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Original article

An integrated multi-pronged reverse vaccinology and biophysical approaches for identification of potential vaccine candidates against Nipah virus

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A B S T R A C T

Nipah virus, a paramyxovirus linked to Hendra virus that first appeared in Malaysia and is the etiological agent of viral lethal encephalitis, has emerged as a strong threat to the health community in recent decades. Viral infections are seriously affecting global health. Since there are now no efficient therapeutic options, it will take considerable effort to develop appropriate therapeutic management for the Nipah virus. The main purpose of this study was to design a messenger RNA-based multi-epitope vaccine construct against Nipah virus. This purpose was achieved through multiple immunogenic epitopes prediction using Nipah virus antigenic protein using the immune epitope database and analysis resource (IEDB) followed by the vaccine construction and processing. As in multi-epitopes vaccine construction we selected immunogenic potential fragments of viral proteins, therefore in host immune stimulation we observed proper immune responses toward a multi-epitopes vaccine. In this study, the Nipah virus V protein was used to identify immunodominant epitopes utilizing several reverse vaccinology, immunoinformatics and biophysical methods. The potential antigenic predicted epitopes were further analyzed for immunoinformatics analysis and only selected probable antigenic and non-toxic epitopes were used in designing a multi-epitope mRNA based in silico vaccine against the target pathogen. In vaccine designing a total number of 03B cell epitopes, 09 Cytotoxic T lymphocytes (CTLs) and 01 Helper T lymphocytes (HTL) were prioritized as a good vaccine candidate. In the vaccine construction phase, the selected epitopes were linked together using EAAAK, GPGPG, KK, and AAY linkers, and B-defensin (adjuvant), and MITD sequences were also added to the vaccine construct to increase the potency. After vaccine construction, the physiochemical properties of the vaccine construct were evaluated which predicted that the vaccine construct comprises 320 amino acids with 34.29 kDa (kDa) molecular weight. The instability index was 36.55 proving its stability with the aliphatic index of 82.88. Furthermore, 9.0 theoretical pI and −0.317, GRAVY (Grand Average of Hydropathy) values were predicted in physicochemical properties analysis. A solubility check was applied against the vaccine construct depicting that the vaccine construct is soluble with its calculated value of 0.6. Additionally, after prediction the 3D structure was modeled and refined for docking analysis, the refined 3D structure of the vaccine candidate was further checked for binding affinity with immune cell receptors through docking analysis, in the docking analysis we observed that the vaccine construct has a good binding affinity with immune cells receptor and can induce a proper immune response in host cells. As we predicted effective binding of the designed vaccine construct, hence it can further facilitate the development of vaccine formulation against the Nipah virus. Additionally, molecular dynamic simulation was done using the AMBER v20 package for analysis of the dynamic behaviour of the docked complexes and we observed proper binding stability of the vaccine with target receptor. In C-immune simulation, different humoral and cellular antibody titer was observed in response to the vaccine. Overall using bioinformatics, immunoinformatics, and biophysical approaches we observed that this mRNA base epitopes vaccine construct could facilitate the proof of concept for the formation of the experimental base vaccine against the Nipah virus, as the in silico predictions indicated that the vaccine is highly promising in terms of developing protective immunity. However experimental validation is required to disclose the real immune-protective efficacy of the vaccine.

1. Introduction

Nipah virus is a unique paramyxovirus related to the Hendra virus which emerged in Malaysia in 1998, the virus was considered for an outbreak of encephalitis in humans and high mortality and morbidity rate, it was also considered an etiological agent for causing respiratory infection and encephalitis in pigs but the very low mortality rate was reported (Gazal et al., 2022). The outbreak subsequently spread to different countries especially in Singapore in the south region due to the infected movement there. The virus also caused systematic infection in humans, pigs, and other mammals (Chua, 2003). The Nipah virus infection in Malaysia frequently spreading to Singapore was considered the main global health problem. In Malaysia, the virus caused tremendous human suffering among human beings, especially among those people who are involved in pig forming, as a result, an excessive amount of loss occurs in the local swine industry (Looi and Chua, 2007).

The Nipah viral disease is mainly characterized by fever with encephalitis with constitutional symptoms while sometimes characterized

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by upper respiratory illness as well. Nipah virus (NiV) has enveloped with nucleocapsid protein and its genome consists of single-stranded negative-sense RNA which is approximately 18.2 kb, overall the genome encodes different six types of proteins including "nucleocapsid (N), Phosphoprotein (P), matrix protein (M), fusion protein (F), glycoprotein (G), and large protein or RNA polymerase (L)" (Eaton et al., 2006).

The Nipah Virus reservoirs include both wild and domestic animals dogs, goats fishes, and chickens, while in wildlife animals rodents, birds, and bats are included, moreover that fruit bats are also considered a reservoir of the Nipah Virus (Bruno et al., 2022). The clinical representation of the virus includes fever, encephalitis in severe conduction, and pulmonary disorder. With the outbreak of the Nipah Virus, the situation can become more worsen and create problems for global health, hence essential efforts are required to tackle the problem and save costs and lives (Alam, 2022). One of the strategies for overcoming viral infections in the body is to create proper immunity (both humoral and cellular immunity) inside the host against the target virus. Through producing immunity, the infection and its related pathogenesis can be countered and can keep the person healthy.

To create immunity against target viruses different types of vaccines could play a vital role, and due to the cost-effective, time-saving, and production of specific types of immunity, reverse vaccinology plays an important role in making an effective vaccine against several viral and bacterial species (Rappuoli, 2001).

Vaccination process has conclusively aided in the improvement of public health around the globe, vaccination has saved millions of lives, with lower public health care expenses and improve quality of life, and vaccination strategy is currently being used for several types of infection diseases (Jahangirian et al., 2021; ud-din et al., 2022).

Reverse vaccinology (RV) is a new paradigm in vaccine development against different pathogens in which pathogens do not need to be cultivated (Albaqami et al., 2023; Kelly and Rappuoli, 2005). In the RV approach bioinformatics and immunoinformatics screening are involved, in the immunoinformatics approach screening of different antigenic proteins could be selected and utilized for multi-epitopes vaccine construct (Gul et al., 2022; Sanami et al., 2023). The in silico base multi-epitope vaccine pipeline include different biophysical approaches like molecular docking and molecular dynamic simulation approaches in which the binding affinity and stability of the designed vaccine with immune cell receptors (Ismail et al., 2022; ul Qamar et al., 2020).

Immunoinformatics or computational immunology is mainly integrate the computational power with the huge amount of proteomics and genomics data of the pathogens collected from pathogens in order to understand their immuno efficacy and that information is further is utilized for vaccine development (Althurwi et al., 2022; Rawat et al., 2023).

Protection from infectious pathogen can be accomplished by two arms of immune system which are antibody dependent and cellular dependent immune responses, The antibody dependent immune response helps in the neutralization of the pathogen while the cellular immune response provide immunological memory to the host, and these immunity could be achieved by using of immunodominant epitopes in the vaccine development (Akbari et al., 2021).

Immunoinformatics has revealed immense potential application in vaccine designing and currently application of immunoinformatics is globally recognize in the selection of good vaccine candidate against different targeted bacterial and viral pathogens (Alharbi et al., 2022; Oli et al., 2020).

Biophysical approaches such as molecular docking and molecular dynamic simulation analysis which are computational base analysis that allow the understanding of binding ability and dynamic behaviour of the vaccine with immune cells receptors. Molecular docking and simulation analysis could make it possible to generate additional information like binding interactive amino acids residues and different types of binding (Khan and Kumar, 2021; Naz et al., 2021), In this work for binding interaction and dynamic behaviour of vaccine and receptor was analyzed through docking and simulation analysis.

