International Journal of Zoology and Applied Biosciences **ISSN: 2455-9571**
Volume 9, Issue 6, pp: 33-42, 2024
https://doi.org/10.55126/ijzab.2024.v09.i06.006 **http://www.ijzab.com**

Research Article

DEVELOPING A MULTI-EPITOPE VACCINE AGAINST MR766 STRAIN OF ZIKA VIRUS: A BIOINFORMATICS APPROACH

***Rutuja A. Satarkar, Mahadev A. Jadhav, Dipak P. Chavan, Shivani S. Dhadge**

Department of Biotechnology and Bioinformatics, Deogiri College (Autonomous),
Chhatrapati Sambhajinagar India-431005

Article History: Received 21st September 2024; Accepted 29th October 2024; Published 20th November 2024

ABSTRACT

The Zika Virus has emerged as a significant global health concern, particularly due to its association with severe neurological complications such as microcephaly and Guillain-Barré syndrome. Given the urgent need for effective intervention strategies, this study aims to develop a targeted vaccine leveraging bioinformatics methodologies to identify B- and T-cell epitopes within the Zika virus Strain MR766 structural proteins. Through comprehensive epitope prediction algorithms, novel epitopes were identified and selected based on criteria including toxicity, immunogenicity, and antigenicity. These epitopes were then integrated into a multiple epitope vaccine constructs, incorporating various linker sequences (EAAAK, AAY, GPGPG) to optimize immunogenicity. Subsequent molecular docking simulations facilitated the design of a vaccine structure capable of effectively interacting with its target receptors. The vaccine construct was cloned into a pET-28a (+) vector for expression in Escherichia coli using SnapGene software. Evaluation of the expressed vaccine demonstrated a robust immune response in preclinical studies. Computational tools were employed to analyze the vaccine's efficacy against diverse Zika virus strains. The resulting Multi-Epitope Vaccine (MEV) emerges as a promising candidate for combating Zika virus infections, offering potential benefits in global public health efforts against this pathogen.

Keywords: Zika Virus MR766, Vaccine Development, Epitope Prediction, Bioinformatics Analysis, Immunoinformatics.

INTRODUCTION

The Zika virus (ZIKV) has emerged as a significant global health concern due to its association with severe neurological complications, including microcephaly in newborns and Guillain-Barré syndrome in adults (Dawes, *et al.,* 2016). Originating in the Zika forest of Uganda in 1947 (Dick *et al.,* 1952), sporadic outbreaks were reported in Africa and Asia until a major epidemic occurred in the Americas in 2015. The unprecedented spread of ZIKV underscored the urgent need for effective preventive measures, including vaccines (Hickman and Pierson, 2017), Traditional vaccine development strategies, like inactivated or live-attenuated vaccines, faced challenges with ZIKV due to safety concerns and scalability limitations. Epitope-based vaccine design, leveraging specific antigenic regions recognized by the immune system, emerged as a promising alternative. These epitopes offer precise targets for inducing protective immune responses while minimizing adverse effects (de Araújo *et al.,* 2018). Efforts to combat Zika include vector control measures, public health campaigns, and research into vaccines and treatments (Krauer *et al.,* 2017). ZIKV is primarily transmitted by Aedes mosquitoes, particularly *Aedes aegypti* and *Aedes albopictus* (*Musso et al.,* 2019) .Its association with birth defects, especially microcephaly, heightened concerns, particularly among pregnant women and those planning to conceive. Bioinformatics-driven epitope prediction revolutionized vaccine development by enabling the identification of immunogenic epitopes within viral proteins with high specificity and efficiency. Analyzing ZIKV strain MR766 proteins allows computational tools to predict B-cell and T-cell epitopes, facilitating the rational design of vaccines tailored to target key antigenic determinants while minimizing non-essential immunogenic regions. The polyprotein encoded by the Q32ZE1 gene initiates ZIKV infection within the host. This precursor

*Corresponding Author: Rutuja A. Satarkar, Department of Biotechnology & Bioinformatics, Deogiri College (Autonomous), Chhatrapati Sambhajinagar, Maharashtra, Email: rutusatarkar@gmail.com.

molecule undergoes proteolytic cleavage to generate functional viral proteins, including structural (capsid, envelope, membrane) and non-structural proteins (NS1, NS3, NS4A, NS4B, NS5). The envelope (E) protein plays a critical role in viral entry into host cells, facilitating attachment to cellular receptors and membrane fusion. Researchers target conserved and immunodominant epitopes within polyprotein-derived proteins to develop effective vaccines and therapeutic interventions against ZIKV infection. Understanding the role of the ZIKV polyprotein in infection initiation is crucial for elucidating viral pathogenesis and developing targeted antiviral strategies. A comprehensive overview of epitope-based vaccine development strategies for ZIKV focuses on rational design and computational analysis of multi-epitope vaccines. Through interdisciplinary collaboration and innovative methodologies, researchers aim to identify novel epitope candidates capable of inducing robust and long-lasting immunity against ZIKV. Challenges and opportunities associated with epitope-based vaccine design include antigenic variability, host immune responses, and vaccine delivery platforms. Harnessing the power of computational biology and bioinformatics accelerates the translation of epitope-based vaccine candidates from bench to bedside, contributing to global efforts to control and eradicate ZIKV infections (WHO, 2014).

MATERIALS AND METHODS

The Zika virus (ZIKV) strain MR766 polyprotein emerges as a promising candidate for vaccine development, primarily due to the adhesive properties of its envelope (E) protein and its relatively low number of transmembrane helices. These attributes facilitate the efficient presentation of immunogenic epitopes to the host immune system, enhancing the potential for a robust immune response upon vaccination. Additionally, the lack of significant similarity between the viral E protein and human proteins reduces the risk of autoimmune responses, thus positioning it as a safe vaccine candidate. Moreover, the cellular localization of the ZIKV polyprotein, including the E protein, ensures accessibility for immune recognition and response (Prasasty *et al.,* 2019). Its pipeline as shown in Figure 1.

