

Revisiting the structural basis and energetic landscape of susceptibility difference between HLA isotypes to allergic rhinitis



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ABSTRACT

The human leukocyte antigen class II (HLA II) molecules are implicated in the immunopathogenesis of allergic rhinitis (AR). The HLA II contains three allelic isotypes HLA-DR, -DQ, and -QP that exhibit considerably different susceptibility to AR. Here, we investigated the structural basis and energetic landscape of the susceptibility difference between the three HLA II isotypes to AR by combining computational analysis and experimental assay. Multiple sequence alignment revealed a low conservation among the three subtypes with sequence identity of ~10% between them, suggesting that the peptide repertoires presented by HLA-DR, -DP and -DQ are not overlapped to each other, and they may be involved in different immune functions and dysfunctions. Structural analysis imparted that the antigenic peptides are rooted on the peptide-binding groove of HLA molecules and hold in a PPII-like helical conformation. Subsequently, the interaction behavior of 17 AR allergen-derived peptides with HLA-DR, -DP and -DQ was investigated using a statistics-based quantitative structure-activity relationship (QSAR) predictor. It was found a significant difference between the binding capabilities of these antigenic peptides to HLA-DR and to HLA-DP/-DQ; the former showed a generally higher affinity than the latter with *p*-value of 0.02 obtained from 2-tailed Student's *t*-test. The computational findings were then confirmed by HLA II-peptide stability assay, which demonstrated that the AR allergen-derived peptides have a high *in vitro* selectivity for HLA-DR over HLA-DP/-DQ. Thus, the HLA-DR isotype, rather than HLA-DP and -DQ, is expected to associate with the pathological process of AR.

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1. Introduction

Allergic rhinitis (AR) is the most common chronic condition seen in the outpatient practice of medicine and characterized by one or more symptoms including rhinorrhea, nasal congestion, nasal itching and sneezing (Berger, 2003). AR is typically triggered by environmental allergens such as pollen, pet hair, dust or mold. Inherited genetics and environmental exposures contribute to the development of allergies. Growing up on a farm and having multiple siblings decreases the risk (Wheatley and Togias, 2015). Epidemiological investigations revealed that AR affects an estimated 20–40 million people in the United States alone, and the incidence is increasing; an estimated 20% of cases are seasonal AR, 40% of cases are perennial rhinitis, and 40% of cases are mixed (Skoner, 2001). Oral H1-antihistamines is described as the first-

line pharmacotherapy for mild-to-moderate AR. Some of the newer oral antihistamines such as cetirizine and desloratadine have been shown to relieve the symptom of nasal congestion (Rosenwasser, 2002).

Immunologic mechanism is implicated in the immunopathogenesis of AR. The underlying mechanism involves immunoglobulin E (IgE) antibodies attaching to the allergen and causing the release of inflammatory chemicals such as histamine from mast cells. Cross-linking IgE bound to its receptor on cells by multivalent allergens initiates a chain of events resulting in allergic immune responses. Antigen-presenting cells are also activated by allergen-loaded IgE, resulting in immunomodulation of T-cell responses through allergen peptides presented by human major histocompatibility complexes, also known as human leukocyte antigen (HLA) (Poole and Rosenwasser, 2005). The HLA class II (HLA II) molecules have been observed to closely associate with the AR, which containing three allelic isotypes HLA-DR, -DQ, and -QP that, however, exhibit considerably different susceptibility to AR. In particular, clinical evidences supported a strong association of AR with HLA-DR, but no significant correlation between the AR and

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Table 1

The 17 allergen peptides involved in AR.

Peptide	Allergen	Origin
CPAVKRDVDFLT (Patel et al., 2013)	Fel d 1	Cat
EQVAQYKALPVVLENA (Patel et al., 2013)	Fel d 1	Cat
KALPVVLENARILNCV (Patel et al., 2013)	Fel d 1	Cat
RILKNCVDAKMTEEDKE (Patel et al., 2013)	Fel d 1	Cat
KENALSLDKIYTSPL (Patel et al., 2013)	Fel d 1	Cat
TAMKKIQDCYVENGLI (Patel et al., 2013)	Fel d 1	Cat
SRVLDGLVMTTISK (Patel et al., 2013)	Fel d 1	Cat
MGETLLRAVESYLL (Bauer et al., 1997)	Bet v 1	Birch pollen
KIVATPDGGSIL (Bauer et al., 1997)	Bet v 1	Birch pollen
KQVTIRIGCKTSSS (Yoshitomi et al., 2002)	Cry j 1	Cedar pollen
HTFTKVDGIIAAYQ (Yoshitomi et al., 2002)	Cry j 2	Cedar pollen
RAEVSYPVHNGAKF (Yoshitomi et al., 2002)	Cry j 2	Cedar pollen
TVNGTTRTVNPLGFFKKEALPK (Cárdaba et al., 1998)	Ole e 1	Olive pollen
KVLTTGLPALISW (King et al., 1998)	Api m 4	Bee
KNEILKRHNDFRQNVAKGLE (King et al., 1998)	Dol m 5	Hornet
NYKVLQNSNFRKVGHYTQM (King et al., 1998)	Dol m 5	Hornet
IEDNWYTHYLVCNYGPGGND (King et al., 1998)	Dol m 5	Hornet

HLA-DR or HLA-QR was observed. For example, a proportion of nasal epithelial cells (NECs) in patients with AR are known to express HLA-DR (Takizawa et al., 2007). The HLA-DR positive dendritic cells (DCs) have been found to express claudin-1 and penetrate beyond occludin in the epithelium of the nasal mucosa with AR (Takano et al., 2005).

