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Article *in* Molecular Simulation · June 2020

DOI: 10.1080/08927022.2020.1772970

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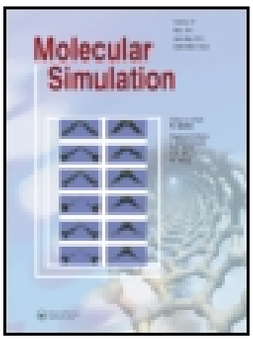

Molecular Simulation

ISSN: 0892-7022 (Print) 1029-0435 (Online) Journal homepage: https://www.tandfonline.com/loi/gmos20

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To cite this article: Sunil Krishnan G, Amit Joshi & Vikas Kaushik (2020): T cell epitope designing for dengue peptide vaccine using docking and molecular simulation studies, Molecular Simulation, DOI: 10.1080/08927022.2020.1772970

To link to this article: https://doi.org/10.1080/08927022.2020.1772970

Published online: 03 Jun 2020.

MOLECULAR SIMULATION
https://doi.org/10.1080/08927022.2020.1772970

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T cell epitope designing for dengue peptide vaccine using docking and molecular simulation studies

Sunil Krishnan G, Amit Joshi and Vikas Kaushik

Domain of Bioinformatics, School of Bio-Engineering and Bio-Sciences, Lovely Professional University, Punjab, India

ABSTRACT

Around the world, emergence and re-emergence of Dengue Virus (DENV) are one of the serious public health concerns. The infection of DENV to human population is mainly caused by the bite of an *Aedes* mosquito. In an estimate, it is found that annually around 100–125 million new dengue incidences are reported from approximately 120 endemic countries. The lone licensed dengue vaccine was not effective to prevent the disease globally. Many dengue vaccines are under clinical trial but there are concerns about the trial reports on safety and efficacy of vaccine. In these circumstances, an epitope-based peptide vaccine is expected to be safe and efficacious against dengue. In this study, the computational prediction of T cell epitope-based peptide vaccine for dengue virus strain BR/97-111 Envelop protein is performed. Total 28 CTL epitopes were predicted using NetCTL 1.2 server. Out of these, five were found to possess antigenicity through VaxiJen 2.0. Further, toxicity prediction using ToxinPred and their conservancy prediction resulted in three epitopes, i.e. TSEIQLTDY, IGIGILLTW and IAVGMVTLY epitope peptide. All these were physically docked and studied for molecular dynamics simulation. In-depth analysis of these results suggests that the predicted three epitopes could be used as a potent vaccine candidate against global dengue disease challenges, although an experimental validation is required for final confirmation.

ARTICLE HISTORY
Received 6 March 2020
Accepted 16 May 2020

KEYWORDS
Dengue virus; antigen; MHC class I; T cell epitope; simulation; docking

1. Introduction

Viral infection diseases spread through either direct or indirect mode of transmission. In indirect mode, mosquitoes are the major culprit, especially female *Aedes aegypti* infect millions of people every year with Dengue Virus which belongs to a family *Flaviviridae*. Female *Aedes aegypti* is a carrier of fast-spreading Dengue, Chikengunya, Yellow fever, Zika in tropical areas [1]. The origin of word dengue is unclear; however, it may have been derived from the Swahili phrase 'Ka-dinga pepo' or 'Dandy Fever' [2]. Ren Kimura and Susumu Hotta reported the first isolation of dengue virus (DENV) in 1943 from Nagasaki, Japan. A first report of DENV exists as early as third century AD in Chinese records. DENV transmits quickly to humans with considerable morbidity and mortality [3]. Dengue fever's clinical presentation is triphasic with symptoms including hyperpyrexia, cephalalgia, nausea, generalised lymphadenopathy, myalgias and arthralgia. Ocular indications include macular oedema, chorioretinits, cotton wool spots, sub conjunctival haemorrhage thrombocytopenia, etc. [4]. Mucocutaneous indications include confluent erythema, haemorrhagic lesions and exanthemas. Ineffective treatment often leads to severe dengue haemorrhagic fever (DHF) or dengue shock syndrome (DSS) [5]. DENV 1 through 4 are the major serotypes and are closely related as evident from their 65–70% genomic sequence similarity [6]. Many genotypes have been identified within the same serotype. Most of the tropical and many subtropical climatic areas have reported dengue spread. DENV has positively stranded RNA as its genetic material which is encapsulated and codes for three structural protein, i.e. nucleocapsid core (C), an enveloped protein (E), membrane-associated protein (M) and seven non-structural (NS) proteins. Severe dengue incidences were less known and it affected only nine countries until 1970 [7]. In the past five decades, DENV incidence has enhanced to 30 fold [8]. In India, dengue disease has been a serious public health issue for decades. There is no effective licensed dengue vaccine available. The first commercialised dengue vaccine, Dengvaxia (CYD-TDV) [9], is yet to be licensed in India and has not proved to prevent this disease effectively. Additionally, this vaccine is now being not accepted by many countries due to its efficacy and safety concerns. Dengue vaccine hesitancy has been due to secondary heterotypic infection, ineffective in long-lasting protection, cross-protection, side effects and other safety-related issues. Traditional vaccine's processing technology is time-consuming, involves safety evaluation, re-virulence concerns and is uneconomical. These diseases are often affecting impoverished regions of the world and require a cheaper alternative. *In silico* methods to design a peptide-based vaccine may be an alternative to the traditional methods of high experimental costs. Considering this, the present study predicts and designs an epitope peptide vaccine candidate for immunisation against dengue viral infection. The logical flow of the computational tools adopted is represented below in Figure 1.

