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Docking Peptides into MHC Class II Complexes

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The interaction between T-cell receptor (TCR) complexes exposed on $CD4^+$ T lymphocytes and exogenous antigenic peptides (p) displayed by dimeric, major histocompatibility complex class II protein complexes (MHCII) constitutes a significant step in the activation of humoral immunity. As TCRs can only bind to MHCII-presented antigenic peptides, the binding of peptides to MHCII is a crucial bottleneck within adaptive immune responses. Therefore, the knowledge about which peptides bind to a particular MHCII is of interest in many medical applications. The high sequence variation of MHCII and the great reservoir of potential MHCII-binding peptides significantly raise costs for experimental approaches aiming to determine MHCII-peptide binding data. Therefore, a plethora of sequence-based bioinformatics approaches has been developed. Here, we present a new structure-based approach to computationally model the p-MHCII interaction on the basis of the previously published IRECS algorithm in combination with the ROTA potentials[2,3]. The method allows modeling high quality complex structures and shows a good correlation between the RMSD of the modeled structures and the corresponding peptide binding score.

1 Introduction

Major histocompatibility complex proteins (MHC) are highly polymorphic, cell surface-exposed transmembrane glycoproteins which present different populations of peptide fragments to T-cell receptors located on the surfaces of T lymphocytes. They are coded within the major histocompatibility complex (MHC) genome region of vertebrates. In humans this region is called human leukocyte antigen (HLA). Every MHC allele shows a characteristic peptide binding preference. Binding of p-MHC complexes to TCRs activates the corresponding T-cells, which is pivotal for the initiation of an adaptive immune response against the protein containing the triggering peptide fragment or cells presenting it. As the TCR protein complex can only recognize MHC bound peptides, the affinity between a MHC and an antigenic peptide is a crucial prerequisite for the antigenic peptide to act as a potential TCR epitope. This phenomenon is known as MHC restriction. Due to the central role of the p-MHC interaction during the stimulation of the adaptive immune system, it is important for many medical applications to know the binding specificity of a particular MHC. Examples are rational vaccine design[4–6], treatment of autoimmune diseases like diabetes[7], immunogenicity predictions of protein biopharmaceuticals[8–10], and cellular-based immunotherapies like combined adoptive T cell therapy and tumor antigen-specific TCR gene transfer[11–13]. However, it is costly and laborious to scan the high amount of candidate peptides for interaction with a particular MHC by using wet-lab facilities only. Therefore, many different bioinformatics algorithms have been developed for the prediction of MHC binding peptide sequences, mainly to reduce the number of necessary screening experiments. Most of these approaches are sequence based and computationally fast, but strongly rely on experimentally determined peptide-binding motifs and data for training. Further, they lack insights into molecular mechanisms guiding a certain p-MHC interaction. Other approaches try to exploit the growing pool of available experimental X-ray crystal structures of p-MHC complexes for the prediction of MHC-binding peptidic ligands applying concepts and methods from molecular modeling. These approaches provide the possibility to interpret the results on a molecular, structural

level[14]. This study is part of a greater effort aiming to model the TCR-p-MHC interface applying structure-based computational biology methods in order to establish a method which can robustly predict T-cell receptor epitopes within amino acid sequences of proteins. The present work addresses the modeling of the interaction between peptide ligands and dimeric MHC class II protein complexes by means of structural bioinformatics. MHCIIs are a special subtype of MHC, which are only found on the surfaces of specialized antigen presenting cells. MHCIIs display peptides derived from processed extracellular proteins of the endocytic pathway to TCRs of $CD4^+$ T lymphocytes, which play an essential role in the generation of a humoral immune response[15]. Within the scope of this study, special focus is given to the correct prediction of the binding conformations of antigenic peptides. For this purpose a new approach has been developed, which uses the homology modeling capabilities of the IRECS[2,3] algorithm in combination with the knowledge-based scoring function ROTA[2,3], both implemented in the DynaCell software package[1].