In order to explore the structural basis and energetic property underlying the susceptibility difference of HLA II molecules to AR, we herein performed a systematic analysis of the intermolecular interaction between AR allergen-derived antigenic peptides and HLA-DR, -DQ and -QP isotypes. The three isotypes were compared at sequence and structure levels, with paying attention to residue difference in peptide-binding groove. The molecular mechanism and interaction energetics of HLA isotypes with peptide ligands were characterized in detail using computational complex modeling and empirical affinity scoring. Subsequently, a standard protocol of HLA II–peptide stability assay was also employed to substantiate conclusions and findings from the computational investigations.

2. Materials and methods

2.1. Class II HLA isotypes

The HLA genes are highly polymorphic and have many variants, which can be divided into two classes HLA I and II. The HLA II is encoded by three different isotypes, namely HLA-DR, -DP, -DQ; they exhibit low sequence conservation and high residue variability in peptide-binding groove (Klein and Sato, 2000). The differential expression of these isotypes can be either protective or risk factors for infectious diseases, immune dysfunction and autoimmunity (Van Lith et al., 2010); the distribution and association of HLA isotypes with the AR risk differs across populations. The HLA-DR has been reported to involve in the pathological process of AR through epithelial cells (NECs) (Takizawa et al., 2007) and dendritic cells (DCs) (Takano et al., 2005). In contrast, there is no clinical evidence supporting the apparent relationship of AR with HLA-DP and -DQ. Here, the primary sequences of HLA-DR, -DP, -DQ were retrieved from the UniProt database (UniProt, 2010).

2.2. AR allergen-derived peptides

A total of 17 allergen peptides involved in AR were collected from literatures (Bauer et al., 1997; Cárdbaba et al., 1998; King et al., 1998; Yoshitomi et al., 2002; Patel et al., 2013) and listed in Table 1. They were derived from a number of typical allergens such as Fel d 1, Bet v 1, Api m 4, Dol m 5, Cry j 1 and 2 originated from cat, birch pollen, cedar pollen, Olive pollen, Bee and Hornet that are detectable in many public places at levels capable of sensitizing or exacerbating symptoms in AR susceptible subjects. These peptides are T-cell epitopes and some of them were used as immunotherapy to induce antigen-specific tolerance by targeting allergen-specific T cells (Worm et al., 2011). For example, seven of these peptides were derived from Fel d 1, a key pet allergen that in cats is encoded by the CH1 (chain 1/Fel d 1-A) and CH2 (chain 2/Fel d 1-B) genes (Morris, 2010). The allergen peptide segments distributing in the crystal structure of Fel d 1-A (PDB: 1zkr) are shown in Fig. 1. As can be seen, these peptides are overlapped to each other in primary sequence and spatially independent in

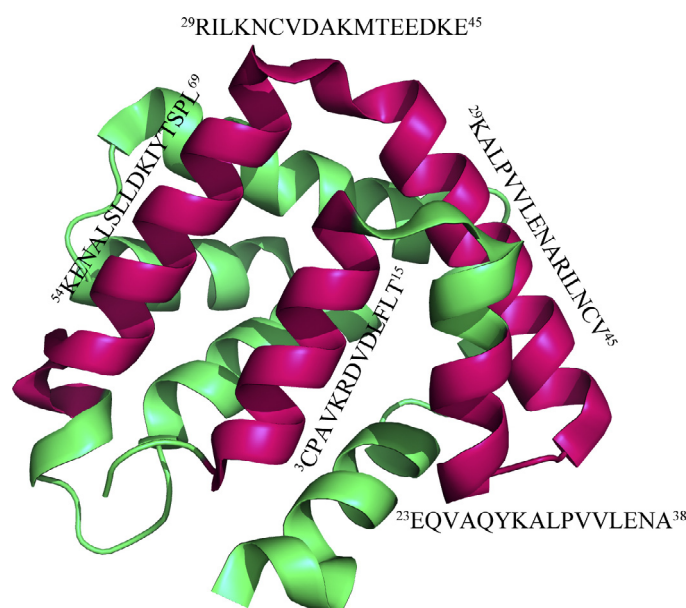


Fig. 1. Distribution of allergen peptides in Fel d 1-A crystal structure (PDB: 1zkr).

three-dimensional structure, which can be generated by chemical or enzymatic hydrolysis of the Fel d 1-A.