DENV-1 Type 1 strain BR/ 97-111 was selected for the epitope prediction due to its global presence. Envelope protein (E)

CONTACT Vikas Kaushik vikas.14664@lpu.co.in

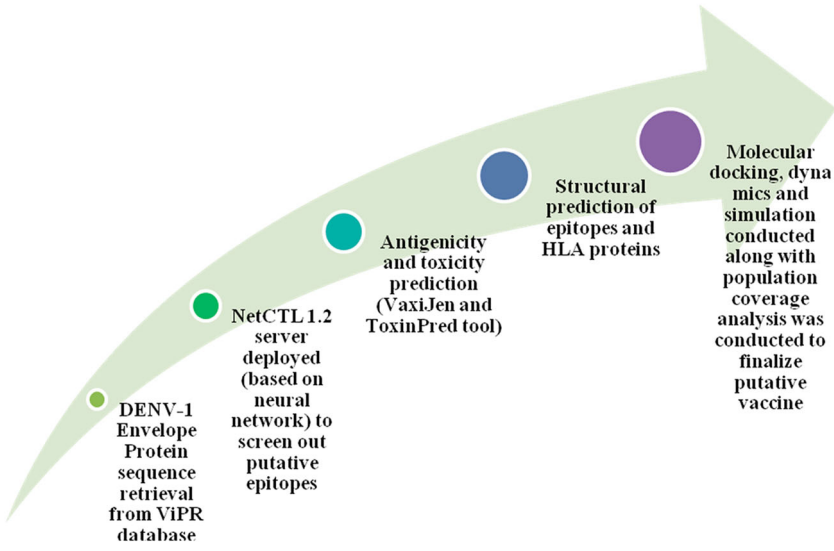

Figure 1. (Colour online) Flow chart representing the computational tools adopted for the study.

was selected for epitope designing due to its importance in vaccine design and development and conservation across the serotypes. Dengue viral morphogenesis, infectivity and tropism are associated with E protein [10]. E protein sequence data of six dengue serotype 1 were retrieved from UniProt. Protein's physical and chemical properties were studied and the secondary structure of DENV strain BR/97-111 was predicted. CTL and antigenicity were calculated and further their toxicity predicted. Finally, linear conservancy prediction was done [11] and the MHC–peptide complex was studied using molecular dynamics simulation. Population coverage study predicts one highly conserved immunogenic stable T cell epitopes for vaccine candidate design at global level.

2. Methodology

2.1. Retrieval of serotype 1 strain details and protein sequences

Serotype 1 strain (BR/97-111) details of DENV were retrieved from ViPR database [12] DENV-1 envelope protein sequence was retrieved from UniProtKB. This database is a comprehensive and manually curate secondary database for protein sequences [13].

2.2. Epitope prediction using integrative approach

CTL epitope prediction was done through an integrative approach employing MHC class 1 binding affinity, C-terminal proteasomal cleavage and TAP transport efficiency using NetCTL 1.2 online server. The chosen parameters included *C* value as 0.15, TAP value as 0.05 and threshold value as 1.25 for optimal predictive performance on an average combined score prediction [14]. Carboxyl (C) terminus proteasomal cleavage and TAP transport Ag-processing and -presentation machinery preceding MHC binding, such as Transporter associated with antigen processing (TAP) protein complex belongs to the ATP-binding-cassette transporter family [15]. TAP delivers cytosolic peptides into the endoplasmic reticulum (ER), where they bind to nascent MHC class I molecules. TAP value used as weight on TAP transport efficiency [16]. The proteasome generates the exact C-terminal of CTL epitopes and the N-terminal with a possible extension. C-terminal, in particular, of CTL epitopes, is cleaved precisely by the proteasome, whereas the N-terminal is produced with an extension, and later trimmed by peptidases in the cytoplasm and in the ER CTL responses may diminish if the epitopes are destroyed by the proteasomes. Therefore, the prediction of the proteasome cleavage sites is important to identify potential immunogenic regions in the proteomes of pathogenic microorganisms. The method for predicting proteasomal cleavage is a matrix-based algorithm called the Stabilised Matrix Method (SMM) trained on *in vitro* cleavage data [17]. The constitutive proteasome data learns a specificity that differs from that of the networks trained on MHC Class I ligands, i.e. the specificity of the immunoproteasome is different than the constitutive proteasome. The tools developed in this study in combination with a predictor of MHC and TAP binding capacity should give a more complete prediction of the generation and presentation of peptides on MHC Class I molecules [18].

2.3. T cell epitope's antigenicity and toxicity prediction

Alignment independent method based on auto cross covariance (ACC) transformation of protein sequences developed by Wold et al. and implemented in VaxiJen 2.0 server was used to predict antigen prediction. This method significantly overcomes the limitations faced by the alignment-dependent methods for antigen prediction due to their limitation in proteins sharing similar structural and biological features but divergent or convergent evolution resulting in poor sequence alignment and ambiguity in results. VaxiJen 2.0 server [19] predicted the antigenic protein sequence from a set of FASTA sequences against the cut-off antigenicity value 1.1. Prediction of non-toxic epitopes from a pool of antigenic epitopes was done using ToxinPred [20]. ToxinPred is machine learning and quantitative matrix-based technique to predict properties

of peptides for their toxicity prediction. This tool was trained on ~2100 known toxin peptides and ~5000 non-toxin peptide sequences with length less than 35 amino acids.

2.4. Epitope conservancy and immunogenicity analysis

Online IEDB's [11] epitope conservancy server was used to predict the degree of the linear conservancy of epitopes. In this study, we used linear conservancy analysis. Immunogenic peptides are very crucial for designing T cell epitope-based vaccine against DENV. IEDB class I immunogenicity analysis tool predicted immunogenicity score for the peptide sequence and positions masked 1st, 2nd, and C-terminus amino acids [21]. High immunogenicity score indicates immunogenic peptide which can become a T cell epitope eliciting an immune response.

2.5. Prediction of epitope and MHC I 3D structures

De novo peptide structure prediction by PEP-FOLD 2.5 Hidden Markov Model derived Structural Alphabet was used for the determination of 3D structures of the selected epitope's amino acid sequences using a discrete set of structural prototypes, and sOPEP coarse-grained force field [22,23]. Structure of the MHC class I alleles was obtained from protein structure database RCSB-PDB (https://www.rcsb.org). The 3D structures of peptide MHC alleles are visualised with pyMOL software (pymol.org).

2.6. Molecular docking

PatchDock molecular docking algorithm (shape complementarity principles) was used for docking [24,25]. The MHC alleles and epitopes PDB files obtained from PEP-FOLD structure prediction used for molecular docking study. The molecular structure of MHC 1 alleles was defined as a receptor and identified peptides as a ligand in the docking program. Clustering RMSD value 1.5 was used to predict the accuracy of docking.