2 Materials and Methods

First, information about the data set used to evaluate the p-MHCII docking approach is presented. Then, the newly developed IRECS/ROTA-based docking workflow is outlined.

2.1 Test set

The approach was validated on a test set of 37 processed p-MHCII complex structures obtained from the Protein Data Bank (PDB; http://www.pdb.org/pdb/home/home.do)[16] comprising MHCII alleles from *Homo sapiens* (HLA-DRA*0101/HLA-DRB1*0101, HLA-DRA*0101/HLA-DRB5*0101, HLA-DRA*0101/HLA-DRB1*0401, HLA-DRA*0101/HLA-DRB3*0101, HLA-DRA*0101/HLA-DRB1*1501, HLA-DRA*0101/HLA-DRB3*0301, HLA-DQA1*0102/HLA-DQB1*0602, HLA-DQA1*0302/HLA-DQB1*0302, HLA-DQA1*0301/HLA-DQB1*0302, HLA-DQA1*0501/HLA-DQB1*0201) and *Mus musculus* (I-E^k, I-E^k [αE11Q,αD66N], I-E^k [βS8C], I-A^b, I-A^d, I-Ag^7, I-A^k, I-A^u). All selected test set structures have a resolution of at least 2.7 Å and do not contain any TCR molecules. Water molecules, small organic molecules, ions and all other biomacromolecules than MHCII (e.g. superantigens) within a biological assembly were removed from the crystal structures. Only the peptide-binding site of the MHCII and the nonameric binding core amino acids of the bound peptide ligands occupying the MHCII peptide-binding clefts[17] were considered during docking simulations. Amino acids included within the applied MHCII peptide-binding sites during docking simulations were selected based on the results of multiple sequence alignments of the MHCII amino acid sequences found in the 37 test set structures performed with MAFFT version 6[18]. Coordinates of missing amino acid side chains in the processed experimental test set structures were calculated with the side chain placement program IRECS[2].

2.2 IRECS/ROTA-based peptide docking

MHCIIs show a strong structural conservation between isotypes and even between MHCIIs of different species[19]. Further, the antigenic peptides bound to MHCIIs display as common feature an extended, polyproline type II helix-like secondary structure guaranteed by the open ends of the MHCII binding groove[20] and stabilized by hydrogen bonds established between conserved MHCII residues in the peptide-binding domain and the peptide backbone[21,19,20]. The IRECS/ROTA-based peptide docking workflow takes advantage of these structural constraints, thereby massively decreasing the conformational space of the peptide ligand to be sampled.

2.2.1 Workflow

In general, the method is based on a library containing different backbone conformations of bound peptides and a repository holding the corresponding holo-MHCII conformations, both deduced from a set of p-MHCII input complexes structurally aligned to a MHCII reference structure. The docking process is performed by selecting a holo-MHCII structure out of the supplied repository and combining it with all possible peptide backbones from the library (Figure 1).