Sequence retrieval and analysis of polyprotein sequence

In our study, we accessed the complete genomic sequence information of ZIKV Strain MR766 from the National Centre for Biotechnology Information NCBI (https://www.ncbi.nlm.nih.gov/) and the polyprotein sequence from UniProt (https://www.uniprot.org/) (Kuno and Chang, 2005)]. Physiochemical properties of the protein were assessed using the ExPASyProtParam tool (https://web.expasy.org/protparam /) Antigenicity and allergenicity were evaluated using VaxiJen 2.0 (http://www.ddgpharmfac.net/vaxiJen/VaxiJen/VaxiJenhtml) and AllerTOP v2.0, respectively. Protein secondary structure prediction was performed with the GOR (https://www.ddg-pharmfac.net/AllerTOP /method.html) method (https: //npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.), and 3D protein modeling was accomplished using SWISS-MODEL (https://swissmodel.expasy.org /).

Epitope prediction of T-cell and B-cell using IEDB and BCEPRED server

For epitope prediction, we utilized the Immune Epitope Database (IEDB) (http://tools.iedb.org/ main /) and the BCEPRED server (https://webs.iiitd.edu.in/raghava/bcepred/bcepred_ submission. html) for B-cell epitopes and the IEDB server for T-cell epitopes. Population coverage analysis was carried out using IEDB's Population Coverage tool (http://tools.iedb.org/population/) (Dhanda *et al.,* 2019).

Vaccine construction

Multi-epitope vaccine construction involved the incorporation of an adjuvant sequence from Uniprot (UniProtKB-P60438) (https://www.uniprot.org/) and the assembly of B- and T-cell epitopes with different linker types such as CPGPG, AAY and EAAAK were arranged manually. To streamline the vaccine design, overlapping B-cell epitopes were fused together, resulting in a consolidated sequence for the final vaccine formulation. The assembled vaccine sequence was evaluated for antigenicity, allergenicity and toxicity using toxin-pred (http://crdd.osdd.net/raghava/ toxinpred) bioinformatics tools (Gupta *et al.,* 2013).

Analysis of physiochemical properties of vaccine construct and its solubility

Additionally, we analyzed the physiochemical properties and solubility of the vaccine construct using ExPASy ProtParam and protein-sol tools.

Secondary & tertiary structure extrapolation

The secondary structure of the constructed vaccine was predicted from GOR and the 3D structure of the constructed vaccine using the online server SWISS MODEL. Both software predict the main helix, coils, and plates in the protein.

Refinement or validation of vaccine tertiary structure

The vaccine construct's 3D structure underwent refinement via the GalaxyRefine web server (https://galaxy.seoklab.org/cgibin/submit.cgi?type=REFINE), incorporating repacking, side chains rebuilding, and molecular dynamics simulation. Validation utilized PROCHECK (https://saves.mbi.ucla.edu/), accessible through PROCHECK-generated PostScript plots for protein structure analysis, while PROCHECK-NMR ensured NMR-solved structure quality. These combined methods ensure the accuracy of the vaccine's tertiary structure, crucial for efficacy and safety evaluation (Ko *et al*., 2012).

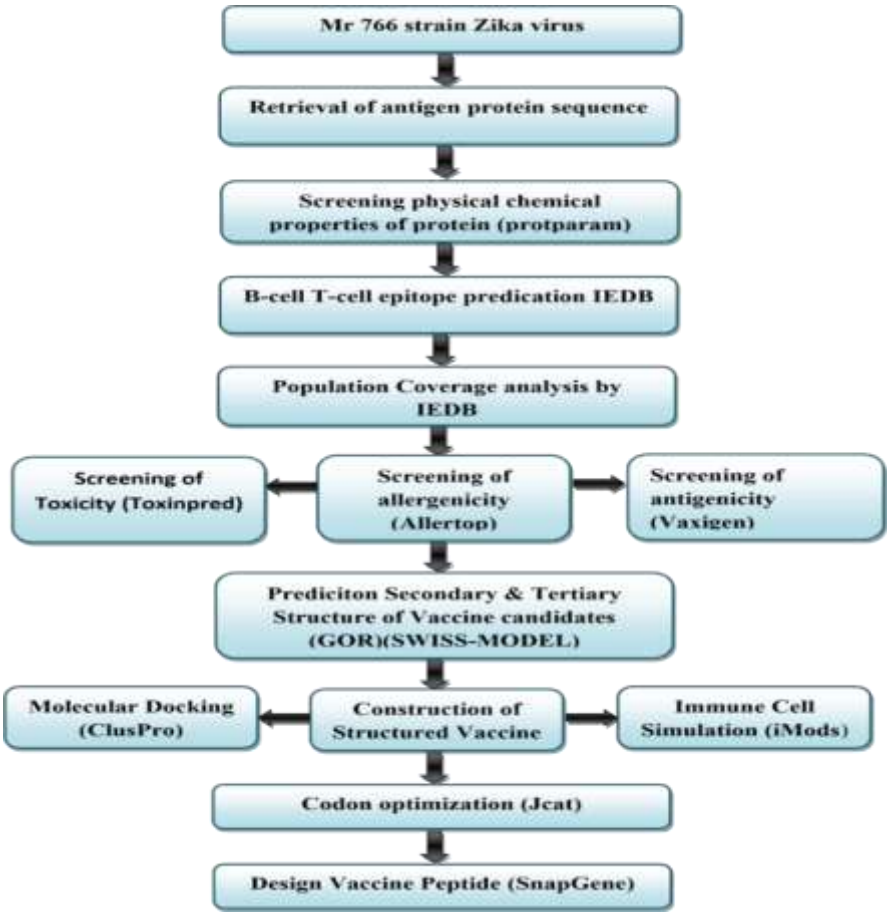


Figure 1. Pipeline of Vaccine design

Docking analysis

Molecular docking between the ligand binding domain of the TLR3 receptor (PDB ID: 2A0Z) and the designed vaccine was carried out using an online server ClusPro 2.0 (https://cluspro.bu.edu/). The server initially necessitates two Protein Data Bank (PDB) files for its fundamental functions. However, it also offers a range of advanced search customization options. These encompass integrating data from small-angle X-ray scattering (SAXS), constructing homo-multimers, implementing attraction or repulsion forces, incorporating pairwise distance constraints, eliminating unstructured protein segments, and identifying heparin-binding sites (Kozakov *et al*., 2017).

