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Immunoinformatics-driven design of a multi-epitope vaccine against nipah virus: A promising approach for global health protection

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ABSTRACT

This study focuses on developing a multi-epitope vaccine against the highly pathogenic Nipah virus using immunoinformatics. It aims to design a vaccine targeting the viral nucleoprotein to elicit robust immune responses. The approach integrates epitope prediction, vaccine construction, and validation through computational tools to address the lack of effective vaccines and mitigate global health threats posed by Nipah virus outbreaks. Immunoinformatics approaches have been utilized for epitope prediction, focusing on B-cell and T-cell epitopes of the Nipah virus nucleoprotein. The multi-epitope vaccine was constructed using linkers and adjuvants to enhance immunogenicity. Structural refinement, molecular docking with human ephrin B2 receptor, and immune simulations were performed to validate the vaccine's stability, binding efficiency, and immune response potential. The designed multi-epitope vaccine exhibited high antigenicity (0.56), non-allergenicity, and non-toxicity. Docking analysis showed a strong binding affinity with the ephrin B2 receptor (binding energy: −920 kcal/mol). Immune simulations indicated significant immune responses with high IgG and IgM levels and memory B-cell activation. Population coverage analysis revealed a global coverage of 88.3 %, supporting its potential for broad immunization. The designed vaccine against the Nipah virus demonstrates promising antigenicity, stability, and strong binding with the ephrin B2 receptor. With global population coverage and a robust immune response, it holds potential for clinical development. Further experimental validation and in vitro studies are recommended to confirm its efficacy as a viable vaccine candidate for the Nipah virus.

1. Introduction

From Sungai Nipah (Nipah River Village), from which the initial extracts were obtained, the name 'Nipah virus' originated. Nipah virus (NiV) stems from the genus Henipavirus, family Paramyxoviridae, and is classified among the zoonotic viruses.[1] Its size varies from 40 to 600 nm in diameter, and it is an enveloped single-stranded, non-segmented RNA virus with a helical arrangement.[2] The RNA encodes for six major proteins: fusion protein (F), glycoprotein (G), matrix protein (M), nucleocapsid (N), phosphoprotein (P), and polymerase protein (L). The replication of the Nipah virus needs the complex of phosphoprotein with unassembled nucleoprotein.[3] The phosphorylation of viral proteins is an essential step for replication and transcription of viral genome catalyzed by RNA-dependent RNA polymerase (RdRp), which is constituted by P and L proteins[4].

In 2001, the first outbreak of NiV was reported in Siliguri, followed by other areas like Nadia, West Bengal, and other areas of Bangladesh in 2007. At that time, the intermediate host remains unidentified, suggesting that it could be bat-to-human and human-to-human transmissions.[5] Bats are considered the host of many emerging viruses, including Hendra virus, Marburg virus, Sosuga virus, and Nipah virus.[5] In South Asia and Southeast Asia, 640 cases of NiV have been reported. As the reservoir of this infection is fruit bats, humans are infected by contact with infected bats, eating contaminated fruits, or exposure to body fluids of infected animals.[6] Previously, reports indicated the bats of Pteropus spp. Often, feed on the shaved bark, potentially contaminating the sap with saliva, urine, and excrement. It is also believed that Pteropus spp. Bats shed NiV in their secretions and excretions.[7]

The NiV enters the host through the F and G proteins. These proteins are the main targets of antibody response.[8] The genomic RNA of the core is tightly binding to the nucleocapsid and phosphoprotein proteins. This virus uses its spikes created by F protein trimers and G protein for

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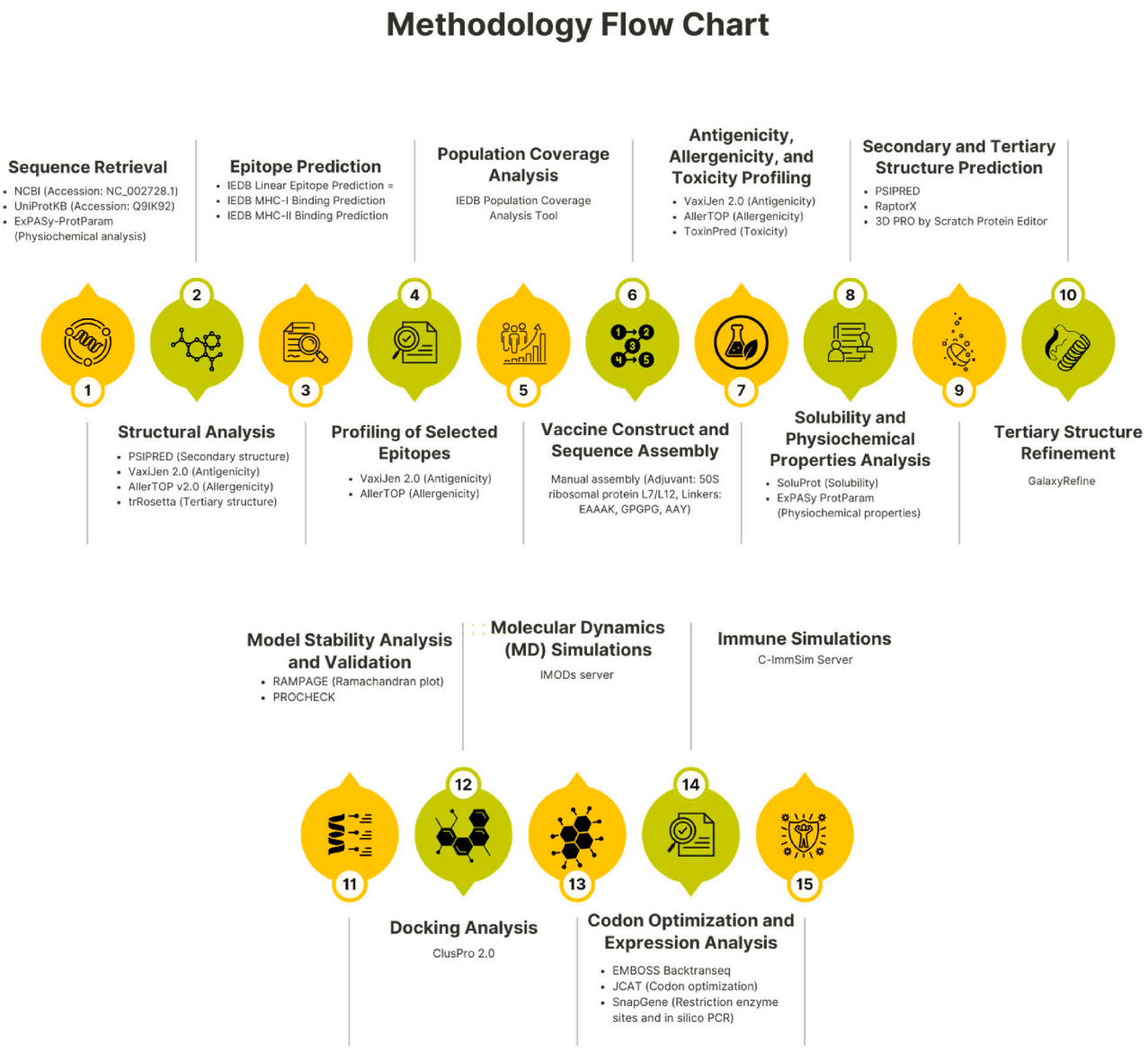


Fig. 1. The flowchart of methodology indicating the steps and tools used for designing this vaccine construct.