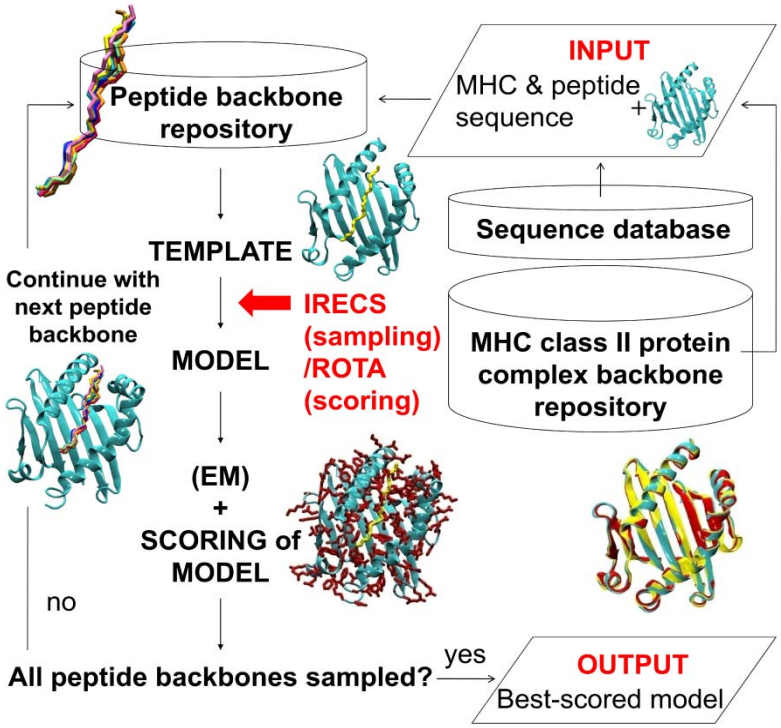

Figure 1: *Diagram of the IRECS/ROTA-based docking workflow.*

First, the backbone (or whole structure) of the holo-MHCII molecule for which the docking is to be performed is chosen from the library. If no structure for this HLA is available the sequence-wise closest related MHC structure is chosen. Subsequently, all peptide backbones within the library are placed in the binding groove. For each peptide backbone-MHC assembly side chain rotamers representing the amino acid sequence of the peptide nonamer core region located inside the binding site are modeled onto the peptide backbone via the IRECS side chain placement algorithm[2]. Initial rotamer sets representing the side chain conformational space within the IRECS algorithm are taken from the BBDEP (backbone-dependent rotamer library) rotamer library[22]. The side chain placement process is influenced by these conformations, the native conformation of the supplied peptide backbones and the holo-MHCII structure. It is guided by the knowledge-based ROTA potential used in the $IRECS_{score}$ scoring function[3]. Finally, each model is submitted to a final scoring step for evaluation and ranking. Depending on the size of the peptide backbone library, the computational costs for a single peptide docking run are reduced to a relatively small number of fast side chain conformer prediction calculations. Comprehensive sampling of all possible ligand conformations within the peptide-binding groove is ensured by the application of the different peptide backbone conformations and the side chain flexibility due to the IRECS side chain conformer prediction capabilities. The softness of the applied ROTA potentials guiding the IRECS procedure further approximates side chain flexibility during docking, allowing the formation of docked complexes containing atoms of slightly overlapping van-der-Waals radii. Possible clashes can be removed by the application of a subsequent energy minimization step prior to scoring. Two different docking procedures were examined: First, docking runs were performed using a rigid-receptor representation, in which the whole experimental MHCII structure from the library was taken and only the side chains of the peptide were placed with IRECS. Second, a semi-flexible receptor representation was examined in which only the experimental MHCII backbone is used and the peptide + MHC side chains are placed with IRECS. The latter allows for a better accommodation of different peptide ligands due to possible adaption of the peptide-binding domain side chains of the MHCII during docking. Moreover, this procedure allows for docking into MHCIIs, which lack a X-ray structure, by modeling the target MHCII amino acid sequence onto the supplied template MHCII backbone conformation of a closely related MHC molecule.

2.2.2 Specific setup

In the current study, 37 p-MHCII X-ray structures were used as test set. All structures were aligned to the peptide-binding site of the X-ray structure with the PDB ID 1KLU[23] using the PyMOL align functionality[24]. 1KLU was chosen as reference, as it is the best resolved (1.93 Å) experimental 3D model of the MHCII HLA-DRA*0101/HLA-DRB1*0101, which represents the biggest MHCII group within the test set. The applied peptide backbone library includes all 37 peptide backbones extracted from the aforementioned structurally aligned complexes. Rotamers were built using the IRECS method together with the CHARMM force field parameter set[25]. For the IRECS algorithm, the target rotamer density was set to 1.0, ensuring that only one rotamer was assigned per amino acid residue. The parameter w1 to scale the BBDEP-dependent rotamer self-score was chosen to be 1.0. The parameter w2 to scale the ROTA terms was set to 0.4. The distance cutoff defining the local residue neighborhood for the computation of pairwise interaction scores between all rotamers of different side chains was set to 10 Å. Energy minimization of docked complexes was performed with functionality from the DynaCell software package[1] using the OPLSA all-atom parameter set[26]. The energy optimization was executed using a step size of 0.002 nm and an energy convergence criterion of 1 kJ/mol. The tool pdb2gmx from the GROMACS software package[27] was applied to protonate IRECS/ROTA-based docking output models prior to energy minimization. The final scoring of the energy minimized models was performed as described in Antes[1], using the OPLS all-atom force field based interaction energy between the peptide and the protein together with the internal energy of the peptide and the optimized DynaDock peptide interaction score (pepscore). Additionally, the differential $IRECS_{score}$ (ΔE^{IRECS}) was used for the final scoring of the non-minimized models. It represents the difference between the $IRECS_{score}$, introduced by Hartmann et al.[2], of the entire p-MHCII complex ($\Delta E^{IRECS}_{p-MHCII}$) and the isolated protein structure MHCII (ΔE^{IRECS}_{MHCII}) and isolated peptide (ΔE^{IRECS}_{p}):

