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In-Silico Approach for Zika Vaccine Designing by Using ZIKA Protein Serine Protease ns3

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Abstract **Original Research Article**

The mosquito-borne flavivirus known as the Zika virus is now a growing worldwide public health problem. An RNA virus called (ZIKV) has spread through mosquito bites. There are currently no Zika vaccinations on the market, and the handful that are are still in scientific studies. Since little is currently recognized about the toxicity, genetic variety, and effects of ZIKV contamination, there's an urgent want for a preventive vaccination against the virus. The traditional vaccination strategy administers live-attenuated or inactivated vaccinations, which are dangerous and cause the illness to worsen. An immunoinformatics method changed into used to design and create a multi-epitope vaccine against Zika, taking into account the necessity for a more secure vaccination. In order to anticipate MHC class-I and class-II epitopes, the ZIKA protein serine protease ns3 was taken from the database and employed in various analyses which include physiochemical analysis, allergenicity, antigenicity, and toxicity. A 50S ribosomal protein L7/L12 became inserted on the N-terminal of the vaccine, followed by CTL and HTL related by corresponding linkers. For the developed vaccine, linear B-cell epitopes were predicted after which physiological parameters had been evaluated. The development was non- allergic and antigenic. It became located that the vaccination becomes safe for allergic reactions and produced an antigen response. After that, the vaccine design became modeled and docked on the TLR4 receptor to see if it would cause an immunological response. For fifty ns, the docked complex turned into further simulated. Ultimately, the vaccine design was cloned in-silico into the pet28a (+) vector in order to use His-tag for affinity purification. It's likely that the vaccine will be advanced as a Zika vaccine. To make certain the legitimacy of its expression efficiency, the construct underwent in silico cloning. The creation of vaccines may continue greater speedy thanks to those computational methods. Additionally, they aid in enhancing the effectiveness of vaccinations. Additional research, which include immunological and experimental trials, can be required to confirm the construct in a real-time situation.

Keywords: Zika · Multi-epitope vaccine · MD simulation · In-silico cloning - Vaccine Immune response.

INTRODUCTION

The Zika virus, belonging to the Flaviviridae family, this virus has sparked curiosity due to its sudden emergence and rapid spread, particularly during the outbreaks that began in Brazil in 2015. Initially found in the Zika Forest in Uganda in 1947, the virus remained largely unfamiliar until it caused outbreaks in the Americas, bringing it to the attention of the public. (Younger, 2016)

One of the main reasons for fear about the outbreaks was the association that was made between the Zika virus and severe neurological disorders, particularly in pregnant women and their fetuses. Pregnancy-related Zika virus infection has been linked to microcephaly, a disorder characterized by abnormally tiny heads and associated developmental abnormalities in a developing child. It has been associated with birth defects and certain

neurological disorders, such as adult Guillain-Barré syndrome. (Massad *et al.,* 2017)

Biting an infected Aedes mosquito, especially an Aedes aegypti or an Aedes albopictus, is the primary approach of Transmission of the Zika virus (Musso & Gubler, 2016) Nonetheless, the virus is also able to be transmitted via sexual intercourse, receiving blood from an infected individual, and from a mother to her unborn child during pregnancy. This complex way of spreading the virus created difficulties for public health efforts, requiring thorough plans to manage mosquito populations and reduce the virus transmission through other methods. Extensive measures were taken to investigate the epidemiology of Zika virus, create diagnostic tools, and speed up research on vaccines and treatments in reaction to the outbreaks. Public health initiatives promoted the significance of controlling mosquitoes, practicing personal protection, and safe sexual behaviors, especially for pregnant women and individuals intending to conceive. (Freitas *et al.,* 2016)

To evaluate a vaccine's toxicity, antigenicity, allergenicity, and physiochemical analysis, many methods are used. Any vaccine's allergenicity can be assessed the usage of a bioinformatics method. A bioinformatic tool can check out a vaccine's toxicity, and Allertop v2. zero and vaxijen v2.0 can be used to assess a vaccine's antigenicity. the use of expasy (protparam), the physiochemical evaluation of the vaccine is performed. This entails figuring out the molecular weight, theoretical ph, instability index, atomic composition, and predicted half-life of every amino acid in the vaccination series. To make certain that the vaccination won't have any dangerous outcomes on human cells, toxinpred is applied. The approach called iedb is used to expect vaccine epitopes on B and T cells. (Antonelli *et al.,* 2022) Next, adjuvant is used to unite the various vaccine additives, and linkers like EAAK, AAY, and GPGPG are used to connect these components collectively to create the final vaccine. The advent of vaccines may additionally continue extra fast way to those computational methods. (Ezzemani *et al.,* 2023)

There is still a chance for another Zika virus outbreak come even though the intensity of outbreaks has decreased in some regions from their height. Planning, monitoring, and research activities must continue to understand the dynamics of the virus, identify the outbreaksmore quickly, and put effective control and prevention measures in place. In addition, one of the main priorities for protecting public health and lessening the effects of upcoming epidemics continues to be the development of vaccines and therapies. (Bogoch *et al.,* 2016)

MATERIAL AND METHODS

Sequence Retrieval from serine protease ns3 for vaccine desiging

serine protease NS3 in Zika virus is essential for viral replication by cleaving viral polyprotein. It processes the viral genome, facilitating the production of individual viral proteins. Immunoinformatics methodologies were employed to formulate an immunogenic multi-epitope subunit vaccine targeting Zika virus infection. (Pruitt *et al.,* 2009) The amino acid sequence of ZIKA serine protease ns3 was obtained protein with accession number (8AQB) from NCBI. Protein sequence was further subjected to the various epitope's prediction (Van Regenmortel & De Marcillac, 1988)

Linear B cell epitopes prediction

From the Immune Epitope Database (IEDB) the B cellular epitope prediction service becomes capable of predict linear B cellular epitopes. This method significantly improves the accuracy of B cellular epitope prediction.

MHC-II specific HTL epitope prediction

MHC molecules have an impact on host immune responses and are essential for adaptive immunity. Derived from extracellular overseas proteins, MHC magnificence II peptides spark off CD4+ T-lymphocytes, coordinating humoral and adaptive immune responses to combat infectious illnesses. Because HTL epitopes are involved in robust immune responses, predicting them is essential.(Greenbaum *et al.,* 2007) Viral protein sequence turned into examined the usage of the Immune Epitope Database MHC-II prediction module (http://gear.iedb.org/mhcii/) and the netmhciipan-4.1 method to locate HTL epitopes, which gave records on how nicely they bound to sure MHC-II peptides..(Nielsen *et al.,* 2010)

MHC-I specific CTL epitope prediction

Cytotoxic T lymphocytes (CTLs) are critical additives of the adaptive immune reaction and are critical within the destruction of diseased or malfunctioning cells. CD8+ T cells are capable of become aware of peptides that are provided by way of MHC-I complexes. at the same time as overseas antigens prompt CTLs to start a cytotoxic immune reaction, self-peptides regularly do now not elicit an immunological response. for this reason, the identity of CTL epitopes is important for the improvement of vaccines and immunotherapeutic strategies to fight infectious problems together with Zika fever.(Kar *et al.,* 2018) The Immune Epitope Database MHC-I prediction module and the NetMHCPan 4.1 EL (epitope prediction) method, which predicts CTL epitopes, were used to evaluate all the Zika virus protein a good way to locate those epitopes..(Alam *et al.,* 2019)

Designing Multi-epitope Vaccine

The peptides and crucial linkers were progressively combined to generate the final vaccine design. To boost immunogenicity against viral

infections, the N-terminal of CTL epitopes was fused with 50S ribosomal protein L7/L12. Adjuvant and CTL epitopes seemed to be connected by linkers such as EAAAK and AAY, which allowed for appropriate folding and functioning. The vaccine design was also completed by identifying and coupling HTL epitopes with the last CTL epitope using the GPGPG linker and the 6X His-tag.(Meza *et al.,* 2017)

Assessment of Antigenicity, Allergenicty, and Physiological parameters

To verify a vaccine's allergy safety, allergen predictions are done using the AllerTOP v. 2.0 server Antigenicity was determined using the VaxiJen v2.0 server which has a high accuracy range of 70% to 89% depending on the target organism. Wu et al. (2016) to be able to verify the vaccine's layout, physiochemical metrics along with putative pI, instability index, half of-existence, balance profile, aliphatic index, and Grand not unusual of Hydropathy (GRAVY) were tested the usage of the ProtParam service. (Costa *et al.,* 2022).