attachment with the human receptor ephrin b2.[9] Epidemiological surveys revealed that the latent period in humans ranges from 4 days to 2 months and in 90 % of cases at 2 weeks or less. Encephalitis (brain swelling) may occur, causing drowsiness, disorientation, and mental confusion, and within 24–48 h it can lead to coma. In 40–75 % of cases, death may occur. Long-term side effects like enduring convulsions and alterations in personality have been noted in survivors of NiV infection.[10] Nipah virus replication takes place in various organs. Initially, it begins by infecting the epithelium of the upper respiratory tract. From there, the virus travels to the pharynx to a lesser extent, reaching the trachea, lungs, and CNS via the blood–brain barrier.[3] At present, there is no vaccine accessible for Nipah virus (NiV) infection. Treatment primarily involves supportive care, including rest, hydration, and treatment of symptoms as they arise.[11] Intensive care is needed in severe cases with neurologic and respiratory complications. Moreover, individuals need to be mindful of both food hygiene and public hygiene.[12]

In-silico approaches are employed to design a potential vaccine against the Nipah virus using its N-protein. Steps include sequence retrieval, physiochemical analysis, epitope prediction for B and T cells, and antigenicity assessment. Vaccine construction involves incorporating 50S ribosomal protein, linkers, and a 6x histidine tag, followed by antigenicity, allergenicity, and toxicity profiling. Structural predictions and docking with the ephrin B2 receptor are conducted for validation. The vaccine design aims to mimic natural immune responses efficiently and promises broad population coverage. Its computational design allows for rapid development and potential for clinical trials, marking a significant advancement in combating NiV infection [13].

2. Materials and methods

A computational approach was used to select the candidate protein, design the vaccine construct, in-silico validation, and immune simulations to design this computational vaccine construct. The flowchart of the steps is given below (Fig. 1).

2.1. Sequence retrieval

The genome sequence of the *Nipah* virus was retrieved from the National Center for Biotechnology Information (NCBI) under the accession number NC_002728.1 (https://www.ncbi.nlm.nih.gov) NCBI's Entrez Molecular Sequence Database System was used for search and retrieval procedures [14] The antigenic nucleoprotein was retrieved

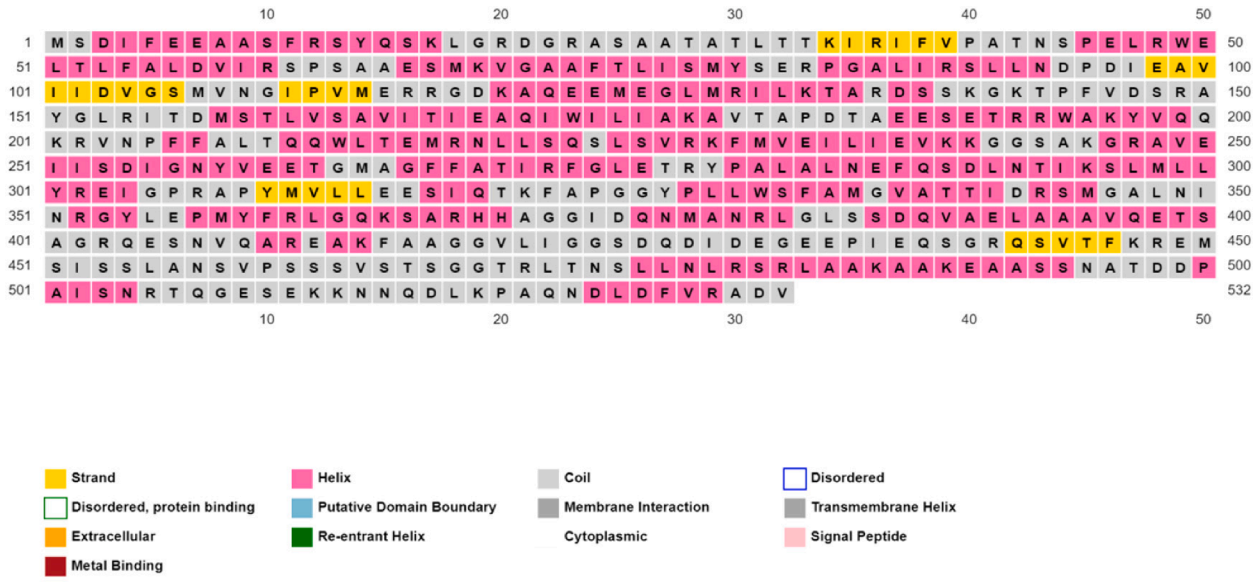


Fig. 2A. Secondary structure of the Nipah virus nucleoprotein, highlighting the coils, helices, and beta-strands.

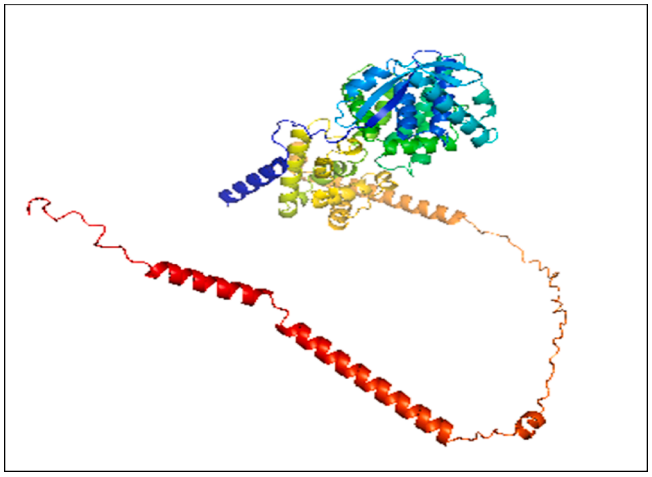


Fig. 2B. The secondary structure of nucleoprotein of Nipah virus.

under the accession number Q9IK92 from UniProtKB [15] ExPASy-Protparam (https://web.expasy.org/protparam) was utilized for the physiochemical analysis of the selected protein. The viral nucleoprotein was selected as the candidate protein because of its critical function in Nipah virus replication and its high antigenic properties. It generates strong B- and T-cell immunological responses while being largely conserved, providing broad protection. Furthermore, antigenicity, allergenicity, and toxicity studies showed its eligibility as a safe and efficacious vaccination candidate [16].

2.2. Structural analysis

2.2.1. Secondary structural analysis & physiochemical parameters

For the study of protein functions, the secondary structure prediction tool PSIPRED (https://bioinf.cs.ucl.ac.uk/psipred) was used.[17] Vaxijen 2.0 (https://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) and allerTOP v2.0 (https://www.ddg-pharmfac.net/AllerTOP) online server was utilized to determine the Antigenicity and Allergenicity. The candidate protein was finalized based on antigenicity, toxicity, allergenicity, and no resemblance to human-like proteins [18,19].

2.2.2. Tertiary structure prediction

The tertiary structure of the nucleoprotein was predicted using the online tool trRosetta (http://yanglab.nankai.edu.cn/trRosetta). This tool also relies on deep learning techniques with which it predicts the structures of proteins using sequence co-evolution complemented with structural templates. The model obtained here helps to explicate the three-dimensional arrangement of the nucleoprotein to allow for structural and functional studies [20].

2.3. B-cell epitopes prediction

The B-cell epitopes on the selected protein were predicted using IEDB Linear Epitope Prediction Tool v2.0 (https://tools.iedb.org/main/bcell), which uses crystal protein structure data to help predict antigenic region. This approach offers knowledge of the immunogenicity of the protein, which guides vaccine and therapeutic development [21].

Table 1
B-Cell epitopes sequences with their respective start and end positions, along with the length of each peptide.

Start	End	Peptide	Length
109	130	NGIPVMERRGDKAQEEMEGLMR	22
133	158	KTARDSSKGKTPFVDSRAYGLRITDM	26
398	474	ETSAGRQESNVQAREAKFAAGGVLIGGSDQDIDEGEEPIEQSGRQSVTFKREMISSLANSVPSSSVSTSGGTRLTN	77
487	528	AAKEAASSNATDDPAISNRTQGESEKKNNQDLKPAQNDLDFV	42

Table 2
MHC-I binding epitopes with their respective antigenicity scores, associated alleles, start and end positions, and IC50 values.