The current research study involved RV, bioinformatics, immunoinformatics, biophysical approaches (Molecular docking and molecular dynamic simulation), and C-immune simulation to make multi-epitopes base vaccine construct against Nipah Virus viral infection using their multi-antigenic proteins (Yousaf et al., 2022).

This research study aimed to design an mRNA-based multi-epitope vaccine construct against the Nipah virus. The above aim was achieved through different objectives, the first objective of the current study was to screen the Nipah virus proteins for prediction of Cytotoxic T-cell (CTL) and Helper T-lymphocyte (HTL). The second objective of the study was to screen the predicted epitopes for immunoinformatics analysis and probably antigenic nonallergic good water-soluble and nontoxic epitopes were used in multi-epitopes vaccine designing, followed by the second objective the third objective was to analyze the binding affinity of vaccine construct with immune cells receptor to ensure the immune response evoking ability of the vaccine. The fourth objective was to observe in silico host immune responses (humoral and cellular immune response) toward the designed vaccine.

The multi-epitope vaccine construct is a promising strategy for the prevention and treatment of the virus (Tahir ul Qamar et al., 2021, 2020). The current work will be for the benefit of the common man and public health, as this work will speed up the vaccine development process against target pathogens that in turn will result in protecting the human population from the Nipah virus, furthermore, the vaccine candidate could save millions of dollars which are need for identification of proper vaccine target in pathogens.

2. Research methodology

The following Fig. 1 depicts the overall methodology flow for designing mRNA epitopes base vaccine construct against the Nipah virus.

2.1. Proteins sequences retrieval and epitopes prediction

In protein sequences retrieval phase, Nipah Virus proteins V and C were retrieved from National Center for Biotechnology Information (NCBI) https://www.ncbi.nlm.nih.gov/. Furthermore physiochemical properties analysis was done using the ProtParam tool (ProtParam, 2017). In physiochemical properties analysis number of amino acids, molecular weight, Theoretical pi instability index and grand average of hydropathicity (gravy) were analyzed (Nawaz et al., 2022), The retrieve proteins were analyzed for antigenicity and allergenicity analysis using vaxijen 2.0 (Doytchinova and Flower, 2007) and allertop tool 2.0 (Dimitrov et al., 2013) web tools respectively. Furthermore the antigenic proteins were subjected for epitopes prediction using the Immune Epitope Database (IEDB) webserver (Greenbaum, 2007), in epitopes prediction cytotoxic T-lymphocytes (CTL) and Helper T lymphocyte (HTL) (Mahmoodi et al., 2023) were predicted in different number and ranked through percentile rank. The CTL and HTL epitopes were further used for the prediction of B-cell epitopes also using IEDB (Vita et al., 2019).

2.2. Epitopes prioritization phase

The predicted epitopes CTL and HTL were further evaluated for antigenicity allergenicity, toxicity and solubility analysis using vaxijen 2.0 https://www.ddg-pharmfac.net/vaxijen/, Allertop 2.0 https://www.ddg-pharmfac.net/AllerTOP/, and ToxinPred 2.0 (Gupta et al., 2013) and INNOVAGEN PepCalc https://pepcalc.com/peptide-solubility-calculator.php tools respectively (Khan et al., 2022).

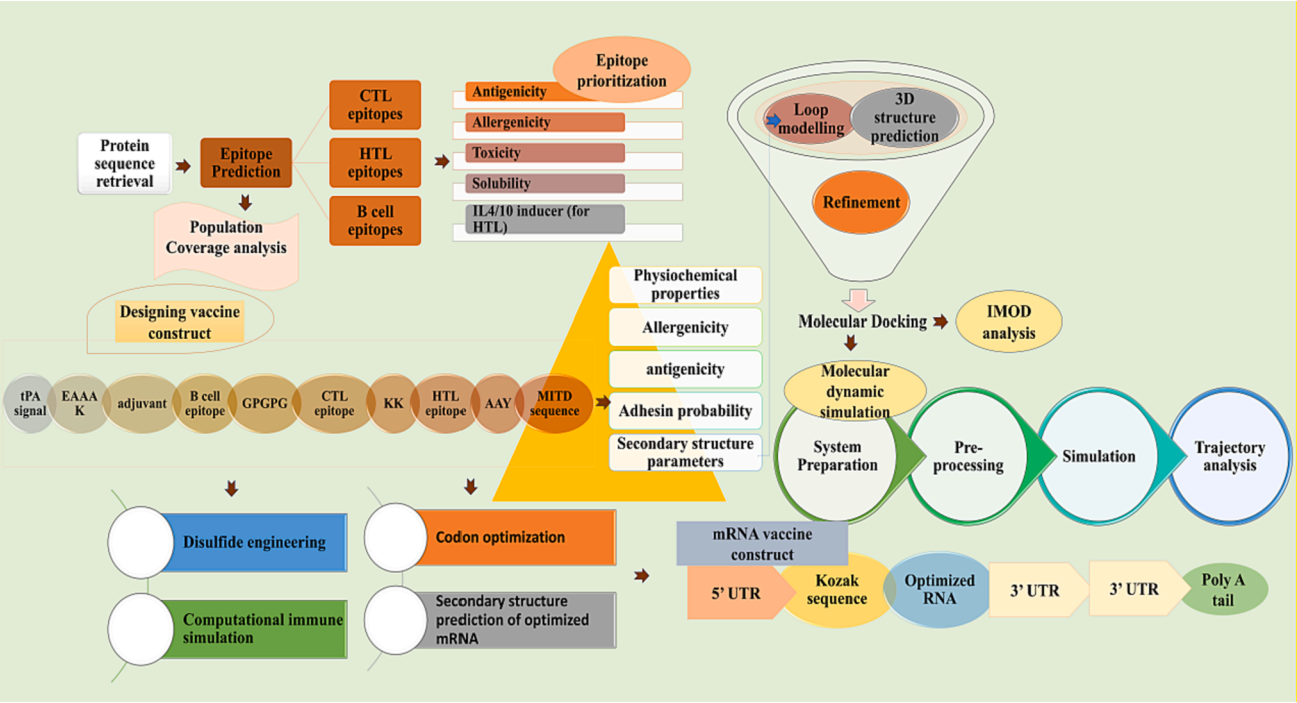

Fig. 1. Illustration of schematic diagram followed for designing of mRNA base multi epitopes based vaccine against Nipah virus, the study was commenced from protein sequence retrieval, followed by epitopes prediction (CTL & HTL and B-cell epitopes prediction), in the third phase of the study Epitopes prioritization different immunoinformatics analysis(Antigenicity, allergenicity toxicity solubility interleukins 4/10 binding ability for HTL, The fourth phase was designing vaccine constructing phase followed by physiochemical properties analysis of designed vaccine construct. After designing of vaccine construct 3D structure, loop modelling and refinement, Disulfide engineering, codon optimization secondary structure prediction of optimized mRNA, and computational immune simulation were done, and then subjected vaccine construct for biophysical analysis (like Molecular docking and Molecular dynamic simulation analysis was performed).

2.3. Population coverage analysis

In population coverage analysis the predicted epitopes (CTL, HTL, and B- cells epitopes were analyzed for their population coverage and predicted how much the predicted epitopes could cover population around the world and create immunity against the target pathogen (Zahroh et al., 2016). This analysis was done through the IEDB online webserver https://tools.iedb.org/tools/population/iedb_input.