2.3. Structures of HLA-DR, -DP and -DQ in complex with peptides

The crystal structures of HLA-DR, -DP and -DQ in complex with antigenic peptides KPVSKWRMATPLLMQALPM (CLIP102-120), QAYDGGKDYIALKG (self-peptide) and PSGEFSFQPSQENPQGS (DQ8-glia- α 1) were retrieved from the PDB database (Berman et al., 2000) with the accessions 4 $\times$ 5W, 4P5M and 4Z7V, respectively. The latter two peptides can be regarded as self-binding peptides that are connected to HLA proteins in primary sequence in the co-crystallized complex structures (Yang et al., 2015, 2016). These structures have been solved at high resolution (1.34, 1.7 and 2.65 Å, respectively) using X-ray crystallography; the bound peptides are either exogenous antigens or endogenous self-peptides associated with autoimmune disorders such as chronic beryllium disease and celiac disease. Here, we adopted the three crystal structures as templates to model the complex structures of HLA-DR, -DP and -DQ with the 17 AR allergen-derived allergen peptides listed in Table 1 using a computational strategy described by Oliveira et al. (2000). Briefly, the HLA II-peptide complex template structures were subjected to structural minimization using the Rosetta all-atom force field (Rohl et al., 2004), resulting in a little deviation between the crystal and refined structures of three templates. This suggests that the refined structures are an excellent starting point for modeling HLA complexes with other antigenic peptides. Subsequently, the residues corresponding to the target peptides were substituted at specific positions of template peptides with keeping peptide backbone immobile. The resulting systems were then subjected to a round of structural minimizations. All computational modeling were performed using the Rosetta software suite (Rohl et al., 2004).

2.4. Peptide binding affinity scoring

The binding affinity of antigenic peptide to HLA protein was estimated using three different approaches based on their computationally modeled complex structures, including force field-based Rosetta scoring (Combs et al., 2013), knowledge-based DFIRE potential (Zhang et al., 2005) and statistics-based QSAR predictor (Wu et al., 2013). The force field-based Rosetta scoring is derived empirically through analysis of observed geometries of high-quality protein structures, consisting of Lennard-Jones term, electrostatic potential, orientation-dependent hydrogen bonding, Lazaridis-Karplus implicit solvation model, rotamer preference probabilities and the reference energies approximating amino acids in unfolded state (Combs et al., 2013). The knowledge-based DFIRE is a distance-dependent atomic contact energy generated from the high-quality crystal structures of protein-ligand, protein-protein, and protein-DNA complexes by using 19 atom types and a distance-scale finite ideal-gas reference state (Zhang et al., 2005). Statistics-based QSAR predictor is a combination of knowledge-based statistical potential derived from 505 interfacially diverse, nonredundant protein-peptide complex structures and supervised quantitative structure-activity relationship (QSAR) modeling trained by 250 protein-peptide interactions with known structure and affinity data (Wu et al., 2013).

2.5. HLA II-peptide stability assay

The peptide binding stability to HLA proteins were measured using a protocol described previously (Texier et al., 2000). Briefly, the immunopurified HLA molecules were diluted in 200 mM NaCl, 2 mM DM, 15 mM phosphate, and 0.005% thimerosal buffer with an appropriate biotinylated tested peptides and competitor peptides.