Peptide	Antigenicity Score	Allele	Start	End	IC50
KVGAAFTLI	0.8481	HLA-A*02:06	66	77	22.87
YPLLWSFAMG	0.9224	HLA-B*35:01	327	336	23.46
LLWSFAMGVA	0.6718	HLA-A*02:01	329	338	26.66
TLVSAVITI	0.5071	HLA-A*02:03	160	168	31.65
LNLRSRLAAK	1.8482	HLA-A*03:01	477	486	35.38
SAATATLTTK	0.9689	HLA-A*11:01	25	34	38.41
AVIIDVGSMV	0.5495	HLA-A*02:03	99	108	27.27
FAMGVATTI	0.7167	HLA-B*58:01	333	341	27.32

Table 3
MHC-II binding epitopes with their associated binding alleles, antigenicity scores, start and end positions, and IC50 values.

Peptide	Antigenic score	Allele	Start	End	IC50
RLTNSLLNL	0.4833	HLA-DPA1*03:01/ DPB1*04:02	469	483	22.4
FATIRFGLE	0.9265	HLA-DPA1*01:03/ DPB1*04:01	262	276	14.8
FAMGVATTI	0.7167	HLA-DRB1*09:01	330	344	8.4
LLNLRSRLA	1.3452	HLA-DRB1*01:01	437	451	3.4
MLLYREIGP	0.927	HLA-DRB1*15:01	295	309	36.9
LMLLYREIGP	1.1695	HLA-DRB1*11:01	296	306	24.6
LIEVKKGGS	1.1058	HLA-DRB1*11:01	232	246	24
VIIDVGSMG	0.8659	HLA-DRB1*13:02	96	110	17.7
ELTLFALDV	0.8396	HLA-DRB4*01:01	46	60	45.3
ASAATATLT	0.5526	HLA-DQA1*05:01/ DQB1*03:01	24	38	21.6
LLWSFAMGV	0.6308	HLA-DRB1*07:01	329	343	20.1

2.4. T-Cell epitopes prediction

2.4.1. MHC- binding prediction

For the MHC-I conserved epitope prediction, the IEDB MHC-1 (https://tools.iedb.org/main/tcell) binding prediction tool was used. For accurate prediction, the sequence was provided in FASTA format. All parameters were set as default. Alleles were selected at the length of 9, and the format of the output table was chosen as XHTML. This table was further used for prediction, and epitopes were screened based on antigenicity and IC50 values.[21]

2.4.2. MHC- binding prediction

For MHC-II epitopes prediction, IEDB MHC-II (https://tools.iedb.org/main/tcell) binding prediction tool was used. Submission of sequence was done in FASTA format, and all possible human loci were selected. All parameters are kept as default, and the output table was selected in XHTML format. The epitopes were screened based on antigenicity and IC50 values for further procedures.[21]

2.5. Profiling of selected epitopes

Antigenicity testing of selected B-cell and T-cell epitopes was performed using the Vaxijen 2.0 online server (https://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html), which utilizes physiochemical properties of proteins. The input sequence was provided in FASTA format, with all parameters kept at their default settings. For allergenicity analysis, the AllerTOP online server (https://www.ddg-pharmfac.net/AllerTOP) was used, which also predicts the most likely route of exposure.[22,23]

2.6. Population coverage analysis

IEDB's Population Coverage Analysis Tool (https://tools.iedb.org/population) was used for the calculation of population coverage of selected epitopes. This analysis is important to find out how many populations can be covered by the vaccine construct. Graph format was used to save the result.[24]

2.7. Vaccine construct and sequence Assembly

The construction of a multiepitope vaccine was followed by the selection of all the potential epitopes. As an adjuvant to bind on the N terminal of the vaccine, 50S ribosomal protein L7/L12 with the UniProt ID P9WHE3 was used. Three types of linkers, EAAAK, GPGPG, and AAY, were used for the joining of epitopes. At the C terminal of the vaccine, 6x His tags were added for binding and to carry out expression. The vaccine was constructed manually by following the arrangement, at the start, the adjuvant sequence was placed, then EAAAK, AAY, and GPGPG linkers with B-Cell epitopes, and then MHC- and MHC- were arranged.[25,26]

2.8. Antigenicity, Allergenicity, and toxicity profiling

VaxiJen 2.0 (https://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) online server was used to analyze the antigenicity of vaccine construct. The default threshold value of 4.0 was used for antigenicity prediction. AllerTOP (https://www.ddg-pharmfac.net/AllerTOP) tool was used to calculate the allergenicity. The toxicity prediction of the vaccine construct was done using the Toxinpred toxicity prediction tool. The vaccine construct should be non-toxic.[22,23]

2.9. Solubility and physiochemical properties analysis

For the analysis of solubility, the SoluProt tool (https://loschmidt.chemi.muni.cz/soluprot) was used. In the construction of a vaccine, solubility is an important factor. Furthermore, the physiochemical properties of the vaccine construct were analyzed using ExPASY Protparam (https://web.expasy.org/protparam).[27,16].

2.10. Secondary and tertiary structure prediction

For the secondary structure prediction of the multiepitope vaccine, two tools were used. These tools were PsiPred (https://bioinf.cs.ucl.ac.uk/psipred) and RaptorX (https://raptorx.uchicago.edu). The PsiPred tool requires the submission of protein sequences, and the main coils, helix, and plates were determined by this tool. RaptorX is a tool used to predict the structural elements as well as the ratios of amino acids that play a role in the disordered conformational randomness of the secondary structure of the protein. For the prediction of the tertiary structure of the vaccine construct, 3D PRO by Scratch Protein Editor (https://scratch.proteomics.ics.uci.edu) was used. The best-predicted model was selected based on the C-score.[17,28]

2.11. Tertiary structure refinement

To verify the tertiary structure of the vaccine construct, the

Table 4
Assembly of the vaccine construct, incorporating B-cell and T-cell epitopes, adjuvant, linkers, and a 6X histidine tag for enhanced immunogenicity.

VACCINE CONSTRUCTION	
Adjuvant	50 s ribosomal protein L7/ L12 *Mycobacterium tuberculosis*
Linker	**EAAAK**
	NGIPVMERRGDKAQEEMEGLMR
	KTARDSSKGKTPFVDSRAYGLRITDM
B-Cell Epitopes	IQTKFA
	ETSAGRQESNVQAREAKFAAGGVLIGGSDQDIDEGEEPIEQSGRQSVTFKREMSISSLANSVPSSSVSTSGGTRLTN
	AAKEAASSNATDDPAISNRTQGESEKKNNQDLKPAQNDLDFV
Linker	**CPGPG**
	KVGAAFTLI
	YPLLWSFAMG
	LLWSFAMGVA
	TLVSAVITI
MHC-1 Epitopes	LNLRSRLAAK
	SAATATLTTK
	AVIIDVGSMV
	FAMGVATTI
Linker	**AAY**
	RLTNSLLNL
	FATIRFGLE
	FAMGVATTI
	LLNLRSRLA
MHC- Epitopes	MLLYREIGP
	LMLLYREIGP
	LIEVKKGGS
	VIIDVGSMG
	ELTLFALDV
	ASAATATLT
	LLWSFAMGV
6X Histidine tag	HHHHHH