2.4. Vaccine construction and processing phase

The selected epitopes, AAY, GPGPG linkers, and adjuvant were used in designing of multi-epitopes vaccine, the selected epitopes were first linked together by GPGPG linkers, CTL epitopes were linked through KK linkers, and HTL through AAY linkers, additionally Beta –defensin was used as an adjuvant, The tPA single sequences were added to the vaccine construct by EAAAK to B-defensin and MITD was also added to the end (Al Tbeishat, 2022). After vaccine construction, the physiochemical properties were checked using the protparam tool https://web.expasy.org/protparam/. Furthermore, the sequences were used for structure prediction, and 3D structure was predicted using Scratch Protein Predictor online web tools https://scratch.proteomics.ics.uci.edu/. The structure was used for further refinement using Galaxy Refine. Disulfide engineering analysis was done using a design 2.0 online web server.

2.5. Molecular docking analysis and in silico immune simulation

Molecular docking analysis was done using online ClusPro 2.0 (Kozakov et al., 2017), before docking analysis of the Toll-like receptor (TLR-3). The receptors and vaccine construct were prepared (Vaccine_TLR-3) using the UCSF CHIMERA tool https://www.cgl.ucsf.edu/chimera/ (Pettersen et al., 2004). To check the immune efficacy of the vaccine construct, the C-ImmSim online web server was utilized (Banerjee et al., 2020).

2.6. Normal mode immune simulation

The docked complexes were further checked for molecular dynamic simulation, for the said analysis iMod online webserver was utilized https://imods.iqfr.csic.es/.The top-docked complexes on the base of the lowest docking energies score were submitted to the online web server and analyzed the binding affinity of the vaccine with immune cells receptors.

3. Results

3.1. Protein sequence retrieval

Nipah Virus proteins V and C were opted out for the analysis. Protein sequences for V and C proteins were retrieved from NCBI https://www.ncbi.nlm.nih.gov/. Both the protein sequences were evaluated for their physiochemical properties. V protein being antigenic and non-allergen was selected to be used to design an mRNA peptide vaccine against the Nipah virus infection. V protein was subjected to BLAST search for Coding Sequence (CDS) leading to the multiple sequence alignment performed to determine the global consensus sequence of V protein.

3.2. CTL epitope prediction

CTL epitopes were predicted for V protein via the IEDB database of MHC-I. A total of 71 epitopes were predicted which were further evaluated for their antigenicity, allergenicity, toxicity, and solubility. Nine epitopes (Table 1) were shortlisted to be part of the peptide vaccine construct.

Table 1
CTL epitopes and their properties.

CTL epitopes	Antigenecity		Allergencity	Toxicity	Solubility
GSDDIQLDP	1.1344	Antigen	Non allergen	Non toxin	Soluble
QLDPVVTDV	1.2435	Antigen	Non allergen	Non toxin	Soluble
PTDGTIGKRV	1.5113	Antigen	Non allergen	Non toxin	Soluble
FTLRNLSDPA	1.1207	Antigen	Non allergen	Non toxin	Soluble
RETDLVHLEN	1.7568	Antigen	Non allergen	Non toxin	Soluble
NVCLVSDAK	0.8561	Antigen	Non allergen	Non toxin	Soluble
MLAEEFECS	0.5013	Antigen	Non allergen	Non toxin	Soluble
DQLEFEDEFA	0.9655	Antigen	Non allergen	Non toxin	Soluble
QTSRNVNLD	1.1893	Antigen	Non allergen	Non toxin	Soluble

3.3. HTL epitope prediction

HTL epitope prediction was performed by MHC-II of IEDB, HTL epitopes were obtained while overlapping was ignored. Predicted epitopes were analyzed for their properties like antigenicity, allergenicity, toxicity, solubility, IL10 and IL4 inducing, and IFN-gamma positive ones. Only 1 HTL as present in (Table 2) epitope had all the previously mentioned properties and was thought to be a good candidate for the peptide vaccine.

3.4. B cell epitope prediction

B cell epitopes were predicted using ABCpred (Saha and Raghava, 2007). A total of 32B cell epitopes were obtained and prioritized using various filters. Antigenic, non-allergen, non-toxic, and soluble were opted out as good candidates, and they were 3 in number given in (Table 3).

3.5. Population coverage analysis for the shortlisted epitopes

Population coverage for the CTL and HTL epitopes against the set of alleles was evaluated from the Population coverage of IEDB. A total of 10 epitopes were subjected to calculate the world population coverage resulting in the 99.74% Class combined coverage Fig. 2.

3.6. Peptide vaccine construct

The Peptide vaccine construct was designed using adjuvants and linkers. To design the mRNA peptide vaccine construct B cell epitopes were linked together via the GPGPG linker CTL epitopes by the KK linker and HLT by AAY linker. Adjuvant B-defensin was used. The tPA signal sequence was added to construct and connected via EAAAK to B-defensin. MITD sequence was also the part of vaccine construct added at the end. The designed sequence was tPA signal-EAAAK-Adjuvant (B-defensin)-GPGPG-B cell epitopes-KK-CTL epitopes-AAY-HTL epitopes-AAY-MITD sequence. Protparam by expasy protparam server was used to evaluate the physiochemical properties of the construct, results stated the vaccine construct consisted of 320 amino acids with 34.29 kDa molecular weight. The instability index was 36.55 proving it stable with the aliphatic index of 82.88. The theoretical pI was obtained as 9.0 and gravy was −0.317. A solubility check was applied against the vaccine construct depicting that the vaccine construct is soluble with its calculated value of 0.6 (Fig. 3A). The Ramachandran plot was obtained for the vaccine (Fig. 3B) depicting 94.9% of the residues in the most favored region. The secondary structure for the vaccine was predicted to consist of 1 beta hairpin, 2 strands, 25 helices, 11 beta turns, and 4 gamma turns (Fig. 3C).

3.7. Tertiary structure prediction and loop modeling

The peptide vaccine construct's (Fig. 4A) 3D structure (Fig. 4B) was obtained using the Scratch predictor. The amino acid sequence was submitted to the online server. Using Chimaera, the resulting 3-D model was visualized for the presence of the loops in the protein structure. Loop modeling was performed on the vaccine model followed by galaxy refinement to refine the structure, scores for the predicted model are given in (Table 4). Model 1 from the galaxy refinement with its RMSD of 0.446 and Rama favored value of 96.9 was selected as the vaccine model for further analysis.

3.8. Molecular docking

The Peptide vaccine model after loop modelling and refinement was subjected to molecular docking. TLR-3 was used as a receptor for the vaccine and docking was performed using cluspro 2.0 online server Fig. 5. The top 10 models with their scores were obtained and 1st model (Table 5) with the best properties was selected for further analysis.

3.9. Computation immune simulation

C-ImmSim webserver was used to predict the immune simulation profile for the vaccine. Computational immune simulation results showed the vaccine is capable of inducing immune responses. Fig. 6A graphs highlight the production rate of primary antibodies and the rate IgM and IgG increased with the number of days, which continued increasing till the 15th day of the vaccine. Cytokine release has been also observed as the graph (Fig. 6B) showed the IFN-gamma release of 400,000 ng/ml with the number of days. Vaccines are observed to induce the release of interleukins also.

3.10. Conformational B cell epitope prediction

The tertiary structure for the peptide vaccine model was submitted to Ellipro of IEDB https://www.iedb.org/ Sto predict conformational B cell epitopes. Five Discontinued B cell epitopes were predicted, with a score higher than 0.6.