Molecular dynamics simulations

iModS (https://imods.iqfr.csic.es/) functions as an online platform utilized for conducting molecular dynamics simulations and predicting torsional angles within complexes. Furthermore, it delves into the collective motions exhibited by proteins and nucleic acids through Normal Mode Analysis (NMA) in internal coordinates. Additionally, it assesses structural deformations, eigenvalues of interacting residues, Root Mean Square Deviation (RMSD) values, covariance among individual residues, and the stability of the complex. These analyses are all conducted within the framework of iModS (López-Blanco *et al*., 2014).

Optimizing codons for a designed vaccine peptide to facilitate expression analysis

Codon optimization was then conducted via the Java Codon Adaptation tool (JCat) (http://www.jcat.de /), tailored for Escherichia coli K12 strain expression. Selection criteria included avoiding transcription termination, ribosome binding, and restoring cleavage sites. GC content percentage and codon adaptation index (CAI) were assessed, aiming for CAI scores between 0.8–1.0 and GC content within 30–70%. The E. coli pET-28(+) vector was chosen for expression. SnapGene tool facilitated vector selection, restriction site analysis (utilizing ECO53KI and BstZ17I sites), and in silico PCR for construct amplification (SnapGene, 2023).

RESULTS AND DISCUSSION

The ZIKV strain 766 protein sequence, extracted from the UniProt database, was assessed for antigenicity using VaxiJen 2.0. With a cutoff point adjusted to 0.425, the full-length polyprotein exhibited an antigenicity of 0.5191, indicating potential as an antigen. Physiochemical properties, analyzed via Protparam, revealed a molecular

weight of 378736.26 Da and 3419 amino acids. The theoretical isoelectric point (pI) was 8.64, suggesting a positive charge. The stability assessment showed a low instability index (II) of 37.33 and high thermostability with an aliphatic index of 86.93. Elemental composition analysis yielded C16829H26701N4687O4864S194.

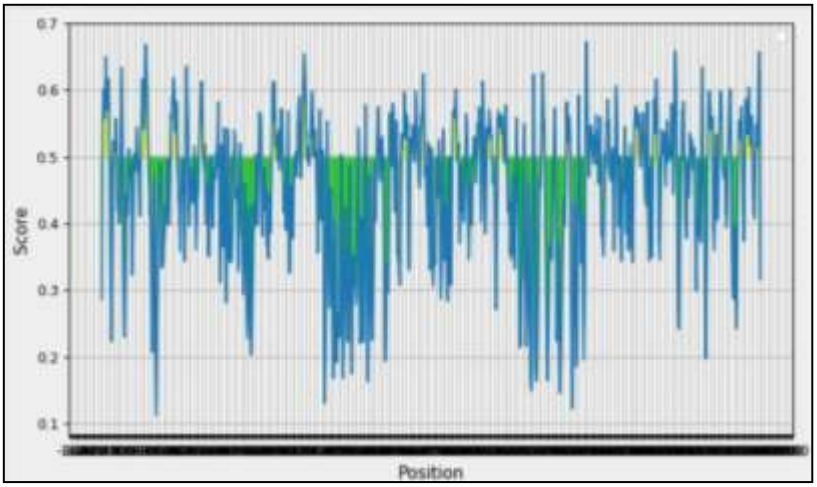


Figure 2. Antigenicity analysis by Kolaskar and Tongaonkar's method.

Historically, vaccine design primarily targeted MHC I and MHC II classes & B-cells using reverse vaccinology. However, our study expands this approach by including MHC-B and MHC-T classes with human leukocyte antigen (HLA) to broaden the spectrum. Recognizing the potential for antigenic drift, we meticulously curated a database of all conceivable E protein epitopes. Employing various prediction algorithms, including IEDB database approaches, we identified Bcell epitopes based on parameters like flexibility, hydrophilicity, and antigen propensity. Kolaskar and Tongaonkar's linear epitope calculations provided visual representations aiding epitope selection the antigenicity maximum propensity was 0.672 while the minimum tendency of antigenicity was 0.112. A threshold value greater than 0.500 was the candidate for antigenic dynamic and the value was set as 1.00. Additionally, T-cell epitopes with high activity against targeted alleles were isolated for further analysis Critical to vaccine development is ensuring antigenic and nontoxic properties of epitopes. We utilized VaxiJen2.0 for antigenicity assessment, where peptides with values above 0.4 were deemed effective. To predict toxicity, an SVM classifier in ToxinPred was employed. AllerTop v.2.0 evaluated allergenicity, filtering out epitopes with conserved protein sequences and high allergen probabilities. Furthermore, IEDB facilitated population coverage analysis for T-cell epitopes, considering the polymorphic nature of MHC molecules.

Table 1. B-cell epitope with their allergenicity, antigenicity, and toxicity for vaccine construction.