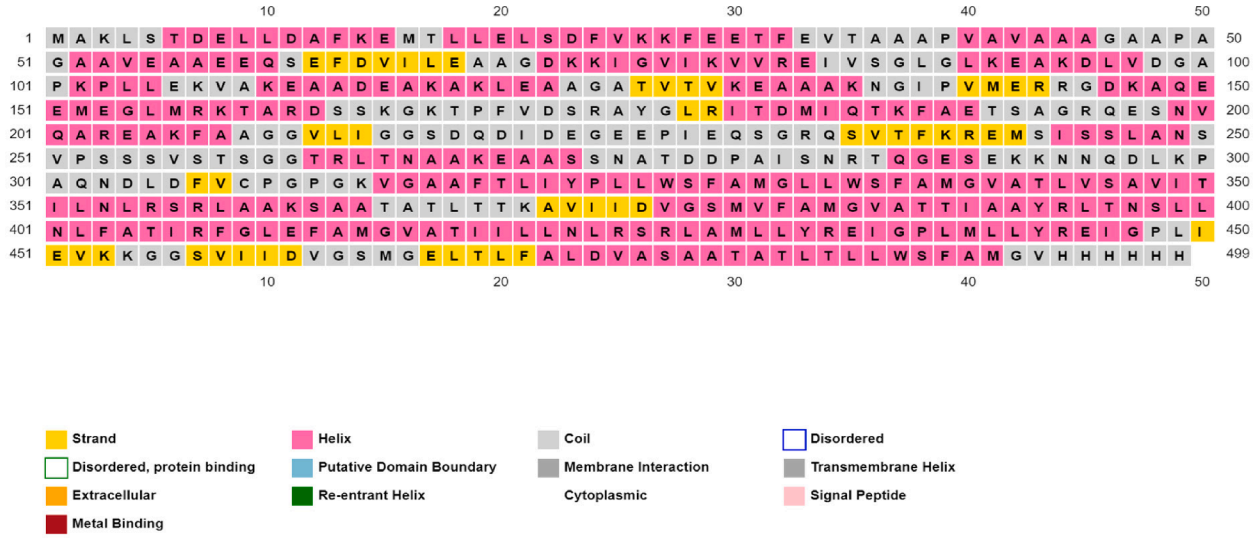


Fig. 3. Primary structure of vaccine construct.

GalaxyRefine online web server (https://galaxy.seoklab.org/cgi-bin/submit.cgi?type=REFINE) was used. The GalaxyRefine server was employed to improve the accuracy of the predicted protein tertiary structure generated by 3Dpro.[29]

2.12. Model stability analysis and validation

The stability and validation of the vaccine are important steps to check the authenticity of the construct. The sequence of the vaccine construct was validated through a Ramachandran plot using RAMPAGE (https://github.com/kepbod/rampage_alu), which assesses the quality of the predicted protein based on its stereo-chemical properties. Ramachandran plot was further validated by PROCHECK (https://saves.mbi.ucla.edu).[30]

2.13. Vaccine solubility analysis

The solubility of the designed vaccine construct was predicted using Protein-Sol (https://protein-sol.manchester.ac.uk), an online tool that evaluates protein solubility based on amino acid composition and physicochemical properties. The FASTA sequence of the vaccine candidate was uploaded, and the tool provided a solubility score compared to the solubility of the E. coli proteome. A solubility score above 0.45 indicated good solubility, suggesting the vaccine's potential for efficient expression and purification in recombinant systems.[31]

2.14. Docking analysis

The ClusPro 2.0 server (https://cluspro.bu.edu/login.php) was

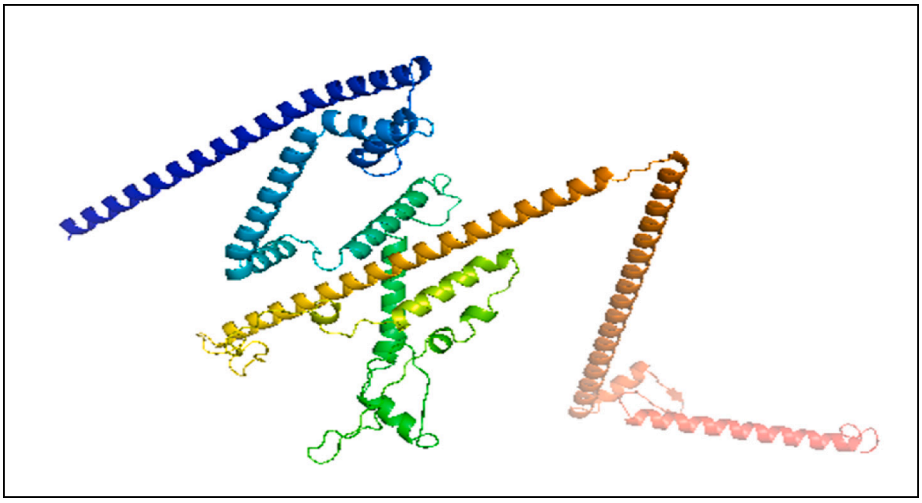


Fig. 4. Tertiary structure of vaccine construct.

utilized for the docking analysis. For protein–protein docking, two PDB files were provided as input, whereas for protein-peptide docking, a single PDB file and a peptide sequence were used. The server generated ten models representing potential interaction complexes. Docking was performed between the human receptor Ephrin B2, obtained from the Protein Data Bank (PDB), and the vaccine construct, with the resulting models providing insights into the interaction dynamics and binding conformations.[32]

2.15. MD simulations

The protein structural flexibility and stability were analyzed using Molecular dynamics (MD) simulations done through the IMODs server (https://imods.iqf.csic.es). IMODs use NMA to model protein motions and to estimate the conformational variations likely to be induced by fluctuations in the protein environment, thus providing the design scientist with functional clues into possible changes in the protein function.[33]

2.16. Codon optimization of vaccine construct and expression analysis

The Backtranseq program EMBOSS (https://www.ebi.ac.uk/jdispatcher/seqstats/emboss_pepstats) was used for protein-to-nucleotide translation of vaccine construct. After nucleotide translation, the codon optimization process is carried out using the Java Codon Adaption Tool (JCAT) (https://www.prodoric.de/JCat). The tool provides GC content and Codon Adaption Index (CAI) as an output to estimate the vaccine expression. The vector p2III A-B was used for the expression because it is considered ideal for experimental purposes. We used Snapgene to insert restriction enzyme sites (*BamHI and HindIII*) and amplified by performing *in silico* PCR. Then, the amplified construct was further analyzed for immune simulations.[34,35]

2.17. Immune simulations

For immune simulations, the online server C-ImmSim (https://kraken.iac.rm.cnr.it/C-IMMSIM/index.php) was employed to assess the response triggered by the vaccine construct. This server utilized a position-specific scoring matrix (PSSM) to determine the immune epitopes and immune system interactions. All the parameters were set as default, and the results were obtained in the form of graphs.[36]

3. Results

3.1. Sequence and structural analysis

Characterization of the physiochemical properties of the retrieved Nucleoprotein of Nipah virus was done using ExPASY-Protparam tools. It can be inferred that the analyzed protein is unstable, as indicated by an II of 52.33, an indication that the protein may not be stable under physiological conditions as suggested by any value >40. The aliphatic index was calculated to be much average as 86.28, which represents the relative volume occupied by aliphatic side chains, so the protein appears to be thermostable in the medium range or with a higher aliphatic index protein found to be more thermostable in various temperatures. Furthermore, a GRAVY score of 0.236 is the most significant significance, which represents the overall hydrophobic or hydrophilic nature of the protein, which is rather slightly hydrophobic. GRAVY predictions can be used to estimate the hydrophobicity of a protein with values between 0 and 1 regarded as having balanced hydrophobicity synonymous with membrane interactions usually found with viral proteins.

3.2. Structural analysis

The secondary structure of the nucleoprotein defined in the context of the A-DNA, shown in Fig. 2A., offers directions into the localized folding features, including alpha-helix and beta-sheeting. The antigenicity and allergenicity analyses are useful in determining how the protein will trigger immune reactions or induce allergies. Fig. 2B. demonstrates the predicted tertiary structure, which is suggestive of the overall conformation that the protein would have and is vital for determining the functional and structural characteristics of the macromolecule.

3.3. Prediction of B-cell epitopes

IEDB Linear Epitope Prediction tool v2.0 was used for B-Cell Epitope prediction. The threshold for antigenic determinations of the protein was 1.05. All the values higher than 1.0 were selected as potential antigenic determinants. Five epitopes were found to be capable of expressing the B-cell response. Predicted B-cell epitopes are given below in Table 1.