3.11. IMOD analysis

IMODS server used for the molecular dynamic simulations of the dock complex (the vaccine construct and TLR-3). The ability of the molecule to acquire residual modification is referred to as deformability represented by the highest peaks in the graph Fig. 7A. The b-factor graph determines the PDB structure and runs it through the normal mode analysis and is obtained by multiplying the NMA mobility by 8pi (Fig. 7B). Fig. 7C graph for the variance of NMA inversely proportional to eigenvalue. Eigenvalue is related to motion stiffness and is related to the energy that is required for the deformation of the structure. The smooth deformation process is supported by the lower eigenvalue (Fig. 7D). Coupling between pairs of residues is plotted on the graph of covariance as shown in Fig. 7E. Pairs of atoms in the structure are linked by springs and determined by the elastic network model (Fig. 7F).

3.11.1. Codon optimization

The amino acid vaccine sequence was reverse-translated by the JCat codon optimization tool. The DNA sequence obtained was with a CAI value of 1.0 and the GC content for the improved sequence was 49.89. The improved sequence for the designed vaccine is

Table 2
HTL epitope and properties.

HTL epitope	Antigenecity		Allergencity	Toxicity	IL 4 inducer	IL 10 inducer
ETDLVHLENKLSTTG	1.167	Antigen	Non Allergen	Non toxin	IL 4 inducer	IL 10 inducer

Table 3
Predicted B cell epitopes along with their properties.

B- cell epitopes	Antigenecity		Allergencity	Toxicity	Solubility
HWSIERSISPDKTEIV	0.6304	Antigen	Non allergen	Non toxin	Soluble
ELVNDGLNIIDFIQKN	0.6411	Antigen	Non allergen	Non toxin	Soluble
NVCLVSDAKMLSYAPE	0.7654	Antigen	Non allergen	Non toxin	Soluble

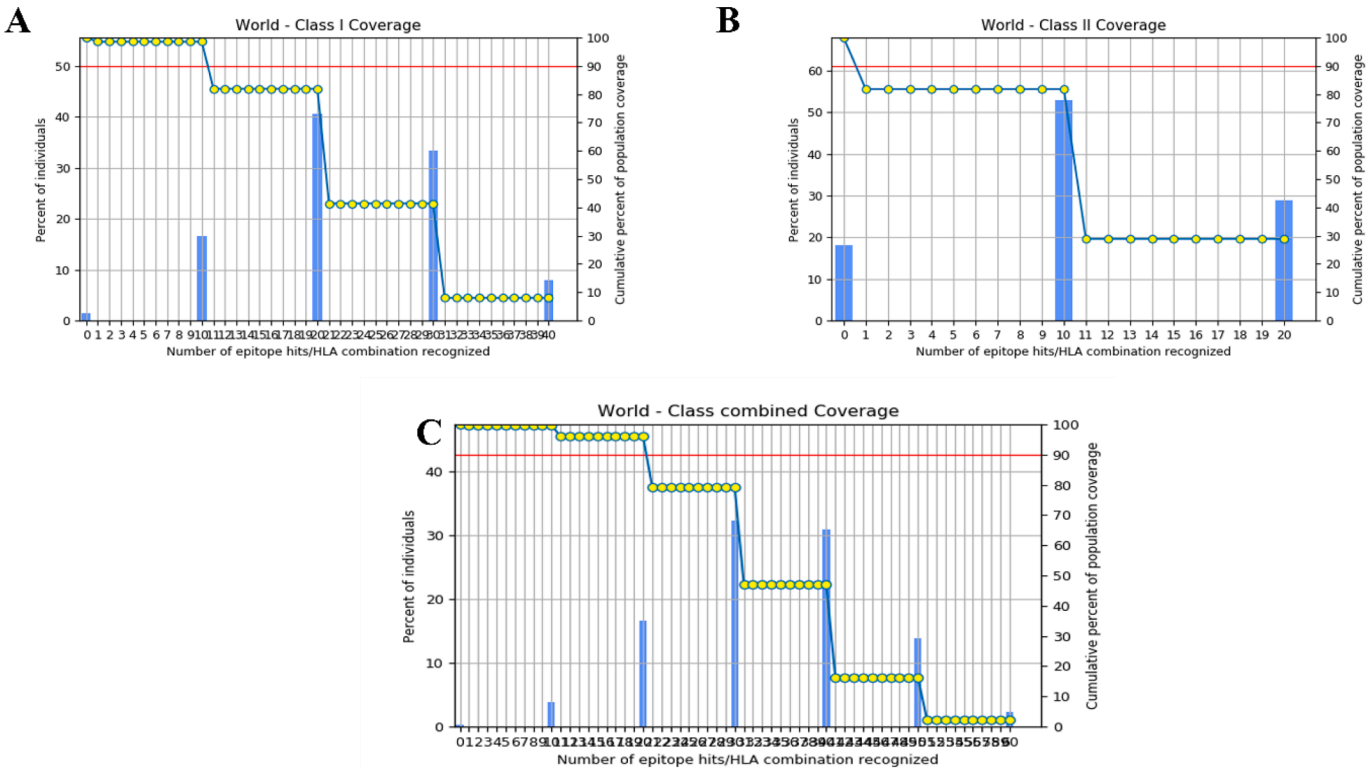

Fig. 2. Population coverage of the shortlisted epitopes. A) World population coverage of MHC class I with 98.55%. B) World population coverage of MHC class II with 81.81%. C) Class combined world population coverage of CTL and HTL epitopes.

(TCTCCGATGCGTGTTACCGCTCCGCGTACCCT-
GATCCTGCTGCTGTCTGG
TGCTCTGGCTCTGACCGAAACCTGGGCTGGTTCTGAAGCTGCTGC-
TAAAG
GTATCATCAACACCCTGTGCAAA-
TACTACTGCCGTGTTCGTGGTGGTCGT
TGCTGCGTTTGCTCTTGCTGCCCGAAAGAAGAACA-
GATCGGTAAATGCTC
TACCCGTGGTCGTAAATGCTGCCGTCG-
TAAAAAAGGTCCGGGTCCGGGTC
ACTGGTCTATCGAACGTTCTATCTCTCCGGA-
CAAAACCGAAATCGTTGGT
CCGGGTCCGGGTGAACTGGTTAACGACGGTCTGAA-
CATCATCGACTTCAT
CCA-
GAAAAACGGTCCGGGTCCGGGTAACGTTTGCCTGGTTTCTGACGCTA
AAATGCTGTCTTACGCTCCGGAAAAAAAAGGTTCTGACGA-
CATCCAGCTG
GACCCGAAAAAA-
CAGCTGGACCCGGTTGTTACCGACGTTAAAAAACCGAC
CGACGGTACCATCGGTAAACGTGTTAAAAAATTCACCCTGCG-
TAACCTGT
CTGACCCGGCTAAAAAACGTGAAACCGACCTGGTTCACCTG-
GAAAACAAA
AAAAACGTTTGCCTGGTTTCTGACGCTAAAAAAAAAATGCTGGCT-
GAAGA
ATTCGAATGCTCTAAAAAAGACCAGCTGGAATTCGAA-
GACGAATTCGCTA
AAAAACA-
GACCTCTCGTAACGTTAACCTGGACGCTGCTTACGAAACCGAC
CTGGTTCACCTGGAAAACAAACTGTCTACCACCGGTGCTGCTTA-
CATCGT
TGGTATCGTTGCTGGTCTGGCTGTTCTGGCTGTTGTTGT-
TATCGGTGCTG
TTGTTGCTACCGTTATGTGCCGTCGTAAATCTTCTGGTGG-
TAAAGGTGGT
TCTTACTCTCAGGCTGCTTCTTCTGACTCTGCTCAGGGTTCT-
GACGTTTC
TCTGACCGCT)

The optimized sequence was further subjected to a rare codon analysis Tool and with a CAI value of 0.86 GC content of 49.39 and CFD value of 0% supported its translation efficacy.