Tertiary structure prediction, refinement, and validation

The SCRATCH Protein Predictor service changed into used to run the amino acid series in order to expect the three-dimensional structure of the vaccine construct. that utilizes threading assembly simulations for automated structure prediction. Next refinement was performed using the Galaxy Refine server, employing mild and aggressive relaxation methods to enhance the overall protein structure quality by over 50%. The best model was chosen after validation by the SAVES server, which employs various tools like ERRAT, Verify-3D, and Ramachandran plot to refine and validate the structure for further analysis.

Docking of the vaccine candidate and immune receptor TLR4

using the Cluspro 2.0 the silico method of molecular docking became used to determine the binding affinity among our proposed peptide vaccine and immune receptor TLR-four (PDB identity: 5AWA). (Farhadi *et al.,* 2016) A protein-protein docking analysis changed into carried out to expose that the vaccine would possibly engage with numerous crucial molecular immunological components, including TLRs. The analysis of protein-protein docking blanketed importing the PDB record containing the vaccination protein and TLR-four receptor to the server. ClusPro is a molecular docking algorithm that find on geometry-based principles to create docking transformations, resulting in best molecular shape complementarity between interacting molecules. (Honegr *et al.,* 2018)

Molecular dynamics simulation of designed vaccine candidate and TLR4 recepter

In molecular dynamics simulations different softwere are used to find out the behavior and structural dynamics of biomolecular systems. we used the dashmond server tool for molecular dynamics simulation the link of this softwere are showing following One such term is Root Mean Square Deviation (RMSD), which define the average deviation between the atomic positions of a reference structure and those in a trajectory of the simulation.(Albekairi *et al.,* 2022) since lower RMSD values advise extra structural balance, RMSD is a beneficial tool for tracking the steadiness and conformational modifications of biomolecules over the years. The foundation suggest square Fluctuation (RMSF) approach is the subsequent helpful technique. It calculates the common deviation of atomic locations from their suggest positions at some stage in the simulation. Higher RMSF values suggest more flexibility. RMSF gives records at the dynamic behavior and versatility of individual residues or atoms in a biomolecular device. Locating the residues containing Secondary structure elements (SSE) is essential for studying the steadiness and conformational changes of secondary structure elements at some point of simulation, which include alpha helices and beta strands. (Khan *et al.,* 2021). Through the evaluation of SSE residues, scientists can also check out the impact of mutations or ligand interaction on the steadiness of secondary systems. Moreover, they could pick out alterations inside the secondary shape composition and times of folding or unfolding. These skills greatly contribute within the expertise of the structural dynamics and behavior of biomolecular structures using molecular dynamics simulations. (Honegr *et al.,* 2018).

High-fidelity simulation of the proposed Zika vaccine

The C-immsim server is a important computational device inside the region of in silico vaccine layout. It allows researchers to simulate and ascertain the immune responses that could reply favorably to vaccine applicants. Some of the several elements of the immune system, innate and adaptive immunity alter the tricky biological techniques called immunological responses. (Usman Mirza *et al.,* 2016) when T and B cells, kinds of immune cells, recognize antigens and end up activated, they proliferate rapidly. This outcomes in the production of memory immune cells and antibodies. In the field of vaccine development, scientists simulate the interactions between antigens and immune cells using in silico simulations the use of programs such as C-ImmSim to predict and improve vaccine candidates. the use of antigen sequences or structures on the server, researchers may also mimic the processing of antigens, T cell activation, antibody production, and the development of memory immune responses (Ezzemani *et al.,* 2023). Nothing in the way of material is obtainable. The study demonstrated that the vaccine successfully elicited a noteworthy initial immune response, as seen through a modern rise in the concentrations of many immunoglobulins, consisting of igg1, igg2, igg, and igm antibodies. The use of a computer to fast examine and select capacity vaccine candidates primarily based on their expected immunogenicity and effectiveness increases the

performance of vaccine development. Effective immunization towards infections and different illnesses can be developed greater without difficulty and with a higher information of immune responses way to the use of computer simulations in vaccine introduction procedures. (Prasasty *et al.,* 2019)

In silico cloning of final vaccine construct

To clone and produce the multiepitope-based totally subunit vaccine, the protein collection of the very last vaccine construct became opposite transcribed into an optimized coding sequence using the Java Codon model program. .(Ghosh *et al.,* 2021) since there was no possible virus to use as an origin, Escherichia coli became decided on as the host organism for the expression. other possibilities had been also taken into consideration To assure perfect expression situations, the proportion guanine-cytosine (GC) awareness, codon adaptive index (CAI), and codon use distribution have been evaluated. (Zheng *et al.,* 2021)

The pET28a (+) vector was selected due to its most use for high-yield protein expression and its inclusion of advantageous features like the His-tag and lac operon. The map of the vector along with the construct is showed with the help of Snap Gene viewer. In silico cloning of the optimized DNA sequence into the pET28a (+) vector was performed, utilizing the BssSI and EcoNI restriction sites present in the vector. (Ghosh *et al.,* 2021)

RESULTS AND DISCUSSION

Selection of antigenic protein for the sequence retrieval

The zika virus proteome often consists of both structural and non-structural proteins, and following a review of the literature, we discovered that the serine protease non-structural proteins of the virus are mostly in control of their replication in host. Here, in this work, we have focused on the non-structural proteins, in order to create a vaccine. The amino acid sequence of ZIKA serine protease ns3 was obtained protein with accession number (8AQB) from NCBI in the fasta format (Smiline Girija, *et al.,* 2020)

Antigenic B cell epitopes prediction from Zika virus protein

B cell epitopes that were forecasted through the IEDB (Immune Epitope Database) server. the b cell epitopes showing in two way one is the table and anather is the graph form the table provides details like peptide length and epitope start/end points. Epitopes are particular antigen regions identified by B cells, a white blood cell type in the immune system. The peptide's size indicates the quantity of amino acids contained within each epitope. (Table 1) Beginning and ending locations indicate the points in the antigen sequence where each epitope starts and finishes. The table provides valuable information on predicted B cell epitopes, aiding in the understanding of immune responses and informing the design of vaccine development strategies (Bhattacharya, 2020) Next the B cell epitopes prediction representation in graph Yellow lines on the graph represent B cell epitopes with a score exceeding 0.50. Certain epitopes are believed to have a greater likelihood of triggering an immune response. (Fig 1)

B cell epitopes that have a score higher than this threshold are depicted in yellow lines on the graph, indicating these specific B cell epitopes. Epitopes with a score lower than 0.50 appear in green. Non-B cell epitopes are illustrated by green lines on the graph. The graphs are generated using the IEDB server to assist researchers in pinpointing the crucial areas for the immune system's detection (Kumar, 2024)

Table 1: Predicted B-cell Epitopes from IEDB server

No.	Start	End	Peptide	Length
1	6	29	WDVPAPKEVKKGET	14
2	29	35	RRLLGST	7
3	63	73	EGRLDPYWGDV	11
4	82	93	GPWKLDAAWDGL	12
5	105	121	ERAKNIQTLPGIFKTKD	17
6	131	140	YPAGTSGSPI	10