3.4. Prediction of T-cell epitopes

3.4.1. MHC-I binding profile prediction

Based on interaction with MHC-I, a Total of 399 epitopes were

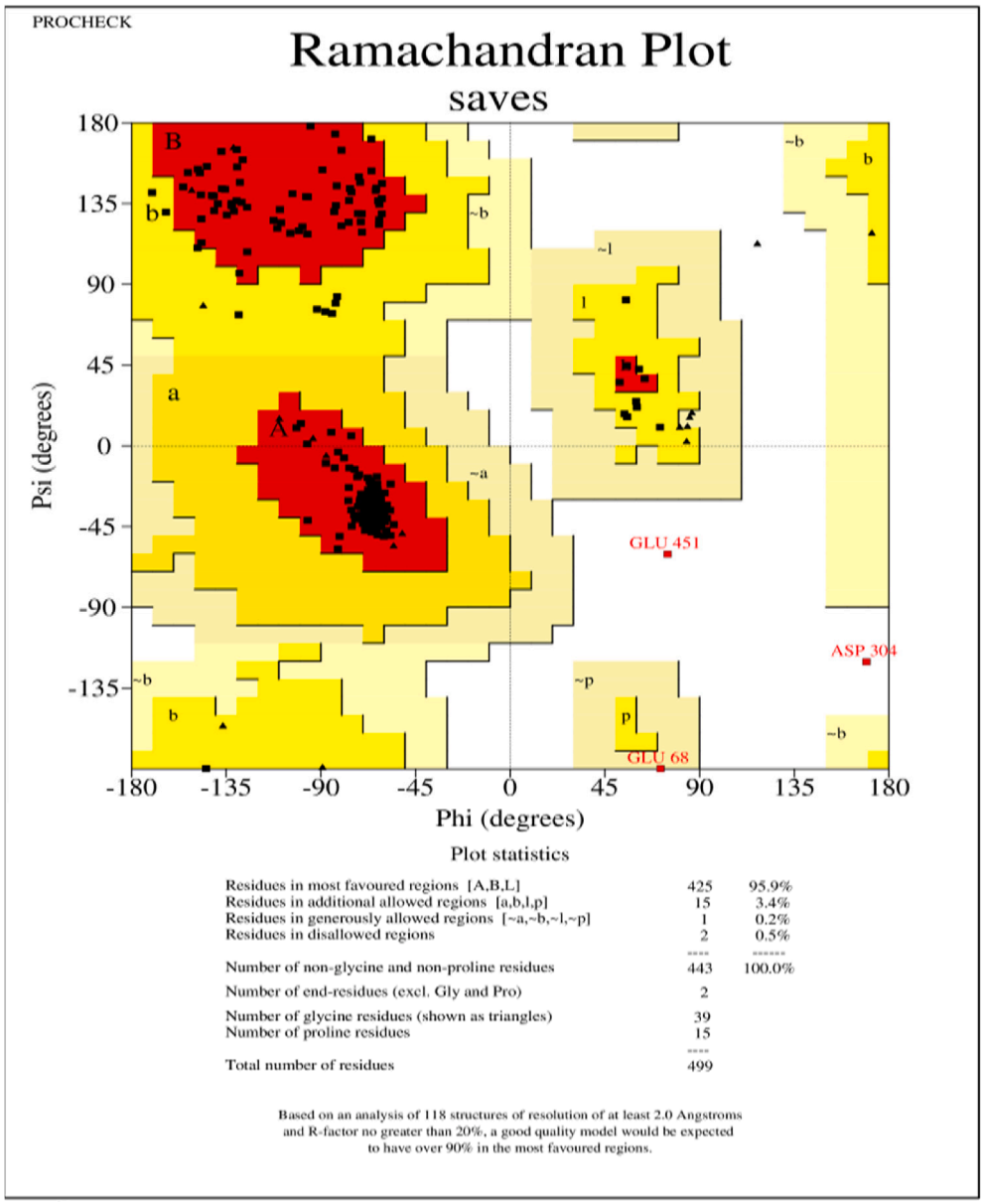


Fig. 5. Ramachandran plot of vaccine construct.

selected. Then, the 39 epitopes were chosen from the original 399 epitopes based on their highest level of interaction. The 39 epitopes were further filtered based on various properties like antigenicity, toxicity, IC50 value, and allergenicity. The higher binding affinity of epitopes with alleles depends on the lower value of IC50. The toxic and allergic epitopes were excluded. The remaining eight epitopes were chosen for further analysis. The epitope LNLRSRLAAK showed a higher antigenic score. The epitopes, along with their alleles, antigenic score, and IC50 values, are presented in Table 2.

3.4.2. *MHC-II binding profile prediction*

A total of 889 epitopes having IC50 values less than 180 were predicted, and they had strong interactions with MHC-II alleles. Only 40 epitopes were selected from all these epitopes. Based on antigenic nature, allele binding, and surface accessibility, the epitopes were further screened out to construct a potential vaccine and to increase the efficacy of the construct. Toxic and allergic epitopes were removed during screening, and around 11 potential epitopes were selected based on antigenic score and non-toxic nature. One epitope, LLNLRSRLA was found to contain the higher antigenic value that is 1.3452. After screening, these epitopes were combined to construct a vaccine. All epitopes with their respective alleles are shown in Table 3.

3.5. *Feature profiling of selected epitopes*

The epitopes were analyzed and selected based on higher antigenicity. Prediction of antigenicity was done using VaxiJen 2.0. The selected epitopes were further analyzed by ToxinPred and AllerTOP v2.0 for toxicity and allergenicity prediction. The epitopes having non-toxic and non-allergic characteristics were selected, and epitopes that showed toxic and allergic characteristics were excluded from the selection.

3.6. *Population coverage analysis*

The next step involved assessing population coverage to determine the interactions of epitopes and their global coverage using the IEDB tool, focusing separately on their interactions with MHC-I and MHC-II

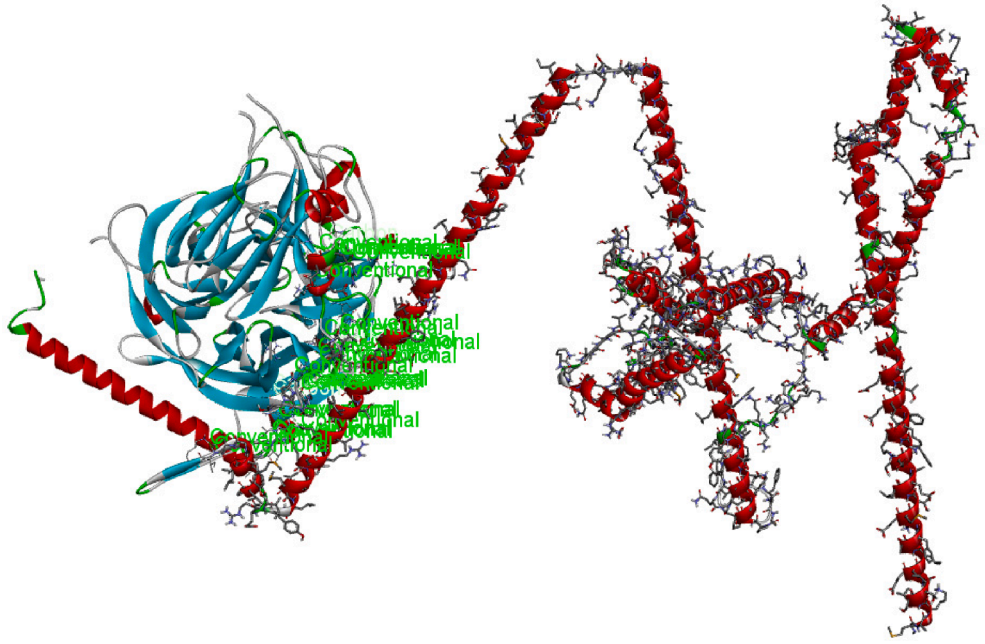


Fig. 6. Docking complex of aphrin B2 receptor and vaccine construct.

alleles. The global coverage of HLA allele distribution could affect the vaccine efficacy. The maximum value of population coverage of MHC-I all over the world was observed to be 69.8 %, and in the case of MHC-II, population coverage values was 60.98 %.