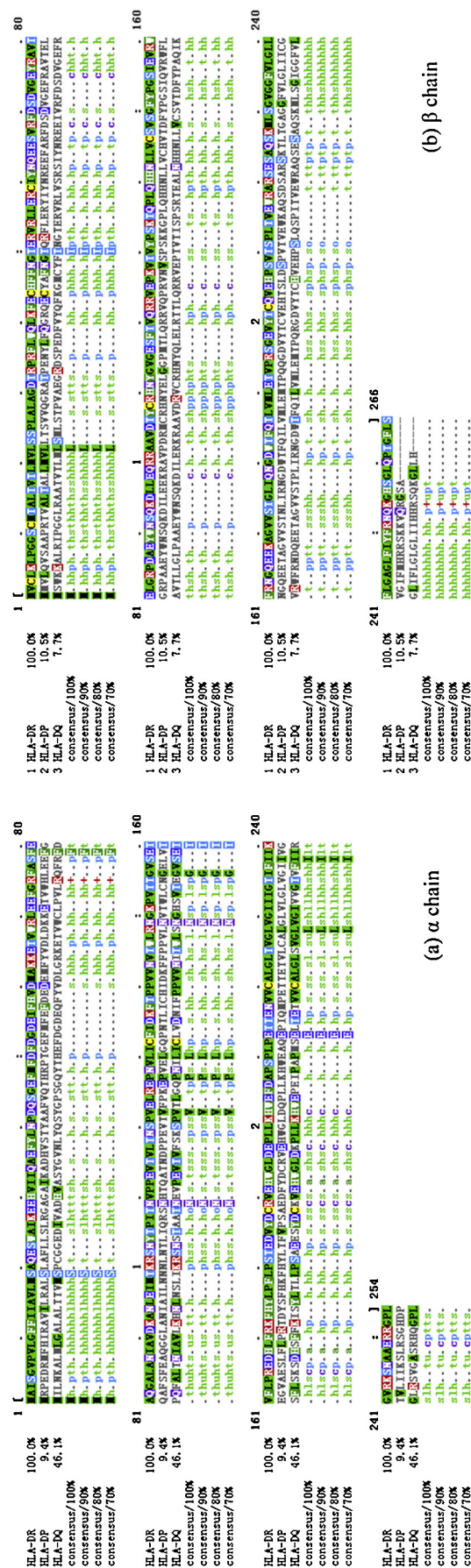


Fig. 2. Multiple sequence alignment between the primary sequences of HLA-DR, -DP and -DQ using MView program (Brown et al., 1998). (a) HLA α chain. (b) HLA β chain.

Mixture was incubated at 37 °C for 48 h and applied to 96-well ELISA plates saturated with 100 mM Tris-HCl (pH 7.5) buffer. Bound biotinylated peptides were detected by adding 4-methyl-umbelliferyl phosphate substrate. Emitted fluorescence was measured and the maximal binding was determined by incubating tested peptides with HLA molecules with or without competitor. The peptide binding stability was characterized by the peptide concentration that inhibiting binding of 50% of the labeled peptide (IC_{50}).

3. Results and discussion

3.1. Sequence and structural comparison of HLA-DR, -DP and -DQ

The extracellular regions of HLA class II molecules consist of two variable Ig-like chains α and β that are responsible for the activation of T helper cells through the interaction with the CD4+ on the cell surface of the T helper cells. Here, primary sequences of the α and β chains of HLA-DR, -DP and -DQ were retrieved from the UniProt database (UniProt, 2010). The α or β chain of different subtypes contains ~260 amino acids, which exhibit high sequence variability according to MView multiple sequence alignment (Brown et al., 1998). As can be seen in Fig. 2, the sequence identities among the α chains and the β chains of three HLA isotypes are only ~10%, suggesting a low evolutionary homology shared by them. This finding implicates that the antigenic peptide repertoires presented by HLA-DR, -DP and -DQ are not overlapped to each other, and the three isotypes may be associated with different immune functions and dysfunctions in clinic. For example, the HLA-DP, -DQ, and -DR were early observed to exhibit differential expressions associated invariant chain (Ii) in immune disorders (Degener et al., 1988). In addition, the three isotypes have different requirements for Ii and HLA-DM, an intracellular protein involved in peptide presentation by class II HLA (Van Lith et al., 2010).

Subsequently, the crystal structures of HLA-DR, -DP and -DQ in complex with co-crystallized peptide ligands using MultiProt server (Shatsky et al., 2005). The superposition result is shown in Fig. 3. Interestingly, despite a low sequence conservation among the three HLA isotypes, their spatial structures and binding modes with antigenic peptides are highly consistent with root-mean-square deviations (*rmsd*) of 0.45 and 0.57 Å through the backbone C α atoms of HLA molecules and peptide ligands, respectively. This is not unexpected if considering that all the HLA class II proteins

possess a similar structural architecture that presents a sandwich profile when bound with peptide ligands. The antigenic peptides are rooted on the peptide-binding groove of HLA molecules and hold in a PPII-like helical conformation (Adzhubei and Sternberg, 1993). However, residue distribution in the groove is highly polymorphic between the three HLA isotypes, which provides a molecular evidence for the non-overlapping peptide repertoires presented by these isotypes that should associate with differential immune events. This can be further confirmed by the considerable difference in co-crystallized peptides KPVSKWRMATPLLMQALPM (CLIP102-120), QAYDGKDYIALKG (self-peptide) and APSGEGSFQP-SQENPQGS (DQ8-glia- α 1) in complex with the three HLA molecules; these peptides are either exogenous antigens or endogenous self-peptides involved in autoimmune disorders such as chronic beryllium disease and celiac disease.

3.2. Testing of peptide binding affinity scoring

It is valuable to assess the performance of three peptide affinity scoring methods, i.e. force field-based Rosetta scoring (Combs et al., 2013), knowledge-based DFIRE potential (Zhang et al., 2005) and statistics-based QSAR predictor (Wu et al., 2013), in reproduction of the experimentally measured activity of HLA II-peptide binding. A total of 18 HLA-DR binding peptides were collected from a previous report (Yin et al., 2012). These antigenic peptides were derived from major core protein A10L and other known vaccinia epitopes bound to HLA-DR; half of them are CD4⁺ T-cell epitope, and remaining half are not. Their biological activity (IC_{50}) was measured using fluorescence polarization assay (Table 2). The complex structures of HLA-DR with the 18 peptides were one-by-one modeled and minimized computationally using the strategy described in method section, based on which the HLA II-peptide binding affinity was scored separately with the Rosetta, DFIRE and QSAR methods. The predicted binding affinities against experimental values for different methods are shown in Fig. 4, and data details are listed in Table 2. It is evident that the force field-based Rosetta and knowledge-based DFIRE performed much worse as compared to statistics-based QSAR predictor. In fact, the DFIRE was originally developed for fast evaluation of small-molecule ligand affinity to protein receptor, and Rosetta was commonly used to model and analyze the protein folding phenomenon. Given the moderate or modest correlations between the two theoretical scores and experimental activity over the 18 peptide samples (Pearson coefficients $r_p = -0.319$ and -0.264 for Rosetta and DFIRE,