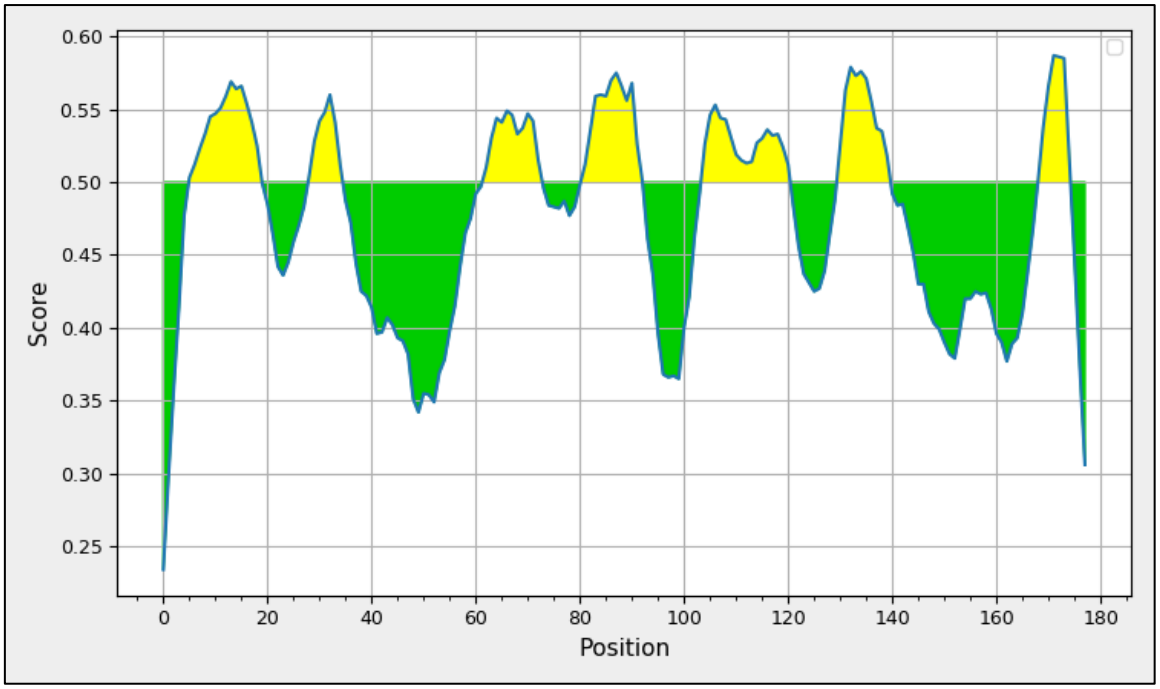


Fig. 1: showing the B cell epitopes in graphically

Antigenic MHC class I epitopes prediction from serine protease ns3

Using the IEDB MHC class I server, non-structural proteins for human alleles were used to predict the MHC-I binding epitopes. The best epitopes were selected for every a stronger affinity for the receptor molecule by obtaining the lowest percentile rank cutoff score of 0.40 for the protein. we selected total ten epitopes that show antigenicity and eligible for vaccine design to enhance our immune system we found MHC class 1 epitopes that are antigenic, or able to elicit the production of antibodies, by targeting the serine protease NS3 protein. **(Table 2**) Another objective of mine is to choose epitopes that do not result in toxicity or allergic reactions. These features are necessary for developing a vaccine, hence the epitopes that shown solubility were also chosen (Agarwal, 2022)

Table 2: Selected Epitopes of MHC Class I Epitopes

MHC Class I Epitopes	Antigenicity	Solubility	Toxicity	Allergenicty
AITQGKREE				
AITQGKREEE				
APKEVKKGE				
APKEVKKGET				
AVPPGERAKN	Antigenic	Good Water Soluble	Non-Toxic	Non-Allergic
DGDIGAVAL				
DIGAVALDY				
DIGAVALDYP AKNIQTLPGI				
ALDYPAGTS				

Antigenic MHC class II epitopes prediction from serine protease ns3

MHC-2 specific HTL epitopes were also predicted by using IEDB server with their start and end details. For the objective of predicting antigenic epitopes (B-cell, HTL, and CTL) that are probably maintained across the various zika virus species and that will need to recognize human T-cell and B-cell receptors in order to potentially elicit an immune response against the simplest method is offered by immunoinformatic servers, which reduces the amount of work required for epitope identification experiments **(Table 3)**. Numerous researchers in the field of vaccination are showing the antigenicity of peptide vaccines against a variety of infectious illnesses with the use of the reverse vaccination strategy. MHC class II peptides activate CD4+ T-lymphocytes; they are produced from extracellular foreign proteins. we selected total seven epitopes that show antigenicity and eligible for vaccine design to enhance our immune system we found MHC class 2 epitopes that are antigenic, or able to elicit the production of antibodies, by targeting the serine protease NS3 protein. (Bhattacharya, 2020)

Table 3: Selected Epitopes of MHC Class II Epitopes

MHC Class II Epitopes	Antigenicity	Solubility	Toxicity	Allergenicity
AKNIQTLPGIFKTKD				
ALWDVPAPKEVKKGE				
APKEVKKGETTDGVY				
APKEVKKGETTDGVY	Antigenic	Good Water Soluble	Non-Toxic	Non-Allergic
DGDIGAVALDYPAGT				
DIGAVALDYPAGTSG				
AGTDGPQTESTENSK				

Assessment of Antigenicity, Allergenicity, toxicity, and Physiological parameters:

The physiochemical properties were determined based on a number of parameters, including the instability index, which was 29.89 (it should have been less than 40), indicating the vaccine candidate's stability. Next, the half-life of the protein was estimated using models such as in vitro mammalian reticulocytes, yeast in vivo, and Escherichia coli in vivo, using the N-terminal residue (N-end rule) as a foundation. This indicates the time after protein production occurs when a portion of the protein disappears from the cell. Consequently, we found that the estimated half-life 30 hours (mammalian reticulocytes, in vitro) >20 hours (yeast, in vivo). >10 hours (Escherichia coli, in vivo) **(Table 4)**

VexiGen 2.0 makes use of a method that selects epitopes according to their antigenicity. This ensures that the epitopes are capable of producing antibodies, which is an essential necessary condition for an immune response to be effective. AllerTOP was used in order to assess the possibility for allergic reactions, with the goal of ensuring that the selected epitopes do not elicit allergic responses from the immune system. The use of SoluProt allowed for the evaluation of solubility, which confirmed that some epitopes are capable of dissolving in solution. This is an essential component in the process of developing vaccines. The evaluation of toxicity: To ensure that the vaccination is safe, Toxin Pred was used to identify epitopes that did not possess hazardous qualities. This was done in order to examine the vaccine's toxicity. Through the use of these techniques, I am able to narrow down the selection from thousands of epitopes to those that possess the necessary characteristics of being antigenic, non-toxic, non-allergenic, and soluble. There is a significant possibility that these antigenic areas might be used to develop a vaccination against the particular enzyme NS3 protein. (Mohammadi, 2023)

Table 4: Physiochemical properties of vaccine construct

Number of amino acids	333
Theoretical pI	5.43
Estimated half-life	30 hours (mammalian reticulocytes, in vitro) >20 hours (yeast, in vivo). >10 hours (Escherichia coli, in vivo).
Instability index	29.89 (stable)
Total number of atoms	4890
Aliphatic index	79.25
Grand value of hydropathicity	-0.458
Molecular weight	35051.80

Structure prediction and model quility assesment of the designed zika vaccine candidate

Predicting the 2D and 3D structure of a designed Zika vaccine candidate is a complex process that involves multiple computational methods and tools. The representation shows a protein's two-dimensional structure, with various structural components being highlighted in different colors. To be exact, the coil regions are gray, the beta strand portions are yellow, and the alpha helix portions are pink. This structural image was obtained using the PSIPRED tool. help researchers visualize the protein's structure. A two-dimensional structural diagram presents the predictions of PSIPRED's output, which include alpha helices, beta strands, and coil regions. **(Fig 2A)** Understanding the two-dimensional (2D) structure of proteins is the first step in creating in silico vaccines. It guides the rational design of effective vaccine candidates bydirecting adjuvant attachment, antigen presentation optimization, epitope selection, and structural stability assessment. (Ezzemani, 2023)

A two-dimensional cartoon representation of a protein has been generated. In order assist in the intuitive comprehension of the protein's overall structure and organization, this visualization attempts to present the structural parts of the protein in an easier to understand way. The creation of this cartoon image was probably made simpler by tools or software created just for visualizing protein structures. **(Fig 3B)**