$$\Delta E^{IRECS} = \Delta E^{IRECS}_{p-MHCII} - (\Delta E^{IRECS}_{MHCII} + \Delta E^{IRECS}_{p}) \qquad (1)$$

Raw docked peptide poses as well as energy minimized docked peptide poses were directly compared to the corresponding peptide conformations from the X-ray crystal structures. For this purpose the heavy-atom RMSD values between the docked and the experimental peptide poses were calculated (referred to simply as RMSD of peptide RMSD throughout the remainder of the manuscript).

3 Results and Discussion

3.1 Placement of the peptides

Different docking experiments were performed to evaluate how accurately our newly introduced IRECS/ROTA-based docking approach can reproduce native conformations of the binding-core regions of MHCII-bound antigenic peptides.

Re-docking studies were performed for all 37 structures to assess the principal capability of the procedure to correctly dock peptides into experimental MHCII structures (Figure 2). In the re-docking experiments RMSD values better than 1.0 Å were obtained for all systems (Figure 2A). The results did not differ substantially between the rigid-receptor representation and the semi-flexible receptor model, in which all side chains of the complex were remodeled. The minimum RMSD values do not exceed 1.0 Å, the average RMSD values are below or at least equal to 2.0 Å, and the maximum RMSD values do not exceed 3.0 Å for all 37 peptide models. In almost all cases the minimum RMSD value was reached for the peptide model containing the native backbone conformation from the original experimental structure. Energy minimization did not considerably influence the RMSD values.

Next to the re-docking experiments, 70 cross-docking studies of peptide ligands were performed to validate the capability of the docking method to reproduce a specific reference X-ray peptide conformation through docking the peptide to a different MHC structure of the same MHCII type. This allows assessing the dependency of the results on different MHCII template conformations of the same MHCII type.