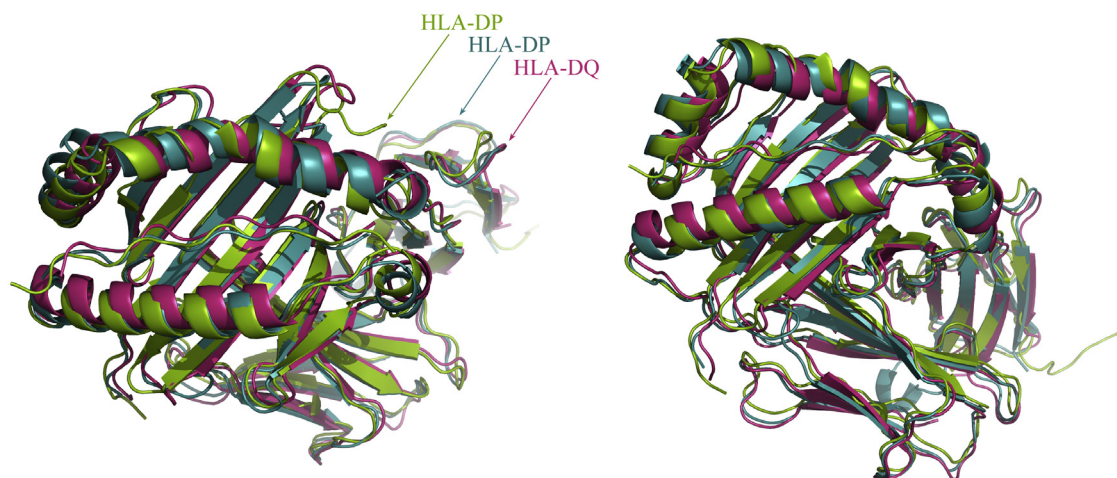


Fig. 3. Multiple structure superposition between the crystal structures of HLA-DR, -DP and -DQ in complex with co-crystallized peptide ligands using MultiProt server (Shatsky et al., 2005).

Table 2

Comparison of the performance of Rosetta scoring, DFIRE potential and QSAR predictor in reproduction of the experimentally measured activity of 18 HLA-DR binding peptides (Yin et al., 2012).

Peptide	CD4 ⁺ T-cell epitope	IC ₅₀ (nM)	Rosetta	DFIRE	QSAR
KFSFMYIESIKVDRI	Yes	192	−8.2	−33.5	5.6
AVSLFIQGYSIYEN	Yes	212	−7.4	−24.2	4.9
TTQSMNIMESIPANT	Yes	44	−9.7	−30.8	7.2
VFRYMSSEPII	Yes	12	−7.6	−33.3	8.1
NKIPFLLSGSPITN	Yes	4	−5.4	−25.7	8.6
LVFNSISARALKAYF	Yes	15	−10.2	−32.2	5.3
GRYLVLKAVKVSD	Yes	37	−8.5	−35.4	8.0
REYLKLGITAIM	Yes	99	−6.7	−25.5	7.5
FDMRFLNSLAIHE	Yes	150	−8.4	−30.6	6.5
LIYYQLAGYILTVL	No	431	−7.0	−27.3	7.9
KVKIVPLDGNKLFNI	No	86	−8.3	−33.4	6.7
HPDYAILAARIAVSN	No	62	−9.2	−34.0	7.4
SLGFKVLDGSPISLR	No	17	−6.9	−31.6	8.0
TPIGIISAQVLSEKF	No	21	−9.2	−29.2	7.9
WFVVVRAIASM	No	360	−5.8	−34.7	5.6
VNVYLLAAVYSDF	No	425	−6.0	−28.5	5.8
EPFVNQSGIEILL	No	106	−8.4	−31.4	6.3
IGVILALALFMYAK	No	7360	−6.2	−25.5	5.2

respectively), the two scoring methods may therefore not be well applicable for the flexible peptide interaction with rigid HLA protein. As can be seen in Fig. 4ab, the Rosetta and DFIRE scores exhibited a similar predictive profile, that is, a low negative correlation with experimental activity. Thus, the two scoring methods can only be used for qualitative purpose to classify between the binders and nonbinders of specific HLA protein, but are not able to give a quantitative description for the affinity and activity of HLA-binding antigenic peptides, albeit the two methods can directly calculate the change in binding free energy. In contrast, the QSAR exhibits a significant correlation ($r_p = 0.628$) with peptide activity, although there are two outliers observed in the scatter plot (Fig. 4c). The QSAR predictor was originally developed specifically for quantitatively predicting protein–peptide interaction stability, which was based on potential of mean force and training by machine learning approach (Wu et al., 2013). Thus, the method is particularly suitable for estimating the binding strength of peptide ligand to HLA receptor

