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ORIGINAL ARTICLE

MHC binding peptides for designing of vaccines against *Japanese encephalitis* virus: A computational approach

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Abstract *Japanese encephalitis* (JE), a viral disease has seen a drastic and fatal enlargement in the northern states of India in the current decade. The better and exact cure for the disease is still in waiting. For the cause an *in silico* strategy in the development of the peptide vaccine has been taken here for the study. A computational approach to find out the Major Histocompatibility Complex (MHC) binding peptide has been implemented. The prediction analysis identified MHC class I (using propred I) and MHC class II (using propred) binding peptides at an expectable percent predicted IC (50) threshold values. These predicted *Human leukocyte antigen* [HLA] allele binding peptides were further analyzed for potential conserved region using an Immune Epitope Database and Analysis Resource (IEDB). This analysis shows that HLA-DRB1*0101, HLA-DRB3*0101, HLA-DRB1*0401, HLA-DRB1*0102 and HLA-DRB1*07:01% of class II (in genotype 2) and HLA-A*0101, HLA-A*02, HLA-A*0301, HLA-A*2402, HLA-B*0702 and HLA-B*4402% of HLA I (in genotype 3) bound peptides are conserved. The predicted peptides MHC class I are **ILDSNGDIIGLY**, **FVMDEAHFTDPA**, **KTRKILPQIIK**, **RLMSPNRVPNYNLF**, **APTRVVAAEMAEAL**,

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YENVFHTLW and MHC class II molecule are **TTGVYRIMARGILGT**, **NYNLFVMDEAHFTDP**, **AAAIFMTATPPGTTD**, **GDTTTGVYRIMARGI** and **FGEVGAVSL** found to be top ranking with potential super antigenic property by binding to all HLA. Out of these the predicted peptide **FVMDEAHFTDPA** for allele HLA-A*02:01 in MHC class I and **NYNLFVMDEAHFTDP** for allele HLA-DRB3*01:01 in MHC class II was observed to be most potent and can be further proposed as a significant vaccine in the process. The reported results revealed that the immune-informatics techniques implemented in the development of small size peptide is useful in the development of vaccines against the *Japanese encephalitis* virus (JEV).

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1. Introduction

Japanese encephalitis (JE) a major viral disease among human beings of developing countries is caused by *Japanese encephalitis* virus (JEV) that belongs to the Flaviviridae family of dengue virus and yellow fever virus. JE virus causes membrane inflammation in the brain and leads to deleterious effects on central nervous system (CNS). It is a known fact that JEV is a single stranded RNA virus that mainly consist of three structural proteins: capsid protein, precursor membrane protein and envelope protein along with seven nonstructural (NS) proteins: NS1 (NP_775667.1) NS2A (NP_775668.1), NS2B (NP_775669.1), NS3 (NP_775670.1), NS4A (NP_775671.1), NS4B (NP_775673.1) and NS5 (NP_775674.1). In response to JEV infection, the host cell produces virus neutralizing antibodies and cytotoxic T cells (CTLs). It has been shown that defense against JEV infection is primarily antibody dependent, and virus-neutralizing antibodies lacking help are sufficient to convey protection (Mishra et al., 2009; Wu et al., 2003). As our knowledge of the immune responses to a protein antigen has progressed epitope identification has become a challenging immune-informatics problem within vaccine design. To be an epitope, a peptide should have the capacity to bind a Major Histocompatibility Complex (MHC) protein. MHC class I epitopes typically comprises of 8–10 residues while the MHC class II binding site is open-ended, allowing much longer peptides. Many computational methods have been developed for T-cell epitopes (Su et al., 2002).

The currently available epitope prediction methods separately address peptides binding particular MHC proteins, developing models for a single target allele. For T-cell prediction of MHC class-I and MHC class II Immune Epitope Database and Analysis Resource (IEDB) analysis tool is being preferably used. The generalized artificial neural network (ANN)-based stabilized matrix method (SMM) uses both peptide and HLA sequence information for epitope prediction (Konishi et al., 1992) Quantitative predictions of peptide binding to any HLA-DR molecule of known sequence is being done using NetMHCII (Pan et al., 2001).

The immunoinformatics approach utilizes computational algorithms to design potential vaccines or T-cell epitopes. Peptide or epitope based vaccines can be delivered at considerably high doses of potential immunogenicity and has also been estimated to be economical (Danylo et al., 2011). For a development of potential vaccine candidate, such as a JE viral protein, it must be located on the virus such that its surface is exposed externally towards the outer environment, it should be antigenic and a causative agent for pathogenicity.