2.7. Molecular dynamics simulations

The X-ray crystallographically driven 3D structure 5IM7.pdb was optimised by the addition of hydrogen bonds, the reconversion of the selenomethionine to methionine and capping of the free N and C terminal amino acids, assignment of pKa value to the titratable side chains of amino acids at pH 7. Protein preparation wizard tool implemented in the Maestro GUI of the Schrodinger Suite was used for the optimisation of the protein structure. Resulting complexes were solvated with TIP3P water model in the orthorhombic box-shaped system with defined boundary conditions. Neutrality of the solvated system and the physiological salt concentration of 0.15M were achieved through the incorporation of an appropriate number of Na+ and Cl− ions. Periodic boundary condition was set to be at least 10 Å. Each face of the box was at 90° with respect to its neighbouring wall of the box. Each simulation was conducted for 100 ns land total of 72,345 atoms consisted of the solved protein system. Desmond simulation software was used for the classical molecular dynamics studies using default settings and 310 K and OPLS-2005 force field.

2.8. Population coverage analysis

IEDB population coverage analysis tool helped to compute population response to predicted peptide based on HLA allele and T cell MHC binding restriction data. Input data were number of epitope set, area country ethnicity, selected area and population and MHC epitope restriction data class I separate options.

3. Results and discussion

3.1. BR/97-111 strain's encoded E protein

Dengue virus consists of four serotypes DENV 1 through 4, among these, the DENV-1 most frequently causes dengue epidemic worldwide. Dengue virus strain BR/97-111 was obtained from ViPR database. Its genome is composed of RNA with Gen Bank accession number is AF311956. Its Envelop protein E sequence was retrieved (UniProt ID P27909). The envelop protein plays a major role in the fusion of viral and host membranes. The synthesis of envelop protein takes place in the ER. These envelop proteins are generally heterodimeric in nature with protein prM. The prM complex helps in defeating the immune system of the host. In Golgi apparatus, PrM-E heterodimers disassociate and form E homodimers. Five epitopes predicted from dengue prM protein and the immunisation assay resulted in only one epitope that elicited an immune response in mice but with limited neutralising activity [26]. Dengue virus shows different antibody binding affinities due to E proteins amino acids structural variation majorly in the interaction portion with host cell receptors. This antibody affinity variation helps the virus left with non-neutralised antibodies to the host system. The non-neutralising antibodies presence on the virus improve the virus infiltration and cause antibody-dependent enhancement of dengue infection it could develop more complex and severe disease [27]. Due to this, the E protein is important for epitope prediction.

3.2. T cell epitope prediction

NetCTL 1.2 predicted 28 T cell epitopes against MHC I supertypes. Three T cell epitopes were predicted against MHC supertype A1, five against supertype A3, two against supertype A24, two against supertype B7, three against supertype B8, two against supertype B27, two against B39, two against B44, six against supertype B58 and one was predicted against supertype B62. No T cell epitopes were predicted at threshold value 1.25 for supertype A2 and A26 (Table 1).

3.3. T cell epitope antigenicity and toxicity prediction

Based on VaxiJen 2.0 antigenicity score of ≥1.1, four antigenic T cell epitope peptides were selected from the NetCTL-1.2 predictions to possess antigenicity. These peptides are TSEIQLTDY, KLTLKGTSY, IGIGILLTW and IAVGMVTLY

Table 1. NetCTL-1.2 MHC supertype's epitope prediction.

Sl. No	Peptide sequence	Prediction score	MHC supertype	Threshold value
1	TSEIQLTDY	3.1296	A1	1.25
2	LTDYGALTL	2.1842	A1	
3	IVQYENLKY	1.5782	A1	
4	ALKLSWFKK	1.4721	A3	
5	KLTLKGTSY	1.3495	A3	
6	VTFKTAHAK	1.3006	A3	
7	KALKLSWFK	1.2967	A3	
8	YVMCTGSFK	1.2804	A3	
9	SYVMCTGSF	1.6932	A24	
10	SWLVHKQWF	1.6347	A24	
11	TPQAPTSEI	1.3178	B7	
12	LPWTSGAST	1.2846	B7	
13	VGKLVHQVF	1.8752	B8	
14	RGARRMAIL	1.6713	B8	
15	HAKKQEVVV	1.4005	B8	
16	NRQDLLVTF	2.6900	B27	
17	KQWFLDLPL	1.2860	B27	
18	TMAKNKPTL	1.5614	B39	
19	VHKQWFLDL	1.3921	B39	
20	AETQHGTVL	1.9120	B44	
21	QEGAMHTAL	1.7167	B44	
22	KSWLVHKQW	1.9941	B58	
23	IGIGILLTW	1.9352	B58	
24	MAILGDTAW	1.6881	B58	
25	SGASTSQEW	1.5844	B58	
26	QTSGTTTIF	1.3708	B58	
27	IAVGMVTLY	1.3054	B58	
28	KLTLKGTSY	1.3251	B62	

with antigenicity score of 1.6089, 1.7285, 1.5690 and 1.1772, respectively (Table 2). Toxicity prediction of these antigenic peptides was predicted using ToxinPred, an SVM-based prediction tool of toxicity at *E*-value cut-off 10. The prediction based on the above methods confirmed that all the four antigenic peptides are found non-toxic (Table 3).

Table 2. Antigenicity predicted T cell epitopes.

Peptide number	Antigenicity prediction score	Antigen/non-antigen score cut off (≥1.1)
1	1.6089	Antigen
2	1.0464	Non-antigen
3	0.7699	Non-antigen
4	0.6427	Non-antigen
5	1.7285	Antigen
6	0.7458	Non-antigen
7	0.4971	Non-antigen
8	0.5939	Non-antigen
9	0.0374	Non-antigen
10	0.2973	Non-antigen
11	0.3131	Non-antigen
12	0.8900	Non-antigen
13	0.3023	Non-antigen
14	0.4539	Non-antigen
15	0.7677	Non-antigen
16	1.0799	Non-antigen
17	0.1382	Non-antigen
18	0.2137	Non-antigen
19	0.4647	Non-antigen
20	0.5192	Non-antigen
21	0.0986	Non-antigen
22	0.3062	Non-antigen
23	1.5690	Antigen
24	0.7313	Non-antigen
25	0.8547	Non-antigen
26	0.0235	Non-antigen
27	1.1772	Antigen
28	0.1325	Non-antigen

Table 3. Toxicity predicted T cell epitope.

