

# Immunoinformatics designed T cell multi epitope dengue peptide vaccine derived from non structural proteome



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## ABSTRACT

Dengue viral disease has been reported as an *Aedes aegypti* mosquito-borne human disease and causing a severe global public health concern. In this study, immunoinformatics methods was deployed for crafting CTL T-cell epitopes as dengue vaccine candidates. The NS1 protein sequence of dengue serotype 1 strain retrieved from the protein database and T-cell epitopes ( $n = 85$ ) were predicted by the artificial neural network. The conserved epitopes ( $n = 10$ ) were predicted and selected for intensive computational analysis. The machine learning technique and quantitative matrix-based toxicity analysis assured nontoxic peptide selection. Hidden Markov Model derived Structural Alphabet (SA) based algorithm predicted the 3D molecular structure and all-atom structure of peptide ligand validated by Ramachandran-plot. Three-tier molecular docking approaches were used to predict the peptide - HLA docking complex. Molecular dynamics (MD) simulation study confirmed the docking complex was stable in the time frame of 100ns. Population coverage analysis predicted the interaction epitope interaction with a particular population of HLA. These results concluded that the computationally designed HTLWSNGVL and FTTCIWLKL epitope peptides could be used as putative agents for the multi CTL T cell epitope vaccine. The vaccine protein sequence expression and translation were analyzed in the prokaryotic vector adapted by codon usage. Such *in silico* formulated CTL T-cell-based prophylactic vaccines could encourage the commercial development of dengue vaccines.

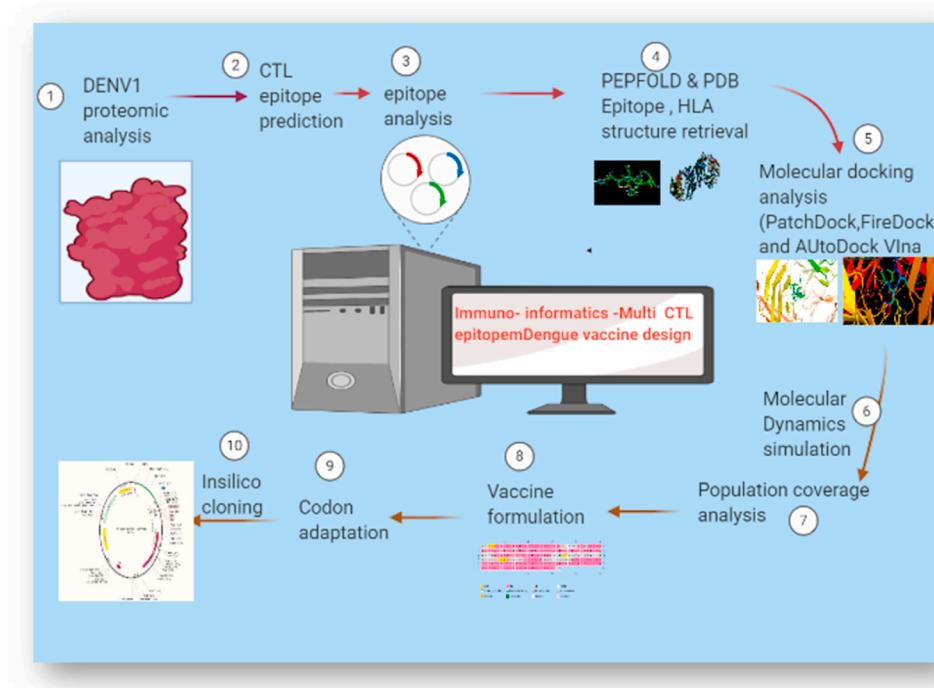
## 1. Introduction

It is a well-known fact that epidemic viral diseases spread through various modes of transmission. Indirect viral transmission through female *Aedes aegypti* mosquitoes makes sick millions of people every year. The emerging and re-emerging viral diseases like Dengue, Chikungunya, Zika, and Yellow fever were spreading by female *Aedes aegypti* [1]. An infected mosquito bite causes the dengue virus (DENV) disease and symptom starts within 3–14 days. An ineffectual treatment may escalate the DENV fever to severe dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS) [2]. Four major DENV serotypes (DENV 1, 2, 3, & 4) were identified so far and they are closely related with 65–70% genomic similarity [3]. There are three known structural and seven non-structural proteins coded by the positive-sense RNA genome (~10, 700 nucleotides). For the past five decades, DENV is a serious public health concern and the infection rates are reported to increase as high as 30-fold [4,5]. The major concern is that the lone commercialized vaccine Dengvaxia (CYD-TDV) is not effective to prevent the disease and

alarmingly, most of the countries have not given usage approval [6,7]. Hence there is an urgent call for an effective, safe, and affordable DENV vaccine. The affordable and efficacious vaccine designing for the most epidemic affected undeveloped regions of the world, traditional vaccine development methods may not succeed. The traditional approach of vaccine designing and development is a lengthy process and huge capital consuming. Recent immunoinformatics studies highlight that, reverse vaccinology and immunoinformatics techniques are highly beneficial for predicting immune-dominant epitope peptide for the development of potential DENV peptide vaccines [8,9]. The DENV non-structural protein (NS1) has a crucial role as a cofactor during viral replication [10]. Hence the epitopes from this protein have immense significance in vaccine design. The objective of this immunoinformatics study was to design a highly conserved, immunogenic, non-toxic, and efficacious multi-epitope CTL T cell epitopes dengue vaccine in less time and at the same time in a cost-effective manner. The NS1 protein sequence data was selected for the epitope prediction based on the reviewed protein sequence dataset and experimental evidence of protein existence. T cell

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**Fig. 1.** Methodology Flow chart.

epitope prediction has an important role in the epitope-based peptide vaccine designing. The immunogenic epitopes are playing an important task in the efficacy of a vaccine and immunization success [11–13]. Low antigenic epitopes are mostly found as a decent active ingredient for peptide vaccine preparation [14]. The multi epitope vaccine formulation, Molecular Dynamics simulation (MD), codon adaptation, and *in silico* cloning were implemented by various computational algorithms ([15–17]).