3.12. Secondary structure prediction

Secondary structure prediction optimized mRNA vaccine was performed using RNAfold web server based on the free energy assessment approach. The secondary structure for stabilized m RNA is obtained as shown in Fig. 8. This step is performed to enhance the stability of the mRNA restraint to endonuclease activity. The calculated free energy of the dynamic ensemble was −292.64 kcal/mol while the minimum free energy centroid secondary structure observed was −220 kcal/mol.

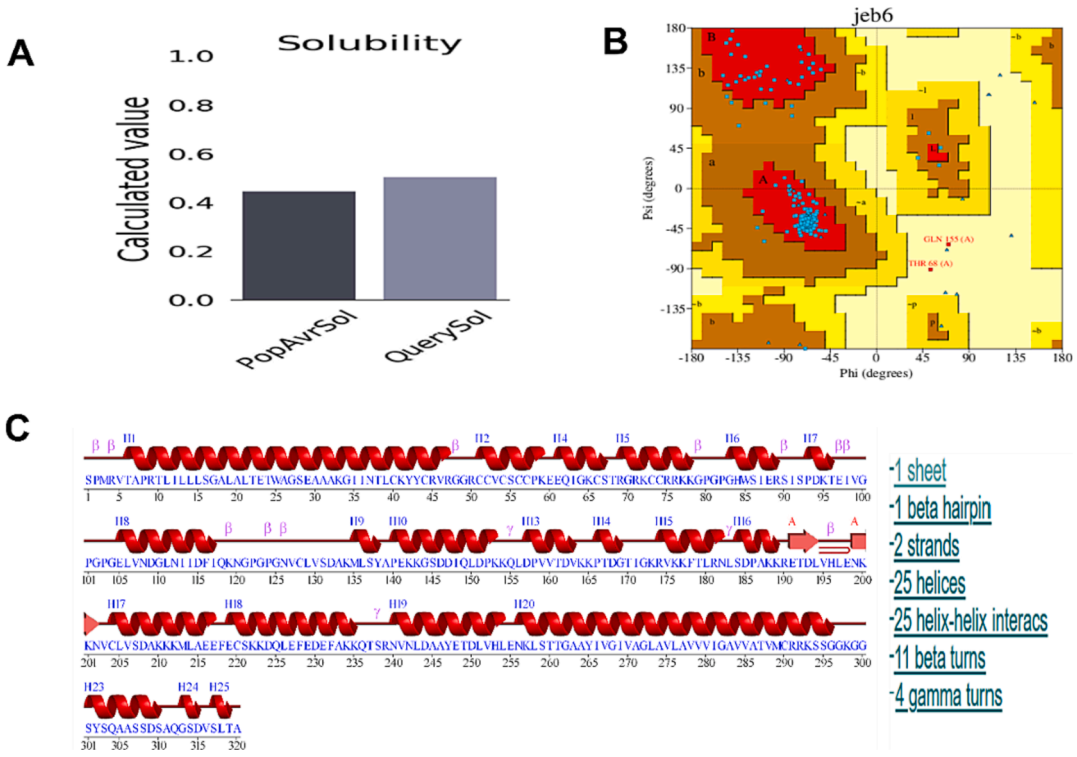

Fig. 3. **(A)** Solubility graph for the Vaccine construct. **(B)** Ramachandran plot depicting the residual properties. **(C)** Secondary structure for the designed vaccine.

Fig. 4. **(A)** detailed components of mRNA vaccine construct. **(B)** The 3D model predicted and modeled (for its loops via the galaxy loop on the galaxy web) the mRNA vaccine.

Table 4
Properties of vaccine models obtained from galaxy refine after refinement.

Model	GDT-HA	RMSD	MolProbity	Clash score	Poor rotamers	Rama favored
Initial	1	0	3.037	43.7	3.8	92.1
MODEL 1	0.9289	0.466	1.752	11.1	0.4	96.9
MODEL 2	0.9328	0.443	1.682	9.3	0	96.9
MODEL 3	0.9391	0.452	1.73	10.5	0.4	96.9
MODEL 4	0.9359	0.443	1.722	10.3	0.4	96.9
MODEL 5	0.9305	0.458	1.675	10.1	0.8	97.2

3.13. Vaccine construction designing

The mRNA vaccine construct based on the multi-epitope vaccine construct was designed as 5′ m7GCap-5′ UTR-Kozak sequence-optimized RNA (tPA signal-EAAAK-adjuvant B-defensin-GPGPG-B cell epitopes-kk-CTL epitopes -AAY- HTL-MITD seq) epitope -stop codon- (3′ UTR) 2-poly-A tail- 3′. 5′ cap prevents the mRNA degradation and is involved in mediating the binding of translation factor Fig. 9. Both the 5′ and 3′ UTRs support the translation. The Kozak sequence initiates the translation while the tPA signal will lead the translation product in its secretion pathways. Poly A tail consisting of 120 bases stabilizes the construct.

3.14. Disulfide engineering

Disulfide engineering is a phenomenon of introducing cysteine residues to provide stability to the structure Fig. 10. Disulfide by design 2.0 was used to specify the unstable regions that were mutated as cysteine residues, 16 pairs were mutated as cysteine are given in (Table 6).

3.15. MD simulations

The molecular dynamic simulation was performed to analyze the dynamic behaviour of the macromolecule (dock complex).Trajectories were determined and analyzed for structural stability and residual flexibility. Root means square deviations (RMSD), Root means square fluctuations (RMSF), and Radius of gyrase (RoG) analysis were carried out depicting the stability throughout the process without any major changes. The average RMSD observed for the complex was 4.09 Å and the maximum RMSD was first observed as 5.19 Å at 9 ns-10 ns and then 5.25 Å at 49.46 ns as presented in Fig. 11A, confirming the overall structure stability with minor changes. The RMSF graph was plotted to determine the residual flexibility. Fluctuations confirm the presence of a high number of flexible loops in the structure, the average RMSF calculated was 1.68 Å while the maximum was observed at the 549th residue with 6.13 Å RMSF as mentioned in Fig. 11B. The RoG is represent in Fig. 11C that is determine the compactness of the structure high RoG value represents high compactness proving the structure is stable. The RoG graph as shown in Figure C remained stable without any major changes, the minimum RoG was 46 Å at 18 ns and the maximum was 53 Å at 12 ns. While the average RoG was 49 Å validating the structure