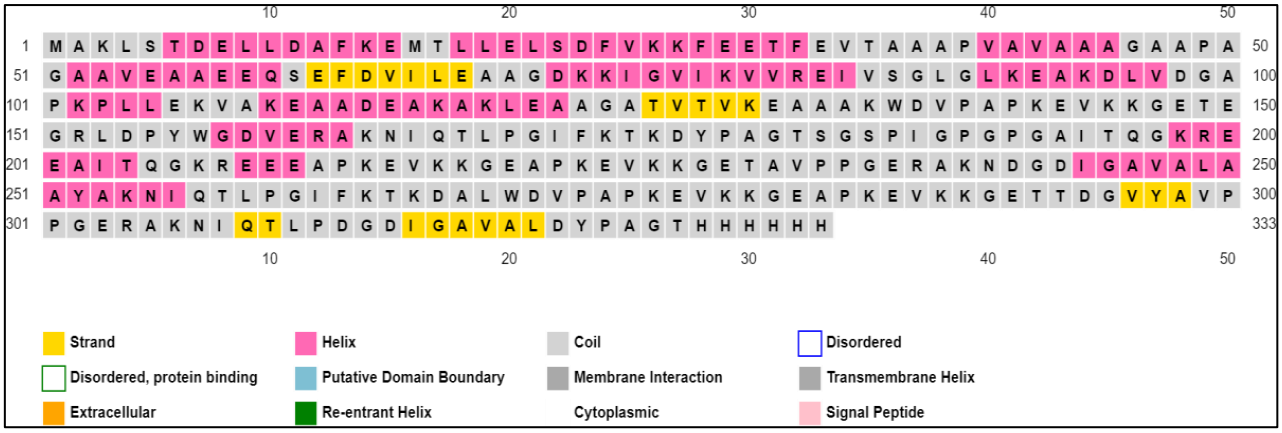


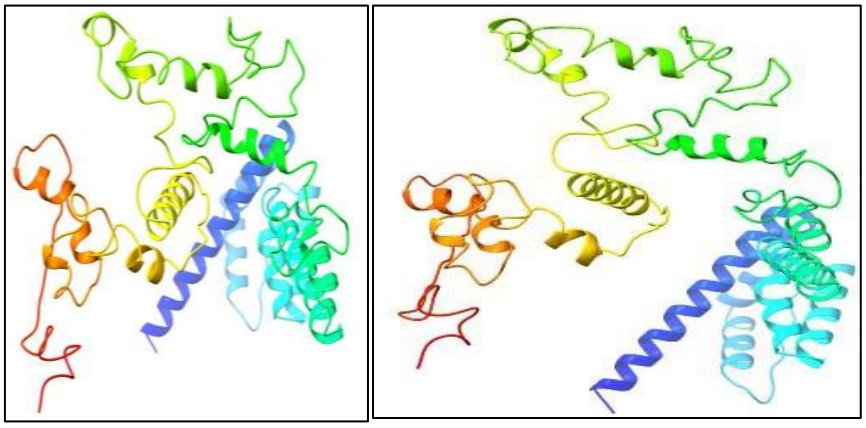
Fig. 2(A): Showing the 2d structure of protein by using the Psipred

Fig. 2(B): secondary satructure are represented in Cartoon generate

Tertiary structure prediction and refinement

The tertiary structure prediction was determined with the help of SCRATCH protien prediction and after that glexy web refine server tool use to refine the 3d structure of protien The image shows the spatial arrangement and folding patterns of a protein in three dimensions. This prognosis helps in comprehending the protein's role, connections with other molecules, and possible medical uses. The Scratch protein predictor is a tool that uses computer calculations to predict protein structures. It employs algorithms and models to mimic the folding and arrangement of amino acid residues in the protein sequence. The expected 3D shape of the protein allows researchers to get a more profound comprehension of its biological properties and behaviours. **Fig 3(A)**

The figure represents the improved three-dimensional configuration of a protein, providing a detailed view of its layout and composition. The enhanced structure is attained by optimising and refining methods to improve the accuracy and quality of the initial structure prediction. The Galaxy web refine server is a computer tool specifically designed for the refinement of protein structures. It uses advanced algorithms and techniques to improve the arrangement of atoms in the protein, leading to an improved and scientifically valid structure. The accurate three-dimensional configuration aids scientists in understanding the functionality of the protein, its interactions with other molecules, and the potential binding sites for medications. **Fig 3(B)**



A **B**

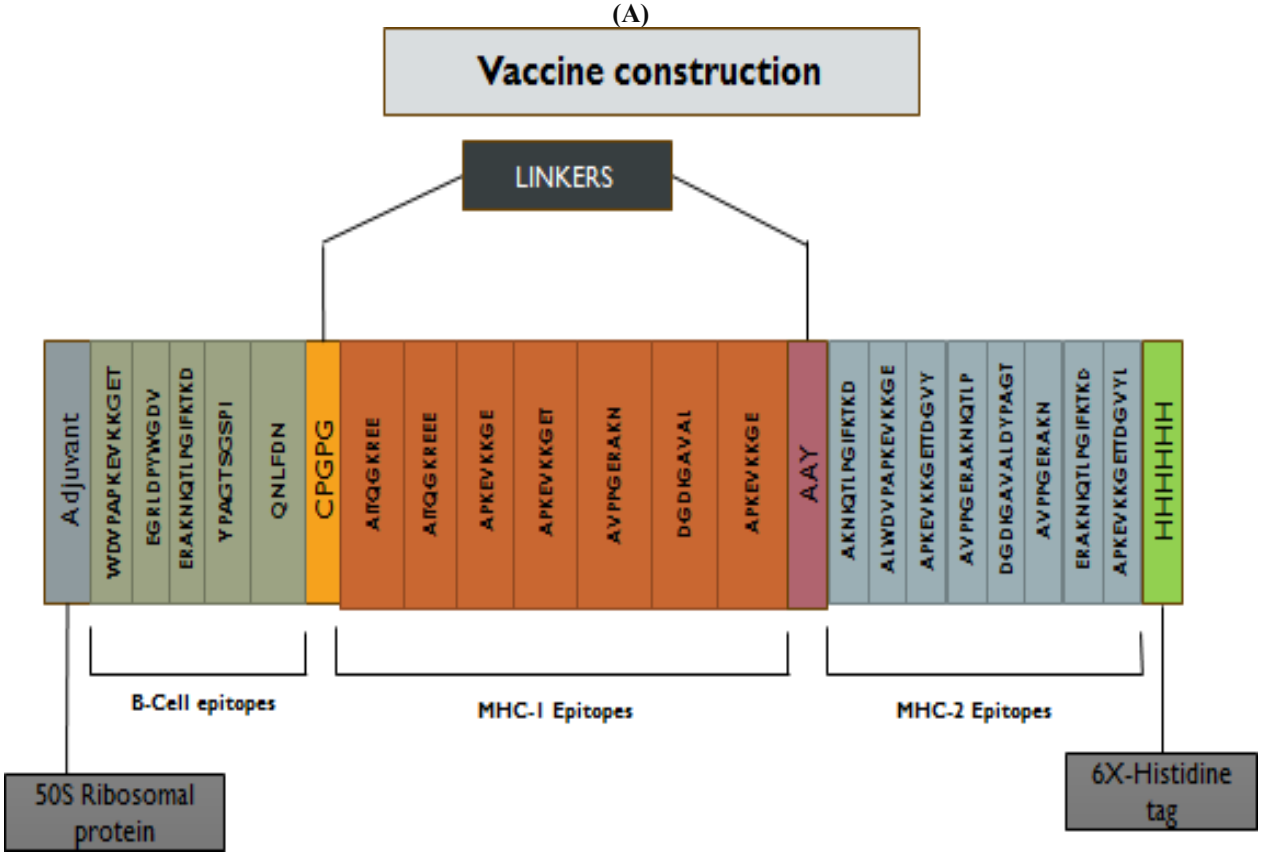
Fig. 3(A): Shows the three-dimensional configuration of a protein that has been predicted using the Scratch protein predictor. (B) Utilise the Galaxy web refine server to ascertain the precise three-dimensional configuration of the protein

Designing of a multi-epitopes antigenic vaccine candidate of zika virus

After the prediction of all antigenic vaccine applicants, they were joined with every other with the usage of linkers so that it will construct a single construct. A vaccine construct was constructed via integrating important components required for a a hit immune reaction. These components consist of B cell epitopes, MHC class 1 epitopes, MHC class 2 epitopes, a linker, an adjuvant, and a histidine tag (His-tag). The purpose of the vaccine is to stimulate the immune system to develop a defensive reaction in response to a specific infection or kind of sickness. The vaccine is made up of many parts that work together to stimulate the immune system. To be more specific, it does this by using combining a range of numerous substances to cause immune reactions primarily based on each cell- and antibody-based responses (humoral reactions).

