



Protein Modelling and Binding Analysis of HLA-DR Promiscuous T cell Epitopes from Pfs-25 Ookinete Surface Antigen

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Abstract

Plasmodium falciparum the agent of malignant form of malaria and in particularly its ability to generate mutant variants makes it a successful pathogen. Due to the increasing drug resistance problem, an effective malaria vaccine is highly desirable. Peptide based vaccine can also be utilized to control malaria but HLA polymorphism is major hurdle in development of peptide based vaccine. Nevertheless promiscuous peptides which have potential to bind with more than one HLA alleles can be utilized to develop vaccine against malaria. Identification of an appropriate T-cell epitope for activation of immune system is of great importance before a strategy for development or trial of malaria vaccine is formulated. In the present study five different *in silico* tools were employed to predict promiscuous peptides by using protein sequence of promoter region of Pfs-25 gene. A total 303 peptides were predicted and by adopting different selection criteria's only 4 peptides were selected for 3D homology modelling. The constructed 3D models of promiscuous peptides and HLA-alleles were further utilized to test for their binding affinity towards HLA alleles by means of peptide protein docking analysis. Docking analysis of these four potential vaccine candidate epitopes revealed that the promiscuous peptide P9 (FLCFLQFIHFFRYLF) showed highest docking affinity with all HLA-alleles and this epitopic region may be utilize as potential vaccine candidate antigen for development of peptide based vaccine against *P. falciparum*.

Keywords: *P. falciparum*; Pfs-25; Promiscuous peptides; 3D Homology modelling; Docking

Introduction

Malaria is still a life threatening parasitic disease with an estimated 212 million cases and 429,000 deaths [1]. The challenge of producing a widely available vaccine that provides a high level of protection for a sustained period is still to be met, although several are under development. The extensive genetic diversity of the malaria parasite constitutes major drawbacks to the development of a successful malaria vaccine [2-6]. *P. falciparum* presents a number of potential vaccine targets because of its complex life cycle with different stages have potential vaccine candidate antigens [7]. Several antigens expressed during the sexual stage of *P. falciparum* are target of antibodies which are capable of preventing the transmission of the parasite from human to mosquito and might be utilize to develop transmission blocking vaccine [8-10]. Malaria transmission blocking vaccine targets the sexual development of the parasite within the mosquito mid gut and antibodies directed against this antigen

will prevent fertilization between male and female gametes, which will further inhibit the development of zygote into motile ookinetes [11]. Pfs-25 is a, cysteine-rich 25kDa surface protein of *P. falciparum*, expressed during the sexual stage of life cycle. It is a potential vaccine candidate antigen which is capable to develop specific antibodies [12].

Peptide based vaccines can also be utilized to develop an efficacious vaccine against malaria. The widespread occurrence of HLA polymorphism is a serious limitation to develop peptide based vaccines for global immunization because, invariably, most of such antigenic peptides bind to a few HLA alleles [13]. Immune responses directed against some epitopes may be genetically restricted and a given epitope may be recognized by one antigen but not by another [14,15]. Hence, an efficacious vaccine against malaria will need to have the potential to induce responses against a number of epitopes that are recognized in the context of many different HLA alleles [14,16]. A conventional approach to discover such promiscuous peptides is to synthesize many possible peptides from an antigen and to test each for their immunogenicity [13]. Experimental identification of vaccine candidate antigens is also costly and time consuming process [17-20]. Bioinformatics or *in silico* tools can also be utilized to predict peptide sequence from the chosen antigen that show optimum binding to HLA alleles that are predominant in the population to be vaccinated. Although the MHC binding prediction algorithm reached high performances but there are reports that suggest these tools may not be very efficient [21]. The efficiency of the predicted promiscuous peptides by sequence based *in silico* methods can be verified by structure based MHC binding *in silico* methods (Kashyap *et al.*, 2017, Khan *et al.*, 2017). One of the main categories in structure-based MHC binding *in silico* methods is docking. The docking analysis of promiscuous peptides with

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HLA alleles to know the interaction between promiscuous peptides with HLA-alleles also reduced the time of wet lab analysis [22]. Keeping these points in view the present study was aimed to predict promiscuous peptides by analyzing the published gene sequences of strong vaccine candidate antigen gene Pfs-25 of *P. falciparum* and to check binding pattern analysis by structure based computational techniques. The immune response of predicted promiscuous peptides was also verified computationally.

Materials and Methods

Recouplement of target sequence

Predominantly and frequently occurring HLA class-I alleles (HLA-A*01:01, HLA-A*02:11, HLA-A*02:01, HLA-A*02:06, HLA-A*11:01, HLA-A*33:03, HLA-B*35:01, HLA-B*40:06) and HLA class-II alleles (DRB1*0301, DRB1*0701, DRB1*1001, DRB1*1101, DRB1*1302, DRB1*1404, DRB1*1502, DRB1*1501) were selected [23-26]. Amino acid sequence of selected HLA alleles was retrieved from NCBI Gen bank database (<http://www.ncbi.nlm>). Protein sequences of Pfs-25 from promoter region of *P. falciparum* with Accession no. (AF030628.1) was retrieved from NCBI data base (<http://www.ncbi.nlm>) and compared with Plasmo DB and Uniprot for conservancy.

Promiscuous peptide prediction

The Immune Epitope Database (IEDB) (<http://www.iedb.org>) [27] and the NetMHCpan web server (<http://www.cbs.dtu.dk/services/NetMHCpan-3.0>) [28] were also used for the prediction of T cell specific epitopes. The NetMHC3.0 server (<http://www.cbs.dtu.dk/services/NetMHC-3.0>) [29] was used to predict the binding of peptides to MHC molecules belonging to the HLA class-I using artificial neural networks. The Net MHCIIpan 2.4, [30]. Immune Epitope Database (IEDB) and Analysis Resource MHC class-II binding prediction tools NN-align [31], SMM-align [32], ARB (average relative binding)²³ and NetMHCII2.2 [33,34] were used to predict peptides specific for HLA class-II alleles. All parameters and their values for individual tools were kept as default. The peptides obtained were classified on the basis of binding pattern based on IC50 value [35,36]. The predicted promiscuous peptides having IC50 value ≤ 500 nM were classified as strong binders, IC50 value in between 500-1000nM were classified as weak binders, IC50 value ≥ 1000 nM classified as non- binders. The predicted promiscuous peptides were further classified for 100% strong binding affinity criteria with all HLA alleles to reduce the number of strong binder promiscuous peptides and for the selection of best promiscuous peptides for 3D Homology Modelling.

Selection criteria's of epitopes for 3D homology modelling

Cluster analysis: The 100% binder promiscuous peptides were subjected to know the relatedness among the peptides by constructing a cladogram with the help of Seaviewversion software [37]. This software utilizes Phylogenetic estimation Maximum Likely hood method and 100 Bootstraps were run to construct a cladogram. This analysis was helpful to reduce

the number of promiscuous peptides because closely related peptides have similar properties and a peptide from one group can be selected as a representative of a respective group.