## 2. Methodology

This experimental immunoinformatics and reverse vaccinology study was conducted using different online web algorithms. In this study we designed epitopes for dengue vaccine candidate through retrieved protein sequence data from UniProtKB/Swiss-Prot reviewed dataset. The epitopes predicted through transporter associated with antigen processing (TAP) and C value. The linear conserved epitope selected for immunogenicity, antigenicity, toxicity and physico chemical properties. Then the identified epitopes and the HLA 3D structure predicted using PEP-FOLD and Research Collaboratory for Structural Bioinformatics (RCSB) protein database respectively. Define the predicted epitope as ligand and HLA as receptor and molecular docking analysis done. The software tools like PatchDock, Firedock and AutodockVina were used in this molecular docking analysis. The resulted docked complexes were visualized in PyMOL. The MD simulation analysis was done for best HLA –epitope docked complex. Population coverage analysis of the selected epitope increases the chance of global coverage. Computational algorithms used for vaccine formulation, analysis, codon adaptation and translation expression. Flow chart given below summarised the methodology design (Fig. 1).

### 2.1. Retrieval of protein sequence data

The UniProt database used for high quality protein sequences and function data retrieval [18,19]. Non structural 1 (NS1) protein sequence of DENV Serotype 1 strain (BR/97-111) retrieved from UniProtKB (ID 'P27909'). The retrieved proteome sequence dataset was reviewed by

UniProtKB/Swiss-Prot database.

### 2.2. CTL epitope prediction

NetCTL version 1.2 predicted CTL epitopes from NS1 protein sequence. MHC class I supertypes binding sites were predicted by Carboxyl (C) terminus proteasomal cleavage value as 0.15, Transporter associated with antigen processing (TAP) value as 0.05 and threshold value as 0.75 for optimal predictive performance on an average combined score helps accurate epitope prediction [20]. In Oropouche virus's CTL epitope prediction helps the combined score [21].

### 2.3. Epitope conservancy

Conserved epitopes are having an important role in peptide vaccine design. The conservancy analysis tool of Immune Epitope Data Base (IEDB) was used to predict the degree of the linear conservancy in epitopes [22]. Only 10% 9 mer conserved T cell epitopes were found potential for vaccine candidate. Potential epitopes predicted from conserved regions had an excellent potentiality for a complete vaccine [18].

### 2.4. Immunogenicity prediction of epitope

IEDB tool uses amino acid properties and position within the peptide to predict the immunogenicity of a peptide MHC complex [23]. Highly conserved epitopes in all the four DENV serotypes were analyzed for MHC class I immunogenicity using Immune Epitope Database (IEDB).

### 2.5. T cell epitopes-antigenicity prediction

VaxiJen 2.0 tool predicted the antigenic peptide epitope from amino acid sequence against the cut off antigenicity value [24]. The antigenicity of T cell epitopes derived from Chikungunya were predicted by VaxiJen server 2.0 [25]. The immunogenic epitopes tested for the antigenicity and low antigenic peptide selected for toxicity study.

**Table 1**

NetCTL-1.2 Predicted Epitopes against MHC supertypes.

Sl. NO	MHC Supertypes	Number of predicted epitopes
1.	A1	3
2.	A2	3
3.	A3	7
4.	A24	8
5.	A26	5
6.	B7	7
7.	B8	7
8.	B27	7
9.	B39	7
10.	B44	9
11.	B58	11
12.	B62	12
Total number of epitopes		85

## 2.6. T cell epitopes-toxicity & physico-chemical analysis

ToxinPred predicted non-toxic epitopes from antigenic epitopes using a main data set of 1805 toxic peptides ( $\leq 35$  residues) [26]. The ToxinPred was used to predict toxicity in different epitope based peptide viral vaccines for Oropouche virus [21], Hepatitis B Virus [27], Epstein-Barr virus [28] Norwalk virus [29], Henipavirus [30], Nipah virus [31], MERS corona virus [32] and SARS-CoV-2 virus [33,34]. Low antigenic epitopes tested toxicity along with physical and chemical properties like hydrophobicity, hydropathicity, amphipathicity, charge, pI and molecular weight predicted using the ToxinPred web tool.

## 2.7. 3D structure predictions

PEP-FOLD (<http://bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD>) was used for the determination of 3D structure of the epitope. PEP-FOLD is a de novo approach aimed at predicting peptide structures from amino acid sequences based on the concept of structural alphabet (SA) letters are assembled by an enhanced greedy algorithm. Optimized Potential for efficient structure prediction (OPEP) parameters are optimized by a genetic algorithm procedure using a large ensemble of protein decoys [35]. RCSB (<https://www.rcsb.org>) was used for the retrieval of validated MHC class I HLA allele's 3D structures. The input file is a FASTA format of amino acid sequence length up to 50 amino acids. The simulation restricted to 36 amino acids using the 3D reconstruction range. This method generated 100 models sufficient to identify the correct fold. The simultaneous validation of ensemble epitope peptide structures were done by MolProbity (<http://molprobity.manchester.ac.uk>) and Ramachandran plot analysis (Christopher et al., 2018; [36]).