The amino acid sequence of the vaccine construct, which consists of the adjuvant (50S ribosomal protein L7/L12), linkers (EAAAK, AAY, GPGPG), epitopes (CTL, HTL), and the histidine tag (His-tag), is proven inside the sequence section. Accurate placement and repetition produce greatest functioning and immunity. Figure 4(A) The vaccine targets to selectively cause a specific pathogen or infection through inducing humoral and cell-mediated immune responses. The vaccine formulation's therapeutic effectiveness is improved by the immune-stimulatory adjuvant capabilities of 50S ribosomal protein L7/L12. Linkers are short peptide sequences used to connect different constituents of the vaccine formulation. Fig 4(B) They give flexibility and distance between epitopes, which allows them to deliver precise immune system display and detection that is accurate.

(A)



(B)

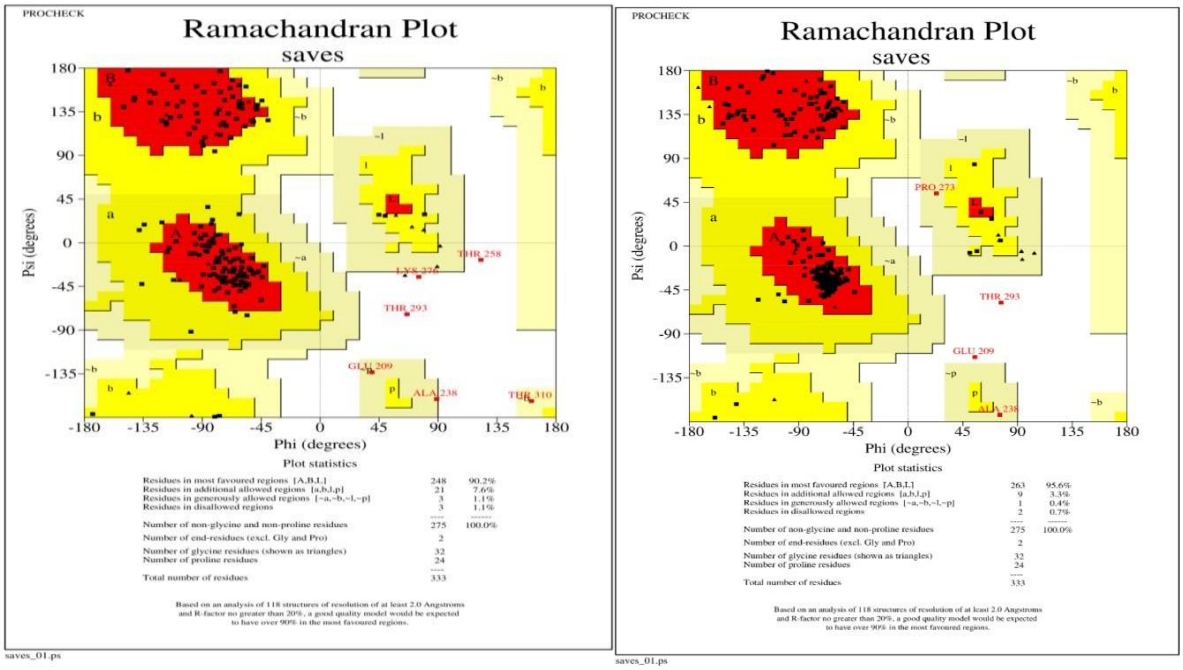
EAAAKWDVPAPKEVKKGETEGRLDPYWGDV
ERAKNIQTLPGIFKTKDYPAGTSGSPI**GPGPG**
AITQGKREEAITQGKREEEAPKEVKKGE
APKEVKKGETAVPPGERAKNDGDIGAVAL
AAYAKNIQTLPGIFKTKDALWDVPAPKEVKKGE
APKEVKKGETTDGVYAVPPGERAKNIQTLP
DGDIGAVALDYPAGT**HHHHHH**

Figure 4(A): displays the combination of T-helper and cytotoxic T-cell epitopes that the server predicted. The potential vaccine constructions with linkers (EAAAK, AAY, GPGPG), adjuvant (50S ribosomal protein L7/L12), sequence, and epitopes (CTL, HTL) arranged consecutively and suitably are schematically shown in Fig. 4(A) and sequenced in Fig. 4(B)

Refinement and validation Ramachandran Plot Before and after refinement

A Ramachandran Plot is displayed, showing the distribution of dihedral angles (phi and psi) for amino acids in a protein structure before refinement**.** While certain amino acids are found in the allowed or prohibited zones, others are found in the more desirable region. The phi and psi dihedral angles of the amino acid sequences of a protein are shown graphically in the image as a Ramachandran Plot. With its phi and psi angles represented on the x and y axes, respectively, each point on the figure represents a distinct amino acid residue. Based on the permitted conformational space for amino acids, the plot is separated into three regions: favored, approved, and forbidden are showing in Fig 5(A)

Ramachandran Plot after refinement more amino acids are located in the favored region, while others are in the allowed or very small in disallowed regions. More amino acids are situated in the most desirable part of the plot after refining, showing that their phi and psi angles match energetically advantageous conformations. Certain amino acids could still be found in the permitted area, suggesting conformations that are allowed but not optimum. The fact that so few amino acids are still in the forbidden area suggests that structural flaws or problems have been solved via refining, leading to greater stereochemical quality. Fig 5(B)



A **B**

Fig. 5(A): Showing the Ramachandran Plot before refinement Some amino acids are located in the favored region, while others are in the allowed or disallowed regions. Fig 5(B) Showing the Ramachandran Plot after refinement more amino acids are located in the favored region, while others are in the allowed or very small in disallowed regions

Molecular docking or protein-protein interaction of the vaccine candidate and TLR4 receptor

We select the TLR4 recepter for docking with vaccine candidate by using the cluspro tool they give the several structure we see and selected the best structure they show maximum docking with the vaccine contruct. After simulation completion, a list of docking models will be sent to you. usually ClusPro provides a number of models that are categorized according to their energy scores. Select the model or models that show strongest docking contacts between the receptor and your vaccination. The vaccine molecule's projected spatial arrangement together with its receptor can be seen in the docking structure. It offers details on the possible interface of contact and binding mechanism between the vaccination and receptor molecules. ClusPro is a web-based protein-protein docking system that forecasts the formation of a complex between two molecules of protein.