Hydrophobicity attribute: The promiscuous peptides were further subjected to check the Hydrophobicity attribute because hydrophobicity index is a measurement of the relative hydrophobicity, or how soluble an amino acid in water. Hydrophobic attribute of promiscuous peptides were calculated by using Peptide property calculator software [38]. Promiscuous peptides with hydrophobicity $>50\%$ are accurately soluble in aqueous solution and hydrophobicity $<50\%$ are partial or insoluble in aqueous phase. Promiscuous peptides with hydrophobicity $>50\%$ were selected for Protein homology modeling.

Interferon-gamma and interleukin-4 inducing MHC class-II binders: The promiscuous peptides selected on the basis of above mentioned criteria's were finally subjected to check whether these selected promiscuous peptides were IFN- γ and IL-4 inducer or non-inducer. IFN epitope and IL-4 pred are *in silico* tools which were utilized to identify MHC class-II binders that can activate IFN- γ and IL-4 inducing T-helper cells [39]. The promiscuous peptides found IFN- γ and IL-4 inducing can be further verified by structure based *in silico* analysis. These tools mainly work on two approaches i.e. Motif based approach which works on software MERCI [40] and second is Machine learning approach which works on Support Vector Machine [41]

Homology modeling of epitopes: The 3D models of promiscuous peptides (epitopes) were required to find out their prominent interaction with HLA alleles. All the promiscuous peptides were modelled using UCSF Chimera Program [42]. UCSF Chimera is a highly extensible program for interactive visualization and analysis of molecular structures and related data, including density maps, supra molecular assemblies, sequence alignments, docking results, trajectories, and conformational ensembles. Primary protein sequence utilized for Peptide Modelling. 3D Models of primary sequence of peptide were generated and all parameters like Φ and Ψ angles were kept as default value. Finally all the Peptide models were minimized by using Amber field with 100 gradient steps.

Protein sequence retrieval for HLA-Alleles: The predominantly and frequently present HLA-Alleles were selected for structure prediction. Amino acid sequences of target proteins from these alleles were retrieved from NCBI Gen bank database (<http://www.ncbi.nlm>) with accession number P04229 (HLADRB1-1), P01912 (HLA-DRB1-03), Q5Y7A7 (HLADRB1-13), Q30167 (HLA-DRB1-10), P13761 (HLA-DRB1-07), Q9GIY3 (HLADRB1-14), P20039 (HLADRB1-11), P01911 (DRB1-15).

Homology modelling of HLA-Alleles

Alignment with template sequence: The first step towards homology modelling was selection of template. For that Blast P was performed with query sequence against PDB Database and the protein with highest similarity for selected HLA molecules were used as template for modelling.

Model generation: For the generation of 3D models of HLA-Alleles the query and template sequences were used as input in



Modler 9v7 Program [43]. Modeller is a program which is based on comparative modelling by satisfaction of the spatial restraints. By using query and template as input 5 models were generated and model with highest score was selected as final structure [43]. Further accuracy of each model was refined and validated with different available tools.

Model optimization and evaluation: Model optimization and evaluation was required because Modelled structure sometimes contains different kinds of errors like bond lengths, bond angles and torsion angles. Therefore, it was essential to minimize the structure constructed by Modeller [44]. Energy minimization was done by using UCSF Chimera 1.8.1 [42] through AMBER and energy minimization should be kept to minimum, so that structure should not be affected. From these models, the most acceptable model was finalized with best fit according to Ramachandran plot generated by PDBSUM program (Laskowski, 1998) and also validated by PROCHECK program. The stereochemical excellence modelled structure was analysed by Z-scores [45,46] and ERRAT analysis [47]. Finally super imposition of template and query sequence was done by using matchmaking in UCSF Chimera Program. After that Modelled beta chain was united with conserved alpha chain of template in chimera with the help of match maker to complete the structure of class-II alleles.

Docking interaction of Modelled structure of HLA-alleles with epitopes: Docking interaction of 3D Models of promiscuous peptides and HLA-alleles was studied by using AutoDock1.4.6 software. In AutoDock, file was prepared by adding polar hydrogens and partial charges, and defining rotatable bonds. Modelled protein was also prepared by adding polar hydrogens and merging nonpolar hydrogens. Kollman charge and atom type parameter were added. Grid map were set around active site and also to required surrounding surface. Lamarckian genetic search algorithm was employed and docking run was set at 250 runs. Maximum number of energy evaluation was 25,000 per run and maximum number of generation in genetic algorithm was set at 27,000 [44].

Results

Epitopic prediction from antigenic protein

Epitopes are mostly derived from no self-protein antigen that interacts with antibodies or T-cell receptors and further activate the immune system [48]. Protein sequence from promoter region of Pfs-25 antigen of *P. falciparum* was utilized to predict T-cell epitope binders with HLA class-I and class-II alleles by using *in silico* approach. A total of 309 and 303 T-cell epitopes were predicted on the basis of binding affinities between the putative epitope regions and HLA-alleles. On the basis of IC-50 value, out of 303 epitopes only 12 were found as 100% binders for HLA class-II alleles. There was not found any Promiscuous peptide for HLA class-I allele because predicted peptides were not fulfilling the criteria of 100% binding affinity.

Selection of promiscuous peptides for 3D Homology modelling

Clustre analysis: All the promiscuous peptides of Pfs-25 antigen were grouped into three major clusters. The peptide

P10 diverge from internode and fall in outgroup in 1st cluster; whereas P9, P11, P12 share a common sub branch and have close similarity with each other. The peptide P4, P5, P6 and P7 share a common branch and sub branch and have close relatedness with each other. The peptide P1 fall in an outgroup and P2, P3 and P8 share common branch and sub branch. The promiscuous peptide showing close relatedness with each other among these one can be selected as a representative of group. These promiscuous peptides were further subjected to check hydrophobicity index, for selection of promiscuous peptides for 3D homology modeling (Figure 1).

Hydrophobicity attribute: On the basis of hydrophobicity index promiscuous peptides P1, P2, P3, P8 comprise <50% hydrophobicity (Table 1) and considered as good promiscuous peptides for synthesis, on the basis of phylogenetic relatedness and hydrophobic attribute of promiscuous peptides P1, P2, P3 and P8 were selected for molecular docking analysis with HLA-alleles.

Interferon gamma and interleukin-4 inducing epitopes: The promiscuous peptides selected on the basis of above mentioned criteria's were finally subjected to check whether these selected promiscuous peptides were IFN- γ and IL-4 inducer or non-inducer. Promiscuous peptides P8, P9, P10, P11 and P12 showed positive IFN- γ inducer. While promiscuous peptides P1, P2, P3, P6, P9, P10, P11 and P12 showed positive IL-4 inducer (Table 1). On the basis of all selection criteria's promiscuous peptides P1, P2, P3 and P9 were selected for 3D homology modelling and finally verified by docking pattern analysis.

