. Efforts are currently underway to manage the necessary resources for experimental validation, and future work will involve pilot-scale animal studies to assess the protective efficacy and immunogenicity of the proposed vaccine candidate.

5. Conclusion

Small ruminant lentiviruses are the main responsible agents behind MV and CAE incidence among goats and sheep, leading to loss in milk and meat production globally. In the present study, a novel multi-epitope vaccine candidate was developed to combat diverse SRLV strains by using bioinformatic and immunoinformatic approaches. A vaccine construct was generated by combining ten B-cell, four CTL, and four HTL epitopes with their respective linker molecules. An adjuvant, Pam3CSK4, and a 6 × His tag were added to generate a more robust immune response and a purified product, respectively. The vaccine showed good antigenicity while being non-toxic and non-allergenic. Also, the vaccine exhibited stable, and high-affinity interactions when docked/simulated with the TLR-2 and TLR-4 receptors. Based on the above findings, the generated vaccine is a potential candidate against SRLV-induced MV and CAE in goats and sheep. While computational analyses indicate favorable structural stability and receptor-binding potential, the proposed vaccine candidate requires comprehensive *in vitro* and *in vivo* validation to establish immunogenicity, safety, and protective efficacy against SRLV infection.

Acknowledgments

None.

Funding

None.

Conflict of interest

The authors declare no conflicts of interest.

Author contributions

Conceptualization: Muhammad Zeeshan Shabbir

Formal analysis: Rumesa Ghazanfar, Zainab Nagari, Muhammad Atiq Ur Rehman, Sourav Chandra

Investigation: Rumesa Ghazanfar, Zainab Nagari, Muhammad Atiq Ur Rehman, Muhammad Zeeshan Shabbir

Methodology: Muhammad Zeeshan Shabbir

Writing–original draft: Muhammad Zeeshan Shabbir, Rumesa Ghazanfar

Writing–review & editing: Muhammad Zeeshan Shabbir

Ethics approval and consent to participate

This study was entirely *in silico*, using publicly available data, and did not involve any animal or human participation. Therefore, ethical approval and consent to participate were not required.

Consent for publication

Not applicable.

Availability of data

The data supporting the findings of this study are available upon request.

References

1. Mosa AH, Zenad MM. Serological and histopathological detection of Maedi-Visna virus in middle Iraq regions. *Plant Arch.* 2020;20(2):6339-6343.
2. Blacklaws BA. Small ruminant lentiviruses: immunopathogenesis of visna-maedi and caprine arthritis and encephalitis virus. *Comp Immunol Microbiol Infect Dis.* 2012;35(3):259-269.
doi:10.1016/j.cimid.2011.12.003
3. Moroz A, Czopowicz M, Sobczak-Filipiak M, *et al.* The prevalence of histopathological features of pneumonia in goats with symptomatic caprine arthritis-encephalitis. *Pathogens.* 2022;11(6):629.
doi:10.3390/pathogens11060629
4. Enache DA, Baraitareanu S, Dan M, *et al.* Preliminary results of MVV and CAEV seroprevalence in Romanian sheep and goats. *Sci Works Series C Vet Med.* 2017;63(1):95-100.
5. Rahman MH, Ahmed E, Haque MN, Hassan MZ, Ali MZ. Major respiratory diseases of goat and their epidemiology, prevention and control. *Bangladesh J Livest Res.* 2022;29(1-2):1-20.
doi:10.3329/bjlr.v29i1.72031
6. Blacklaws BA, Berriatua E, Torsteinsdottir S, *et al.* Transmission of small ruminant lentiviruses. *Vet Microbiol.* 2004;101(3):199-208.
doi:10.1016/j.vetmic.2004.04.006
7. Minardi da Cruz JC, Singh DK, Lamara A, Chebloune Y. Small ruminant lentiviruses (SRLVs) break the species barrier to acquire new host range. *Viruses.* 2013;5(7):1867-1884.
doi:10.3390/v5071867
8. Arcangeli C, Torricelli M, Sebastiani C, *et al.* Genetic characterization of small ruminant lentiviruses (SRLVs) circulating in naturally infected sheep in Central Italy. *Viruses.* 2022;14(4):686.
doi:10.3390/v14040686
9. Santry LA, de Jong J, Gold AC, Walsh SR, Menzies PI, Wootton SK. Genetic characterization of small ruminant lentiviruses circulating in naturally infected sheep and goats in Ontario, Canada. *Virus Res.* 2013;175(1):30-44.
doi:10.1016/j.virusres.2013.03.019