No	Start	End	Length	Peptide	Antigenicity	Allergenicity	Toxicity
1	215	222	8	AYLDKQSD	0.7221	non-allergen	Non-toxic
2	223	229	7	DMASDSR	0.5178	non-allergen	Non-toxic
3	230	236	7	VEVTPNS	1.5477	non-allergen	Non-toxic
4	237	243	7	IVIGVGD	1.5018	non-allergen	Non-toxic
5	244	252	9	LGLNTKNGS	2.2353	non-allergen	Non-toxic

Table 2. T-cell epitope MHC-I with their allergenicity, antigenicity, and toxicity for vaccine construction

No	Start	End	Length	Peptide	Antigenicity	Allergenicity	Toxicity
1	1976	1985	10	AETDEGHAHW	0.9095	non-allergen	Non-toxic
2	740	748	9	KSLFGGMSW	0.7431	non-allergen	Non-toxic
3	1286	1294	9	VPRTDNIAL	0.8391	non-allergen	Non-toxic

4	2425	2423	9	MTIDPQVEK	0.6412	non-allergen	Non-toxic
5	1126	1135	9	RPRKEPESNL	0.4537	non-allergen	Non-toxic

Table 3. T-cell epitope MHC-II with their allergenicity, antigenicity, and toxicity for vaccine construction.

No	Start	End	Length	Peptide	Antigenicity	Allergenicity	Toxicity
1	1487	1501	9	YVYVKTGKR	1.0935	non-allergen	Non-toxic
2	1486	1500	9	LVEFKDAHA	1.3339	non-allergen	Non-toxic
3	525	539	9	YVYVKTGKR	1.0935	non-allergen	Non-toxic
4	2945	2959	9	FWALVDRER	1.2821	non-allergen	Non-toxic
5	2109	2118	9	FKEFAAGKR	0.5479	non-allergen	Non-toxic

After stringent screening, we selected 5 B-cell epitopes table1, 5 MHC Class-II T-cell epitopes Table 2, and 5 MHC Class-I T-cell epitopes Table 3 as vaccine components. To enhance immune response, we incorporated the 50S ribosomal protein L3 as an adjuvant, boosting immune-reactive properties. Linking chosen epitopes with appropriate linkers, including GPGPG and AAY, facilitated multiepitope subunit vaccine construction. Notably, an EAAAK linker was included to attach the adjuvant to the first predicted B-cell epitope, enhancing fusion protein entanglement.

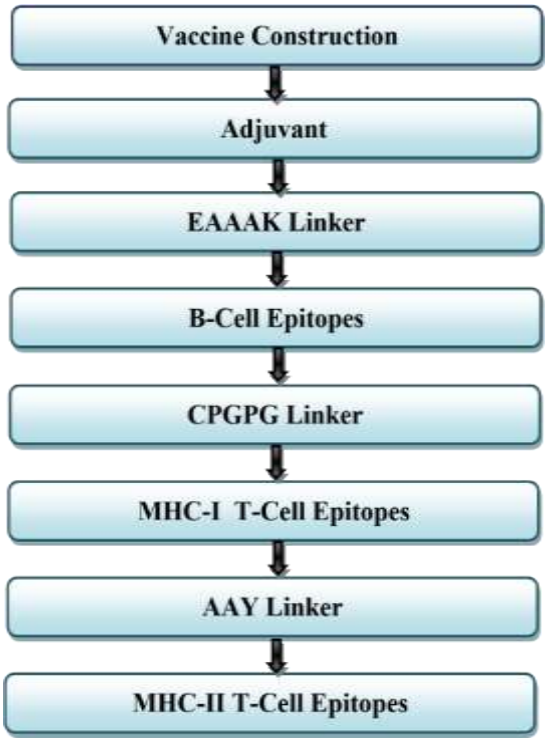


Figure 3. Vaccine Construction using B-cell & T-cell epitopes with linkers (EAAAK, CPGPG, AAY)

Ensuring safety and efficacy, we verified the absence of allergenic or poisonous qualities in the vaccine protein sequence. Despite minimal utility in some investigations, Vaccine protein antigenicity was predicted using VaxiJen 2.0: 0.6284 with adjuvant and 0.8291 without adjuvant, indicating high antigenicity for both formulations. Toxinpred analysis showed non-toxicity, and AllerTop predicted non-allergenicity, Physicochemical properties of the multi-epitope vaccine were analyzed using ExPASY ProtParam. The molecular weight was 38148.49 Da. The calculated pI was 9.55, indicating a basic nature. The instability index (II) was 18.46, suggesting stability. The aliphatic index was 76.96, and The GRAVY index was -0.381, indicating hydrophilicity and stability.The Solubility rate, determined via Protein-Sol, scored 0.678, denoting good solubility. Secondary and tertiary structures play pivotal roles in vaccine efficacy. Secondary structure analysis revealed helix and coil predominance, essential for stability.

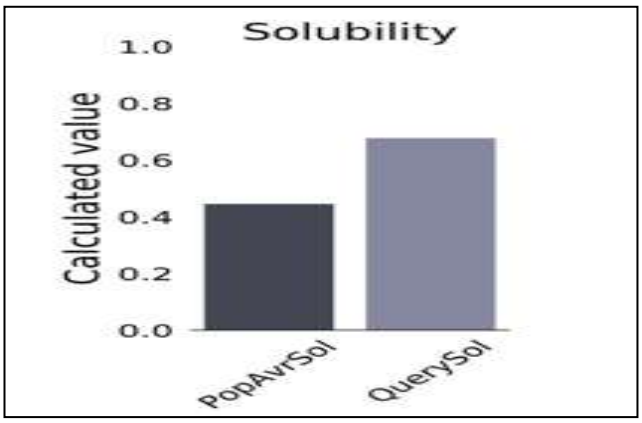


Figure 4. Solubility rate of multi-epitopes vaccine calculated through Protein-Sol.