2. Materials and methods

2.1. Collection of NS protein sequences

The complete protein sequences of nonstructural (NS) protein of JEV were retrieved from the NCBI protein database. It is know that JEV infection of host cells produces seven nonstructural proteins (NS) *viz* NS1 (NP_775667.1), NS2A (NP_775668.1), NS2B (NP_775669.1), NS3 (NP_775670.1), NS4A (NP_775671.1), NS4B (NP_775673.1) and NS5 (NP_775674.1). All these protein sequences were collected from NCBI's genpept protein sequence database.

2.2. Modeling of 3D structure of NS3

Homology modeling method was adopted to generate the Three Dimensional structure of NS3protein using Modeler 10v. The work has been earlier published in the journal Sayeed et al. (2014).

Table 1 MHC-1 best alleles HLA-A*01:01, HLA-A*02:01, HLAA*03:01, HLAA*24:02, HLAB*07:02, HLA-B*44:02.

S.I.	Alleles	Seq num	Start	End	Length	Peptide	Method	Percentile rank	Receptor	Energy
1.	HLA-A*01:01	1	139	150	12	ILDSNGDIIGLY	ANN	0.2	NS3	−597.74
2.	**HLA-A*02:01**	**1**	**282**	**293**	**12**	**FVMDEAHFTDPA**	**ANN**	**0.3**	**NS3**	**−638.11**
3.	HLA-A*03:01	1	200	210	11	KTRKILPQIIK	Consensus (ANN/SMM)	0.2	NS3	−583.94
4.	HLA-A*24:02	1	269	282	14	RLMSPNRVPNYNLF	ANN	0.2	NS3	−519.28
5.	HLA-B*07:02	1	223	236	14	APTRVVAAEMAEAL	ANN	0.3	NS3	−509.21
6.	HLA-B*44:02	1	42	50	9	YENVFHTLW	Consensus (ANN/SMM)	0.15	NS3	−578.85

The bold value indicate best binding affinity and energy score.

Table 2 MHC Class-II best alleles HLADRB1*0101, HLA-DRB3*0101, HLA-DRB1*04:01. HLA-DRB1*01:02, and HLA-DRB1*07:01.

S.I.	Alleles	Start position	End position	Percentie rank	Predicted epitope	Length	Receptor	Energy
1.	HLA-DRB1*01:01	19	33	3.95	TTGVYRIMARGILGT	14	NS3	−556.82
2.	**HLA-DRB3*01:01**	**278**	**292**	**0.01**	**NYNLFVMDEAHFTDP**	**14**	**NS3**	**−694.77**
3.	HLA-DRB1*04:01	310	324	1.31	AAAIFMTATPPGTTD	14	NS3	−572.89
4.	HLA-DRB1*01:02	16	30	0.22	GDTTTGVYRIMARGI	14	NS3	−561.69
5.	HLA-DRB1*07:01	114	128	0.66	FGEVGAVSL	14	NS3	−483.12

Table 3 Interacting amino acid of allele with NS3.

S. No.	Allele	Interacting amino acid residues
MHC class-I		
1.	HLA-A*01:01	I1, L2, D3, S4, N5, G6, D7, I8, I9, G10, L11 and Y12
2.	HLA-A*02:01	F1, V2, M3, D4, G5, A6, H7, F8, T9, D10, P11 and A12
MHC class-II		
3.	HLA-DRB3*01:01	N1, Y2, N3, L4, F5, V6, M7, D8, E9, A10, H11, F12, T13, D14 and P15
4.	HLA-DRB1*04:01	A1, A2, A3, I4, M6, T7, A8, T9, P10, P11, G12, P13, T14 and D15

2.3. Epitope prediction

Initially, the amino acid sequence of the NS3 protein of JEV was obtained from the NCBI protein database. The sequence was obtained in FASTA format. For the prediction of MHC class-I epitope IEDB analysis tool (an online tool) was used (Saini and Vrati, 2003).The sequence was submitted here in FASTA format and with specific selected alleles. The epitopes were predicted for different alleles of MHC class-I. The predicted epitopes were in the form of small peptides. For the prediction of MHC class-II epitopes same IEDB analysis tool was used but with different parameters. All the predicted epitopes were in the form of small peptide sequences.