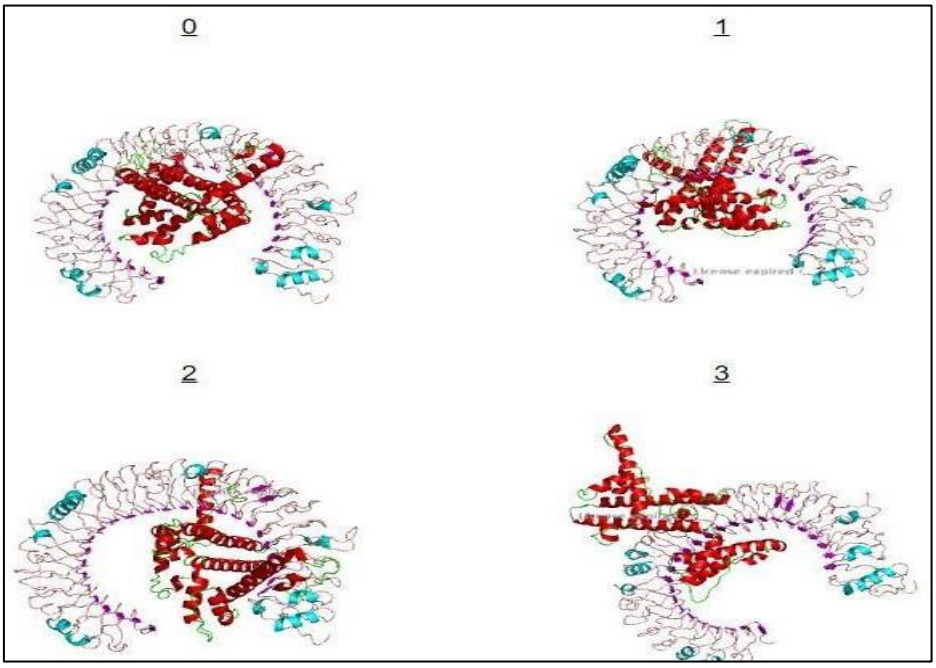


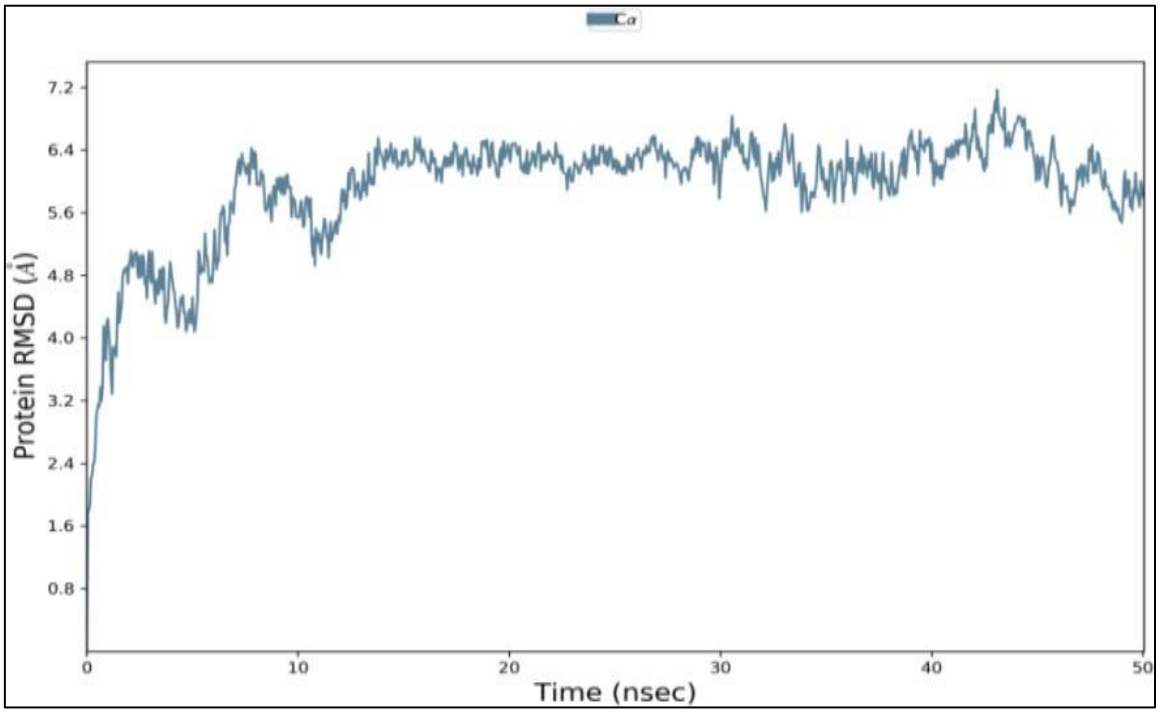
Fig. 6: Showing the docking structure of vaccine and receptor by using the clus pro online tool

Molecular Dynamics Stimulation of the docked complex

An effective technique for analyzing biological systems is MD simulation, which offers a wealth of mechanistic insights into how the system would behave in a biological environment that is simulated. The manufacturing phase MD was performed using Dashmond server. With the objective give a mechanistic knowledge of the structural properties and interactions between the TLR4 receptor and the predicted vaccine protein, the obtained trajectories were studied. The graph shows the docking structure and can give RMSD values during the duration of a 50 ns experiment. The root mean square deviation (RMSD) measures the average distance between atoms in overlapping structures to show the degree of structural variation across time. During a period of fifty nanoseconds, the graph illustrates the stability of the docking structure. The stability of the structure is retained when the graph mostly maintains its level and has low values for the root mean square degree. Conversely, significant changes inside the graph or a rise in the RMSD values suggest that the structure is shifting or losing stability. Scientists can also get a deeper understanding of the docking complex's lengthy-term functioning through using those facts. A significant root imply rectangular deviation (RMSD) of 6.4 angstroms is seen in the simulation, suggesting that changes arise in the first 9 nanoseconds of the run **Fig 7(A).**

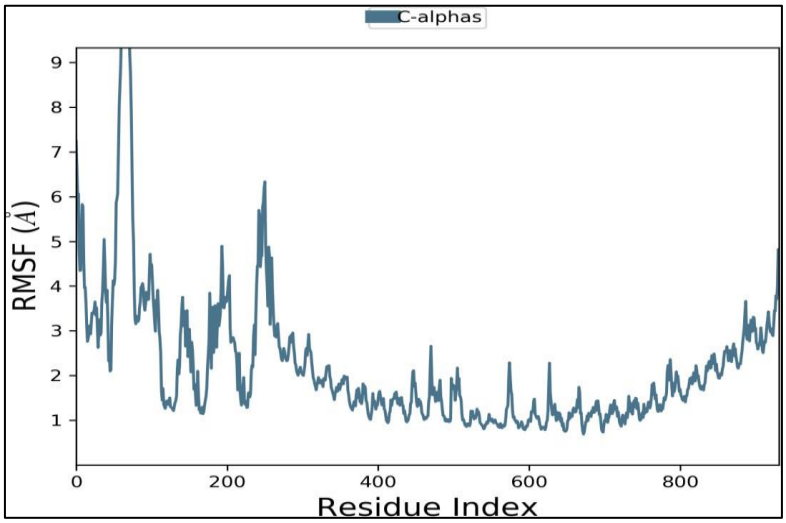
Every amino acid's degree of mobility or variation within the docking structure is proven at the chart. For certain amino acids, the graph's peaks indicate a high degree of mobility, while the troughs imply a reduced degree of movement. Making use of this data makes it easier to understand the various degrees of stability or flexibility shown by various structural elements. Obtaining this knowledge is necessary in order to understand how the docking complex functions. Changes may be seen in the residue indices 50 and 210, which may imply that amino acids are moving extra freely in sure regions. Notwithstanding those changes, the fundamental design is precise and dependable. This examine offers same weight to structurally flexible and strong components, presenting important insights into the dynamic behavior of the docking complex. An extensive examination of the functioning or conduct of the docking complex. **Fig 7(B)**

The graph that was generated by the Dashmond tool displays the proportion of secondary structural elements (SSE), which may range from 0% to 100%, as well as the repetition indices, which can range from 0 to 1000. There are distinct regions of the secondary structure of the protein that are represented by the blue and red lines included within the graph. The parts that are shown by blue lines are those in which the secondary structure of the protein is largely in a conformation that is put together. Examples of such sections include alpha helices and beta strands. In most cases, these regions are linked to structural patterns that are constant and clearly distinguishable. **Fig 7(C)**



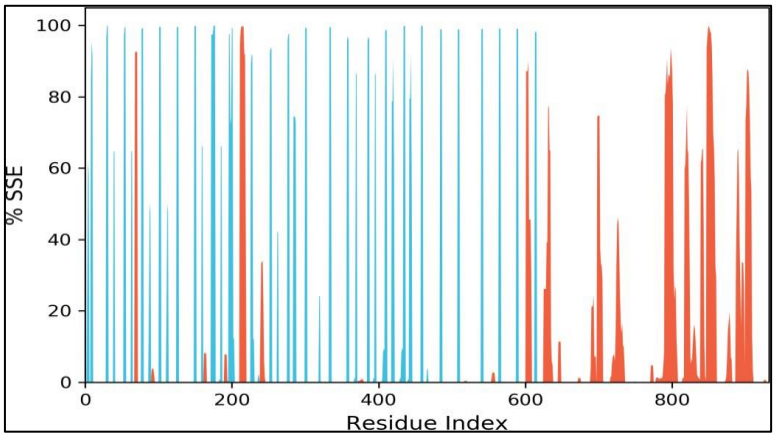
(A)