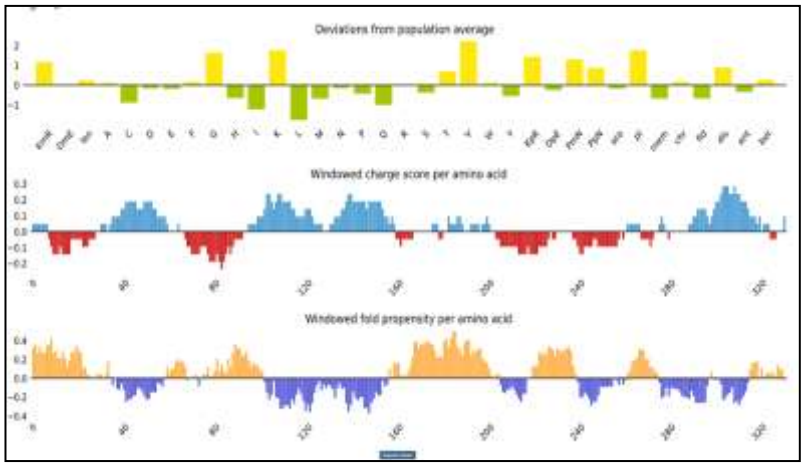


Figure 5. Deviation from population average and charge score of multi-epitopes. vaccine calculated through Protein-Sol server.

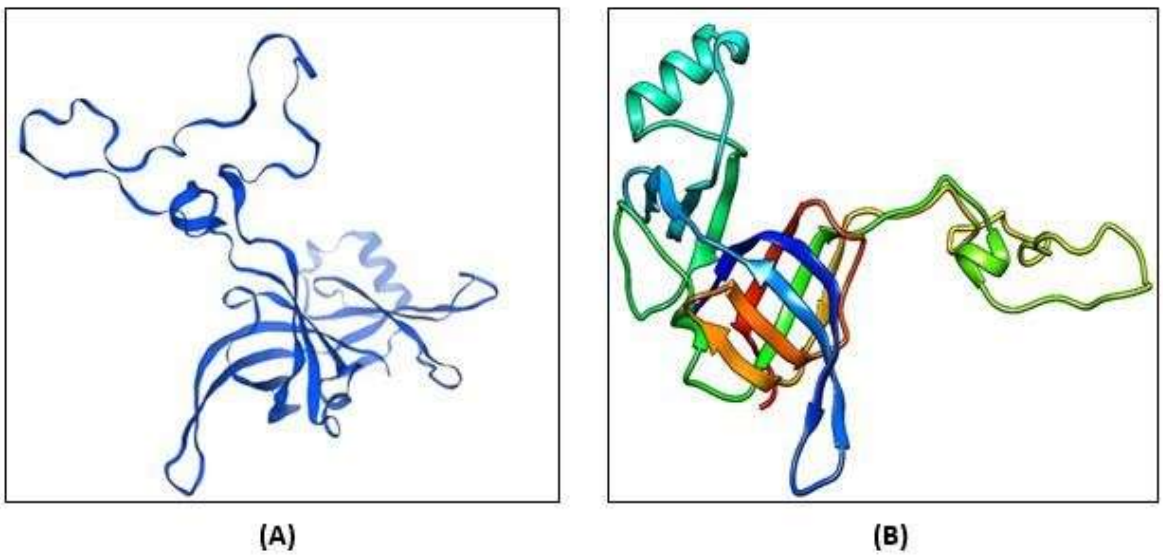
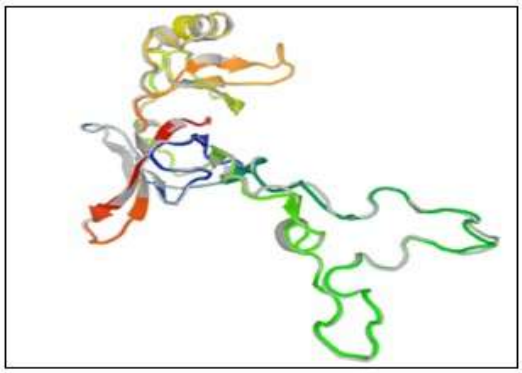


Figure 6. Prediction of the tertiary structure via (A) SWISSMODEL (B) visualization by a CHIMERA.

The vaccine construct was refined using GalaxyRefine, providing 5 models. Parameters included MolProbity (1.355), GDT-HA (0.9797), and RMSD (0.335). The clash score was 6.5, The Ramachandran's score reached 88.4%, and the poor rotamers score was 0.0. Model 4 which is the best was selected for further simulation.



Structure Information

Model	GDT-HA	RMSD	MolProbity	Clash score	Poor rotamers	Rama favored
Initial	1.0000	0.000	1.010	0.6	0.6	95.7
MODEL 1	0.9844	0.314	1.449	8.3	0.6	98.6
MODEL 2	0.9821	0.339	1.449	8.3	0.6	98.6
MODEL 3	0.9773	0.343	1.388	7.1	0.0	99.0
MODEL 4	0.9797	0.345	1.355	6.5	0.0	98.1
MODEL 5	0.9809	0.311	1.489	9.2	0.6	98.6

(A) (B)

Figure 7. (A) Refined 3D-Strucutre of constructed vaccine (B) Table of Refined 3D-Strucutre information.

PROCHECK validated the vaccine's refined structure. The Ramachandran plot showed 97.1% of the structure in the favoured region, 0.0% in the disallowed region, and 2.9% in the allowed region.