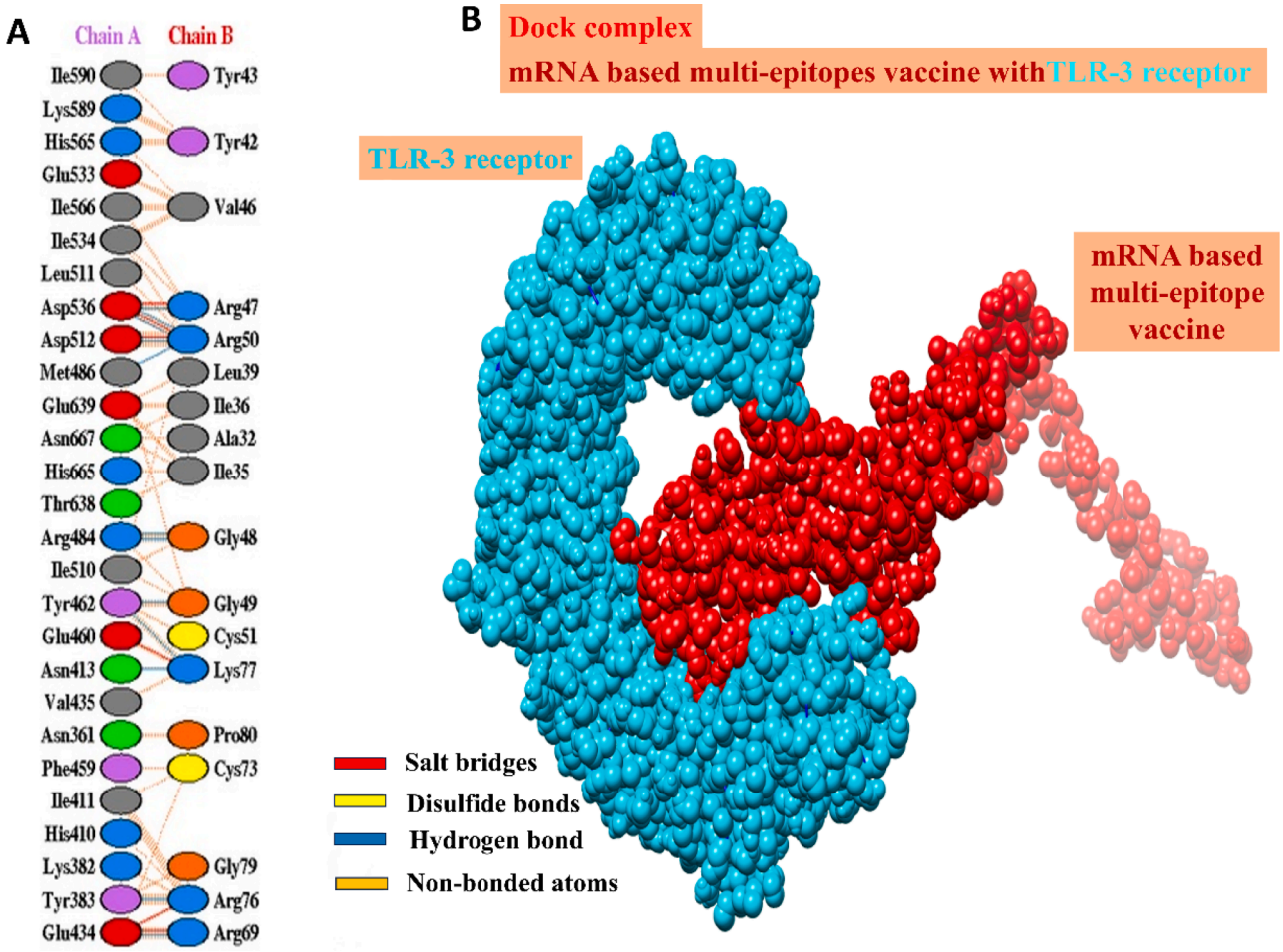

Fig. 5. **(A)** Residual interaction between the residues of the vaccine (Chain A) and TLR-3 (chain B). **(B)** 3D model for the Dock complex (vaccine-TLR3).

Table 5
Dock complex for vaccine model and TLR-3 with their lowest energy scores and energy score at the canter, obtained by cluspro 2.0.

Cluster	Members	Representative	Weighted Score
0	77	Center	−830.7
		Lowest Energy	−984.2
1	49	Center	−883.4
		Lowest Energy	−1026.7
2	49	Center	−861.9
		Lowest Energy	−978.2
3	47	Center	−849.8
		Lowest Energy	−1089.1
4	38	Center	−871.4
		Lowest Energy	−923.4
5	33	Center	−935
		Lowest Energy	−935
6	31	Center	−824.3
		Lowest Energy	−972.6
7	28	Center	−827.9
		Lowest Energy	−921.5
8	28	Center	−811.6
		Lowest Energy	−941.4
9	25	Center	−805.9
		Lowest Energy	−875.5

compactness.

4. Discussion

Nipah virus belongs to the family paramyxoviridae causing encephalitis in humans and can be fatal sometimes. Nipah Virus, the P gene encodes the three accessory proteins W, V, and C proteins have a role in the pathogenesis of the Nipah Virus as reported in the hamster infection model (Singh et al., 2019). We selected V and C proteins retrieved their sequence from NCBI and evaluated them on their physiochemical properties. The c protein sequence was observed as an allergen and discarded while the V protein Sequence was subjected to a Blast run of NCBI to get CDS sequences. The homologous sequences were subjected to MSA for multiple sequence alignment to get the global consensus sequence. The global consensus sequence for the V protein was used for further epitope prediction.

The vaccine is considered the best solution to viral infection, in the era of immunoinformatics and reverse vaccinology the epitope peptide-based mRNA vaccine have advantages over the conventional vaccine in term of cost and efficacy. Immunodominant epitopes are considered the best candidate for the vaccine construct (M. T. Khan et al., 2021). CTL, HTL, and B cell epitopes were predicted and prioritized for antigenicity allergenicity, and toxicity. Potential epitopes antigenic, non-allergen, and non-toxin were utilized to design vaccine construct. Epitopes can initiate cellular immune responses. CTL and HTL epitopes against the set of alleles were analyzed for population coverage, covering 99.6% of the world population. The Peptide vaccine construct was designed by linking the epitopes together via linkers and adjuvants. We used B-defensin adjuvant for our vaccine construct; adjuvant is the one used to boost the efficacy and functioning of the vaccine while B-defensin is important in inducing innate immunity. Linker EAAAK liking the adjuvant with the construct, GPGPG joining the B cell epitopes, KK and AAY connecting the CTL and HTL epitopes respectively. Linkers are used to avoid overlapping and they play a crucial role in provoking the immune responses (Behmard et al., 2020). The peptide construct was based on the tPA signal, adjuvant (B-defensin), linkers (EAAAK, GPGPG, KK AAY), epitopes (B cell, CTL, HTL), and MITD sequence. The designed peptide-based vaccine construct was evaluated for its physicochemical properties. The Molecular weight of the vaccine construct was 34.92kd, lighter in weight and complementing the efficacy, While the Gravy value more negative the more effective the structure would be, was obtained as −0.317.

The 3D structure for Vaccine was predicted followed by loop modelling and refinement. Loop modeling is the prediction of loop regions in the protein structure and is associated with the prediction of structures at loop regions (S. Khan et al., 2022). Molecular docking of the vaccine and TLR-3 (human receptor) was performed by Cluspro. Docking predicts the interaction between the vaccine and human body receptors (Nawaz et al., 2022). TLR-3 (toll-like receptor 3) is a human body receptor that functions as inducing immune response, signal transduction, and causing the release of IFNs (Yujuan et al., 2021). The dock complex was further analyzed for its efficacy and function through computational immune simulation and molecular dynamic simulation (Arwansyah et al., 2022). C-Immsim was used for the computational immune simulation predicting the immune responses induced by the vaccine. Immunoglobulins and interleukins release was noticed proving the vaccine capable of activating the immune system. IMOD analysis is associated with the graph of B-factor variance, co-variance, elastic network model, eigenvalue, and deformability generated for the dock complex. Simulations depict the binding affinity and induction of immune responses against the vaccine and test the efficacy and capability of the vaccine (López-Blanco et al., 2014).