3.7. Vaccine construction and sequence Assembly

For the construction of a multiepitope vaccine, 5B-Cell epitopes, 8 MHC-I, and 11 MHC-II epitopes were used. At the N terminus of the sequence, an adjuvant, which is 50S ribosomal protein L7/L12 with the UniProt ID: P9WHE3, was used. This adjuvant was attached to the first B-Cell epitope through the linker EAAAK. For connecting the B-Cell epitopes to the MHC-I linker, CPGPG was used, and linker AAY was used to link the MHC-I epitopes to the MHC-II epitopes. A 6xHis tag was used at the C-terminus of the peptide sequence. The designed vaccine construct, along with epitopes and linkers, is given below in Table 4.

3.8. Antigenicity and allergenicity assessment of vaccine construct

The antigenicity of the vaccine construct was determined using the VaxiJen 2.0 server, and the antigenicity of the construct with adjuvant was found to be 0.6647. Hence, this protein was antigenic. Antigenicity assessment was also done by excluding the adjuvant part, and it was found to be 0.7624. So, it is concluded that the sequence is antigenic whether it is attached to the adjuvant or not. To analyze the allergenicity, AllerTOP was used, and the sequence was found to be non-allergen with or without adjuvant. The sequence was also non-toxic, and it was analyzed using ToxiPred.

3.9. Analysis of solubility and physiochemical properties

Protein-sol was used to determine the vaccine construct's solubility, and it was found to be 0.575 which indicates it is highly soluble, and the molecular weight was determined by ExPaSy-Protpram. The weight was found to be 52823.69 Da. The Isoelectric Point Value (PI) was found to be 5.54. The instability index (II) was computed as 31.70, which indicates it is a stable protein. The calculated half-life of the vaccine construct was found to be >30 h (mammalian reticulocytes, in vitro), >20 h (yeast, in vivo), and >10 h (Escherichia coli, in vivo). The aliphatic index confirms its thermostability, and it was found to be 95.93. The overall Average Hydropathicity was determined to be 0.088. This positive value shows the non-hydrophilic nature of the protein.

3.10. Secondary and tertiary structure prediction

The predicted secondary structure of the vaccine construct is shown in Fig. 3, which indicates the presence of coils, helices, and beta-strands. The tertiary structure of the vaccine construct is shown below in Fig. 4, which was further utilized for the rest of the analysis.

3.11. Tertiary structure refinement and validation

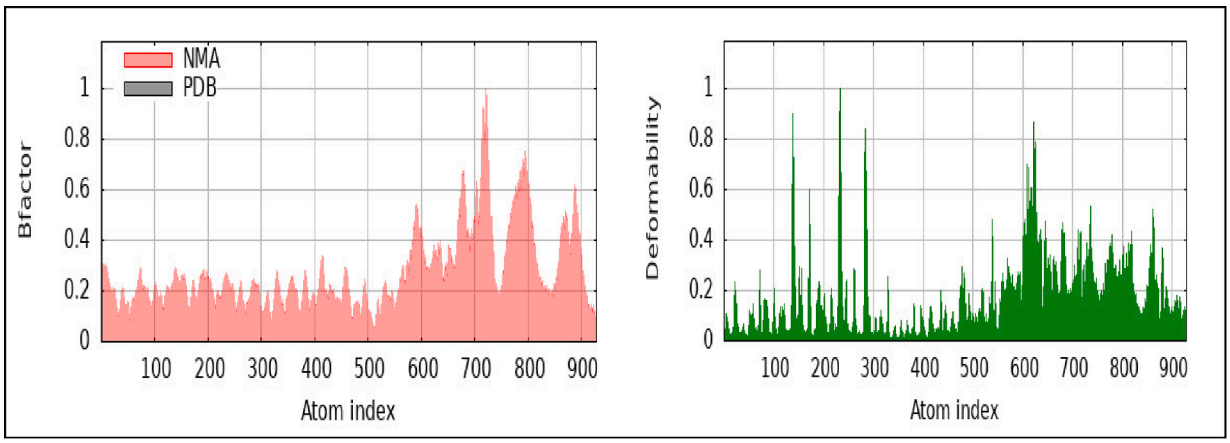
The GalaxyRefine was used for the refinement of the predicted model. It provides five models after refinement, and model one was selected based on the following parameters: GDTHA (0.8913), RMSD (0.555), and MolProbity (1.420). The clash score was found to be 7.6. Poor rotamers score was found to be 0.3. The predicted Ramachandran score was 98 %. The model was analyzed for further investigations. As per the results of the Ramachandran Plot, there were 95.9 % residues in the favored region, 3.4 % in the allowed region, and 0.5 % in the disallowed region. Fig. 5 represents the constructed Ramachandran Plot.

3.12. Docking analysis

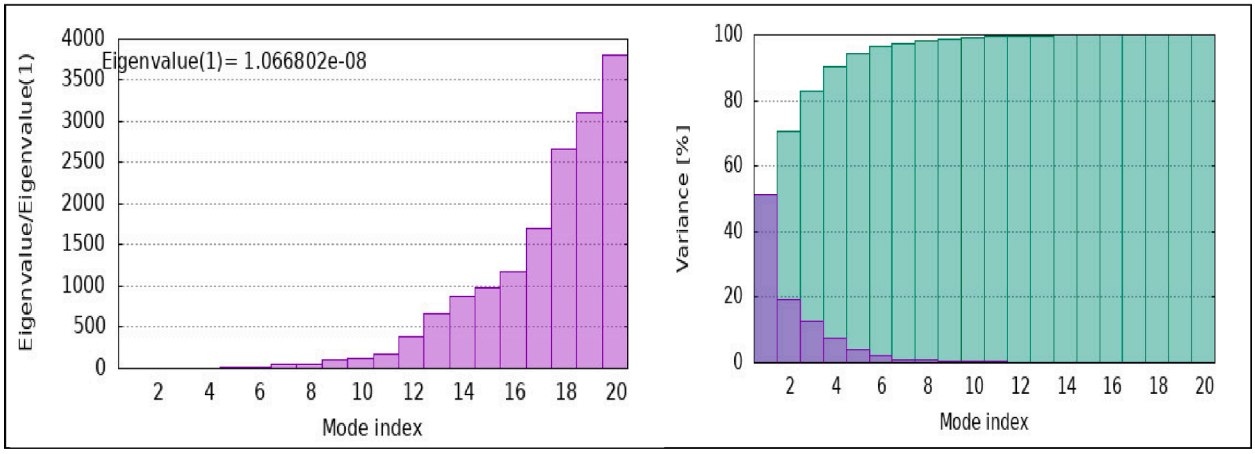
The interaction between the refined structure of the vaccine and the receptor Aphrin B2 was analyzed by performing the docking analysis using the online software ClusPro 2.0. It gives 10 models as a result to analyze. Model 1 was considered to be the best dock model after analyzing all models. The docking complex is shown in Fig. 6.