Sl. No	Peptide sequence	Toxicity score cut off (≥0)	Toxicity result
1	TSEIQLTDY	−1.47	Non-toxin
2	KLTLKGTSY	−1.34	Non-toxin
3	IGIGILLTW	−0.91	Non-toxin
4	IAVGMVTLY	−1.02	Non-toxin

3.4. Analysis of conservancy and immunogenicity

Four epitope peptides predicted to be non-toxic were analysed for their conservancy using IEDB's conservancy analysis. These epitopes analysed for degree of conservancy with six E protein sequences of Singapore/S275/1990, Nauru/West Pac/1974, Brazil/97-11/1997, Thailand/AHF 82-80/1980, Jamaica/CV1636/1977 and Nauru/West Pac/1974) DENV 1 strains covering different geographic regions of the world. The conservancy prediction revealed that three epitopes (TSEIQLTDY, IGIGILLTW and IAVGMVTLY) reported 100% conservancy (Table 4). This suggests that these epitopes peptides regions are mutated least across different serotypes and thus can be used for universal dengue vaccine development. All four epitopes predicted for immunogenicity analysis using IEDB class I immunogenicity analysis. The highest score was 0.20978 for peptide IGIGILLTW. Immunogenicity score indicates that this peptide was highly immunogenic compare to the other predicted conserved peptides. High immunogenic score indicates which would be eliciting immune response. Conservancy and Immunogenicity results of the predicted epitopes are shown in Table 4.

3.5. 3D structure – prediction of epitope and MHC I

Three peptide ligand's 3D structure and PDB file made from amino acid sequence by using PEP-FOLD. The output result was five best models and predicted structures are provided in PDB format Top ranked one model selected for each epitope and visualised in PyMOL. The selected MHC I alleles were two HLA-A1 and one HLA-B5801. The Crystal structure of HLA-B5801 (PDB ID: 5INC and 5IM7) and the Crystal structure of HLA-A1(PDB ID: 1W72) MHC I alleles Predicted peptides corresponding MHC alleles retrieved from PDB and visualised in PyMOL. Figure 2(A–C) representing the 3D model structure's PyMOL visuals of peptide TSEIQLTDY, IGIGILLTW and IAVGMVTLY, respectively. Figure 3 representing MHC I receptor 3D structures of 1w72, 5im7 and 5inc (C) visuals from PyMOL.

3.6. Molecular docking

Following toxicity, conservancy and immunogenicity evaluations, the physical binding of the peptides with the MHC 1

Table 4. Conservancy and immunogenicity results of the predicted epitopes.

Sr. No	Epitope	Peptide length (9 mer)	Conservancy percentage matches	Immunogenicity score
1	TSEIQLTDY	9	100.00% (6/6)	0.0889
2	KLTLKGTSY	9	16.67% (1/6)	−0.24056
3	IGIGILLTW	9	100.00% (6/6)	0.20978
4	IAVGMVTLY	9	100.00% (6/6)	−0.05836

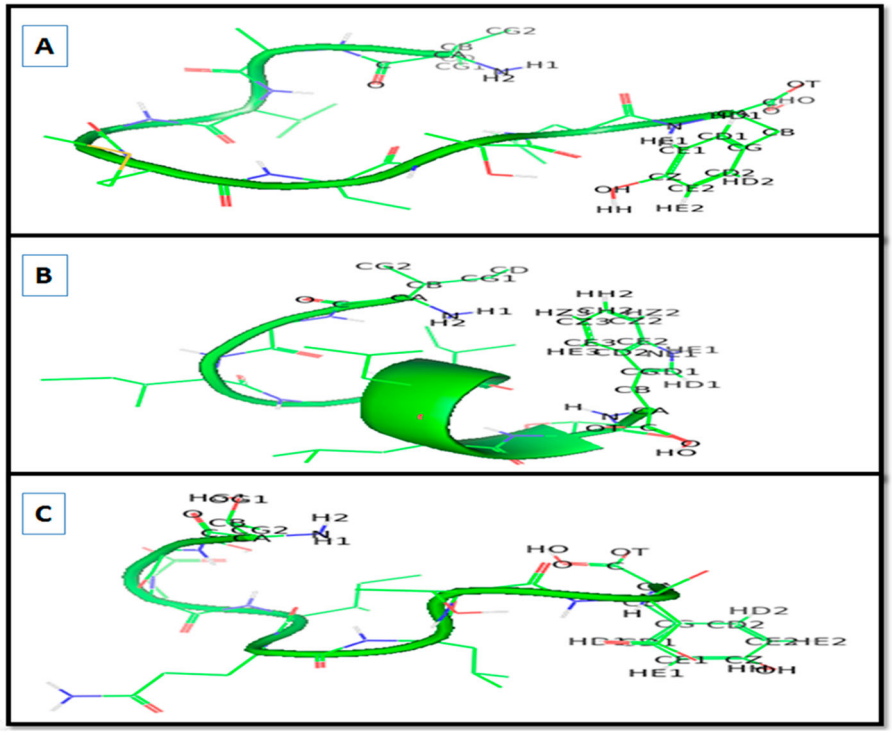

Figure 2. (Colour online) 3D Structure of Epitopes from N-terminus to C-terminus direction: IAVGMVTLY (A), IGIGILLTW (B) and TSEIQLTDY (C) visuals from Pymol.

allele was evaluated using Patch dock molecular docking tool. For each epitope peptide, a total of 200 docked conformation poses were calculated and top conformation for each MHC 1 HLA allele and ligand peptide was selected based on shape complementarity score and area of docking translation, for details, refer Table 5. The analysis was performed for three HLA alleles receptor and the predicted epitopes peptide. Patch dock molecular docking tool was used to find the interacting residues of molecules. The output of 200 docked receptor and ligand complex conformation poses for each set of parameters used. Top one conformation for each MHC 1 allele and ligand peptide was selected based on shape complementarity score and area of docking translation. The output docking results were summarised in Table 5 with column and rows. In column 1: Receptor PDB ID, 2: epitope peptide ligand sequence, 3: Clustering RMSD used for docking, 4: Shape complementarity Score, 5: approximate interface area of the complex, 6: atomic contact energy (ACE) and 7: 3 D Transformation values represented. The solutions are sorted according to this score, Area: approximate interface area of the complex, ACE: atomic contact energy, 3D transformation: 3 rotational angles and 3 translational parameters. The 3D