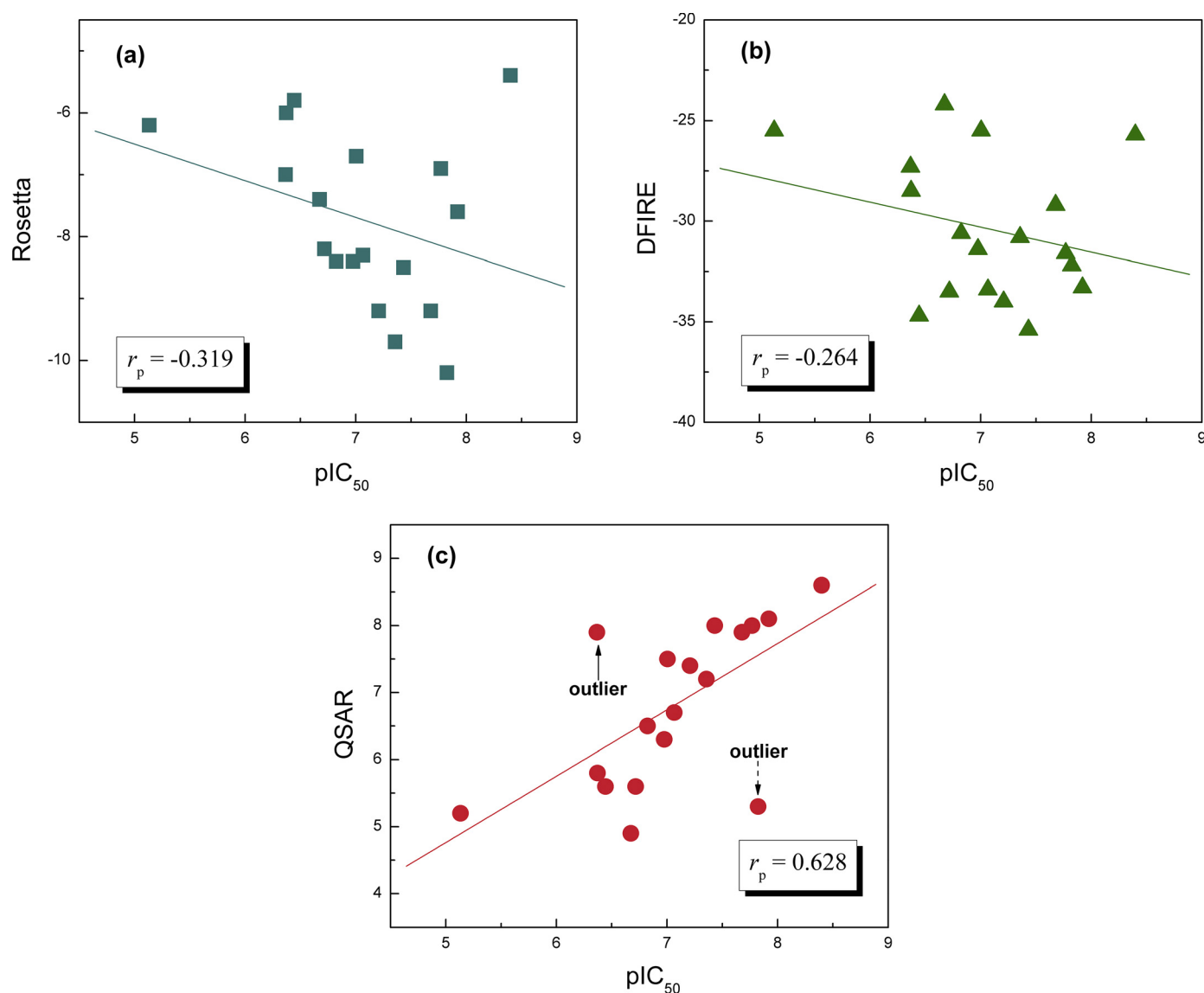


Fig. 4. Scatter plots of Rosetta, DFIRE and QSAR scores against experimentally measured activity of 18 HLA-DR binding peptides.