3.13. MD simulations

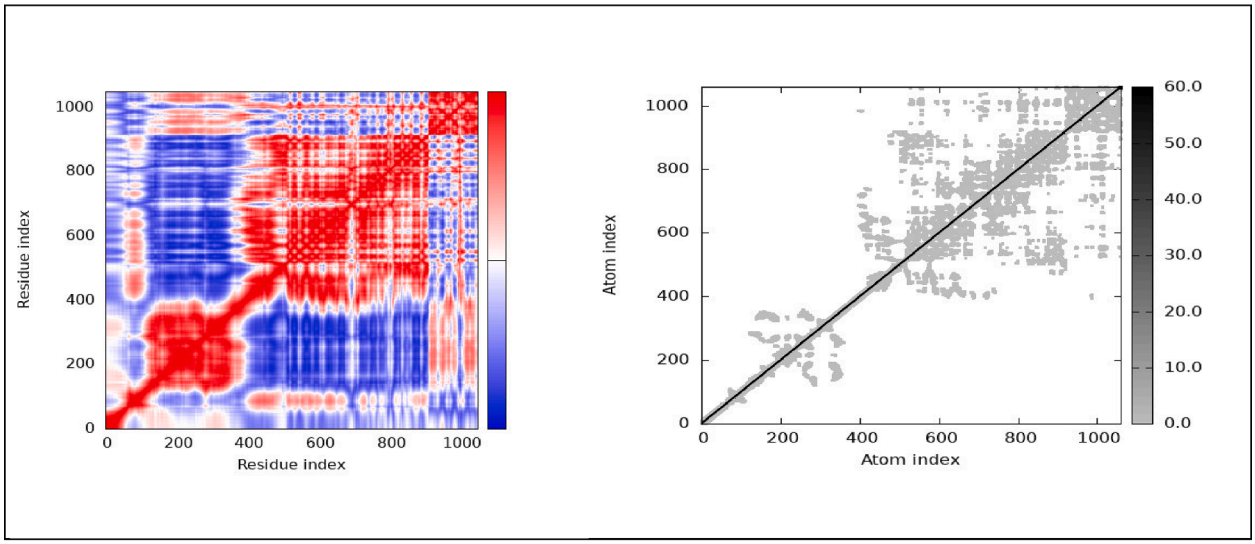
For better interpretations of docking results, the iModS online tool was used to perform molecular dynamic simulations. The docked model is processed to perform energy minimization. The deformability of the docked complex is presented in Fig. 7(A). Fig. 7(B) shows the B-factor of the molecule and nucleoprotein docked complex. The eigenvalue of the docking complex is shown in Fig. 7(C). Variance is shown in Fig. 7(D), where individual variances are shown in purple color, and collective variances are shown in blue color. Fig. 7(E) shows the co-variance map



(A) (B)



(C) (D)



(E) (F)

Fig. 7. MD Simulation of Docked complex of aphrin B2 receptor and vaccine construct. (A) B-Factor, (B) Deformability, B-factor, (C) Eigenvalues, (D) Variance, (E) Covariance map, (E) Elastic network

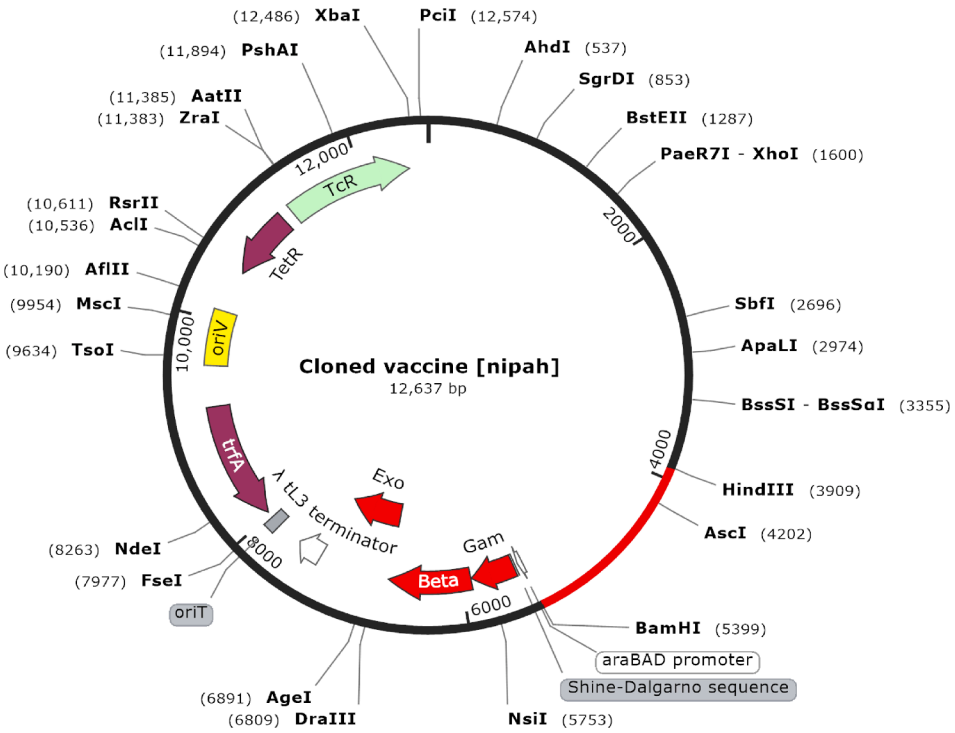


Fig. 8. Recombinant plasmid obtained after cloning of peptide vector.

where the red color shows the correlated area and the blue color shows the anti-correlated area. The elastic network is presented in Fig. 7(F), in which the grey color indicates the stiffer region.

3.14. Codon optimization and expression analysis of vaccine construct

The optimization of Codon through Java Codon Adaption (JCat) found the codon adaption index (CAI) of 0.8 and GC content of 30–70 % was considered optimum. The results indicate the stable expression of *E-Coli*. After codon optimization, the next step was expression analysis, which was performed in vector P221 LIII A-B. The next step was amplification, and it was done by *in-silico* PCR (Polymerase Chain Reaction). In the vector, the restriction sites were added using SnapGene. The old restriction sites were replaced by *Hind*III and *B*amHI. Forward and reverse primers were used, and the fragment of the construct was added to the vector. The resulting engineered fragment was the vaccine construct and was highlighted by red color. The vector is shown in black color. Fig. 8 represents the cloned vaccine construct.

3.15. Immune simulation

The increased level of IgM was used to mark the essential response. The B-cell population was used to mark the auxiliary and tertiary responses. When the antigen's amount decreases, the IgM, IgG_1 + IgG_2, and IgG + IgM amounts increase, and on subsequent exposure to antigen, memory cells are also produced. An increase in the amount of Helper (T_H) and Cytotoxic (T_C) cells was also observed. For the long-term effect of the vaccine, memory cell production was also observed as a good thing.

In Fig. 9, panel (A) illustrates the production of immunoglobulins (Ig), represented by black lines, alongside the different classes of immune cells, which are depicted by colored lines. Panel (B) highlights changes in the B-cell population, including the production of memory cells. Panel (C) shows the generation of cytotoxic T cells, as well as other immune cells, with anergic cells, which lack antigen presence, indicated separately. Lastly, panel (D) focuses on the production of helper T cells, playing a crucial role in orchestrating immune responses.