Protein homology modelling of promiscuous peptides: The 3D structures for P1, P2, P3 and P9 promiscuous peptides (Figure 2-5) for promoter region of Pfs-25 antigen were constructed by using Chimera 1.8.1 software and minimized by

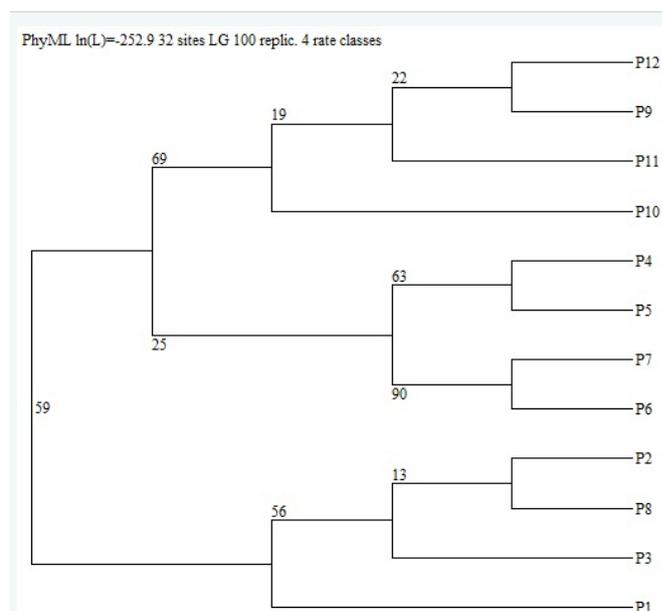


Figure 1 Cladogram tree of 100% binder promiscuous peptides of Pfs-25 antigen.

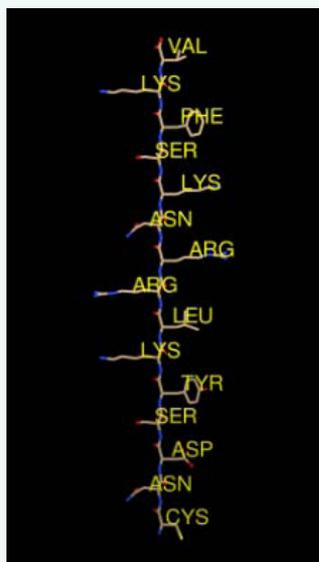


Figure 2 3D structure of promiscuous peptide (P1) (CNDSYKLRNRKSFKV).

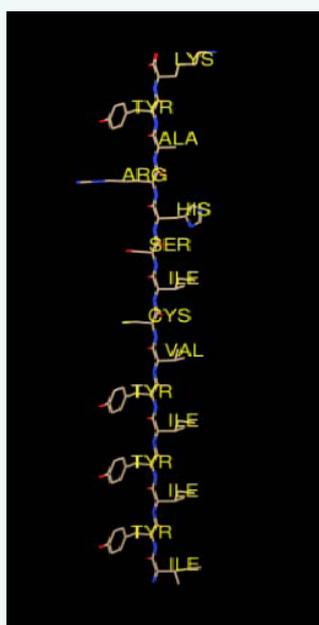


Figure 3 3D structure of promiscuous peptide (P2) (IYIYIVVCISHRAYK).

using 100 gradient steps. Energy minimization was performed to stabilize 3D structure.

Homology modelling of HLA-alleles

Template identification: Large number of sequences shown similarity with HLA Class-II alleles, but sequence with highest similarity was selected as template. Hence 1A6A was selected as a template for HLA DRB*0301, HLA DRB*1301, HLA DRB*1401 and 1ADQ, 2SEB, 1YMM, 1AQD for HLADRB*0701, HLA DRB*1101, HLA DRB*1501, HLA DRB*1001 respectively because

these templates shown highest similarity with respective HLA-alleles and X-ray crystallographic structure of these templates is available on RCBS database.

Verification of HLA class-II structure: By using above mentioned specific template for specific HLA-allele, five models were generated by using Modeller9V7 program. After optimization and energy minimization process, among five 3D models best model was selected on the basis of Modeller score. Optimization and energy minimization of 3D-models was done to provide maximum stability to the protein structure. The accuracy of structure was checked by Ramachandran plot drawn through Rampage program. Ramachandran plot analysis showed that

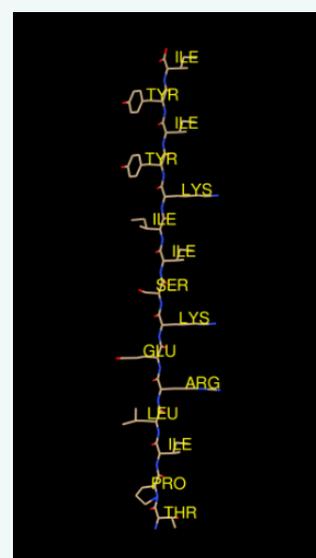


Figure 4 3D structure of promiscuous peptide (TPILREKSHIKYIYI).

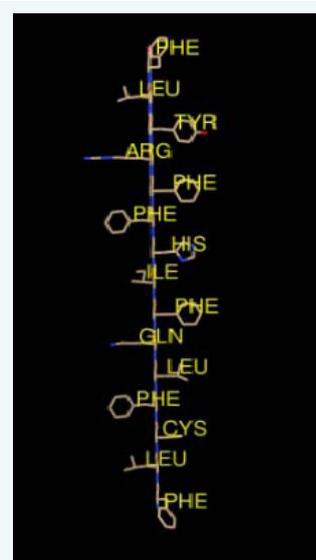


Figure 5 3D structure of promiscuous peptide (P3) peptide (P9) (FLCFLQFIHFFRYLF).

Table 1: Hydrophobicity Index, interferon-gamma and IL4 inducing MHCclass-II binders promiscuous peptides from promoter region of Pfs-25 antigen.