2.4. Immune Epitope Database Analysis Resource

This server provides a collection of tools for the prediction and analysis of immune epitopes MHC class-I and MHC class-II epitope. The T-cell MHC class-I epitopes of NS3 was predicted for six alleles i.e., HLA-A*01:01 allele, HLA-A*02:01 allele, HLA-A*03:01 allele, HLA-A*24:02 allele, HLA-B*07:02 allele, and HLA-B*44:02 allele as well as for five alleles i.e., HLADRB1*0101, HLA-DRB3*0101, HLA-DRB1*04:01. HLA-DRB1*01:02, and HLA-DRB1*07:01 of MHC class II epitopes of NS3. These small sequence epitopes were obtained from the IEDB analysis tool that uses and cross validated via SVMHC server which uses SYFPEITHI methods of epitope prediction while IEDB analysis tool uses artificial neural network [ANN] and stabilized matrix method SMM method of epitope prediction (Zhang et al., 2008).

2.5. NetMHC-3.0

This server predicts the binding affinity of either a list of peptides with a defined length (8–11 residues) or all possible

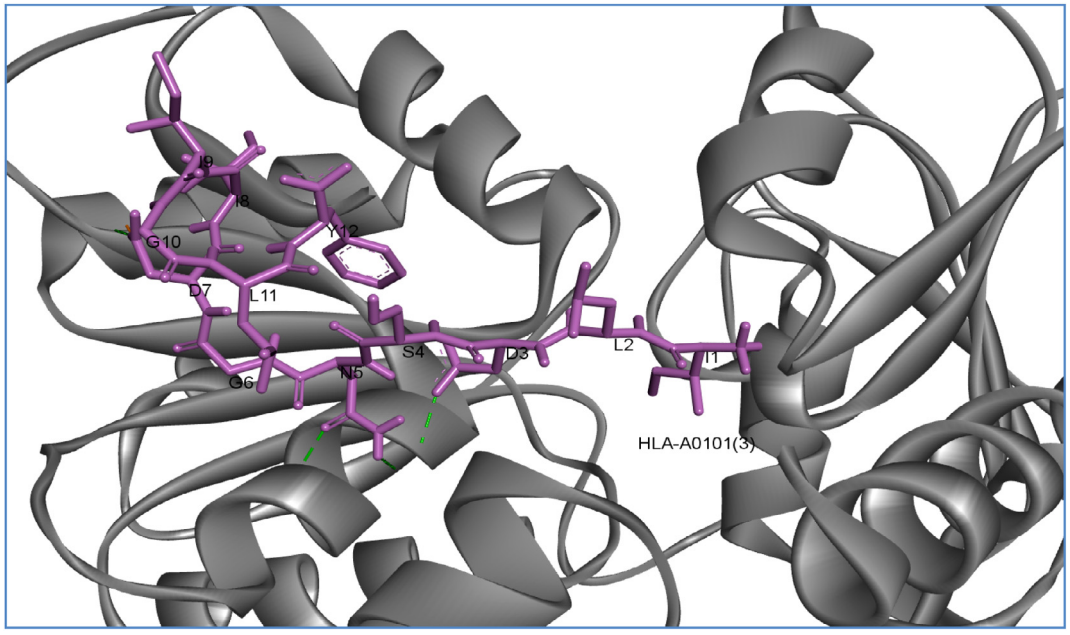

Figure 1 Showing the interaction of HLA-A*01:01 (in purple color) with NS3. The green dotted lines are showing the hydrogen bond. Graphics developed by Discovery Studio Visualizer 4.1.