## 2.8. Molecular docking analysis

We implemented a three-tier docking approaches for docking analysis. Shape complementarity principles based PatchDock molecular docking algorithm was used for analyze of receptor-ligand atom penetrations [37]. Further the protein flexibility refinement and rescoring of rigid-body docking solutions by Firedockserver [38]. Auto Dock Vina was deployed to found the accuracy of average binding of complex and selection of best model [39,40].

## 2.9. MD and simulation analysis for a docked complex of epitope-MHC HLA allele

The whole elements reenactment concentrate for the HLA-epitope complex was conducted in YASARAtool for MD simulation. Before re-enactment, the complex was cleaned and advanced the hydrogen bond network. Further, a cubic cell was made with an intermittent limit condition; also, the particles of the complex were analyzed by utilizing the AMBER14 field. The pKa estimations of protein titratable amino

acids were determined and solvated the simulation box utilizing the transferable intermolecular potential3 points (TIP3P) water model (thickness: 0.998 g/L<sup>-1</sup>). 48905 atoms were minimized energetically from the framework with gradient of 5000 cycles followed by simulated annealing strategy. All the atom molecular simulation was conducted by utilizing PME approach to analyze electrostatic interactions with in complex at a cut off separation of 8 Å at physiological conditions (298 K, pH 7.4, 0.9% NaCl). Multiple times step calculation along with a MD simulation time step 2.50 fs was selected [41,42]. MD simulation was performed for 100 ns long at consistent temperature utilizing a Berendsen thermostat and constant pressure. MDtrajectories were stored for every 250 ps and analyzed. Trajectories were analyzed for stability of complex through RMSD and RMSF plots generated with the help of YASARA MD-simulation tool.

## 2.10. Population coverage analysis

A large number of HLA binding epitope peptide relatively give and increased population coverage. IEDB tool helped to predict the population coverage of the potent epitope interact with HLA allele of a particular population [22,43].

## 2.11. Vaccine formulation and MD simulation

Through different integrative approaches of reverse vaccinology, we selected two CTL epitopes for *in silico* vaccine formulation. The vaccines were formulated by using methods described in Human Papilloma Virus and Herpes Simplex Virus type 1 and 2 vaccine [44–46]. In this study, the multi-epitope vaccines were formulated by the blending of the efficacious CTL epitopes and the adjuvant. ExPASy ProtParam tool helped for the vaccine's physical-chemical analysis [47]. MD Simulation was conducted by using Gromacs ver. 2019 [48] OPLS 2005 force field was used. The equilibration steps were set with constant pressure and temperature (NPT) ensemble. The MD simulations were carried out at standard temperature of 300 K and pressure level of 1.013 bar. The MD trajectories were estimated by analyzing the root mean square deviation (RMSD) and the root mean square fluctuation (RMSF) of the complexes (vaccine construct and TLR5 receptor) for a timescale of 40 ns.

## 2.12. Codon adaptation and *in silico* cloning expression analysis

Formulated Multi CTL epitope vaccine adapts the codon usage to sequenced prokaryotic organism (*E.coli* strain K12). The adaptation is based on Codon Adaptation Index (CAI) values calculated by algorithm of the Java Codon Adaptation Tool (J-CAT) server [49].

## 3. Results and discussion

### 3.1. NS1 protein data retrieval from UniProt

NS1 protein sequence data obtained from UniProt (ID P27909). The NS1 protein plays a major role in immune evasion, pathogenesis and viral genome replication. Assist membrane bending and envelopment of genomic RNA at the endoplasmic reticulum. NS1 protein's hexameric lipo particle excretes function against host immune response. There was a remarkable conservation of the structural proteins and non-structural proteins in dengue strains of Brazil [50].

### 3.2. T cell epitope prediction from the protein sequence

For the prediction of T cell epitope web server NetCTL 1.2 was used. The analysis predicted total 85 T cell epitopes against the entire 12 MHC supertypes (A1, A2, A3, A24, A26, B7, B8, B27, B39, B44, B58 and B62) available in the server. Threshold value 0.75 and combined score was used to predict peptides against each supertype. For summarised details refer Table 1.