S.No.	Promiscuous peptides	Hydrophobicity	IFNepitope	IL4pred
1.	P1 (CNDSYKLRRNKSFKV)	20%	Negative	Positive
2.	P2 (IYIYIVVCISHRAYK)	40%	Negative	Positive
3.	P3 (YIYIYVCISHRAYKC)	33.33%	Negative	Positive
4.	P4 (IIFILYIQGIKKFF)	73.33%	Negative	Negative
5.	P5 (IFILYIQGIKKFFF)	66.67%	Negative	Negative
6.	P6 (VNLNIIMSFLTSLIIF)	66.67%	Negative	Positive
7.	P7 (NLNIIMSFLTSLIIFI)	66.67%	Negative	Negative
8.	P8 (TPILREKSIHKYIYI)	46.67%	Positive	Negative
9.	P9 (FLCFLQFIHFFRYLFF)	66.67%	Positive	Positive
10.	P10 (LCFLQFIHFFRYLFF)	66.67%	Positive	Positive
11.	P11 (CFLQFIHFFRYLFFF)	66.67%	Positive	Positive
12.	P12 (FLQFIHFFRYLFFF)	73.33%	Positive	Positive

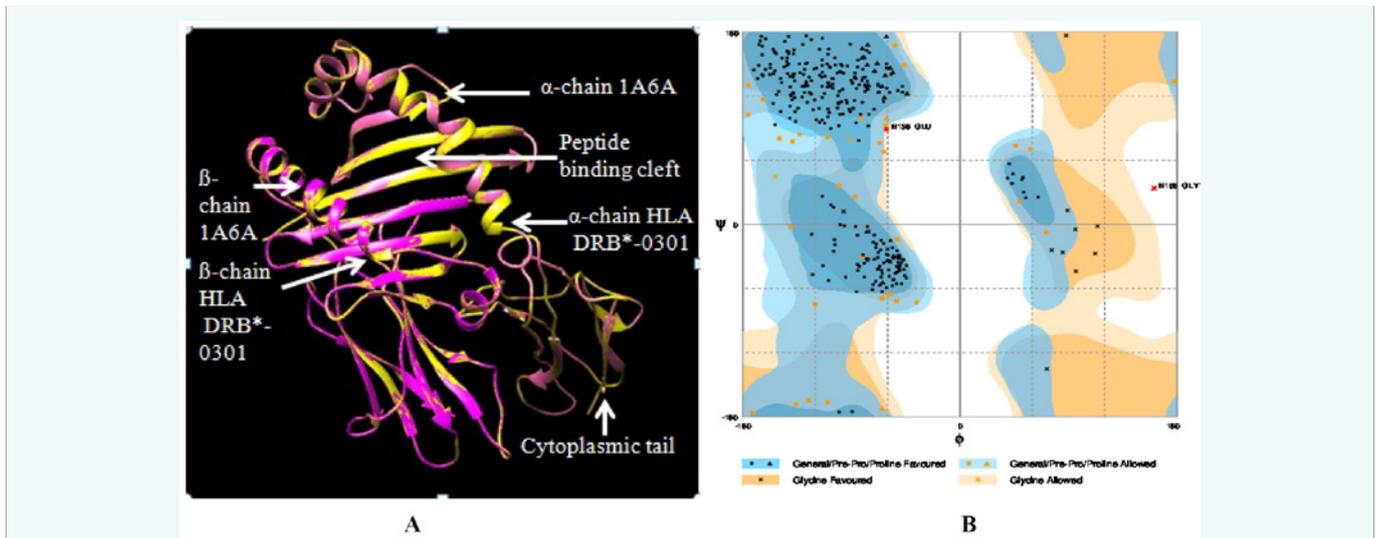


Figure 6 3D structure of HLA DRB*0301 in superimposition with template 1A6A (A) Ramachandran plot of HLA DRB*0301 (B)

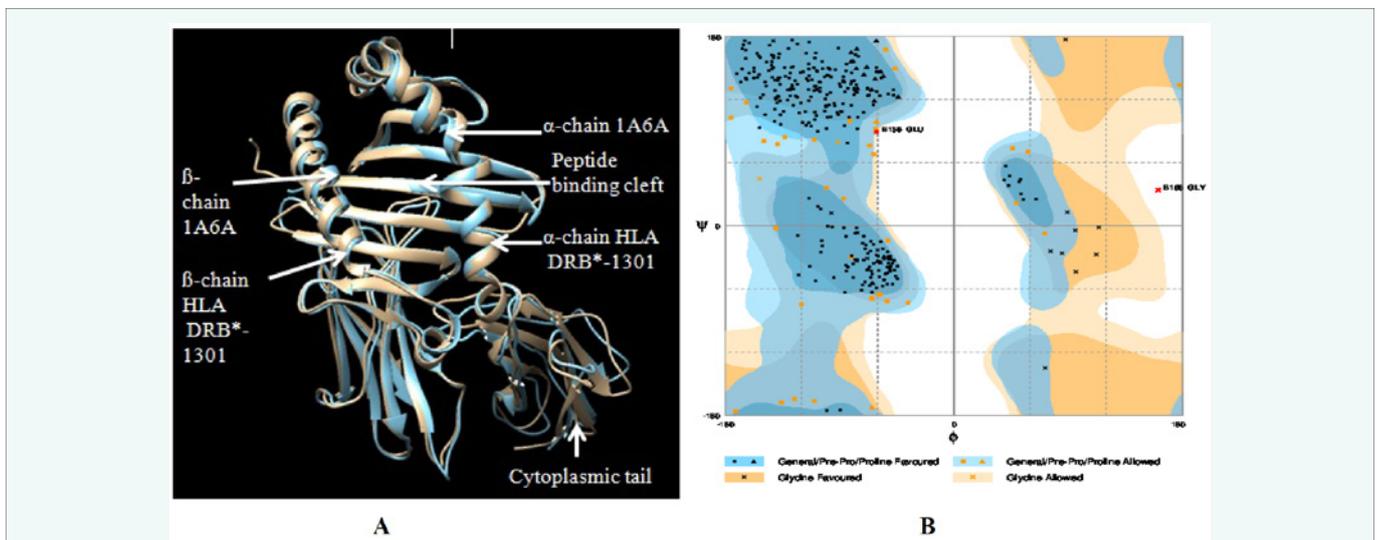


Figure 7 3D structure of HLA DRB*1301 in superimposition with template 1A6A (A) Ramachandran plot of HLA DRB*1301(B).

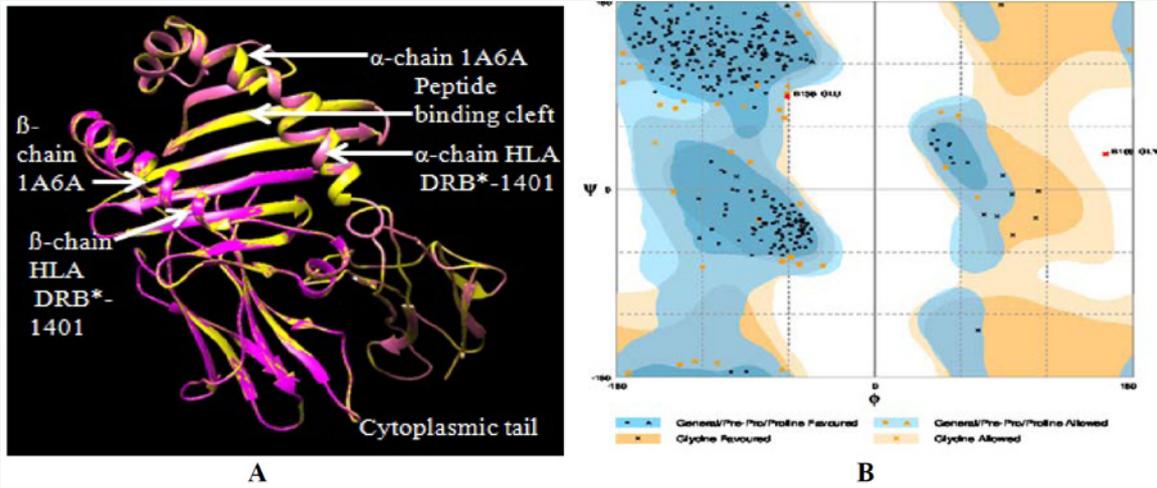


Figure 8 3D structure of HLA DRB*1401 in superimposition with template 1A6A (A) Ramachandran plot of HLA DRB*1401(B)