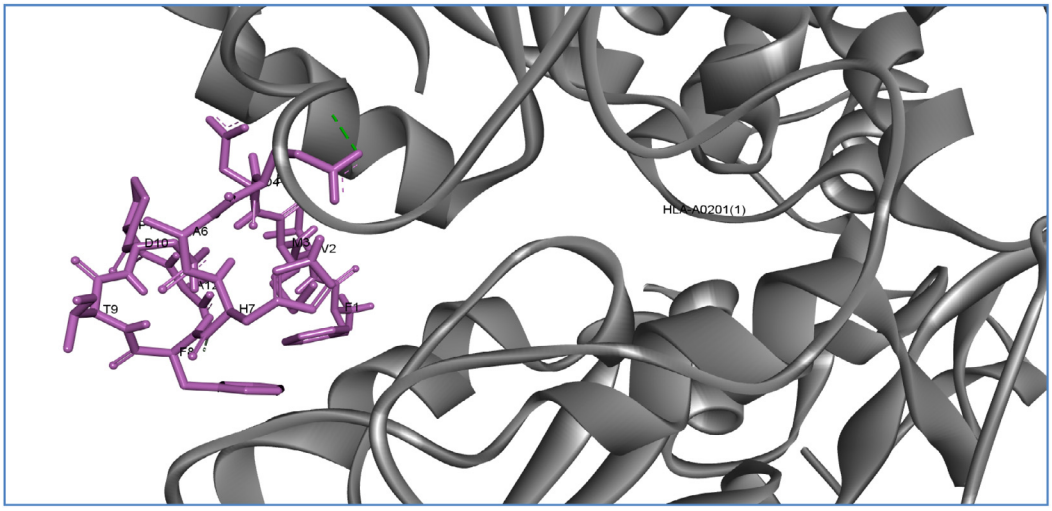

Figure 2 Showing the interaction of HLA-A*02:01 (in purple color) with NS3 .The green dotted lines are showing the hydrogen bond. Graphics developed by Discovery Studio Visualizer 4.1.

sub-peptides hosted within full-length proteins. NetMHC-3.0 is trained on a large number of quantitative peptide data using both affinity data from the IEDB and elution data from SYFPEITHI. The method generates high-accuracy predictions of MHC: peptide binding. The predictions are based on ANN trained on data from 55 MHC alleles (43 human and 12 non-human), and position-specific scoring matrices (PSSMs) for additional 67 HLA alleles. As only the MHC class I prediction server is available, predictions are possible for peptides of length 8–11 for all 122 alleles. Artificial ANN was given as actual inhibition constant IC_{50} values whereas position-specific scoring matrix PSSM predictions are given as a log-odds likelihood scores (Lundegaard et al., 2008).

2.6. Structure-based modeling of T-cell epitopes

The PEPstr server predicts the tertiary structure of small peptides with sequence length varying between 7 and 25 residues (Kaur et al., 2007). The prediction strategy is based on the realization that β-turn is an important and consistent feature of small peptides in addition to regular structures. Thus, the methods uses both the regular secondary structure information predicted from PSIPRED and β-turns information predicted from Beta Turns. The side-chain angles are placed using standard backbone-dependent rotamer library. The structure is further refined with energy minimization and molecular dynamic simulations using Amber (Case et al., 1999).

2.7. Peptide-NS interaction

The Peptide NS Interaction analysis was performed using HEX program (Ritchie and Kemp, 2000) owing to its promising results in the CAPRI (Critical Assessment of Predicted Interactions; http://capri.ebi.ac.uk/) competition with respect to proposing good docking solutions. HEX determines the steric shape, electrostatic potential, and charge density of each

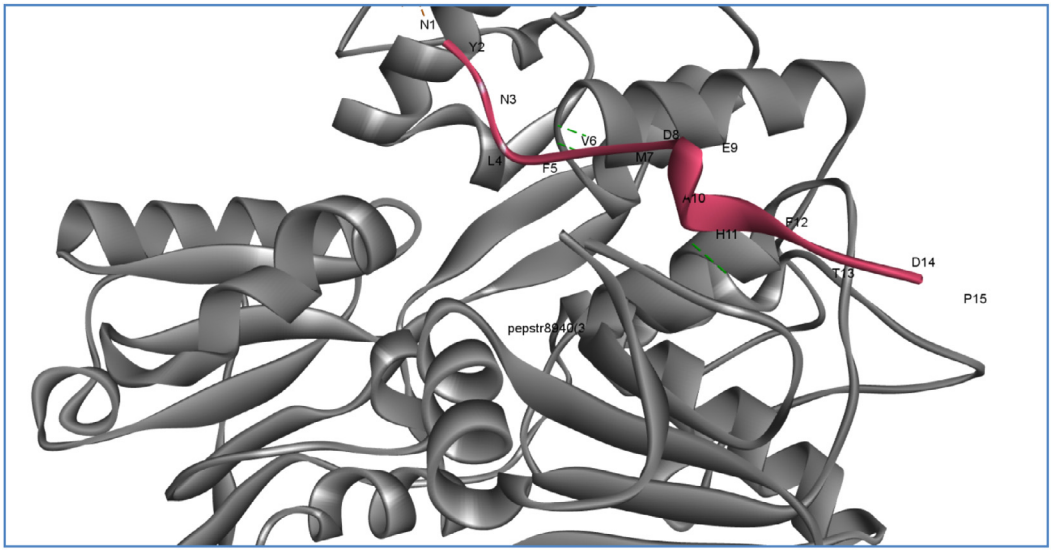

Figure 3 Showing the interaction HLA-DRB3*01:01 (in purple color) with NS3. The green dotted lines are showing the hydrogen bond. Graphics developed by Discovery Studio Visualizer 4.1.