**Table 2**  
Epitope Conservancy results.

Serotypes	NS1 Protein UniProt ID & sequence position	Epitope position	Epitope sequence	Conservancy percentage	MHC Supertype
1	P33478 & 775-1126	12 to 20	ELKCGSGIF	100	B62
2	P07564 & 776-1127				
3	Q6YMS4 & 774-1125				
4	Q58HT7 & 775-1126				
1	P33478 & 775-1126	252 to 260	SQHNYRPGY	100	B27 & B62
2	P07564 & 776-1127				
3	Q6YMS4 & 774-1125				
4	Q58HT7 & 775-1126			88.89	
1	P33478 & 775-1126	26 to 34	HTWTEQYKF	100	B62
2	P07564 & 776-1127				
3	Q6YMS4 & 774-1125				
4	Q58HT7 & 775-1126				
1	P33478 & 775-1126	262 to 270	TQTAGPWHL	100	B44
2	P07564 & 776-1127				
3	Q6YMS4 & 774-1125				
4	Q58HT7 & 775-1126			88.89	
1	P33478 & 775-1126	325 to 333	GEDGCWYGM	100	B39
2	P07564 & 776-1127				
3	Q6YMS4 & 774-1125				
4	Q58HT7 & 775-1126				
1	P33478 & 775-1126	313 to 321	CRSCTLPPPL	100	B39
2	P07564 & 776-1127				
3	Q6YMS4 & 774-1125				
1	P33478 & 775-1126	163 to 171	FTTNIWKL	100	A24
2	P07564 & 776-1127				
3	Q6YMS4 & 774-1125				
1	P33478 & 775-1126	229 to 237	HTLWSNGVL	100	B39
2	P07564 & 776-1127				
4	Q58HT7 & 775-1126				
1	P33478 & 775-1126	310 to 318	EWCCRSCTL	100	A24
2	P07564 & 776-1127				
3	Q6YMS4 & 774-1125				
1	P33478 & 775-1126	314 to 322	RSCTLPPLR	100	A3
2	P07564 & 776-1127				
3	Q6YMS4 & 774-1125				

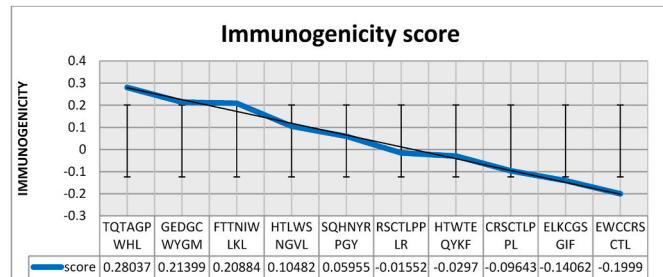


Fig. 2. Graphical representations of Immunogenicity score.

### 3.3. Epitope conservancy- analysis

The IEDB conservancy analysis was performed for all linear sequences of 85 epitopes. These epitopes analyzed for degree of conservancy against NS1 protein sequence of all dengue virus serotypes (1, 2, 3 &4). DENV1 strain Singapore/S275/1990) P33478, DENV 2 strain Jamaica/1409/1983, DENV 3 strain Sri Lanka/1266/2000 and DENV strain Philippines/H241/1956. The conservancy prediction revealed that ten epitopes showing >88% conservancy against NS1 protein sequences all four DENV serotypes. Four epitopes (ELKCGSGIF, HTWTEQYKF, GEDGCWYGM and HTLWSNGVL) showed 100% conservancy in all the four DENV serotypes and other 6 epitopes are 100% conserved in 3 serotypes. DENV serotype, NS1 protein UniProt ID & sequence position, epitope position, epitope sequence, conservancy percentage and MHC supertype details of each epitope summarised in Table 2.

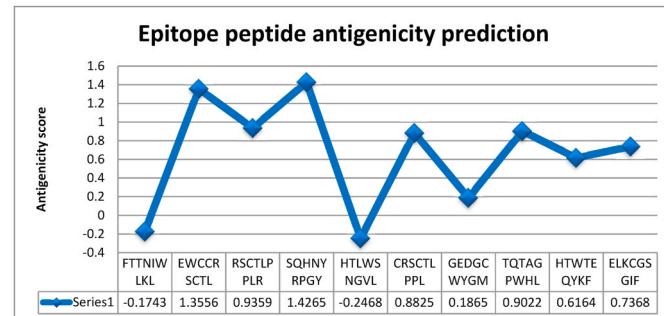


Fig. 3. Graphical representation of antigenicity of peptides.

### 3.4. Immunogenicity prediction of epitopes

The higher immunogenicity score indicates a greater probability of eliciting an immune response. Ten epitopes considered optimal for the immunogenicity prediction. The highest immunogenicity score was 0.2803 and the lowest was -0.1999. While considering low antigenicity score, all the 10 immunogenic epitopes further analyzed for antigenicity. Immunogenicity score graphic details available in Fig. 2.

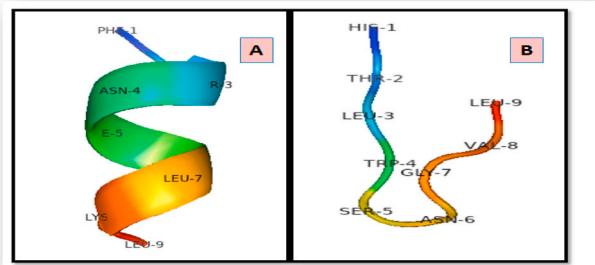
### 3.5. T cell epitope antigenicity prediction

Two low antigenic T cell epitope peptides were predicted from 10 immunogenic epitopes on the basis of VaxiJen antigenicity score. High antigenic epitope peptides were not considered for the further analysis. Fig. 3 exhibits graphical representation of antigenicity of peptide.

**Table 3**

Toxicity, Physical&amp; chemical properties of T cell epitope.

Peptide Sequence	SVM Score	Toxicity	Hydrophobicity	Hydropathicity	Amphipathicity	Hydrophilicity	Charge	pI	Molwt
FTTNIWLKL	-0.58	Non-Toxin	0.07	0.58	0.41	-0.99	1.00	9.11	1135.51
HTLWSNGVL	-0.91	Non-Toxin	0.07	0.26	0.16	-0.99	0.50	7.10	1026.30

**Figs. 4.** 3D Peptide structure - A. FTTNIWLKL epitope B. HTLWSNGVL epitope.

### 3.6. Computation of peptide toxicity & physico-chemical properties

The selected low antigenic peptides with high immunogenicity score and tested in ToxinPred web tool. The test result confirmed the selected epitopes are non-toxin. The tool also predicted physico-chemical properties(Hydrophobicity, hydropathicity, amphipathicity, charge, pI and Molecular weight and support vector machine(SVM) score) for peptides. For the FTTNIWLKL peptide, SVM score was -0.58 and HTLWSNGVL peptide SVM Score was -0.91. The other physical and chemical property of the peptides are summarised in Table 3.