10. Gomez-Lucia E, Barquero N, Domenech A. Maedi-Visna virus: current perspectives. *Vet Med*. 2018;9:11-21.
doi:10.2147/VMRR.S136705
11. Olech M, Valas S, Kuźmak J. Epidemiological survey in single-species flocks from Poland reveals expanded genetic and antigenic diversity of small ruminant lentiviruses. *PLoS ONE*. 2018;13(3):e0193892.
doi:10.1371/journal.pone.0193892
12. Pépin M, Vitu C, Russo P, Mornex JF, Peterhans E. Maedi-visna virus infection in sheep: a review. *Vet Res*. 1998;29(3-4):341-367.
13. Querat G, Audoly G, Sonigo P, Vigne R. Nucleotide sequence analysis of SA-OMVV, a visna-related ovine lentivirus: phylogenetic history of lentiviruses. *Virology*. 1990;175(2):434-447.
doi:10.1016/0042-6822(90)90428-T
14. Molaee V, Bazzucchi M, De Mia GM, *et al*. Phylogenetic analysis of small ruminant lentiviruses in Germany and Iran suggests their expansion with domestic sheep. *Sci Rep*. 2020;10(1):2243.
doi:10.1038/s41598-020-58990-9
15. Michiels R, Van Mael E, Quinet C, Adjadj NR, Cay AB, De Regge N. Comparative analysis of different serological and molecular tests for the detection of small ruminant lentiviruses (SRLVs) in Belgian sheep and goats. *Viruses*. 2018;10(12):696.
doi:10.3390/v10120696
16. Pétursson G, Matthíasdóttir S, Svansson V, *et al*. Mucosal vaccination with an attenuated maedi-visna virus clone. *Vaccine*. 2005;23(24):3223-3228.
doi:10.1016/j.vaccine.2004.11.074
17. González B, Reina R, García I, *et al*. Mucosal immunization of sheep with a Maedi-Visna virus (MVV) env DNA vaccine protects against early MVV productive infection. *Vaccine*. 2005;23(34):4342-4352.
doi:10.1016/j.vaccine.2005.03.032
18. Torsteinsdóttir S, Carlsdóttir HM, Svansson V, Matthíasdóttir S, Martin AH, Pétursson G. Vaccination of sheep with Maedi-visna virus gag gene and protein, beneficial or harmful? *Vaccine*. 2007;25(37-38):6713-6720.
doi:10.1016/j.vaccine.2007.07.004
19. De Andres X, Reina R, Ciriza J, *et al*. Use of B7 costimulatory molecules as adjuvants in a prime-boost vaccination against Visna-Maedi ovine lentivirus. *Vaccine*. 2009;27(34):4591-4600.
doi:10.1016/j.vaccine.2009.05.080
20. Koçkaya ES, Can H, Yaman Y, Ün C. In silico discovery of epitopes of gag and env proteins for the development of a multi-epitope vaccine candidate against Maedi Visna virus using reverse vaccinology approach. *Biologicals*. 2023;84:101715.
doi:10.1016/j.biologicals.2023.101715
21. Cheevers WP, Knowles DP, McGuire TC, Baszler TV, Hullinger GA. Caprine arthritis-encephalitis lentivirus (CAEV) challenge of goats immunized with recombinant vaccinia virus expressing CAEV surface and transmembrane envelope glycoproteins. *Vet Immunol Immunopathol*. 1994;42(3-4):237-251.
doi:10.1016/0165-2427(94)90070-1
22. Sievers F, Higgins DG. Clustal Omega for making accurate alignments of many protein sequences. *Protein Sci*. 2018;27(1):135-145.
doi:10.1002/pro.3290
23. Troshin PV, Procter JB, Barton GJ. Java bioinformatics analysis web services for multiple sequence alignment—JABAWS: MSA. *Bioinformatics*. 2011;27(14):2001-2002.
doi:10.1093/bioinformatics/btr304
24. Hall T. BioEdit: an important software for molecular biology. *GERF Bull Biosci*. 2011;2(1):60-61.
25. Saha S, Raghava GPS. Prediction of continuous B-cell epitopes in an antigen using recurrent neural network. *Proteins*. 2006;65(1):40-48.
doi:10.1002/prot.21078
26. Saha S, Raghava GPS. BcePred: prediction of continuous B-cell epitopes in antigenic sequences using physico-chemical properties. In: Flower DR, Dimitrov I, eds. *Immunoinformatics: Predicting Immunogenicity in Silico*. Berlin, Heidelberg: Springer; 2004:197-204.
27. Jespersen MC, Peters B, Nielsen M, Marcatili P. BepiPred-2.0: improving sequence-based B-cell epitope prediction using conformational epitopes. *Nucleic Acids Res*. 2017;45(W1):W24-W29.
doi:10.1093/nar/gkx346
28. Doytchinova IA, Flower DR. VaxiJen: a server for prediction of protective antigens, tumour antigens and subunit vaccines. *BMC Bioinform*. 2007;8(1):4.
doi:10.1186/1471-2105-8-4
29. Dimitrov I, Bangov I, Flower DR, Doytchinova I. AllerTOP v. 2—a server for in silico prediction of allergens. *J Mol Model*. 2014;20(6):2278.
doi:10.1007/s00894-014-2278-5
30. Sharma N, Naorem LD, Jain S, Raghava GP. ToxinPred2: an improved method for predicting toxicity of proteins. *Brief Bioinform*. 2022;23(5):bbac174.
doi:10.1093/bib/bbac174
31. Singh H, Raghava GPS. ProPred: prediction of HLA-DR