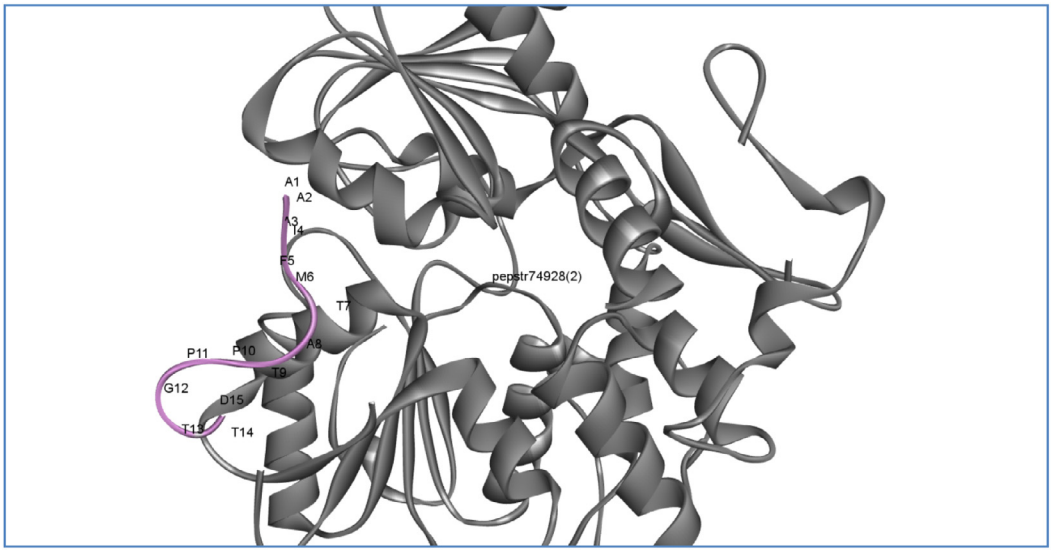

Figure 4 Showing the interaction HLA-DRB1*04:01 (in purple color) with NS3.The green dotted lines are showing the hydrogen bond. Graphics developed by Discovery Studio Visualizer 4.1.

protein as expansions of spherical polar Fourier basis functions. The protein surface shapes are calculated to determine the match potential of two proteins. Then, candidate-docking solutions are refined using a "soft" molecular mechanics energy minimization procedure, and the list of docking solutions is clustered to assist in identifying distinct orientations (Wang et al., 2012).

3. Results and discussion

The antigenic protein retrieved from Uniprot in FASTA format was used to predict the T-cell epitopes for MHC class-I and MHC class-II molecules of *Homo sapiens* using the IEBD analysis resource tool. These predictions were not easy to make because their respective alleles need to be specified. HLA-A*01:01, HLA-A*02:01, HLAA*03:01, HLAA*24:02, HLAB*07:02, HLA-B*44:02, HLADRB1*0101, HLA-DRB3*0101, HLA-DRB1*04:01, HLA-DRB1*01:02, and HLA-DRB1*07:01 alleles were considered for epitope prediction. Docked conformation of both proteins MHC class I and MHC class II with predicted epitopes were analyzed and graphical interpretation has been done using Discovery Studio 2.5 tool (Dassault Systèmes BIOVIA, Discovery Studio Modeling Environment, Release 4.5, San Diego: Dassault Systèmes, 2015). Interacting interface residues have been identified between both biomolecules. Interface residue identification would be helpful for prediction of epitopes to generate a vaccine against JEV.