### 3.7. Peptide 3D structure prediction

Two selected epitope peptides were 9 mer long. Five 3D structures for each epitope were predicted from PEP-FOLD web tool. Onetop ranked model was selected for FTTNIWLKL and HTLWSNGVL epitopes. The 3D structure of FTTNIWLKL and HTLWSNGVL peptidere presented in Fig. 4A and B respectively. The presence of residues in favorable

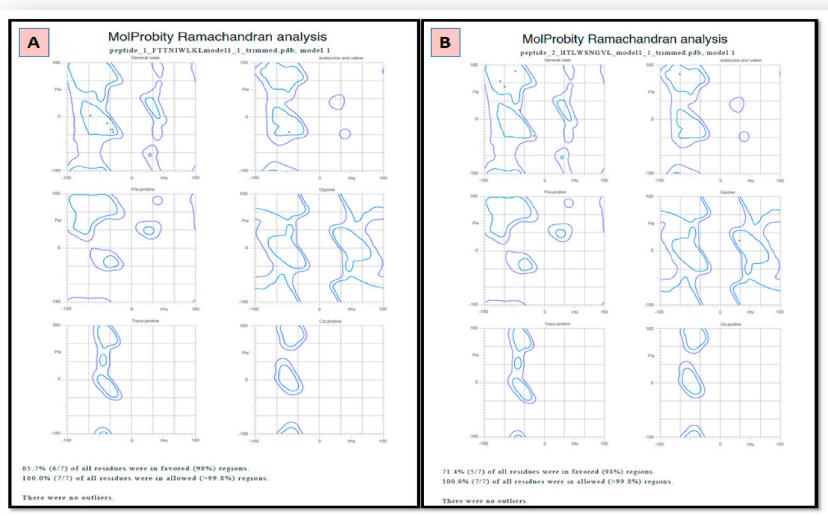
region obtained from Molprobity biochemical structural validation analysis (Fig. 5).

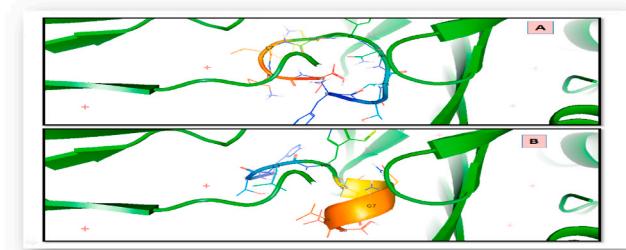
### 3.8. Molecular docking

Patch dock and firedock screening tool was deployed to find out the interacting residues of molecules. Docking complex of FTTNIWLKL and MHC I HLA-A allele resulted in geometric shape complementarity score is 8632 with approximate interface area of the complexes 1037.40 and atomic contact energy (ACE)-241.69. Docking complex of HTLWSNGVL and MHC I HLA-A allele complex resulted geometric shape complementarity score was 7978, approximate interface area of the complex is 1015.50 and atomic contact energy (ACE) -216.43. Epitopes FTTNIWLKL and HTLWSNGVL clearly exhibits best model at rmsd value 0, on the basis of Vina split program which was used to select out best modelled ligand of both epitopes. For summarised docking details refer Table 4. Fig. 6 represents AutoDock-vina docking results for selected epitopes, the binding energies were found -7.1 and -8.1 Kcal/mol for FTTNIWLKL and HTLWSNGVL respectively.

**Table 4**  
Molecular docking results for putative epitopes.

Complex	Geometric shape complementarity score	Atomic contact energy (ACE)	AutoDock-Vina Binding Affinity (kcal/mol)
FTTNIWLKL ligand & MHC I HLA A allele complex	8632	-241.69	-7.1
HTLWSNGVL ligand & MHC I HLA A allele complex	7978	-216.43	-8.1

**Fig. 5.** Ramachandran plot analysis A. 100% of all residues of epitope FTTNIWLKL were in allowed region B. 100% of all residues of epitope HTLWSNGVL were in allowed region.



**Fig. 6.** AutoDock vina results for epitopes interacting with 1w72 (HLA A1 allele) **A.** FTTNIWLKL **B.** HTLWSNGVL.

### 3.9. MDsimulation analysis of HLA and epitope complex

The 100 ns MD simulation of HLA-epitope (HLA- A1 to FTTNIWLKL and HTLWSNGVL epitopes) complexes was carried out using AMBER14 force field, following the energy minimization protocol. The stability of the HLA-epitope complexes by means of RMSD and RMSF was calculated and visualized in Fig. 7.