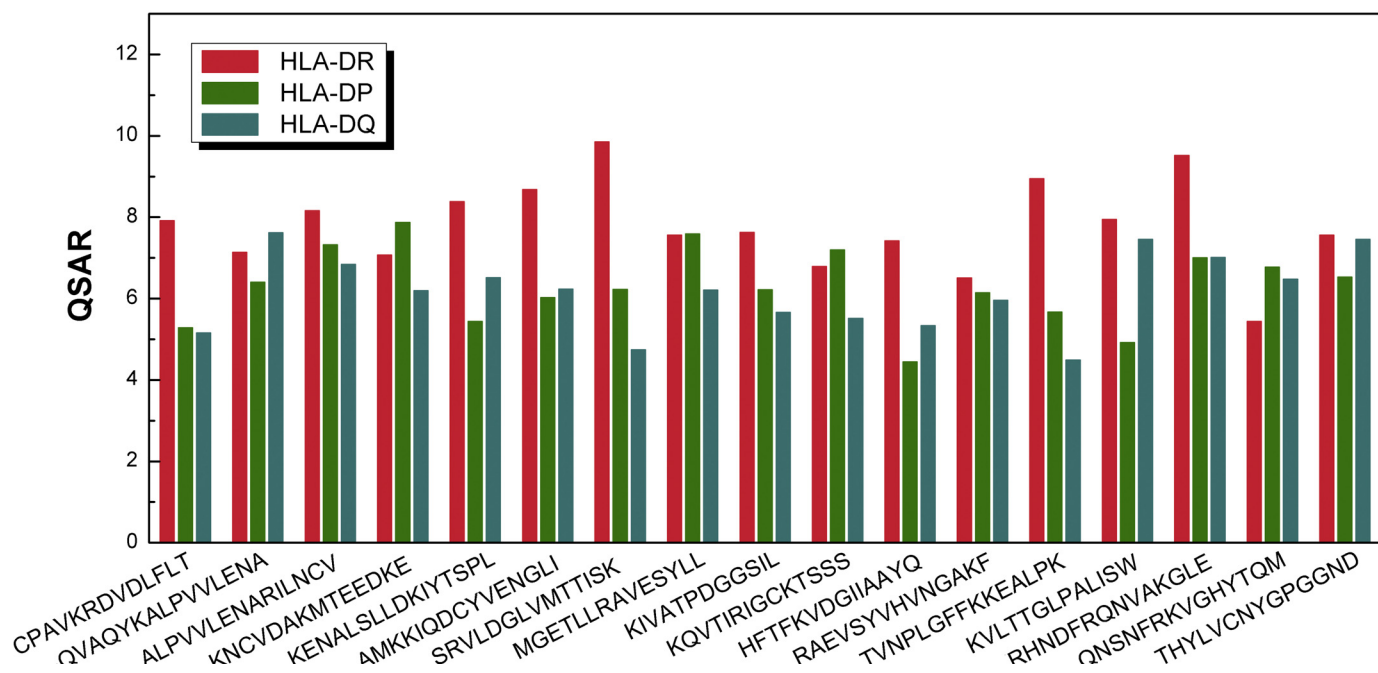


Fig. 5. Diagrammatic comparison of QSAR scores for the binding capability of 17 AR allergen-derived peptides to HLA-DR, -DP and -DQ.