3.1. Results for MHC class-I binding epitope prediction

Table 1 shows the predicted T-cell MHC class-I epitopes for various HLA alleles. These small sequence epitopes were obtained from the IEDB analysis tool using ANN method of epitope prediction and cross validated via SVMHC server which uses SYFPEITHI methods for epitope prediction. The structure of docked complexes of predicted epitopes with MHC class I was visualized using Discovery Studio Visualization tool. There are six alleles i.e., HLA-A*01:01, HLA-A*02:01, HLAA*03:01, HLAA*24:02, HLAB*07:02 and HLA-B*44:02 of MHC class-I molecule for which epitopes are predicted. The complex with best binding affinity and energy score obtained is −638.11 for '**FVMDEAHFTDPA**' epitope against **HLA-A*02:01** allele docked with NS3 protein (Table 1). The important amino acids involved in interaction of epitope with NS3 protein are I1, L2, D3, S4, N5, G6, D7, I8, I9, G10, L11 and Y12 (Table 3).

The structure of '**ILDSNGDIIGLY**' epitope docked with NS3 protein showed highest binding affinity and energy score of −**597.74** kJ/mol while the structure of '**FVMDEAHFTDPA**' epitope docked with NS3 protein showed best binding affinity and energy score of **−**638.11** kJ/mol (Table 1).

The structure of '**KTRKILPQIIK**' epitope docked with NS3 protein produced highest binding affinity and energy score of −**583.94** kJ/mol while the structure of '**RLMSPNRVPNYNLF**' epitope docked with NS3 protein produced highest binding affinity and energy score of −**519.28** (Table 1).

Similarly the structure of '**APTRVVAAEMAEAL**' epitope docked with NS3 protein showed highest binding affinity and energy score of −**509.21** kJ/mol and the structure of '**YENVFHTLW**' epitope docked with NS3 protein showed highest binding affinity and energy score of −**578.85** kJ/mol (Table 1). The predicted epitope of NS3 protein for six allele were arranged in accordance to their start and end positions along with their percentile rank.

3.2. Result for MHC class-II binding epitope prediction

Prediction of MHC-II binding peptides is difficult as compared to MHC class-I binding peptides due to their variable size. There are five alleles i.e., HLADRB1*0101, HLA-DRB3*0101, HLA-DRB1*04:01. HLA-DRB1*01:02, and HLA-DRB1*07:01 of MHC class-II molecule for which epitopes are predicted using IEDB analysis tool and cross

validated by SVMHC server. Only those epitopes having a peptide score above the threshold value and 50% cut off have been selected. The structure of predicted '**NYNLFVMDEAHFTDP**' epitope against allele **HLA-DRB3*01:01** docked with NS3 protein produced the best binding affinity and energy score of −694.77 kJ/mol (Table 2). The important amino acids involved in interation of epitope '**NYNLFVMDEAHFTDP**' with NS3 protein are N1, Y2, N3, L4, F5, V6, M7, D8, E9, A10, H11, F12, T13, D14 and P15 (Table 3).

The structure of '**TTTGVYRIMARGILG**' epitope docked with NS3 protein showed binding affinity and energy score of −556.82 kJ/mol **while** the structure of '**AAAIFMTATPPGTTD**' epitope docked with NS3 protein showed binding affinity and energy score of −572.89 kJ/mol (Table 2).

Similarly the structure of '**GDTTTGVYRIMARGI**' epitope docked with NS3 protein produced binding affinity and energy score of −561.69 kJ/mol while the structure of '**FGEVGAVSL**' epitope docked with NS3 protein produced the binding affinity and energy score of −483.12 kJ/mol Table 2.

Design and development of short peptides as vaccine candidate for JEV is gaining momentum in recent years. Therefore, in the present study we have predicted epitope like region in the NS3 protein having RPN interaction. On the basis of energy score two best dock figure of each MHC-I and MHC-II were attached in this study for better understanding (Figs. 1–4). Hence, these data could be useful in designing candidates capable of producing antipeptide antibodies which are competent of recognizing JEV specific nucleocapsid protein.

4. Conclusions

The T-cell epitopes against MHC class I molecule are **ILDSNGDIIGLY**, **FVMDEAHFTDPA**, **KTRKILPQIIK**, **RLMSPNRVPNYNLF**, **APTRVVAAEMAEAL**, **YENVFHTLW** respectively for each of the alleles. Among these **FVMDEAHFTDPA** epitope has best binding affinity with docking score of **−638.11** kJ/mol. The T-cell epitope for MHC class-II molecule are **TTGVYRIMARGILGT**, **NYNLFVMDEAHFTDP**, **AAAIFMTATPPGTTD**, **GDTTTGVYRIMARGI** and **FGEVGAVSL** out of which **NYNLFVMDEAHFTDP** has the highest binding affinity with docking energy of **−694.77** kJ/mol.

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