- binding sites. *Bioinformatics*. 2001;17(12):1236-1237.
doi:10.1093/bioinformatics/17.12.1236
32. Doytchinova IA, Guan P, Flower DR. EpiJen: a server for multistep T cell epitope prediction. *BMC Bioinformatics*. 2006;7(1):131.
doi:10.1186/1471-2105-7-131
 33. Trolle T, Metushi IG, Greenbaum JA, *et al.* Automated benchmarking of peptide-MHC class I binding predictions. *Bioinformatics*. 2015;31(13):2174-2181.
doi:10.1093/bioinformatics/btv123
 34. Lybeck K, Tollefsen S, Mikkelsen H, *et al.* Selection of vaccine-candidate peptides from *Mycobacterium avium* subsp. *paratuberculosis* by in silico prediction, in vitro T-cell line proliferation, and in vivo immunogenicity. *Front Immunol*. 2024;15:1297955.
doi:10.3389/fimmu.2024.1297955
 35. Gaafar BB, Ali SA, Abd-Elrahman KA, Almofti YA. Immunoinformatics approach for multiepitope vaccine prediction from H, M, E, and N proteins of Peste des petits ruminants virus. *J Immunol Res*. 2019;2019:6124030.
doi:10.1155/2019/6124030
 36. Oladipo EK, Taiwo OR, Teniola FO, *et al.* Immunoinformatics approach to Rift Valley fever virus vaccine design in ruminants. *Biomed Res Ther*. 2024;11(2):6233-6247.
doi:10.15419/bmrat.v11i2.869
 37. Yang Y, Wei Z, Cia G, *et al.* MHCII-peptide presentation: an assessment of the state-of-the-art prediction methods. *Front Immunol*. 2024;15:1293706.
doi:10.3389/fimmu.2024.1293706
 38. Ponomarenko J, Bui HH, Li W, *et al.* ElliPro: a new structure-based tool for the prediction of antibody epitopes. *BMC Bioinform*. 2008;9(1):514.
doi:10.1186/1471-2105-9-514
 39. Crowe J, Masone BS, Ribbe J. One-step purification of recombinant proteins with the 6xHis tag and Ni-NTA resin. *Mol Biotechnol*. 1995;4(3):247-258.
doi:10.1007/bf02779018
 40. Gasteiger E, Hoogland C, Gattiker A, *et al.* Protein identification and analysis tools on the ExPASy server. In: Walker JM, eds. *The Proteomics Protocols Handbook*. Totowa, NJ: Humana Press; 2005:571-607.
 41. Magnan CN, Randall A, Baldi P. SOLpro: accurate sequence-based prediction of protein solubility. *Bioinformatics*. 2009;25(17):2200-2207.
doi:10.1093/bioinformatics/btp386
 42. Geourjon C, Deleage G. SOPMA: significant improvements in protein secondary structure prediction by consensus prediction from multiple alignments. *Bioinformatics*. 1995;11(6):681-684.
doi:10.1093/bioinformatics/11.6.681