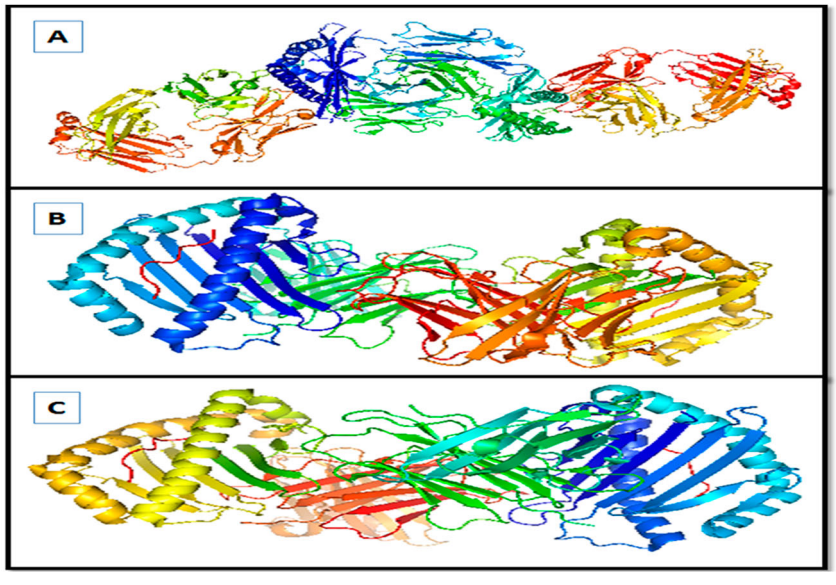

Figure 3. (Colour online) MHC I receptor 3D structures: 1w72 (A), 5im7 (B) and 5inc (C) visuals from PyMOL.

Table 5. Patch dock molecular docking result of MHC 1 receptor and epitope peptide ligand.

Receptor PDB ID	Ligand	Clustering RMSD	Shape complementarity Score	Interface area of the complex	ACE	3D Transformation values
5INC (HLA-B5801)	IAVGMVTLY	1.5	7782	1080.70	−128.42	−1.80, −0.10 −1.27, −20.93, −5.19, −8.51
1W72 (HLA-A1)	TSEIQLTDY	1.5	8192	981.30	20.99	−2.12, 0.35 −0.47, 28.65 25.44, 81.09
5IM7 (HLA-B5801)	IGIGILLTW	1.5	8130	1044.40	−150.87	1.55, 0.31, −1.08, 43.64 89.09 167.27

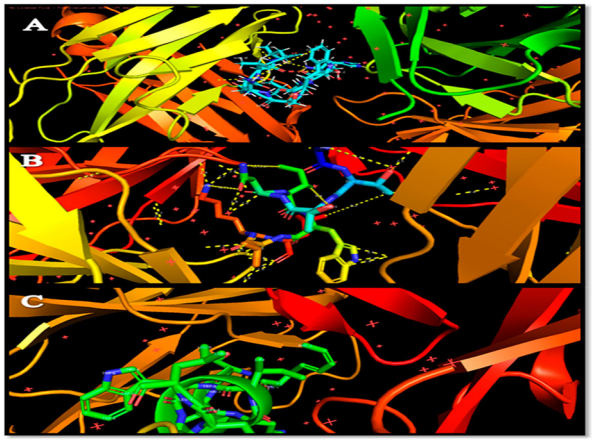

Figure 4. (Colour online) Patch dock molecular docking MHC 1 receptor and epitope peptide ligand IAVGMVTLY (A), TSEIQLTDY (B) and IGIGILLTW (C).

transformation applied on the ligand molecule while the docking complex prediction. Docking complex of MHC HLA allele and IAVGMVTLY epitope resulted in geometric shape complementarity score is 7782, the approximate interface area of the complex was 1080.70 and ACE −128.42. Docking complex of TSEIQLTDY and MHC I HLA allele complex resulted in geometric shape complementarity score was 8192, the approximate interface area of the complex is 981.30 and ACE 20.99. Docking complex of IGIGILLTW and MHC I allele HLA-B 5801 (5IM7) complex resulted in geometric shape complementarity score was 8130, approximate interface area of the complex is 1044.40 and ACE −150.87. IGIGILLTW and MHC I HLA-A1 allele complex selected for further molecular dynamics simulation based on the lowest ACE score.

The best one Patch dock molecular docking MHC 1 receptor HLA-B5801 and HLA-A1 and epitope peptide ligand structures visualised in Figure 4(A–C).

3.7. Molecular dynamics simulations

Among the docked complexes of MHC and peptides, IGIGILLTW–MHC complex was solvated and subjected to an all atom-based production MD run of 100 nanoseconds. For comparison of the stability of the complex, experimentally observed