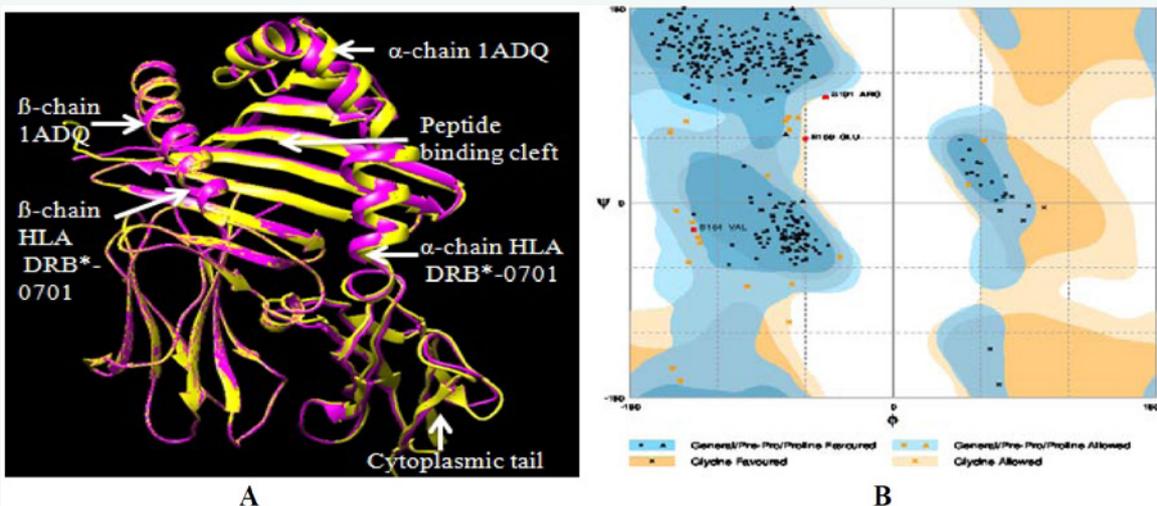


Figure 9 3D structure of HLA DRB*0701 in superimposition with template 1A6A (A) Ramachandran plot of HLA DRB*0701(B)

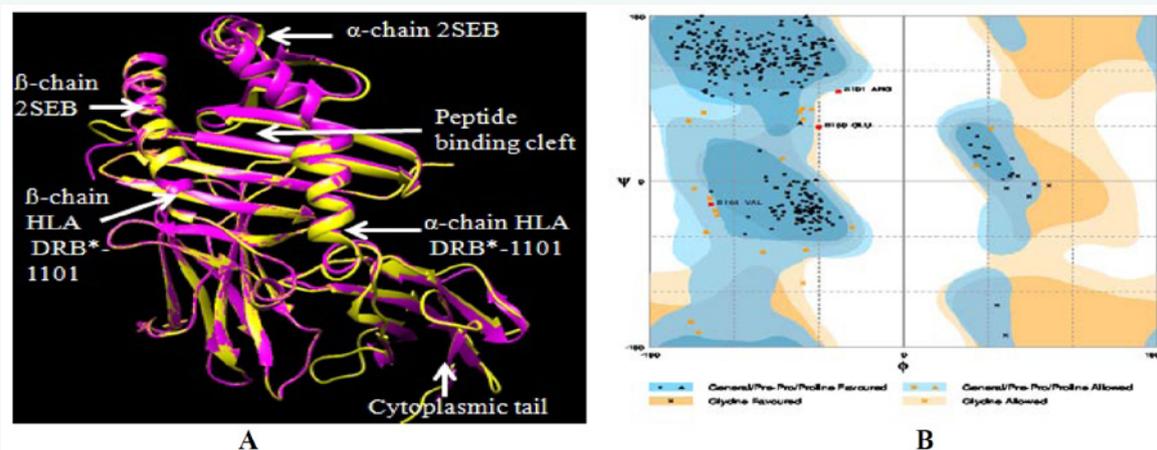


Figure 10 3D structure of HLA DRB*1101 in superimposition with template 2SEB (A) Ramachandran plot of HLA DRB*1101(B)

88% of total residues felt in favoured region and 10.9% in allowed region, 2 residues (0.6%) i.e. GLU and GLY lies in outlier region for HLA DRB*0301, HLA DRB*1301, HLA DRB*1401(Figure 6,7,8). For HLA DRB*0701 and HLA DRB*1101, 93.7% of total residues felt in favoured region and 5.4% in allowed region, 3 residues (0.8%) i.e. VAL, GLU and ARG lies in outlier region(Figure 9,10). For HLA DRB*1501, 85.9% of total residues felt in favoured region and 11.0% in allowed region, 11 residues (3.1%) i.e. GLY, ARG, THR, PHE, ASP, GLN, LEU, VAL, PRO and PRO lies in outlier region (Figure 11). For HLA DRB*1001, 98.9% of total residues felt in favoured region and 1.1% in allowed region, there is no amino acid that lies in outlier region (0%) (Figure 12). This stipulates that phi (φ) and psi (ψ) occupied reasonably accurate positions in the selected 3D-models for all the HLA-alleles.

Z score value: The predicted 3D models of HLA DRB*0301, HLA DRB*1301, HLA DRB*1401, HLA DRB*0701, HLA DRB*1101, HLA DRB*1501, HLA DRB*1001 were also compared against protein database (PDB) using DaliLite V.3 server [49] and illustrated Z score value 20, 23.5, 20.0, 21.7 and 19.4 respectively. The quality of structures was also evaluated by ERRAT analysis and according to that the structures having value > 50 were considered as good. In the current study as shown in the Figure 13 ERRAT scores for modelled HLADRB*0301(Figure 12A), HLADRB*0701(Figure 12B), HLADRB*1301(Figure 12C), DRB*1101(Figure 12D), DRB*1501(Figure 12E) were 88.18, 56.74, 67.41, 70.86 and 68.31 respectively. So the model quality is significant and acceptable.