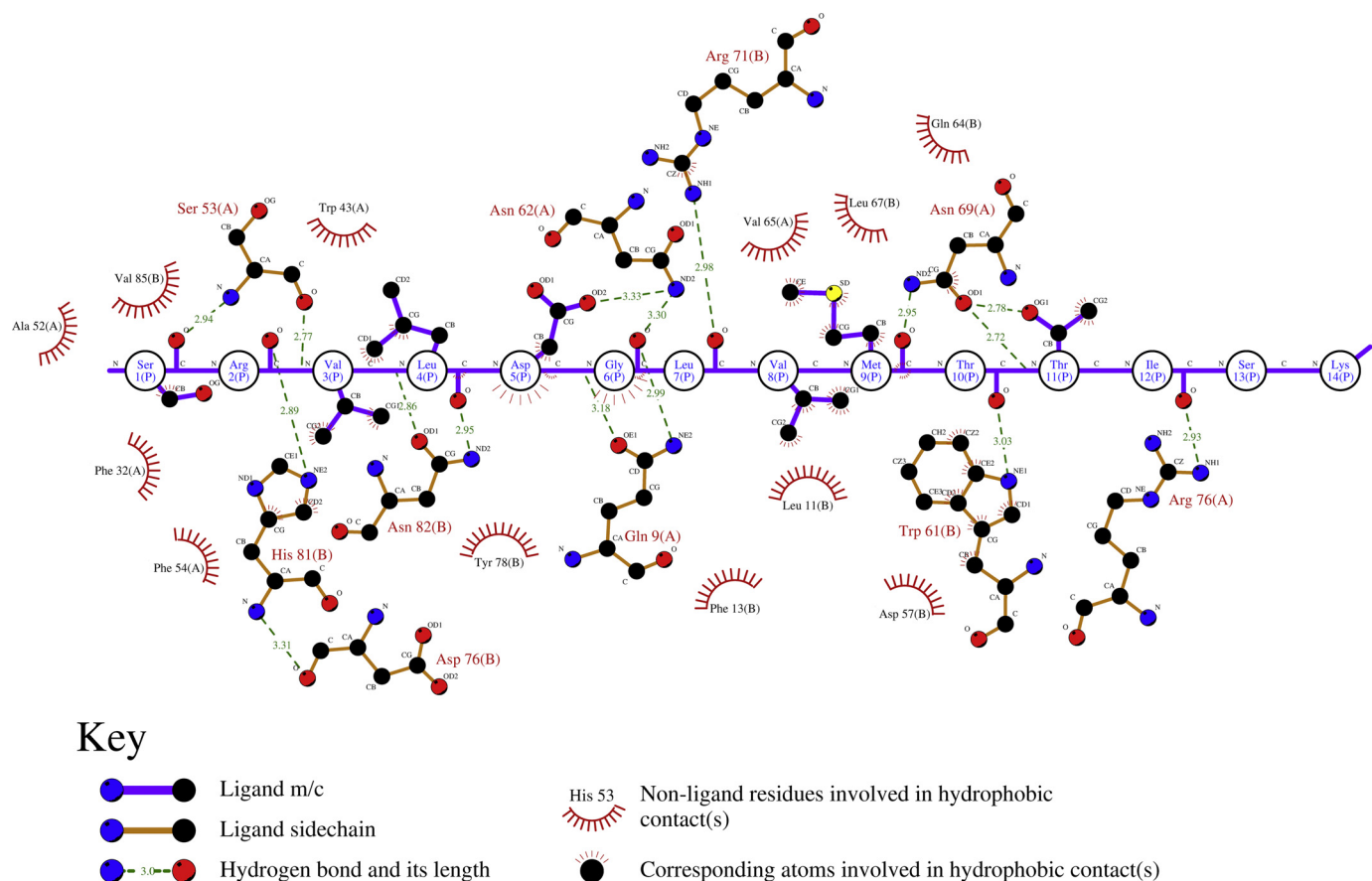


Fig. 6. Schematic representation of hydrophobic contacts and hydrogen bonds at the complex interface of HLA-DR with antigenic peptide SRVLDGLVMTTISK from the AR allergen Cat Fel d 1. The figure was produced using LIGPLOT program (Wallace et al., 1995).

3.3. HLA-DR, -DP and -DQ interactions with AR allergen-derived peptides

Next, the complex structures of HLA-DR, -DP and -DQ with the 17 AR allergen-derived peptides listed in Table 1 were modeled and minimized using computational strategy, based on which the HLA II-peptide binding affinity was scored using the QSAR, and resulting values for the three isotypes were compared in Fig. 5. Evidently, these peptides exhibit generally higher affinity towards HLA-DR than HLA-DP and -DQ, except the two samples EQVAQY-KALPVVLENA and NYKVGLQNSNFRKVGHYTQM. In order to solidify this, we performed Student's *t*-test to determine the significance of difference between the binding potencies of peptides to HLA-DR and -DP/-DQ. As might be expected, peptide binding to HLA-DR generally possesses a high affinity as compared to them binding to HLA-DP and -DQ with *p*-value = 0.02 obtained from 2-tailed Student's *t*-test, indicating that, in general, HLA-DR can bind more tightly to AR allergen-derived peptides than HLA-DP and -DQ. Therefore, the high binding potency is thought as the prerequisite for an antigenic peptide to be a CD4⁺ T-cell epitope presented by HLA-DR rather than by HLA-DP and -DQ. Thus, the HLA-DR is potentially associated with AR immune event as compared to HLA-DP and -DQ.

According to the QSAR-derived binding free energy comparison, the antigenic peptide SRVLDGLVMTTISK from AR allergen Cat Fel d 1 protein has the high selectivity for HLA-DR over -DP and -DQ with 3981 ($10^{3.6}$)- and 125892 ($10^{5.1}$)-folds. This can be confirmed by HLA II-peptide stability assay. The peptide SRVLDGLVMTTISK can competitively bind to HLA-DR, -DP and -DQ with IC₅₀ values of 12, 670 and 3584 nM, respectively, indicating a high selectivity and specificity for the HLA-DR isotype. Therefore, the peptide was selected to investigate its intermolecular interaction with HLA-DR. The three-dimensional structure of HLA-DR-SRVLDGLVMTTISK complex was modeled computationally and minimized using Rosetta full-atom forcefield (Combs et al., 2013). Based on the structure nonbonded interactions at the complex interface of HLA-DR with the peptide were identified using LIGPLOT program (Wallace et al., 1995) and shown schematically in Fig. 6. As can be seen, a number of hydrogen bonds and hydrophobic contacts can be found at the interface, conferring strong specificity and high stability to the complex system. In particular, the hydrogen bonding network is very intensive which distributes along the whole primary sequence of peptide ligand, while a variety of hydrophobic contacts can largely stabilize the complex architecture. In this respect, the AR allergen-derived peptides can be recognized and bound tightly by the HLA-PR molecule with high affinity and strong specificity, which are expected to closely associate with the pathological process of AR.

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