RMSD values and Atomic fluctuation per amino acid residue were obtained for epitopes interacting with HLA-Allele structure; this analysis allows the perfect pair selection and validation. Finally two epitopes i.e. FTTNIWLKL and HTLWSNGVL, were identified as immunodominant T-cell epitope as putative vaccine antigen. Fig. 7A and B shows the RMSD Plot per residue for FTTNIWLKL - HLA-A complex, RMSD plot per residue for HTLWSNGVL - HLA-A complex and RMSF Plots. The average energy of the simulation was  $-578125.270$  kj/mol. The average Coulombic charge and van der Waals interactions was  $-694749.662$  kj/mol,  $77122.511$  kj/mol, respectively. Both the results were positive as best interactions for protein-ligand docked complexes must possess RMSD values from 1.0 to 3.0 Å as a preferred range. RMSF values in plot clearly indicates that there is no alteration in structures of docked complexes.

### 3.10. Epitope population coverage analysis

The epitope population converge analysis was done by IEDB tool using the formula  $AF = a/2n$ . For peptide FTTNIWLKL & HTLWSNGVL used HLA A alleles for the prediction of population coverage. The highest population coverage for peptide found in south Africa 43.73% and the lowest 14.87% in south America. In India predicted 17.40% population coverage. Fig. 8 shows graphical representation of population coverage for both the selected epitopes of CTL peptide.

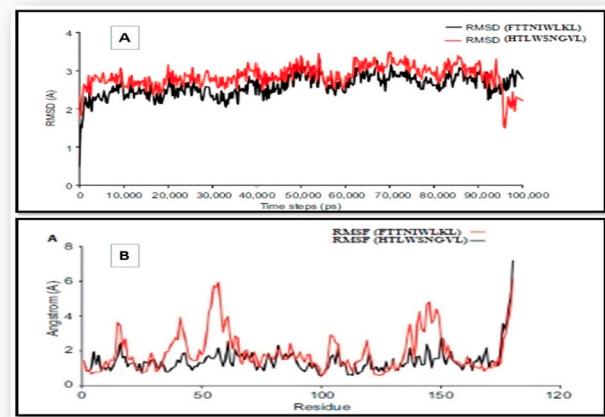
### 3.11. MD simulation of formulated multi T cell epitope vaccine

The vaccine construct was formulated by adjoining multi epitopes with suitable adjuvants using the linkers. Adjuvants act as catalysts of the innate immune response so that they were used in the epitope peptide vaccine formulation to elicit a robust immune response after vaccination. Three adjuvants like RS09 (APPHALS), PADRE sequence (AKF- VAAWTLKAAA) and N-terminal and C-terminal sequence of *Salmonella typhimurium* flagellin protein were linked to the epitopes with the help of GGS linker. The final vaccine was stable sequence analyzed for its physical and chemical properties (The details summarised in Table 5).

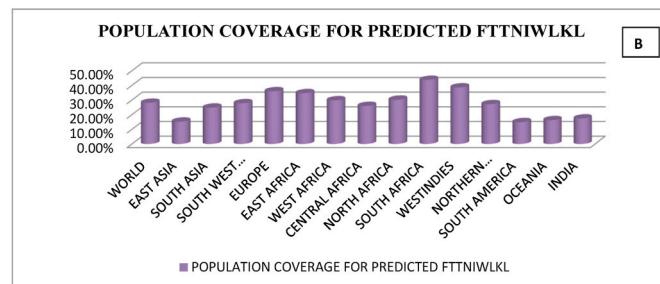
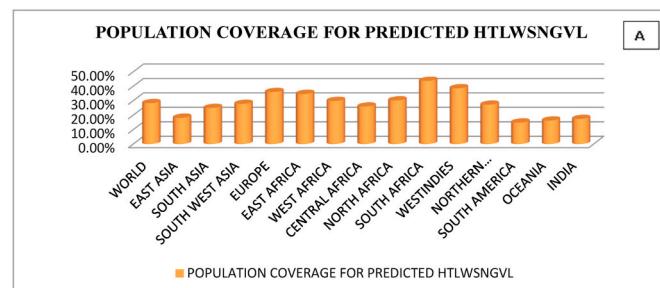
Docking of TLR5 and vaccine construct was conducted by deploying z-dock tool [51]. MD simulation and trajectory analysis for RMSD and RMSF resulted in a constant stability inference for docked complex of TLR-5 receptor and Vaccine construct (Fig. 9) under OPLS force field.

### 3.12. Codon adaptation and in silico cloning

The JCat algorithm was used for optimizing the codon usage of



**Fig. 7.** **A** - RMSD Plot: (Black color) RMSD Plot for FTTNIWLKL - HLA-A1 complex, for each amino acid residue by Molecular dynamics analysis, (Red color) RMSD Plot for HTLWSNGVL- HLA-A1 complex, for each amino acid residue by Molecular dynamics analysis. **B.** RMSF plot: (Red color) RMSF Plot for FTTNIWLKL - HLA-A1 complex, (Black color) RMSF Plot for HTLWSNGVL- HLA-A1 complex.



**Fig. 8.** **A)** Population coverage analysis of selected epitope HTLWSNGVL **B)** Population coverage analysis of selected epitope FTTNIWLKL.

vaccine construct in *E. coli* (strain K12). The optimized codon sequence was composed of 6188 bp. CAI-value of the improved sequence was 0.9419 and 52.414% of GC content in the codon adapted sequence. Codon adaptive index was found to be higher than 0.8 and less than 1 which makes it more susceptible for production purposes via expression vector pET28a (+) (*E. coli* -strain K12). Finally, the adapted codon sequences were inserted and the expression analysis performed by the SnapGene tool. Fig. 10 describes, *in silico* cloning by deploying pET28a (+) vector here CTL vaccine expressing gene was inserted between the two multiple cloning site (MCS) and can visualize in green color.