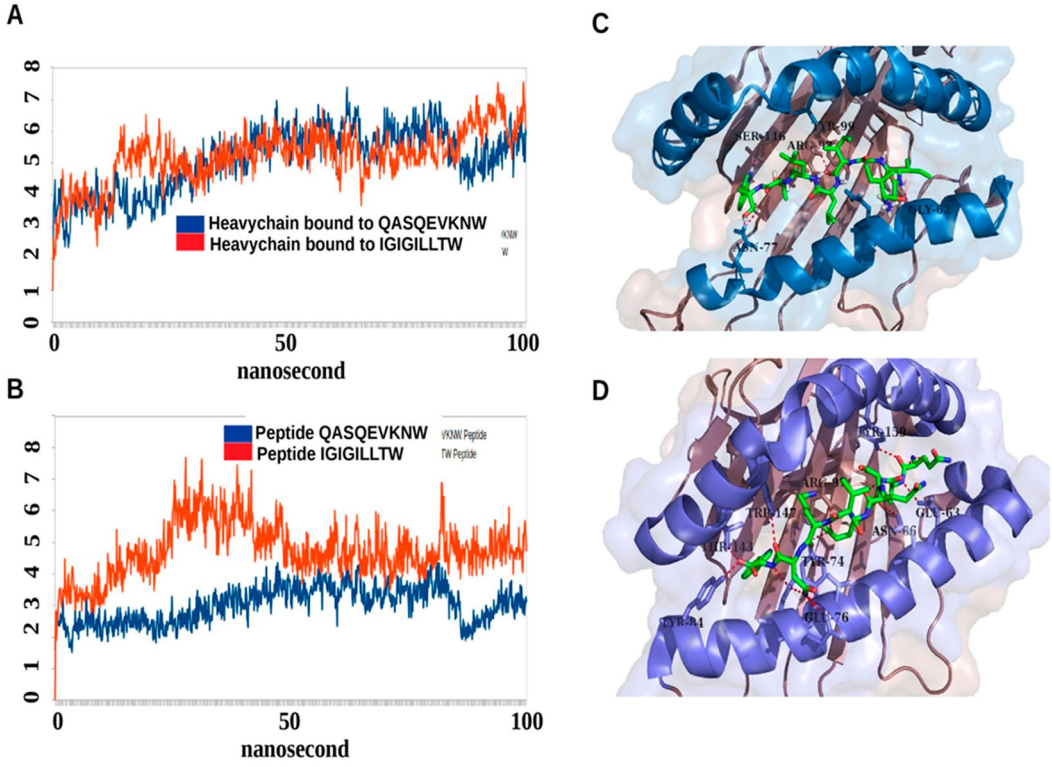

Figure 5. (Colour online) RMSD plots of heavy chains of MHC 1 HLA to crystallographically observed QASQEVKNW (Blue) and docked IGIGILLTW (Red) reporting similar magnitude of deviation (A). RMSD plots of QASQEVKNW (Blue) and docked IGIGILLTW (Red) (B). Binding pose of the IGIGILLTW (C) and QASQEVKNW (D) peptide with MHC-1 Heavy Chain.

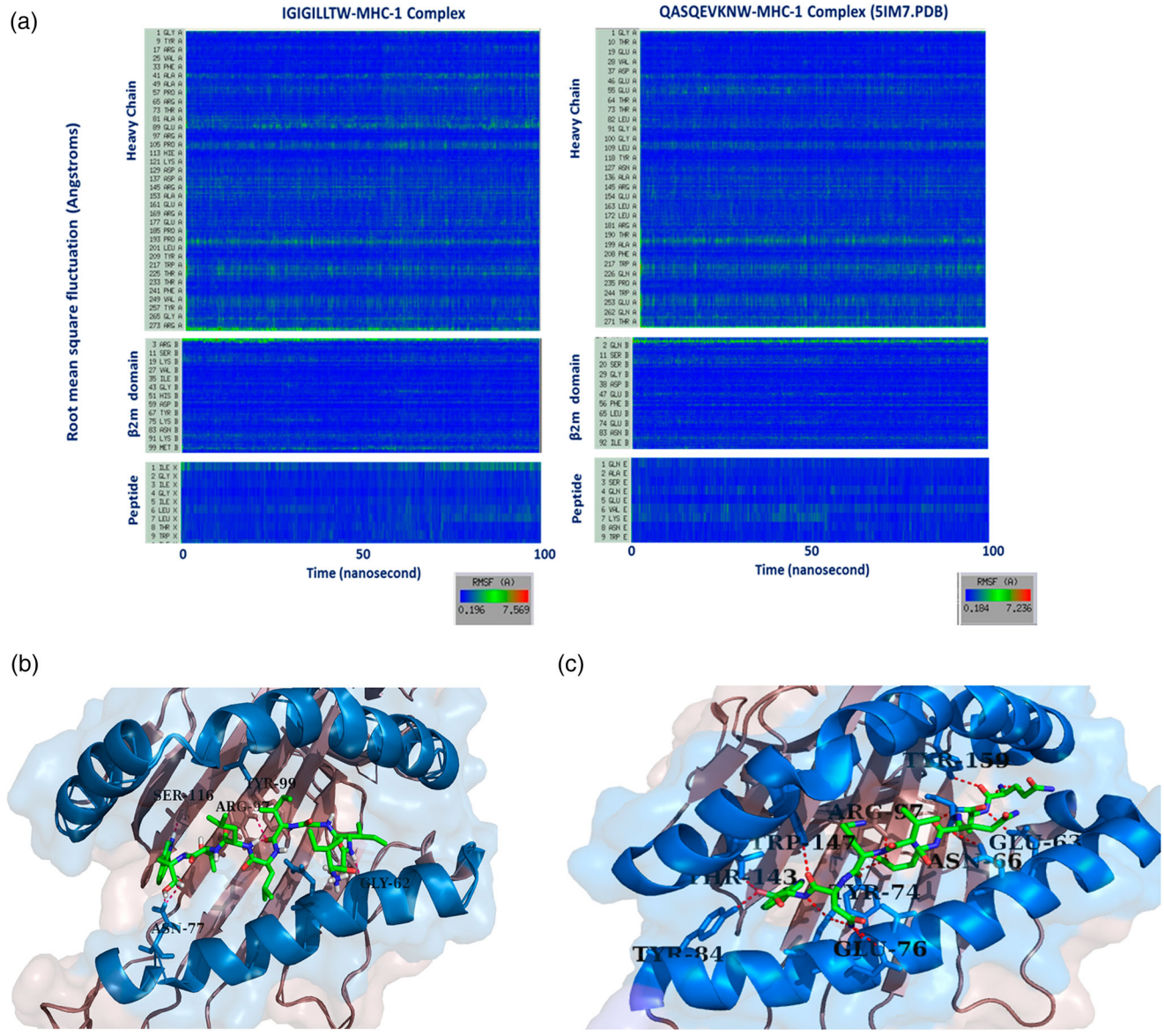

Figure 6. (Colour online) (A) Timeline RMSF analysis of the atoms of Chain A, Chain B and bound peptide IGIGILLTW (Left) and experimentally observed QASQEVKNW (Right). The results show that the fluctuation profile of both experimentally observed peptide QASQEVKNW and computationally identified peptide IGIGILLTW are comparable suggesting the conducive binding between the identified peptide and MHC-1 HLA. (B) IGIGILLTW epitope interacting with MHC class I molecule: ninth position Tryptophan of selected epitope interacting with Asn 77 and Ser 116 via hydrogen bond formation, fifth position Isoleucine of selected epitope interacts with Arg 97 and Tyr 99 via hydrogen bond and third Isoleucine of selected epitope interacts with Gly 62 via hydrogen bond. (Hydrogen bond depicted in dotted red line in image). (C) Reference (Experimental) epitope QASQEVKNW interacting with MHC I molecule: 1st position glutamine interacts with Tyr at 159th position and Asn at 66th position via hydrogen bond formation, 2nd position alanine interacts with Glu 63rd position via hydrogen bond. 4th position glutamine interacts with Asn 66th position, Arg 97th position and Tyr 74th position via hydrogen bond. 8th position asparagine interacts with TRP 147th position and GLU 76th position via hydrogen bond. Tryptophan at 9th position interacts with THR 143rd position and TYR 84th position via hydrogen bond.