Binding pattern analysis of promiscuous peptides with

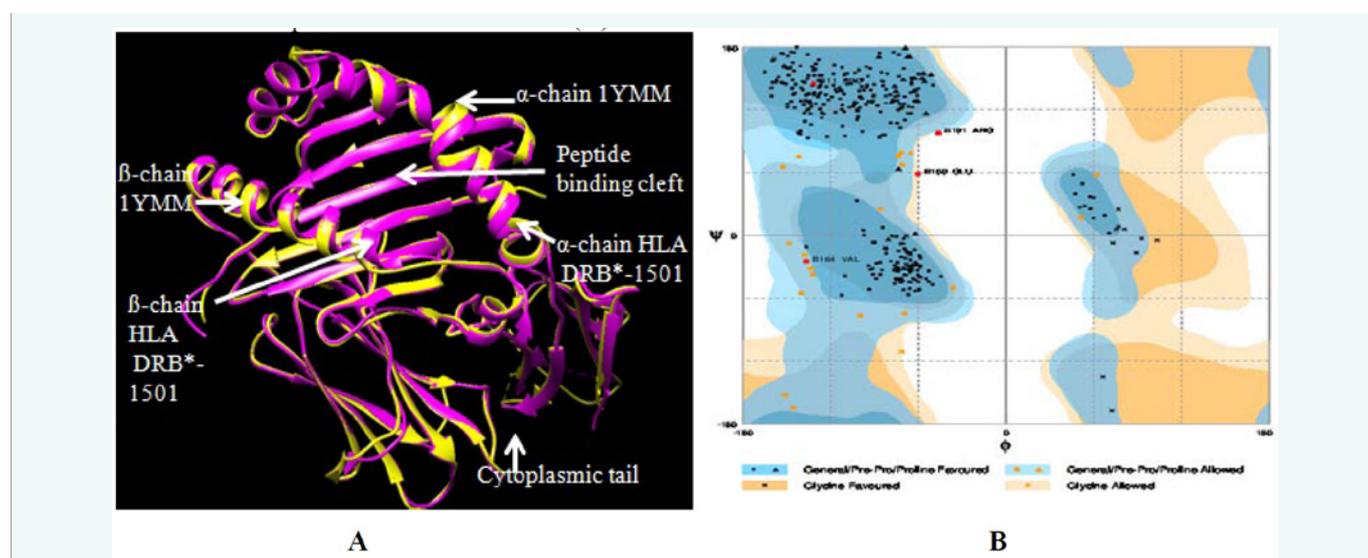


Figure 11 3D structure of HLA DRB*1501 in superimposition with template 1YMM (A) Ramachandran plot of HLA DRB*1501 (B)

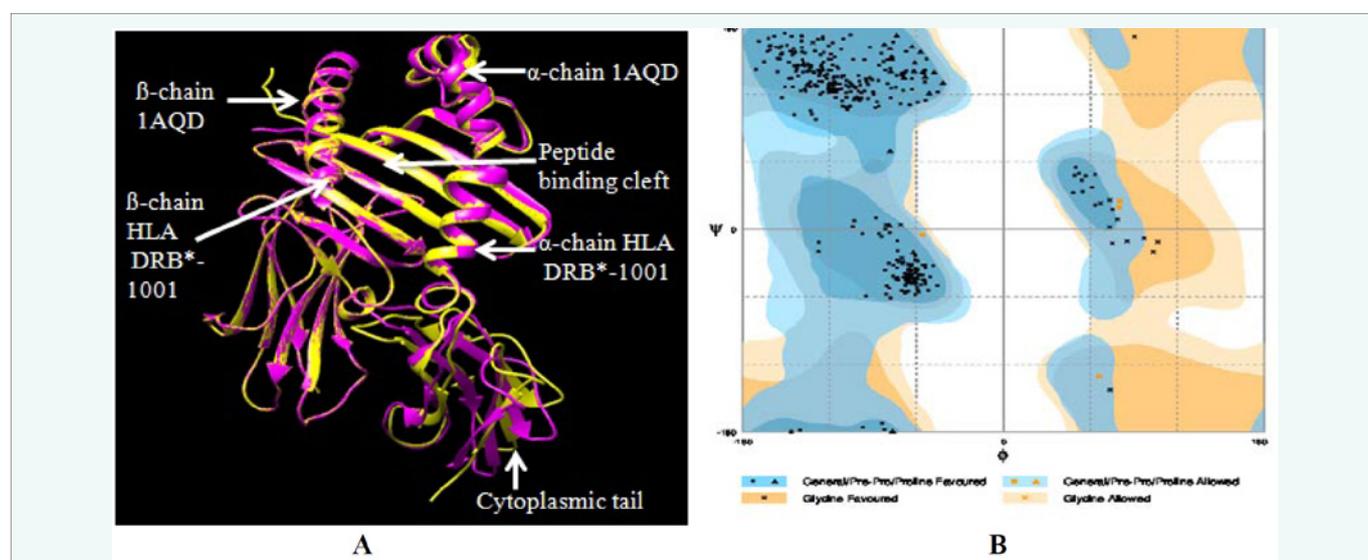


Figure 12 3D structure of HLA DRB*1001 in superimposition with template 1AQD (A) Ramachandran plot of HLA DRB*1001(B)