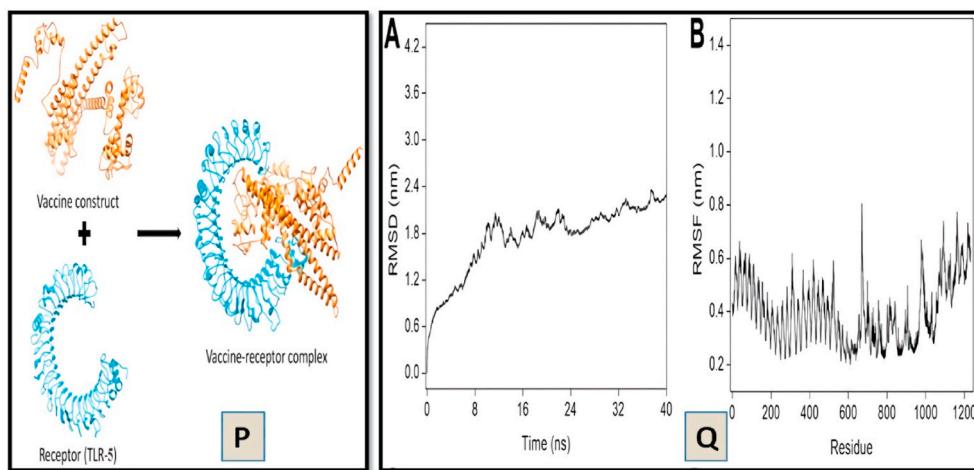
## 4. Discussion

The proteome of DENV is constructed of 10 proteins; out of it 3 are

**Table 5**

Finalized CTL epitope with physicochemical properties.

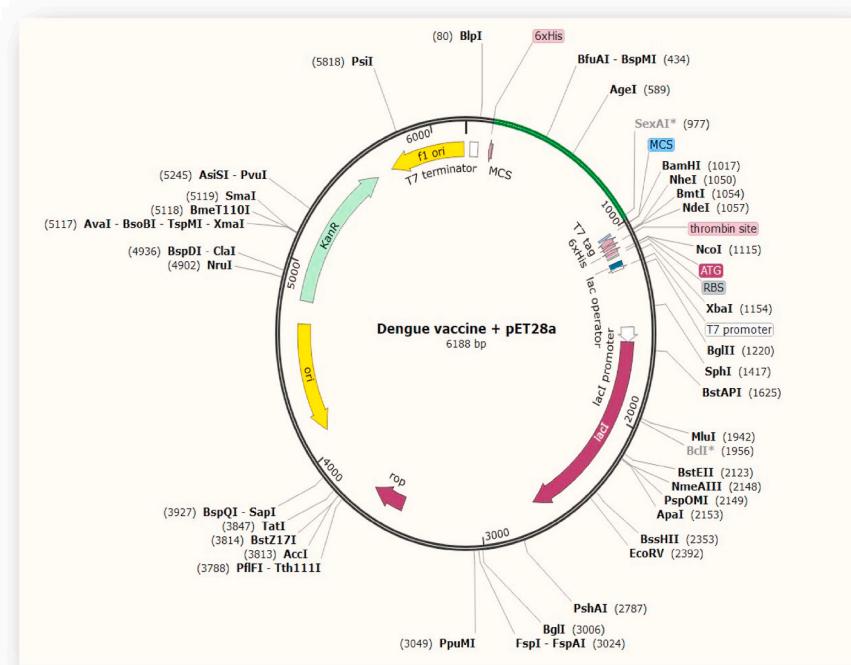
Final vaccine containing selected Epitopes	Instability index	GRAVY SCORE	Estimated Half-life	Aliphatic Index	No. of Amino acids	Molecular weight	Theoretical pI
MAQVINTNSLLTQNNLNKSQ SALGTAIERLSSGLRINSAKDDAA GQAIANRFTANIKGLTQASRNAND GISIAQTTEGALNEINNNLQRQVRELA VQSANSTNSQSDLDLSDIQAEITQRL NEIDRVSGQTQFNGVKVLAQDNT GGSAPPHALSGGSHTLWSNGLGGSAKFVAAWTL KAAAGGSFTTNIWLKLGGSLQKIDAALAQVDTLRSDLGAVQN RFNSAITNLGNTVNNLTSARSRIEDSDY ATEVSNMSRAQILQQAGTSVLAQQA NQVPQNVLSLLR	38.37% (STABLE)	-0.275	30 h (Mammalian reticulocytes)	92.86	283	29741.95	9.09



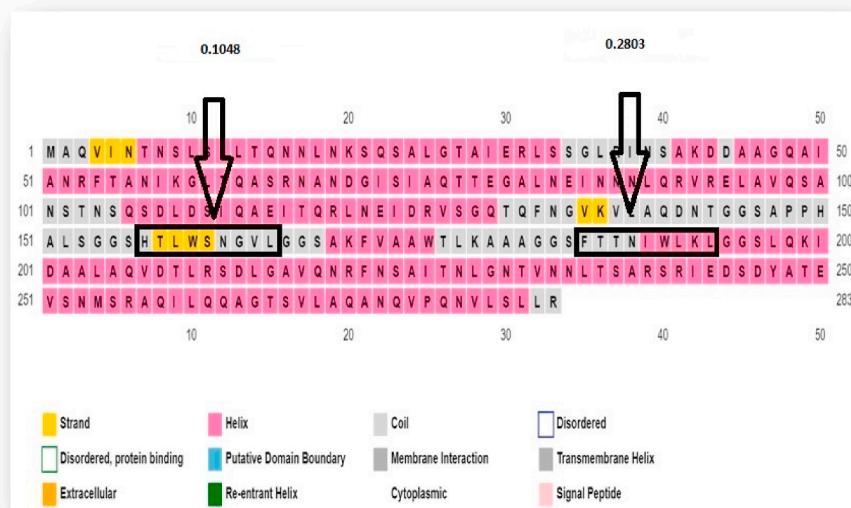
**Fig. 9.** Docking and MD simulation results for finalized vaccine construct with TLR5 receptor: P) finalized vaccine construct interacting TLR5 receptor. Q) MD trajectory analysis: A. RMSD plot for docked complex B. RMSF plot for docked complex.