 43. McGuffin LJ, Bryson K, Jones DT. The PSIPRED protein structure prediction server. *Bioinformatics*. 2000;16(4):404-405.
doi:10.1093/bioinformatics/16.4.404
 44. Zhang Y. I-TASSER server for protein 3D structure prediction. *BMC Bioinform*. 2008;9(1):40.
doi:10.1186/1471-2105-9-40
 45. Laskowski RA, MacArthur MW, Moss DS, Thornton JM. PROCHECK: a program to check the stereochemical quality of protein structures. *J Appl Crystallogr*. 1993;26:283-291.
doi:10.1107/S0021889892009944
 46. Heo L, Park H, Seok C. GalaxyRefine: Protein structure refinement driven by side-chain repacking. *Nucleic Acids Res*. 2013;41(W1):W384-W388.
doi:10.1093/nar/gkt458
 47. 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):W407-W410.
doi:10.1093/nar/gkm290
 48. Kozakov D, Hall DR, Xia B, *et al.* The ClusPro web server for protein-protein docking. *Nat Protoc*. 2017;12(2):255-278.
doi:10.1038/nprot.2016.169
 49. Xue LC, Rodrigues JP, Kastiris PL, Bonvin AM, Vangone A. PRODIGY: a web server for predicting the binding affinity of protein-protein complexes. *Bioinformatics*. 2016;32(23):3676-3678.
doi:10.1093/bioinformatics/btw514
 50. Krissinel E, Henrick K. Inference of macromolecular assemblies from crystalline state. *J Mol Biol*. 2007;372(3):774-797.
doi:10.1016/j.jmb.2007.05.022
 51. Pettersen EF, Goddard TD, Huang CC, *et al.* UCSF Chimera—a visualization system for exploratory research and analysis. *J Comput Chem*. 2004;25(13):1605-1612.
doi:10.1002/jcc.20084
 52. Seeliger D, de Groot BL. Ligand docking and binding site analysis with PyMOL and Autodock/Vina. *J Comput Aided Mol Des*. 2010;24(5):417-422.
doi:10.1007/s10822-010-9352-6
 53. Laskowski RA, Jabłońska J, Pravda L, Vařeková RS, Thornton JM. PDBsum: Structural summaries of PDB entries. *Protein Sci*. 2018;27(1):129-134.
doi:10.1002/pro.3289

54. Kosloff D, Kosloff R. A Fourier method solution for the time dependent Schrödinger equation as a tool in molecular dynamics. *J Comput Phys.* 1983;52(1):35-53.
doi:10.1016/0021-9991(83)90015-3
55. Rapin N, Lund O, Castiglione F. Immune system simulation online. *Bioinformatics.* 2011;27(14):2013-2014.
doi:10.1093/bioinformatics/btr335
56. Grote A, Hiller K, Scheer M, *et al.* JCat: a novel tool to adapt codon usage of a target gene to its potential expression host. *Nucleic Acids Res.* 2005;33(Suppl 2):W526-W531.
doi:10.1093/nar/gki376