MHC-1–peptide complex (5IM7.pdb) was also subjected to 100 nanosecond long MD simulation. Both MD simulations were first analysed for quality analysis including the total energy, potential energy, pressure, volume and temperature of the system throughout the simulation timeline. Root mean square deviation and fluctuation (RMSD and RMSF) analyses were done to compare the structural deviations and fluctuations. The RMSD plots of heavy chains and peptides from both simulations were compared and found to undergo similar deviation through time (Figure 5(A,B)). Root mean square fluctuation plots also reported a comparative fluctuation profile of identified peptide, i.e. IGIGILLTW with the experimentally known peptide QASQEVKNW (Figure 4). These observations suggest that the identified peptide for dengue virus has the capability to bind the heavy chain of the MHC HLA to form a stable complex. RMSF analysis is represented in Figure 6(A) for selected epitope IGIGILLTW and reference epitope QASQEVKNW.

In Figure 6(B), MHC I interaction with selected epitope IGIGILLTW is represented. Third Isoleucine of predicted epitope interacts with backbone oxygen of glycine residue at 62nd position in MHC I complex through hydrogen bond. Isoleucine at the fifth position of our epitope shows two interactions one with hydroxyl group of tyrosine (at 99th position) and another with amino group of Arginine(at 97th position) through hydrogen bond formation. Also, Indole group of tryptophan (9th position in predicted epitope) was found to form hydrogen

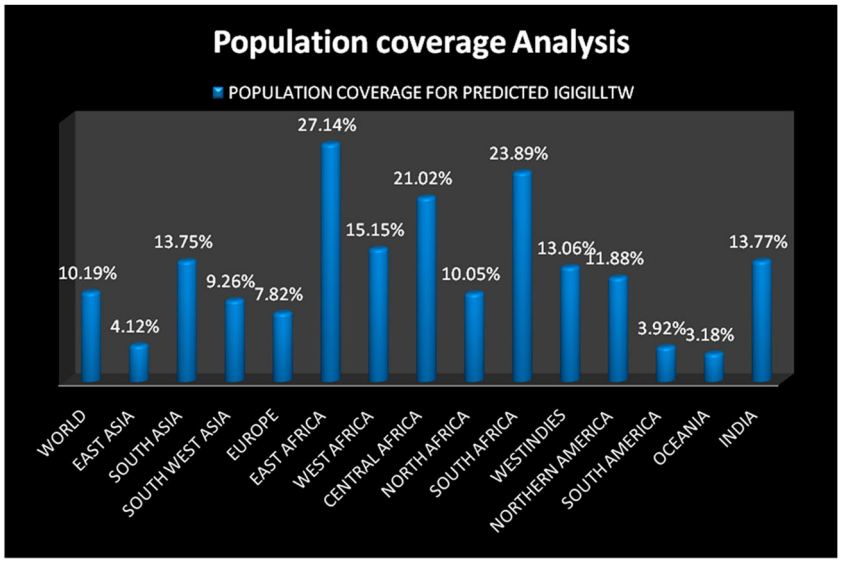

Figure 7. (Colour online) Population coverage analysis for predicted IGIGILLTW peptide.

bond with asparagine 77 and serine 116 isoleucine of predicted epitope forms hydrogen bond. While in Figure 6(C), reference or experimental epitope QASQEVKNW interaction with MHC I via hydrogen bond formation is represented.

3.8. Population coverage analysis

The epitope population converges analysis for 15 different global population using the formula AF = $a/2n$ by IEDB population coverage analysis tool. For peptide, IGIGILLTW used HLA-B*15:16, HLA-B*57:01, HLA-B*58:01, HLA-B*58:04, HLA-B*57:04, HLA-B*58:02 alleles for the prediction of population coverage. The highest population coverage for peptide found in East Africa 27.14% and the lowest 3.18% in Oceania. In India, predicted 13.77% population coverage. The prediction of population coverage details is shown in Figure 7.

4. Conclusion

Four immunogenic, antigenic and non-toxic T cell epitopes (TSEIQLTDY, KLTLKGTSY, IGIGILLTW and IAVGMVTLY) were identified and studied in detail for their binding to the receptor. These epitopes were analysed further for conservancy with six DENV1 protein sequence identified from different geographic regions of the world. The conservancy prediction revealed that the three epitopes (TSEIQLTDY, IGIGILLTW and IAVGMVTLY) have 100% conservancy. PEP-FOLD predicted epitopes 3D structures and PATCHDOCK docking studies revealed potential complexes sorted by ACE value. This result would help to design a T cell-based vaccine candidate against the global dengue viral disease challenge. MD simulation using Desmond simulation software used for 100 ns long IGIGILLTW and MHC I allele complex simulation. RMSD and RMSF analyses suggest that the IGIGILLTW peptide from dengue virus E glycoprotein is capable to bind the heavy chain of the MHC HLA to form a stable complex. Population coverage study results were satisfactory for selected epitopes along the global population scenario as depicted in IEDB coverage analysis. This epitope peptide has the potential to become a candidate vaccine that can elicit immune responses in different population. The study has to be validated by *in vitro* and *in vivo* studies.

Acknowledgements

The authors acknowledge computational services and sound supervision provided throughout the research work by the Department of Bioengineering and Biosciences, Lovely Professional University, Jalandhar, Punjab, India.

Disclosure statement

No potential conflict of interest was reported by the author(s).