Fig. 8(A): Showing the docking structure in graph form in rmsd 50ns



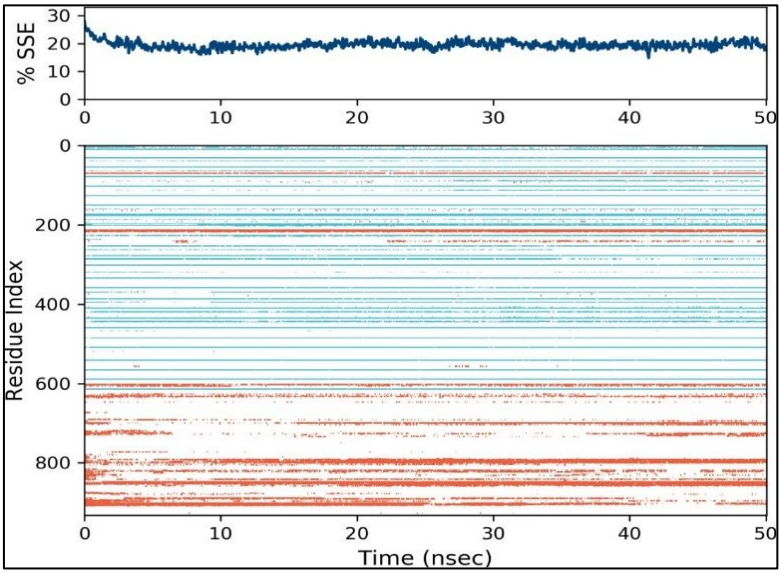
(B)

Fig. 8(B): Showing the docking structure in graph form in Rmsf fluctuation in amino acid



(C)

Fig. 8(C): Dashmond tool-generated graph displaying secondary structure ele ment (SSE) percentages along the y-axis and residue indices along the x-axis, highlighting regions of protein structural order (blue lines) and disorder (red lines)

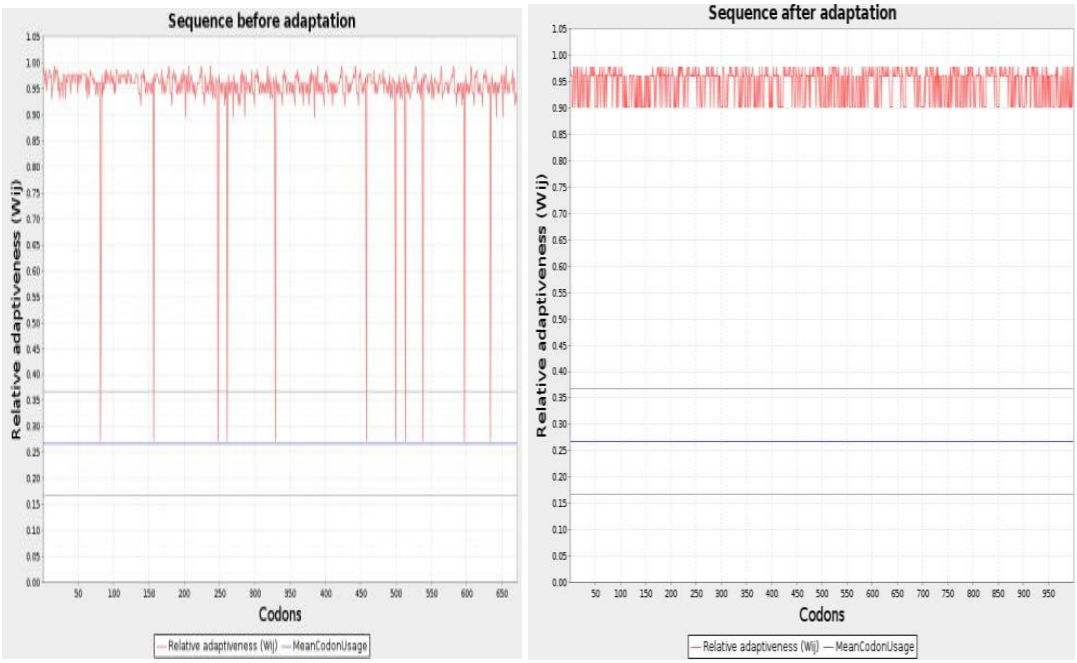


(D)

Fig. 8(D): Dashmond tool-generated graph displaying residues index percentages along the y-axis and time (nsec) along the x-axis

Codon optimization of the protein sequence conducted by for optimal expression

The method of optimizing the protein collection for the excellent expression in Escherichia coli covered codon optimization. The Java Codon model tool changed into used to perform this optimization. The first graph, displaying the distribution of codons along the x-axis and their associated relative adaptiveness on the y-axis, depicts codon use prior to adaptation **Fig 9(A)**. The red line consistently shows a relative adaptiveness range from 0.90 to 1, illustrating excellent codon use throughout the sequence, in the second graph, which shows the results of adaptation. **Fig 9(B)** This consistency shows that the modified codon use is more effective and customized to raise expression levels or fulfill other particular needs.

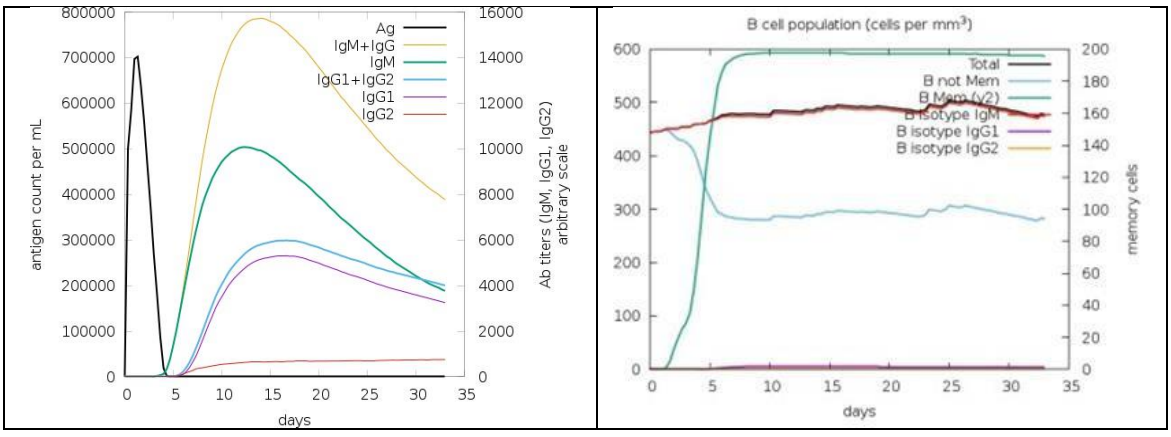


A **B**

Fig. 9(A): Graphs depicting codon optimization using the Java Codon Adaptation tool and the Fig 9(B) representing the codon after optimization

Generation of immune response by zika virus vaccine candidate

The C-ImmSim server, that delivers a precise immune response result, was used to conduct the immune simulation experiment. The results we obtain show that the secondary and tertian immune response—distinguished by IgG1 + IgG2 and IgM—is proliferating. Additionally, when the antigen count dropped, IgG + IgM also shown the proliferation reaction **Fig 10(A)** The result shows how the immune system developed after vaccination. Following vaccination, the B-cell population level was also increased and considered to be extremely high. **Fig 10(B)** The levels rise steadily over time, reflecting the body's ongoing immune response we measure the levels of IFN (interferon), TGN (Tumor Growth Necrosis Factor), and TNF (Tumor Necrosis Factor), with units represented in arbitrary scales. This graph helps us understand how the body's immune system responds to the vaccine by tracking the levels of key immune markers over a 35-day period **Fig 10(C).**