4. Discussion

Nipah virus (NiV disease) has no cure even though it is relatively severe, and there are no FDA-approved drugs for use by humans. Some anticancer agents have displayed this activity with great in vitro efficacy but proved to be quite worthless in test animals.[37,38] Scientists are currently working on the production of NiV vaccine and therapy because of the escalating virulence of the virus and the absence of adequate remedy. There is, therefore, the need to come up with new methods of preventing and controlling future outbreaks of this deadly virus. In the past few decades, the process of getting vaccines for such diseases has involved one of the most promising and potentially efficacious vaccines called mRNA vaccines.[39–41]

Computational vaccinology is quite helpful at present time in the context of mapping and selecting epitopes and antigens and in designing immunogens. For a long time, two- and three-dimensional B-cell epitopes were the prime targets of vaccine development.[42,43] However, in recent immunology, the T-cell epitopes have emerged as significant, especially those concerning MHC and HLA. T-cell epitopes are vital to identify because they enhance immunogenicity and are especially essential when the body is not producing viable antibodies. These epitopes are important to increase the efficiency, protection, and antigens description of vaccines.[44,45] It is for this reason that the future assessment of vaccines against viruses such as NiV must start considering the T-cell epitopes in addition to B-cell epitopes and the cross-conservation between the pathogen's epitopes and the human genome that has recently emerged as a critical fact in future vaccine designs.[46–49]

The development of a multi-epitope-based vaccine against NiV is seen as a potential approach to combat this high pathogenicity virus, owing to the absence of potential treatments. The ability to utilize immunoinformatics for creating vaccines allows for the determination of the important B-cell substrates as well as T-cell receptors from the NiV nucleoprotein, which also has B-epitope properties for stimulating immune reactions.[50,51] The use of computational approaches allows better identification of the most immunogenic epitopes with the subsequent construction of a vaccine provoking the greatest response without significant variation between populations. The present investigation thus

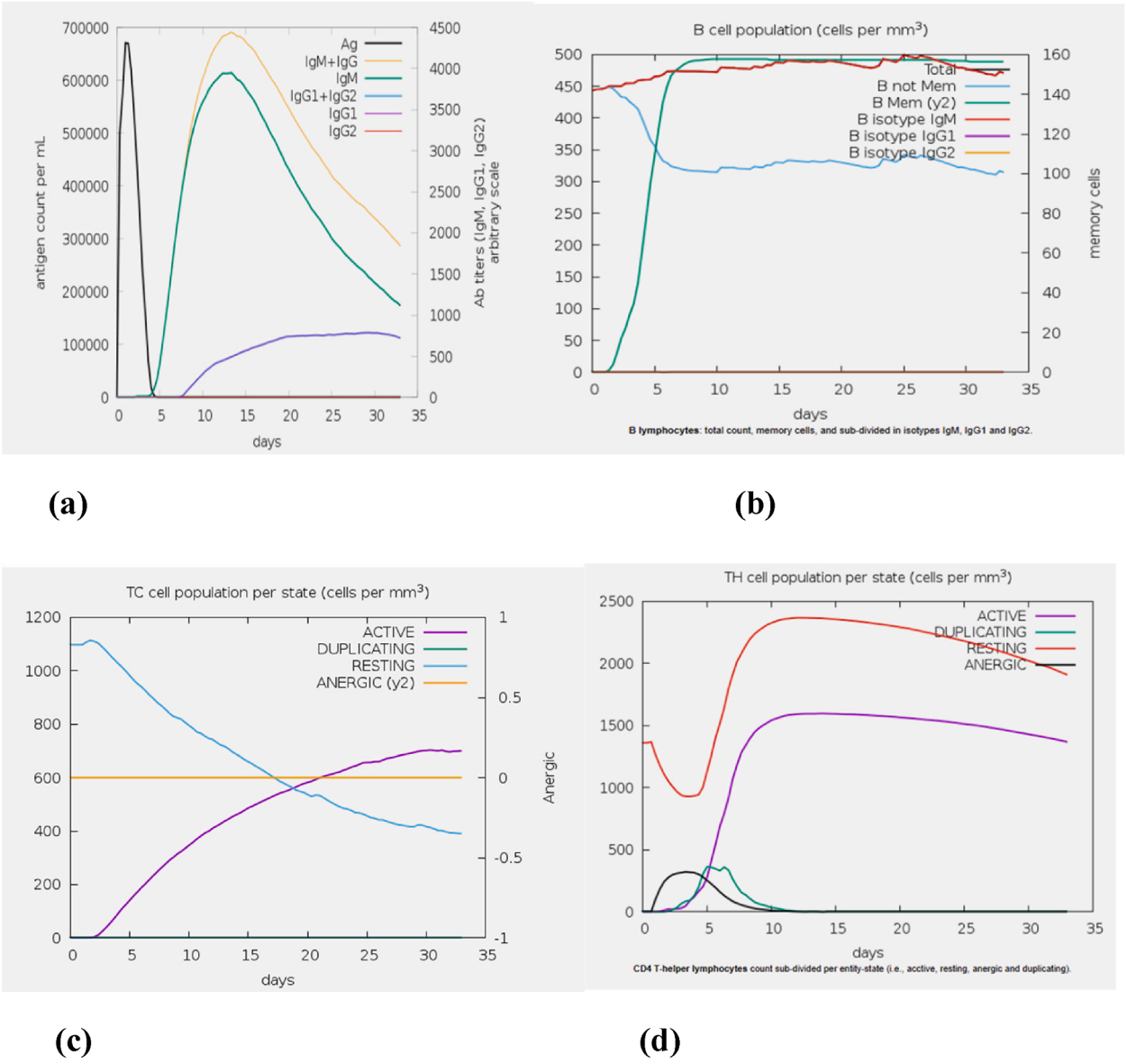


Fig. 9. Immune simulation profile of vaccine construct. **(A)** Immunoglobin production shown by black lines; classes of immune cells are shown by colored lines. **(B)** Changes in B-Cell population and production of memory cells. **(C)** Showing the production of Cytotoxic T cells and other cells represented anergic cells on which antigen are not present. **(D)** Helper T cells production.

showed that the MEV approach can generate high antigenicity, non-allergenicity, and non-toxicity, which are important prerequisites for a safe and effective vaccine.[52] The ability of the designed vaccine to have a high binding affinity for the human ephrin B2 receptor, a key host-cell attachment factor for NiV, also indicates that the vaccine can evoke protective immunity.[53–55]

Furthermore, the results of an immune simulation show that the vaccine produces robust humoral and cellular immunity characterized by high titers of IgG and IgM antibodies and enhanced activity of memory B-cells. Secondly, population coverage analysis reveals that the vaccine proposed here may afford a global population coverage of 88.3 %. Thus its immunization potential is quite high. The long-standing stability of the vaccine, the high efficiency of the binding, the quality of immune reactions, and the induction of long-term immunity put this candidate into the group of vaccines that require further experimental testing and clinical trials. More advanced research should, therefore involve further exploration into in-vitro and in-vivo models to ascertain the protective characteristics of the vaccine.[56,57]

Therefore, the NIV vaccine developed using immunoinformatics from eight multi-epitopes serves as a potential preventive measure to fight the global health issue caused by this virus. The approach provides a novel and cost-effective method of generating vaccines for newly identified infectious diseases, which enhances disease surveillance and prevention.[58,59] Further investigations of the immunological functionality and antigenic relationship of T-cell epitopes to pathogen resilience will remain essential in the progress of immunogenic treatments for NiV and analogous viruses.[60,61]

5. Conclusion

Therefore, the design of an epitope-based vaccine against the Nipah virus by immunoinformatics tool is a suitable strategy to prevent this highly pathogenic virus. Thus, the computational design of the vaccine, which targets selected epitopes from the NiV nucleoprotein, manifested high antigenicity, lack of allergenic properties, and high affinity to the human ephrin B2 receptor, underlines possible protective immunogenicity of the proposed approach. In addition, results of the simulation of immune status show that the effectiveness is received as a result of

inducing significant activation of both humoral and cellular immunity. The protection of a broad population basal and satisfactory immunogenicity suggest that this vaccine approval may be developed clinically to meet the increasing threat of NiV. Nonetheless, more comprehensive experimental testing must be conducted to prove the effectiveness of the procedure as well as to confirm its practical usability.

CRediT authorship contribution statement

Muhammad Aqib Shabbir: Writing – review & editing, Supervision, Methodology, Conceptualization. **Ammara Amin:** Writing – review & editing, Writing – original draft, Investigation. **Ammarah Hasnain:** Writing – review & editing, Resources, Data curation. **Ayesha Shakeel:** Writing – review & editing. **Ambreen Gull:** Visualization, Software.

Ethics approval

No animal/humans has been used in this study, therefore, the ethics approval is not required.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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