ORCID

Vikas Kaushik http://orcid.org/0000-0003-2349-1220

References

[1] Caraballo H, King K. Emergency department management of mosquito-borne illness: malaria, dengue, and West Nile virus. Emerg Med Pract. 2014;16(5):1–23. quiz 23–24.
[2] Rigau-Perez JG. The early use of break-bone fever (Quebranta huesos, 1771) and dengue (1801) in Spanish. Am J Trop Med Hyg. 1998;59(2):272–274. doi:10.4269/ajtmh.1998.59.272.
[3] Bäck AT, Lundkvist A. Dengue viruses – an overview. Infect Ecol Epidemiol. 2013;3. doi:10.3402/iee.v3403i3400.19839.
[4] Powell JR. Mosquito-borne human viral diseases: Why *Aedes aegypti*? Am J Trop Med Hyg. 2018;98(6):1563–1565. doi:10.4269/ajtmh.17-0866.
[5] Singhi S, Kissoon N, Bansal A. Dengue and dengue hemorrhagic fever: management issues in an intensive care unit. J Pediatr (Rio J). 2007;83(2 Suppl):S22–S35. doi:10.2223/jped.1622.
[6] Johnson BW, Russell BJ, Lanciotti RS. Serotype-specific detection of dengue viruses in a fourplex real-time reverse transcriptase PCR assay. J Clin Microbiol. 2005;43(10):4977. doi:10.1128/JCM.43.10.4977-4983.2005.

[7] Murray NEA, Quam MB, Wilder-Smith A. Epidemiology of dengue: past, present and future prospects. Clin Epidemiol. 2013;5:299–309. doi:10.2147/CLEP.S34440.
[8] Bhatt S, Gething PW, Brady OJ, et al. The global distribution and burden of dengue. Nature. 2013;496(7446):504–507. doi:10.1038/nature12060.
[9] Vannice KS, Durbin A, Hombach J. Status of vaccine research and development of vaccines for dengue. Vaccine. 2016;34(26):2934–2938. doi:10.1016/j.vaccine.2015.12.073.
[10] Mondotte JA, Lozach P-Y, Amara A, et al. Essential role of dengue virus envelope protein N glycosylation at asparagine-67 during viral propagation. J Virol. 2007;81(13):7136–7148. doi:10.1128/JVI.00116-07.
[11] Bui HH, Sidney J, Li W, et al. Development of an epitope conservancy analysis tool to facilitate the design of epitope-based diagnostics and vaccines. BMC Bioinformatics. 2007;8:361. doi:10.1186/1471-2105-8-361.
[12] Zhang Y, Zmasek C, Sun G, et al. Hepatitis C virus database and Bioinformatics analysis tools in the virus Pathogen Resource (ViPR). Methods Mol Biol. 2019;1911:47–69. doi:10.1007/978-1-4939-8976-8_3
[13] Mulder NJ, Kersey P, Pruess M, et al. In silico characterization of proteins: UniProt, InterPro and Integr8. Mol Biotechnol. 2008;38(2):165–177. doi:10.1007/s12033-007-9003-x.
[14] Larsen MV, Lundegaard C, Lamberth K, et al. Large-scale validation of methods for cytotoxic T-lymphocyte epitope prediction. BMC Bioinformatics. 2007;8:424. doi:10.1186/1471-2105-8-424.
[15] Daumke O, Knittler MR. Functional asymmetry of the ATP-binding-cassettes of the ABC transporter TAP is determined by intrinsic properties of the nucleotide binding domains. Eur J Biochem. 2001;268(17):4776–4786. PMID 11532014. doi:10.1046/j.1432-1327.2001.02406.x.
[16] Suh WK, Cohen-Doyle MF, Fruh K, et al. Interaction of MHC class I molecules with the transporter associated with antigen processing. Science. 1994;264(5163):1322–1326. PMID 8191286. doi:10.1126/science.8191286.
[17] Nielsen M, Lundegaard C, Lund O, et al. The role of the proteasome in generating cytotoxic T-cell epitopes: insights obtained from improved predictions of proteasomal cleavage. Immunogenetics. 2005;57:33–41. doi:10.1007/s00251-005-0781-7.
[18] Kesmir C, Nussbaum AK, Schild H, et al. Prediction of proteasome cleavage motifs by neural networks. Protein Eng. 2002;15(4):287–296.31. doi:10.1002/eji.200425811.
[19] Doytchinova IA, Flower DR. Vaxijen: a server for prediction of protective antigens, tumour antigens and subunit vaccines. BMC Bioinformatics. 2007;8:4. doi:10.1186/1471-2105-8-4.
[20] Gupta S, Kapoor P, Chaudhary K, et al. In silico approach for predicting toxicity of peptides and proteins. Plos One. 2013;8(9):e73957. doi:10.1371/journal.pone.0073957.
[21] Calis JJ, Maybeno M, Greenbaum JA, et al. Properties of MHC class I presented peptides that enhance immunogenicity. PLoS Comput Biol. 2013;9:10.
[22] Shen Y, Maupetit J, Derreumaux P, et al. Improved PEP-FOLD approach for peptide and miniprotein structure prediction. J Chem Theory Comput. 2014;10(10):4745–4758.
[23] Thévenet P, Shen Y, Maupetit J, et al. PEP-FOLD: an updated de novo structure prediction server for both linear and disulfide bonded cyclic peptides. Nucleic Acids Res. 2012;40(W1):W288–W293.
[24] Duhovny D, Nussinov R, Wolfson HJ. Efficient unbound docking of rigid molecules. In International workshop on algorithms in bioinformatics (pp. 185–200). Berlin, Heidelberg: Springer; 2002, September.
[25] Schneidman-Duhovny D, Inbar Y, Nussinov R, et al. Patchdock and SymmDock: servers for rigid and symmetric docking. Nucleic Acids Res. 2005;33(suppl_2):W363–W367.
[26] Luo Y, Guo X, Yan H, et al. Comprehensive mapping infection-enhancing epitopes of dengue pr protein using polyclonal antibody against prM. Appl Microbiol Biotechnol. 2015;99:5917–5927. doi:10.1007/s00253-015-6538-9.
[27] Modis Y, Ogata S, Clements D, et al. Variable surface epitopes in the crystal structure of dengue virus type 3 envelope glycoprotein. J Virol. 2005;79(2):1223–1231.