The mRNA vaccines are considered a good strategy to combat viral infection as the mRNA vaccine inhibits the nuclear genome integration. The mRNA sequence consisted of the 5′ and 3′ UTR, Kozak sequence, optimized RNA (optimized peptide-based vaccine), and poly-A tail. UTRs enhance the translation efficiency process; the size of the poly-A tail also affects the mRNA. Poly A tail with 120 bases is involved in prolonged expressions. Kozak sequence also contributes to the

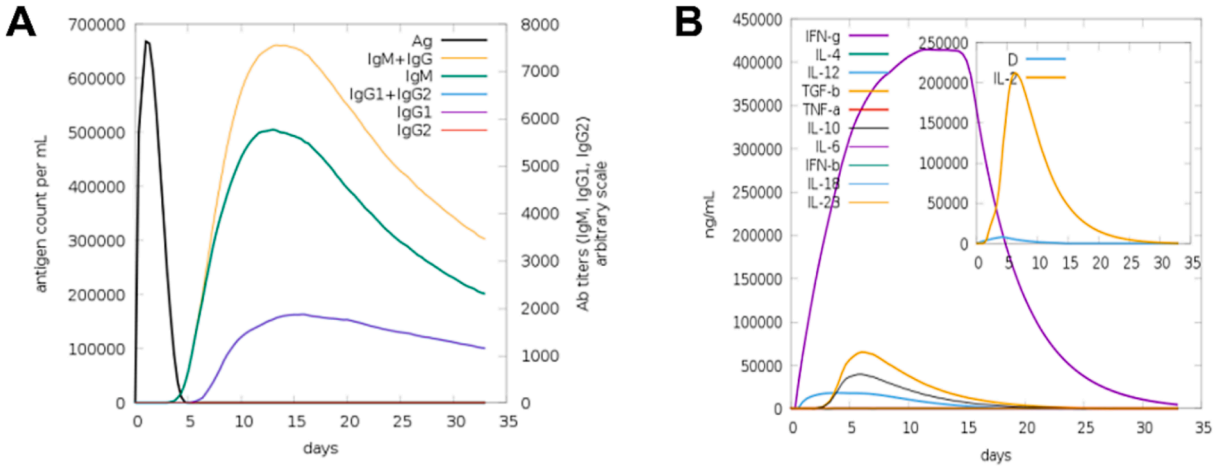

Fig. 6. **(A)** Production of Immunoglobulins in response to vaccine. **(B)** Cytokine release was observed against the designed vaccine.

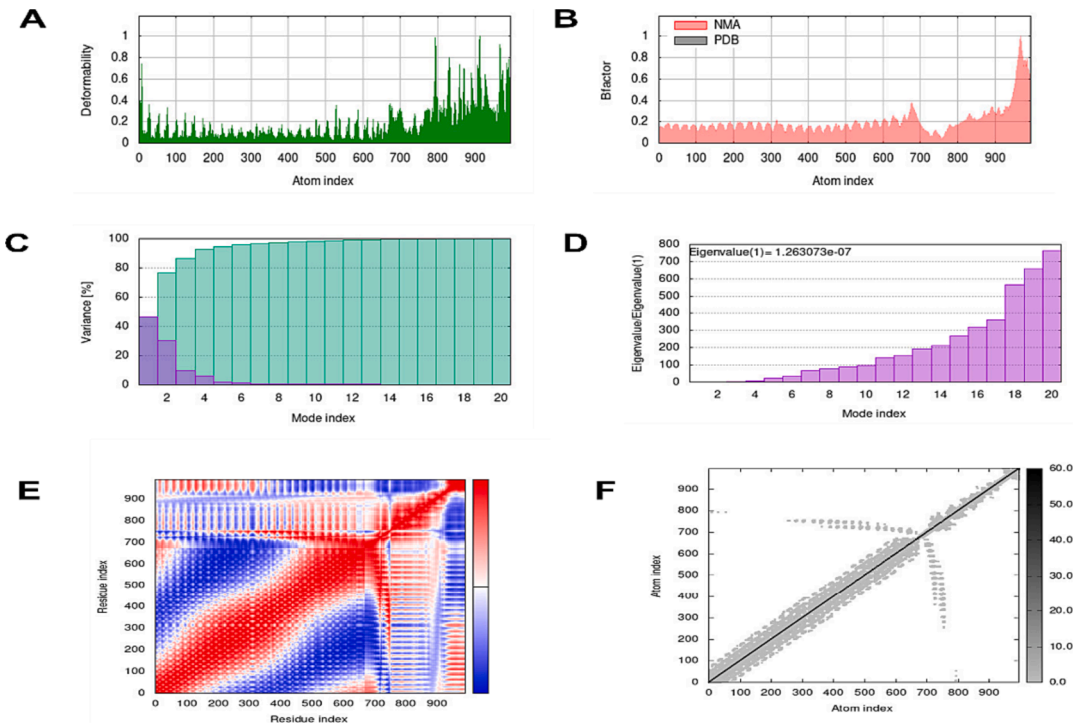

Fig. 7. IMOD analysis. A) Deformability graph, peaks representing the deformability of the structure. B) B-factor graph determining the PDB structure and NMA. C) Variance plot, green color is sowing the cumulative variance. D) Plot for the Eigenvalue, The desired lower eigenvalue has been observed. E) Residues correlation determined by the covariance graph red color shows the correlation while blue highlights the anti-correlated residues and white color is associated with uncorrelated motions. F) Elastic network model grey dots are associated with the springs the darker dotes specify the stiffer springs.

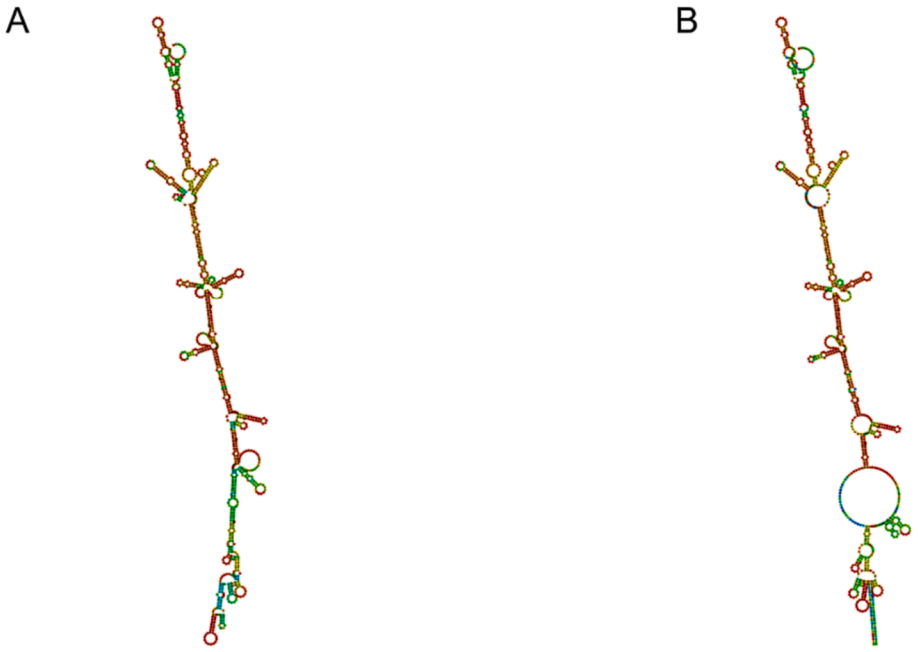

Fig. 8. **(A)** MFE (minimum free energy) secondary structure. **(B)** Centroid secondary structure.

translation (Schlake et al., 2012).