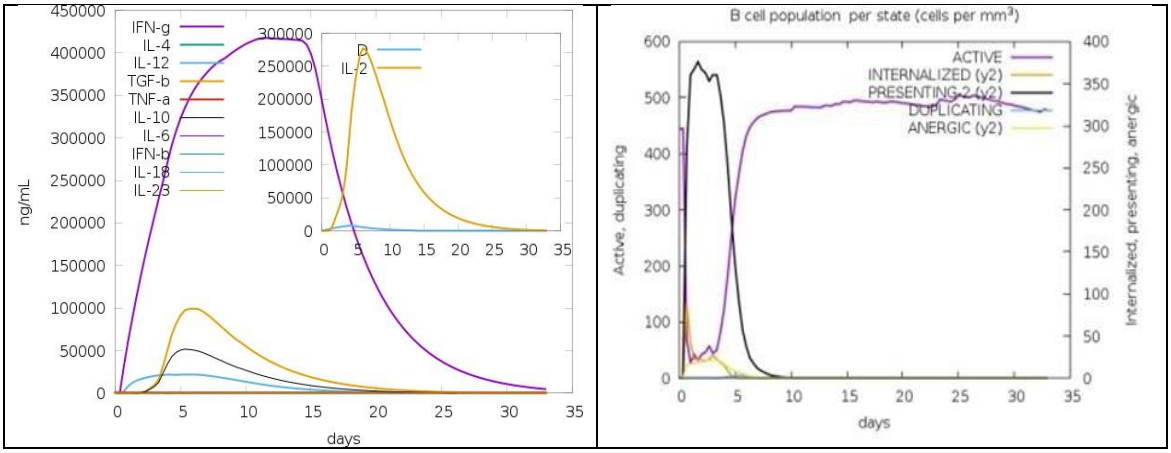


Fig. 10: Immune simulation using C-IMMSIMM as a stand-in for the designed vaccine construct

(A) Colored lines (black-hued lines) show different subclasses as well as the immunoglobulin and immunocomplex reactions to vaccinations. A rise in the B-cell count **(A, B) (D)** growth in the population of helper T-cells and rise in the population of plasma B-cells during the injections

In-silico cloning by vector representation of vaccine construct

A gene sequence, such as the vaccine gene sequence, is computationally inserted into a specified place inside a vector sequence in this case, the pET28a (+) expression vector—between the EconI and BssSI restriction sites during the process of in silico cloning. SnapGene and other bioinformatics tools are used in this method. One advantage of in silico cloning is that it minimizes the need for physical materials allowing researchers to plan and simulate molecular cloning processes. This decreases the possibility of experimental errors while also saving time and money **Fig 11**. Before performing actual tests in the lab, researchers can utilize tools like Snap Gene to examine the cloning process, confirm that the insert is oriented correctly within the vector, and predict any potential problems or limits. Researchers can model and organize cloning operations without tangible resources like DNA, enzymes, or vectors. This saves the time and materials that would have been needed to set up and conduct the tests. Because procedures for cloning are complex and prone to error, doing experiments in silico reduces the chances of mistakes in the lab. When researchers are able to foresee and handle these issues before they exist, research are planned and carried out with more precise results. Generation of immune response by zika virus vaccine candidate.

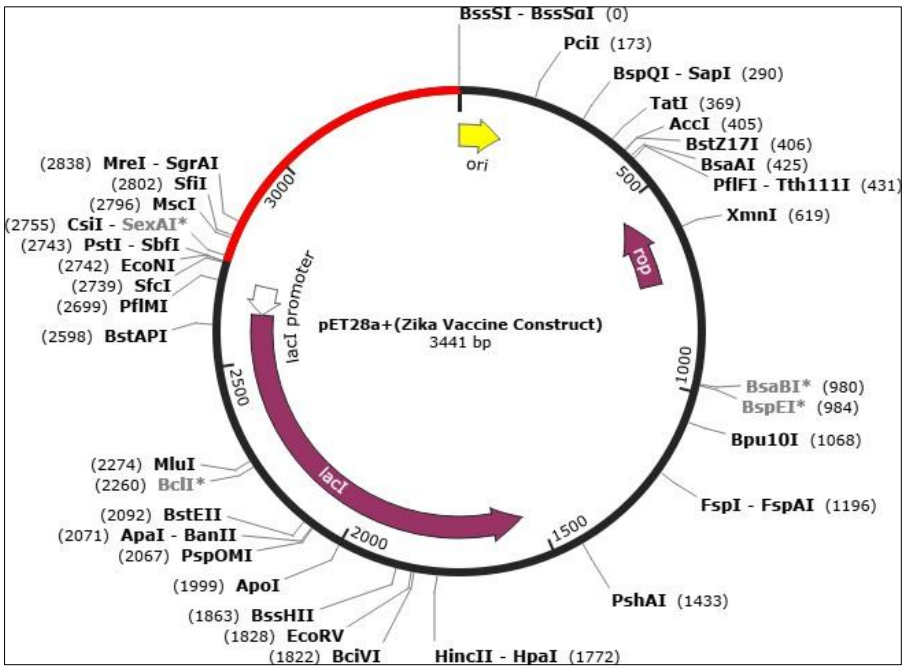


Fig. 11: In silico cloning map showing the insert of vaccine into the pET28a (+) expression vector between EconI and BssSI

DISCUSSION

Zika is one of the maximum horrible infections that has been affecting hundreds of thousands of humans global, especially in 1/3-world nations. While vaccinations are being created, their use vaccinations, both live and attenuated, can result in an occurrence of these viruses and cause lasting damage. One of the main reasons for fear about the outbreaks was the association that was made between the Zika virus and severe neurological disorders, particularly in pregnant women and their fetuses. Pregnancy-related Zika virus infection has been linked to microcephaly, a disorder characterized by abnormally tiny heads and associated developmental abnormalities in a developing child. Public health initiatives promoted the significance of controlling mosquitoes, practicing personal protection, and safe sexual behaviors, especially for pregnant women and individuals intending to conceive. The objective of this study was to create a multi-epitope vaccination that would be considered safe to deliver and not create any negative effects. To evaluate a vaccine's toxicity, antigenicity, allergenicity, and physiochemical analysis, many methods are used. Any vaccine's allergenicity may be assessed the usage of a bioinformatics approach. The vaccine's antigenicity may be assessed the use of vaxijen v2.0; its toxicity may be assessed the usage of the bioinformatic tool toxinpred to make sure the vaccine may not damage human cells; and its physiochemical evaluation can be assessed the usage of expasy (protparam), that may calculate the vaccine's molecular weight, theoretical ph, instability index, atomic composition, and expected 1/2-life.The final vaccine design was created by gradually combining the peptides with the important linkers To predict the three-dimensional structure of the vaccine construct Molecular docking, is a silico technique, was used to find the binding affinity between our designed peptide vaccine and immune receptor TLR-4 A Ramachandran Plot is displayed, showing the distribution of dihedral angles (phi and psi) for amino acids in a protein structure before refinement**.** While certain amino acids are found in the allowed or prohibited zones, others are found in the more desirable region. An effective technique for analyzing biological systems is MD simulation, which offers a wealth of mechanistic insights into how the system would behave in a biological environment that is simulated. The root mean square deviation (RMSD) measures the average distance between atoms in overlapping structures to show the degree of structural variation across time. Codon optimization was done for the best expression of in Escherichia coli the optimization done in Escherichia coli using the Java Codon Adaptation tool. After that the cloning done A gene sequence, such as the vaccine gene sequence, is computationally inserted into a specified place inside a vector sequence in this case, the pET28a (+) expression vector—between the EconI and BssSI restriction sites during the process of in silico cloning. SnapGene and other bioinformatics tools are used in this method. Before to the vaccination being administered, a comprehensive real-time validation of the application is needed. Based to the study's conclusion, the Zika vaccine may offer a different kind of vaccination to combat the oncoming global pandemic.

CONCLUSION

An illness caused by the Zika virus is now recognized as a worldwide health threat, after a decade of being shrouded in mystery. Being bitten by an Aedes mosquito that is infected with the Zika virus is the primary mode of transmission of the virus. The importance of managing mosquitoes, exercising personal protection, and engaging in sexual behaviours that are harmful to one's health was brought to light by public health programmes. As a consequence of this, there is now neither a cure that is permanent nor a vaccine that is really effective for the treatment of illnesses caused by the Zika virus. It has been shown that none of the antiviral medications are especially helpful in treating the virus, despite the significant study that has been conducted. A multiepitope-based subunit vaccine that has the capacity to trigger humoral and cellular immune responses has been produced via the use of reverse vaccinology and computational techniques. This vaccine has the potential to provide protection against cancer. For the purpose of vaccine design, we make use of bioinformatics tools by working with a variety of internet servers to anticipate epitopes, do more research on this topic, and build vaccines in an inslico manner. Even if the severity of epidemics has diminished in certain locations from their peak, there is always a possibility that another Zika virus outbreak may occur in the future. Continued planning, monitoring, and research efforts are required in order to get a better understanding of the dynamics of the virus, discover outbreaks in a more-timely manner, and implement control and preventive measures that are both effective and efficient.

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