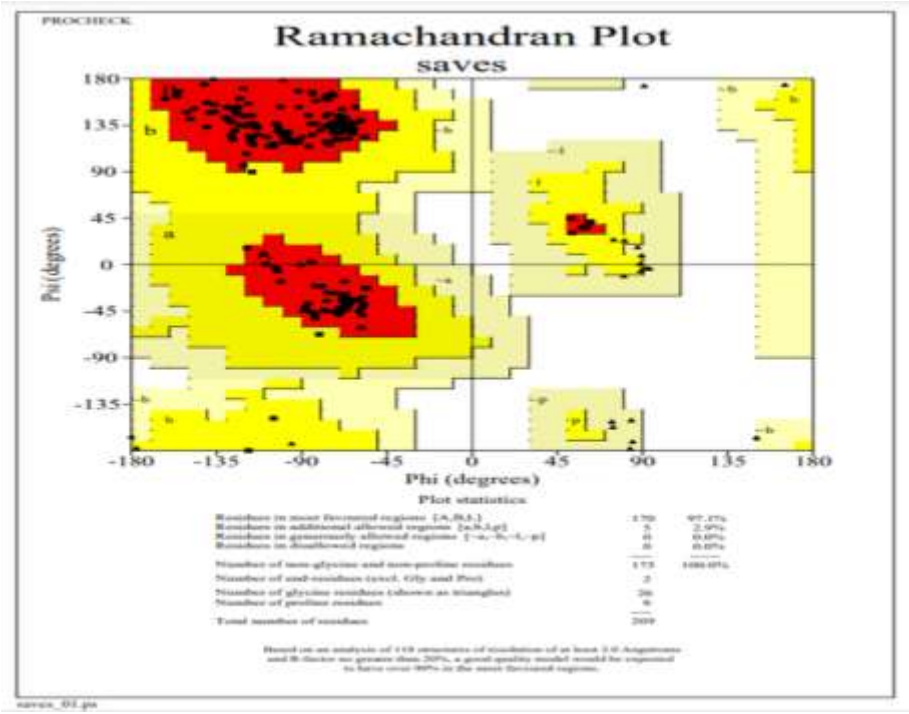


Figure 8. Ramachandran plot for Validation of structure by using PROCHECK Server.

Molecular dynamics simulations provided insights into dynamic behavior, revealing decreased risks of deformation during immune response. Covariance matrix analysis corroborated immune response simulation, indicative of vaccine efficacy. Our proposed V1 Multi Epitope Vaccine presents a significant candidate against ZIKV infections, warranting further in vitro and in vivo validation. iMODS, an online server, analyzes complex structures using adjusted force fields with varied time intervals, revealing minimal deformation at each residue. The complex eigenvalue is 4.368016e-06, indicating improved residue interaction. Low RMSD and correlated areas in heat maps

depict better residue interaction. The figures illustrate protein structure with MNA mobility, low deformability, B-factor, eigenvalue (1.837617e-06), and variance. The elastic network and covariance of the complex are also highlighted. In silico cloning involved designing vaccines with suitable linkers and adjuvants for efficient expression in E. coli. The Java codon adaptation tool optimized the nucleotide sequences for prokaryotic organisms, yielding a CAI of 0.95 for both original and adapted sequences. The optimized sequence, with 1050 nucleotides and 49.52% GC content, was cloned into the pET-28a (+) vector using SnapGene software, resulting in a recombinant plasmid.

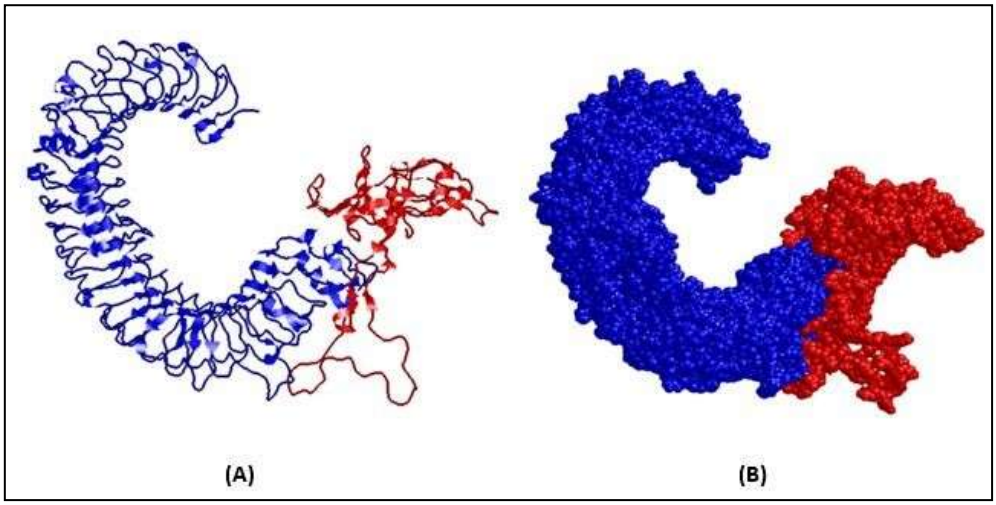


Figure 9: Best docked model of multi-epitope vaccine/receptor using ClusPro visualized by Rasmol (A) Molecular Surface model that blue indicates the receptor protein TLR3 & red indicates the peptide (B) cartoon model that blue indicates the receptor protein TLR3 & red indicates the peptide

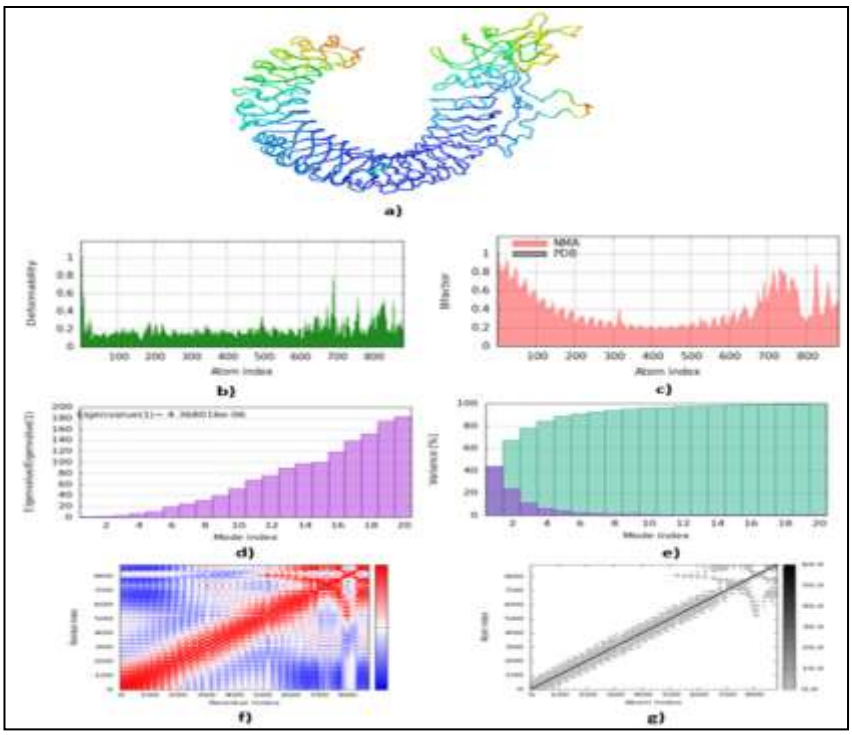


Figure 10. Molecular dynamics simulations of the docked complex. a) MNA mobility of protein structure, b) deformability of complex, c) B-factor, d) Eigenvalue, e) cumulative variance shown in green color and variance in red color, f) correlated, anti-correlated and non correlated variance map indicated by red, blue and white color, respectively, and g) shows elastic network, h) stiffer regions indicated by darker grey color.