5. Conclusion

In the ongoing Nipah virus infection, it is hard to counter the circulating infection through preventative or therapeutic strategies, Vaccination has become a promising approach to tackle various infectious diseases, before the occurrence of the COVID-19 pandemic mRNA technology was mostly used for the development of novel anti-cancer drugs that were showing promising results, while the COVID-19 pandemic adopted mRNA base vaccine approach as a means to tackle and eradicate, and a new generation of vaccines has increasingly reached to common man. In this research work multi-epitopes base mRNA vaccine was developed against the Nipah virus, to overcome the

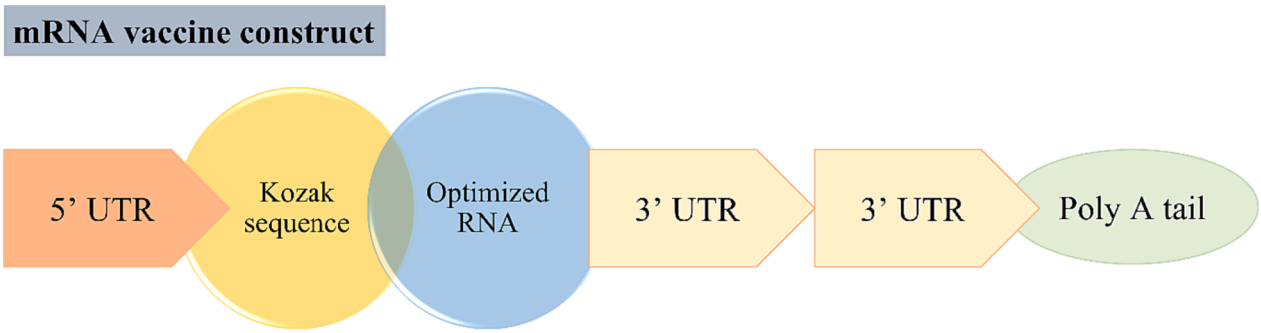

Fig. 9. Final mRNA vaccine design.

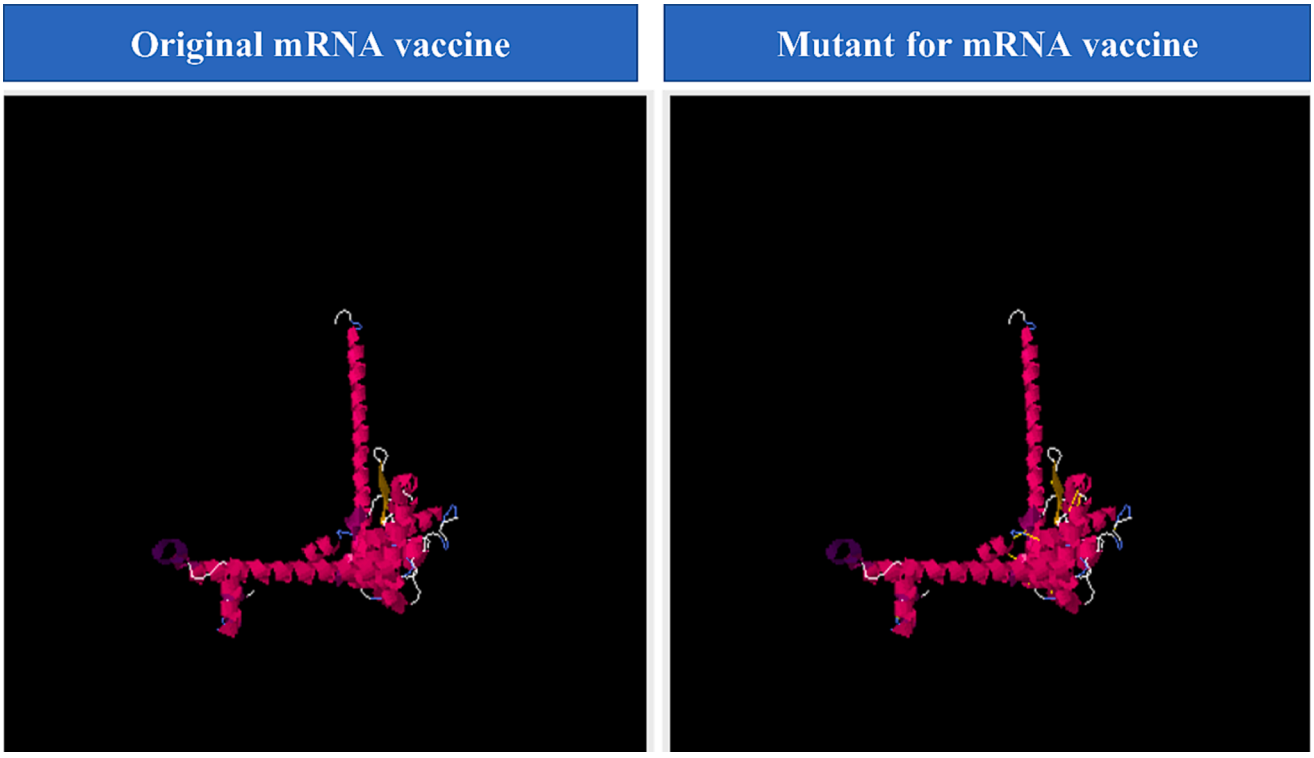

Fig. 10. Original and mutant model of mRNA peptide vaccine.

Table 6
Unstable residue pairs mutated as cysteine residue.

Residue pairs		χ3	Kcal/mol
44 CYS	57 CYS	115.85	3.26
50 ARG	53 VAL	−104.86	3.18
54 CYS	74 CYS	−79.33	7.31
58 PRO	88 GLU	−81.2	3.51
61 GLU	92 SER	67.22	5.78
67 SER	70 GLY	77.7	6.48
104 GLY	107 VAL	80.75	1.75
118 GLN	123 GLY	−66.78	8.1
151 ASP	160 VAL	126.48	5.32
164 LYS	167 THR	−102.95	7.03
166 PRO	181 ARG	118.04	8.16
193 ASP	200LYS	124.02	6.62
215 ALA	222 LYS	−113.4	2.43
218 PHE	221 SER	85.45	0.48
239 ARG	242 ASN	−74.29	5.82
308 SER	313 GLY	104.76	4.27

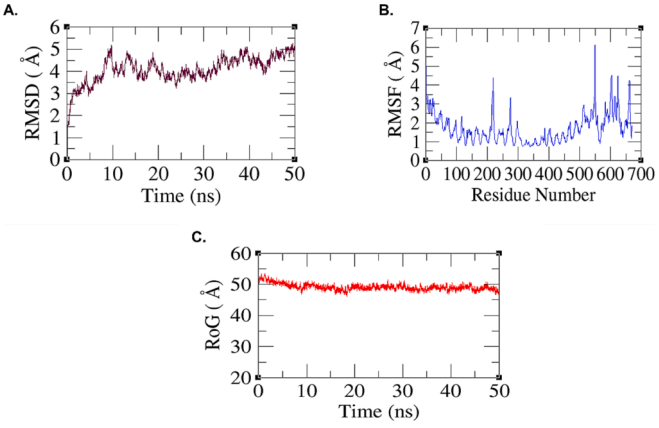

Fig. 11. Simulation trajectories of Molecular dynamic simulation (A) Root mean square deviation (RMSD). (B) Root mean square fluctuation (RMSF) and Radius of gyration (RoG).

limitation that occurs in the experimental laboratory during vaccine formulation Immmunoinformatics and revere vaccinology approaches are recommended to be developed by the research community for targeting potential vaccine targets against different viral and bacterial pathogens.

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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