structural and 7 non structural in nature. NS1 is one of the most mysterious proteins among DENV strains(Scaturroet al., 2015). It has role in immune evasion, pathogenesis and viral replication. Various studies pointed out that the humoral response is primarily directed against the structural proteins and NS1 [52,53]. In the present study, Epitopes predicted from NS1 protein of Dengue virus Strain BR/97-111 are subjected to intensive Immuno-informatics analysis. Protein sequence data was retrieved from UniProt database. NetCTL 1.2 web server epitope prediction resulted in 85 numbers of T cell epitopes against all the 12 MHC supertypes. Linear sequences of all the 85 epitopes were used for IEDB conservancy analysis. Two T-cell epitope peptides predicted from 10 immunogenic epitopes by VaxiJen score and tested in ToxinPred. Toxicity predictions were done for various classes of therapeutic peptides (cell penetrating peptides, tumor homing peptides, anti-viral peptides, anti-bacterial peptides, anti-cancer peptides using ToxinPred [26]. The prediction based on the above methods confirmed that FTTNIWLKL and HTLWSNGL peptides are nontoxic based on SVM score. PatchDock-Firedock screening along with AutodockVina revealed the most stable perfectly docked complexes. After this molecular dynamics-simulation based study assisted in exhibiting good interaction between selected epitopes with binding pocket of MHC-HLA allelic protein. The highest population coverage for peptide was found in south Africa 43.73% and the lowest 14.87% in south America. In India predicted population coverage was 17.40%. Hence the HTLWSNGL and FTTNIWLKL T-cell epitope was selected and it would be a best active ingredient for multi-epitope based dengue vaccine and further vaccine design studies. Classical vaccines are based on the either inactivated or

live attenuated pathogens but in modern era proteome analysis is pre-requisite to save time [54]and previously, similar kind of study was found to be successful in analyzing other non-structural proteins for dengue viruses [55], hepatitis c virusNS5 [56] and NS3 and NS5 proteins of zika virus [57]. *In silico* epitope peptide vaccine predictions for SARS-CoV 2 [58] and Nipah virus [59,60] found immunoinformatics techniques would be helpful. It is a powerful alternative strategy for the experimental discovery of candidate epitopes [61]. The designed multi epitope vaccine was formulated by adjoining CTL T cell multi epitopes with RS09, PADRE sequence *Salmonella typhimurium*flagellin protein adjuvants to elicitinnate and adaptive immune response after vaccination [46,62–64] adjuvant to a robust. Docked complex of TLR-5 receptor and vaccine construct found constant stability inference in MD simulation and trajectory analysis for RMSD and RMSF. The final vaccine sequence (Fig. 11) was found to possess highest immunogenic propensity, stability and the codon adaptive index score, and hence considered more susceptible for vaccine protein production purposes via expression vector. Thus, this approach can be used as a template for the analysis of other pathogens, providing a novel and generalized approach to the crafting of multi epitope based vaccine that are effective against a broad diversity of pathogens [65].



**Fig. 10.** In-silico cloning of CTL Epitope: pET28a vector, CTL vaccine expressing gene was inserted between the two MCS and in green color.



**Fig. 11.** CTL vaccine consisting predicted epitopes HTLWSNGVL and FTTNIWLKL underlies region 151 to 200 amino acid residues had the highest immunogenic propensity for T-cell epitopes.

## 5. Conclusion

Immunoinformatics approaches have been used to find out potent vaccine candidate against dengue virus. On the basis of toxicity, antigenicity, affinity and stability, two vaccine candidates were selected. HTLWSNGVL and FTTNIWLKL could well become a potent vaccine candidate against dengue after validation in wet laboratory as these epitopes have good binding energy –7.1 and 8.1 kcal/mol. Molprobity, Ramachandran plot and ToxinPred biochemical analysis reveals integrity of selected epitopes along with the IEDB population coverage analysis indicates better extant over area. MD simulated vaccine protein sequence successfully *in silico* cloned for the affordable *in vitro* production of the designed vaccine. This vaccine prediction and design

technology can be used for saving time and monetary efforts. The designed vaccine could be reproduced and need to validate the process. The efficacy of the vaccine, potency and safety needed to be validated by *in vitro* and *in vivo* for the probability of large-scale manufacturing.

## Ethical approval

Ethical approval not required since there is no human or animal involvement in this study.

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### Authors' contribution

SKG: data collection, methodology design, and manuscript drafting, AJ: software methodology, NA: visualization and review, Vikas Kaushik: final concept, supervision, reviewing and final editing.

### Declaration of competing interest

The authors declare no conflict of interest.

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