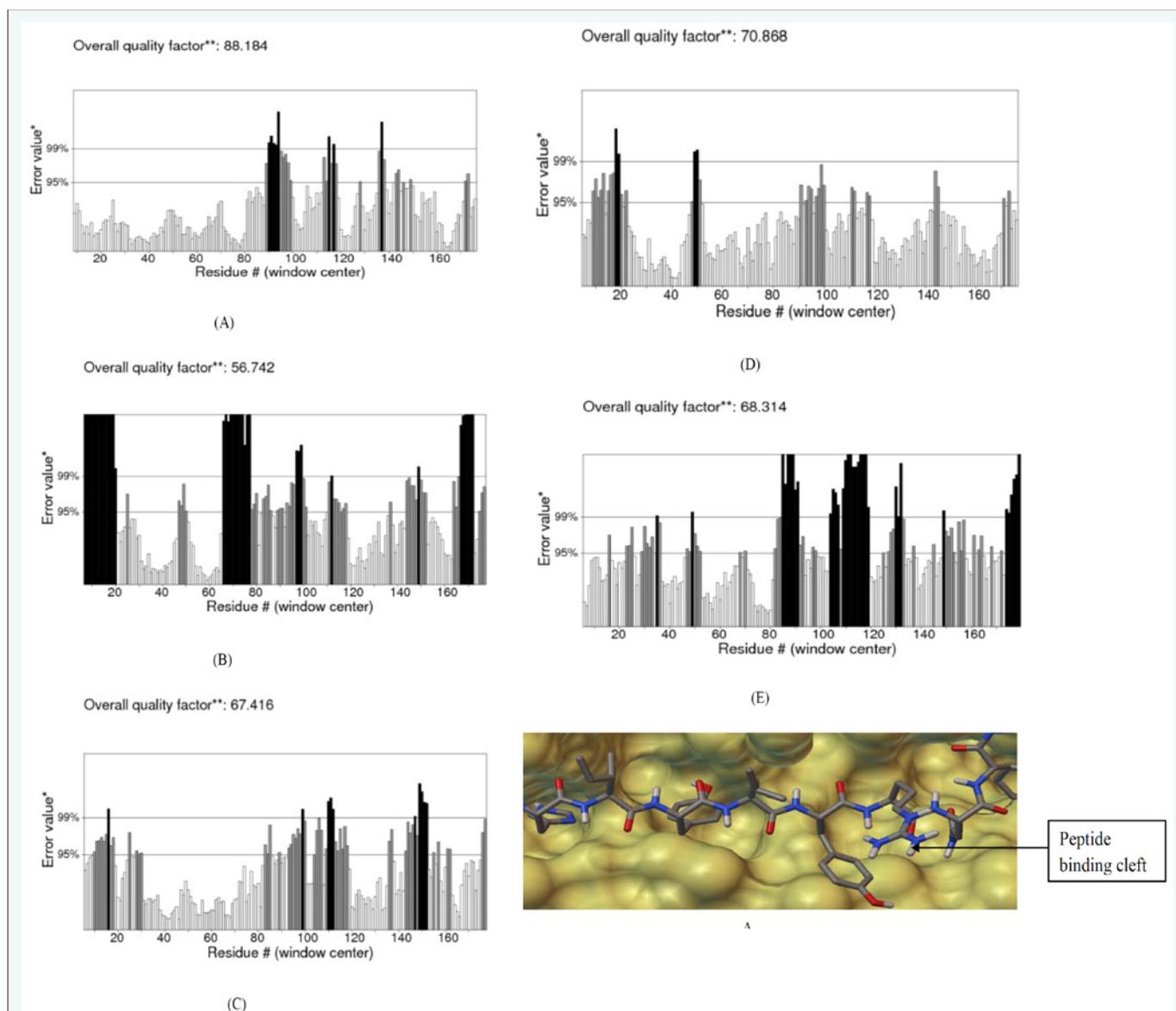


Figure 13 Errat analysis plot for HLADRB*0301(A), HLADRB*0701(B), HLADRB*1301(C), DRB*1101(D), DRB*1501(E).

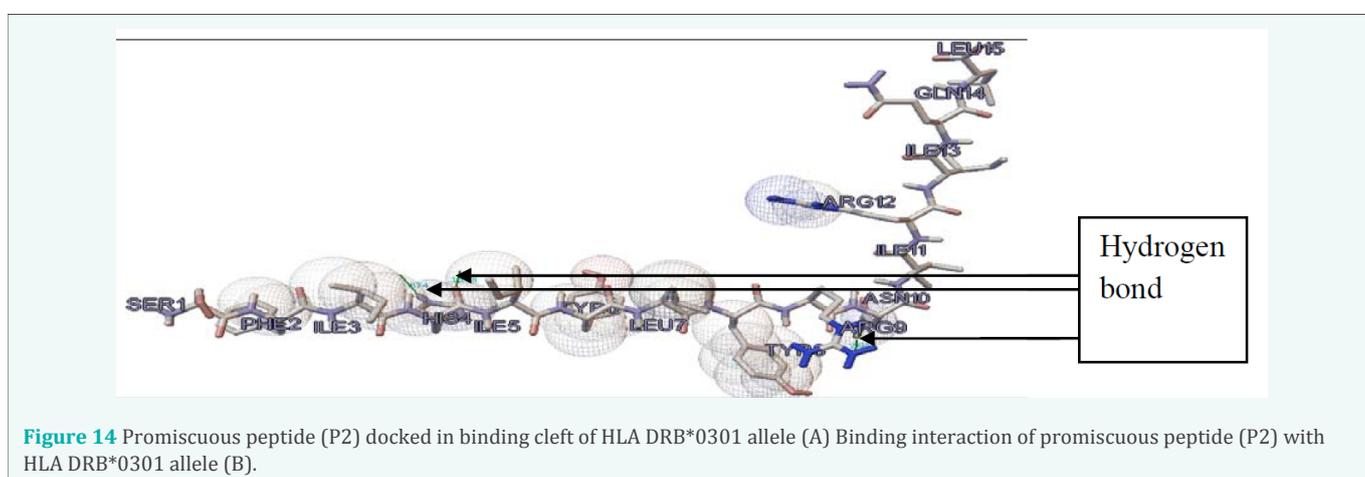
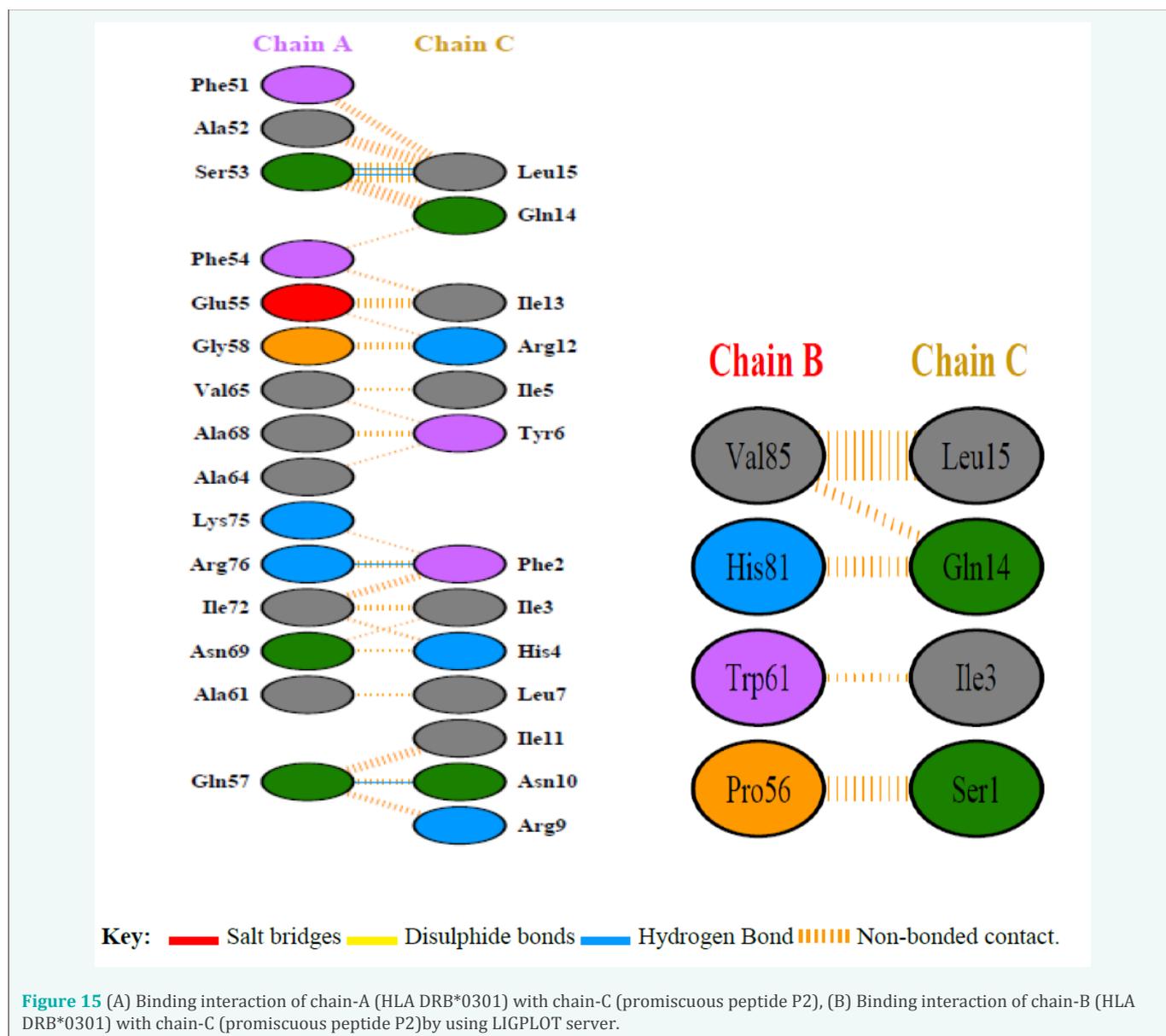