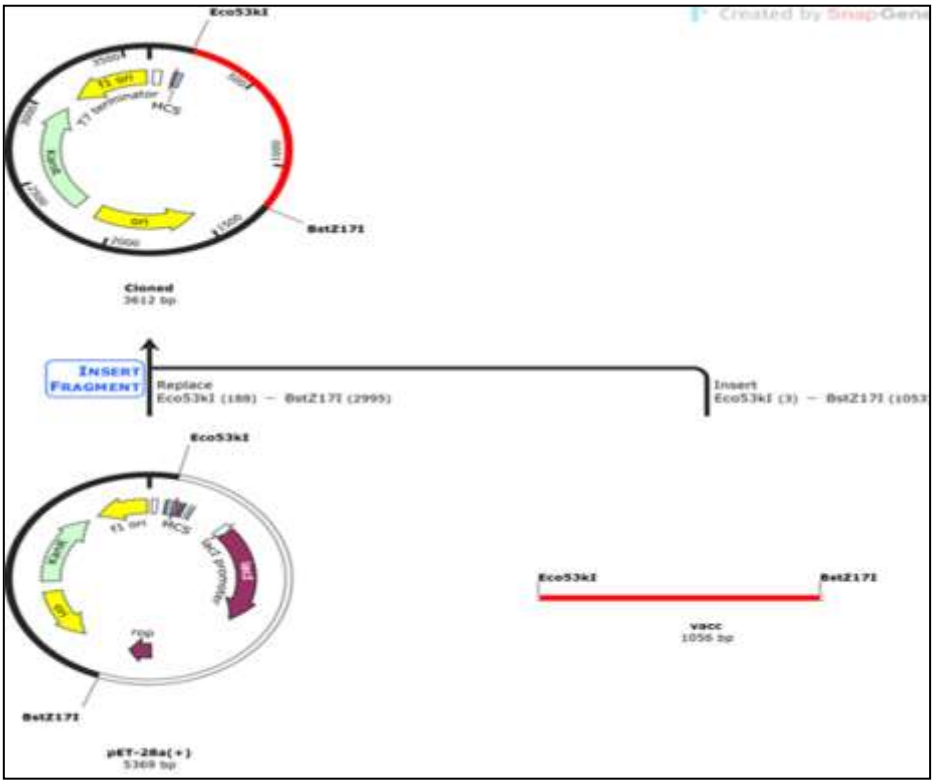


Figure 11. In Silico PCR amplification of vaccine construct followed by addition of restriction sites and cloning in pET-28a (+) vector.

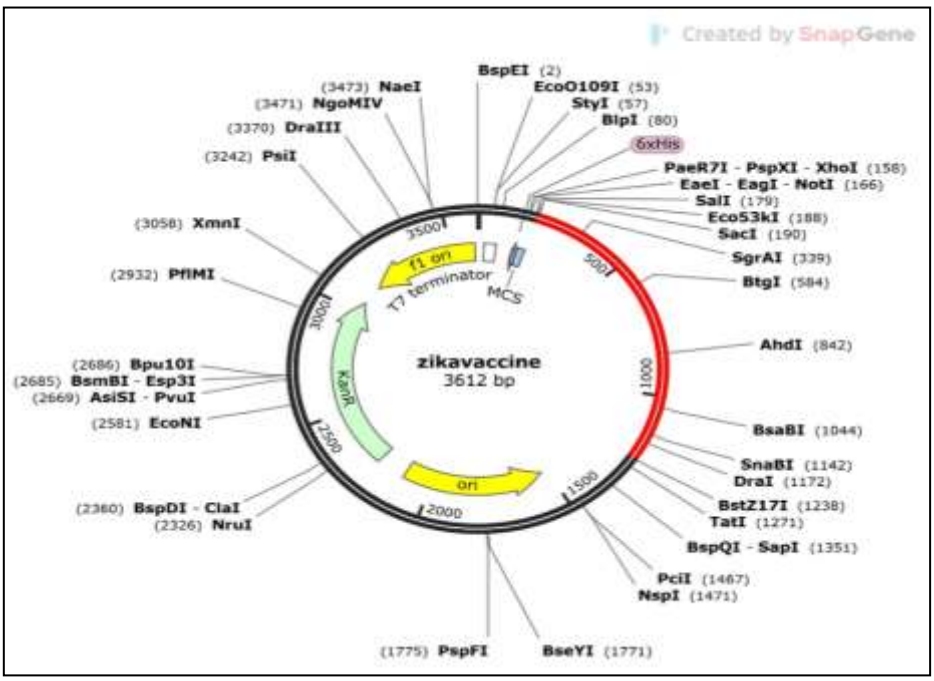


Figure 12. The optimized sequence was cloned in pET-28a (+) vector to make a recombinant plasmid after being amplified through in silico PCR by using SnapGene software.