Figure 14 Promiscuous peptide (P2) docked in binding cleft of HLA DRB*0301 allele (A) Binding interaction of promiscuous peptide (P2) with HLA DRB*0301 allele (B).



HLA-alleles: For promoter region of Pfs-25 antigen, the best docked confirmation of epitope “IYIYIYVCISHRAYK” (P2) in the active site of HLA DRB*0301 allele was found with estimated free binding energy of -2.45Kcal/mol at temperature 298.15K. Binding pattern of epitope “IYIYIYVCISHRAYK” (P2) with HLA DRB*0301 allele shown in Figure 14 A,B. The best docked confirmation was checked for interaction by LIGPLOT server. LIGPLOT counts the numbers of both hydrogen bond and non-bonded contacts. The number of non-bonded contacts in the native HLA DRB*0301 and “IYIYIYVCISHRAYK” (P2) complexes are much higher than the hydrogen-bonded contacts, the number of non-bonded contacts are 89 whereas there was 4 hydrogen bond between complex of chain A of receptor and chain C of “IYIYIYVCISHRAYK” (P2) (Figure 14A), 18 non-bonded contacts and 0 hydrogen bonds in complex of chain B of receptor and chain C of epitope “IYIYIYVCISHRAYK” (P2) (Figure 15A,B).

Discussion

The genetic polymorphism in vaccine candidate antigens and HLA Alleles is the major hurdle in the path of vaccine development against malaria. The identification and use of promiscuous peptides can be potentially employed as vaccine candidate antigen. With the accelerating use of *In silico* tools, the immune dominant areas in the protein sequence with potential binding sites for B and T cells can be analyzed, which in turn leads to the development of an effective vaccine against malaria. Structure based analysis is also a rapid and accurate method. Molecular docking is a key structure-based method of immune informatics and has proved to be a rapid and accurate method for evaluating peptide binding to MHCs [50]. In the present study we have employed a combination of *In silico* analysis and structure based study of HLA specific peptide binding. By employing different



in silico tools 12 promiscuous peptides were found as 100% binder for HLA Class-II, Similarly in our previous publication by Khan et al., 2017. Doolan et al., 2000 also identified peptides from CSP, SSP2, LSA-1 and EXP-1 antigens of *P. falciparum*. These selected peptides were further subjected to cluster analysis, hydrophobicity attribute, IL-4 and IFN γ inducer for the selection of specific HLA-DR motifs for Pfs-25 antigen. Finally promiscuous peptides P1, P2, P3 and P9 were selected for structure based analysis. Interaction of HLA alleles with antigenic peptides lead to T-Cell mediated immune response. Three dimensional structures of HLA alleles and promiscuous peptides are required to study the binding pattern analysis. Predominantly present DRB1 alleles and promiscuous peptides were modeled by using Modler9v7 and Chimera 1.8.1 program respectively. Ramachandran plot analysis for HLA models was performed and were also validated using Z-score and ERRAT analysis, Z score represents the overall model quality and check whether the input structure is found within the range of scores. ERRAT analysis showed that whether the structures were significant or not. Similar study was also reported by [16]. These structures will be further helpful to study binding pattern analysis of promiscuous peptide with HLA alleles by using Autodock program. The Promiscuous peptide P2 predicted to be most promising was docked with HLA allele. The criteria of choosing most promising peptide were based on all criteria used. Similar to the present study docking interaction of promiscuous peptides from PFEMP-1 with different HLA alleles was previously reported in our study Khan et al., 2017. Similarly docking was also performed by [51] to study interaction of *E. granulosus* with HLA alleles. The present study was also able to know about the interactions of promiscuous peptides with HLA alleles. These were, (a) the interaction of chain A of HLA with chain C of P2 (), there were presence of three hydrogen bonds in between Leu 15 and Ser 53, Phe2 and Arg76, Glu57 and Asn10, (b) the interaction of Chain B of HLA with chain C of peptide, there were presence of non-bonded contacts between different residues of peptide and HLA alleles. These structures will further helpful to understand the molecular interactions between the antigenic promiscuous peptides and different HLA alleles. We have predicted structures of some frequently present HLA alleles by using computational methods. Homology modeling is one of the extensively used methods for constructing a reliable 3D structure of HLA alleles and promiscuous peptides. The docking interaction of HLA alleles with potential vaccine candidate antigen reveals that, if further study will carry out then this vaccine candidate antigen can be used further for development of vaccine against malaria.

Conclusion

Peptide based vaccine for *P. falciparum* is based on prediction of antigenic epitopes which can activate immune system and can protect the host from malaria infection. In the present study protein sequence of promoter region of Pfs-25 antigen was screened by using *in silico* tools for prediction of immunogenic HLA class-I and HLA class-II cytotoxic T cell (CTL) epitopes. Structure based approach was also applied to study the interaction of predicted epitopes with HLA alleles by using docking. Out of 303 peptides only 12 promiscuous peptides were obtained as 100% binder. Further on the basis of cluster analysis, hydrophobicity

attribute and IFN- and IL-4 inducer only P1, P2, P3 and P9 were selected for structure based analysis. Promiscuous peptide P2 exhibit minimum binding energy with HLA DRB*0301 HLA class-II allele. This promiscuous peptide is good binder for HLA class-II receptor and can be a key factor for development of a vaccine against malaria. Epitope prediction and binding analysis would be a beneficial tool for designing of a structure based drugs.

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