CONCLUSION

In silico vaccine design presents a promising avenue for identifying potential candidates suitable for clinical trials, especially when aiming for broad population coverage and robust immune responses. In this study, we employed various computational methodologies to develop an effective vaccine targeting the Polyprotein of ZIKV Strain MR766. Utilizing immunoinformatics approaches, we predicted B-cell and T-cell epitopes crucial for eliciting immune responses. Molecular docking analysis conducted with ClusPro revealed a notable binding energy of 794.6.1 kcal/mol. Assessment via the Ramachandran plot demonstrated that 97.1% of the structure resided in the favored region, indicating favorable protein conformation. Furthermore, protein expression analysis using SnapGene indicated promising expression levels for our construct. Through extensive in silico trials, our vaccine exhibited promising immune responses. Nonetheless, further validation through in vitro and in vivo experiments is imperative to consolidate the findings of this study. Our proposed vaccine fulfilled essential criteria, including antigenicity, allergenicity, toxicity, and other physiochemical properties. In conclusion, while our designed vaccine construct appears safe, rigorous preclinical validation is warranted before proceeding to clinical trials.

ACKNOWLEDGEMENTS

The authors would like to thank Prof. Ashok V. Tejankar, Principal, Deogiri College (Autonomous), Chhatrapati Sambhajinagar for providing kind support and infrastructural facility.

REFERENCES

Dawes, B. E., Smalley, C. A., Tiner, B. L., Beasley, D. W., Milligan, G. N., Reece, L. M., Hombach, J., & Barrett, A. D. (2016). Research and development of Zika virus vaccines. *NPJ Vaccines*, 1, 16007. https://doi.org/10.1038/npjvaccines.2016.7.

de Araújo, T. V. B., Ximenes, R. A. A., Miranda-Filho, D. B., Souza, W. V., Montarroyos, U. R., de Melo, A. P. L., Valongueiro, S., de Albuquerque, M. F. P. M., Braga, C., Filho, S. P. B., Cordeiro, M. T., Vazquez, E., Cruz, D. D. C. S., Henriques, C. M. P., Bezerra, L. C. A., Castanha, P. M. D. S., Dhalia, R., Marques-Júnior, E. T. A., Martelli, C. M. T., Rodrigues, L. C., … State Health Department of Pernambuco (2018). Association between microcephaly, Zika virus infection, and other risk factors in Brazil: final report of a case-control study. *The Lancet. Infectious Diseases*, 18(3), 328–336. https://doi.org/10.1016/S1473-3099 (17)30727-2

Dhanda, S. K., Mahajan, S., Paul, S., Yan, Z., Kim, H., Jespersen, M. C., Jurtz, V., Andreatta, M., Greenbaum, J. A., Marcatili, P., Sette, A., Nielsen, M., & Peters, B. (2019). IEDB-AR: immune epitope database-analysis resource in 2019. *Nucleic Acids Research*, 47(W1), W502–W506. https://doi.org/10.1093/nar/gkz452.

Dick, G. W., Kitchen, S. F., & Haddow, A. J. (1952). Zika virus. I. Isolations and serological specificity. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 46(5), 509–520. https://doi.org/10.1016/0035-9203 (52)90042-4.

Gupta S, Kapoor P, Chaudhary K, Gautam A, Kumar R, Open Source Drug Discovery Consortium , Raghava G. (2013) In Silico Approach for Predicting Toxicity of Peptides and Proteins. *PLoS ONE* 8(9): e73957. doi:10.1371/journal.pone.0073957,

Hickman, H. D., & Pierson, T. C. (2017). T Cells Take on Zika Virus. *Immunity*, 46(1), 13–14. https://doi.org/10.1016/j.immuni.2016.12.020.

Ko, J., Park, H., Heo, L., & Seok, C. (2012). GalaxyWEB server for protein structure prediction and refinement. *Nucleic acids research*, 40(Web Server issue), W294–W297. https://doi.org/10.1093/nar/gks493.

Kozakov, D., Hall, D. R., Xia, B., Porter, K. A., Padhorny, D., Yueh, C., Beglov, D., & Vajda, S. (2017). The ClusPro web server for protein-protein docking. *Nature Protocols*, 12(2), 255–278. https://doi.org/10.1038/nprot.2016.169.

Krauer, F., Riesen, M., Reveiz, L., Oladapo, O. T., Martínez-Vega, R., Porgo, T. V., Haefliger, A., Broutet, N. J., Low, N., & WHO Zika Causality Working Group (2017). Zika Virus Infection as a Cause of Congenital Brain Abnormalities and Guillain-Barré Syndrome: Systematic Review. *PLoS Medicine*, 14(1), e1002203. https://doi.org/10.1371/journal.pmed.1002203.

Kuno, G., & Chang, G. J. (2005). Biological transmission of arboviruses: reexamination of and new insights into components, mechanisms, and unique traits as well as their evolutionary trends. *Clinical Microbiology Reviews*, 18(4), 608–637. https://doi.org/10.1128/CMR.18.4.608-637.2005.

López-Blanco, J. R., Aliaga, J. I., Quintana-Ortí, E. S., & Chacón, P. (2014). iMODS: internal coordinates normal mode analysis server. *Nucleic acids research*, 42(Web Server issue), W271–W276. https://doi.org/10.1093/nar/gku339

Musso, D., Ko, A. I., & Baud, D. (2019). Zika Virus Infection - After the Pandemic. *The New England Journal of Medicine*, 381(15), 1444–1457. https://doi.org/10.1056/NEJMra1808246.

Prasasty, V. D., Grazzolie, K., Rosmalena, R., Yazid, F., Ivan, F. X., & Sinaga, E. (2019). Peptide-Based Subunit Vaccine Design of T- and B-Cells Multi-Epitopes against Zika Virus Using Immunoinformatics Approaches. *Microorganisms*, 7(8), 226. https://doi.org/10.3390/microorganisms7080226.

SnapGene. (Version 7.1) [Computer software]. (2023). *GSL Biotech LLC*. Available at https://www.snapgene.com

World Health Organization. (2014). A global brief on vector-borne diseases. *World Health Organization*. https://iris.who.int/handle/10665/111008