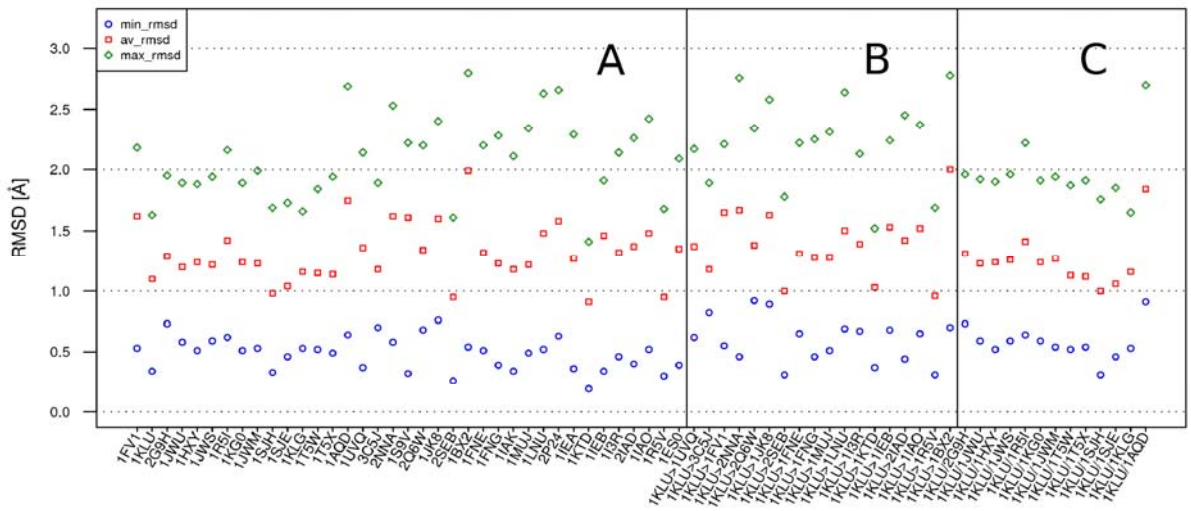

Figure 2: *Heavy-atom peptide RMSD values for the IRECS/ROTA-based docking approach using the semi-flexible receptor description and energy minimization: Minimum and maximum RMSD values of the best (blue) and worst (green) sampled peptide model, respectively, and average RMSD values (red) from A) all re-dockings, B) all cross-MHCII dockings (templatePDB-ID>targetPDB-ID) and C) one example of cross-docking (receptorPDB-ID/ligandPDB-ID).*

This allows assessing the dependency of the results on different MHCII template conformations of the same MHCII type. For this experiment structures from the MHCII type HLA-DRA*0101/HLA-DRB1*0101 were taken as this MHCII is represented by six different p-MHCII complexes and 14 different MHCII structures within the test set. The values for the peptide RMSD obtained by the cross-docking runs are comparable to those found for the corresponding re-docking experiments (Figure 2C). Again, energy minimization has nearly no effects on the RMSD values. These results indicate that the docking procedure allows docking and therefore screening of different peptides with high accuracy if one experimental template MHCII structure of a given MHCII type is available. Figure 3 depicts an example of a cross-docked peptide model and its corresponding experimental X-ray structure.

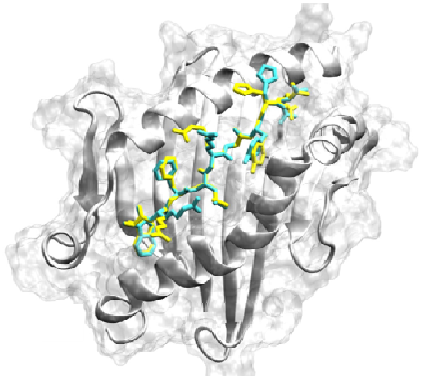

Figure 3: *Cross-docking pose of the 1AQD peptide into 1KLU receptor (silver) (yellow: X-ray conformation; cyan: docked model).*

To evaluate the performance of the IREC/ROTA-based docking procedure if no experimental structure of the corresponding HLA type is available and a homology model must be built and used as MHCII template structure, cross-MHCII docking experiments were performed. For this the 1KLU MHCII structure was used as MHCII template backbone together with the semi-rigid docking procedure. Cross-MHCII dockings were executed for 18 test set systems. Again, minimum peptide RMSD values better than 1.0 Å could be obtained for all test systems. In general, the sampling shows a similar performance as the corresponding re-and cross-docking runs (Figure 2B).

3.2 Scoring of the docked poses

In a real application scenario the experimental conformation of the peptidic ligand is not known and the conformation with the best docking score must be used. Therefore we examined the RMSD values for the poses with the best binding scores for all three scoring schemes. Scoring with the differential $IRECS_{score}$ led in 35 cases of re-docking runs to best-scoring poses that had RMSD values better than 2.0 Å and in 24 cases to best-scoring poses that had RMSD values better than 1 Å. If scored with the OPLS force field based peptide interaction energy score, in 35 cases of re-docking runs best-scoring poses that had RMSD values better than 2.0 Å and in 21 cases best-scoring poses that had RMSD values better than 1.0 Å could be observed. In 34 cases of re-dockings, the pepscore could identify a pose with a RMSD value better than 2.0 Å and in 17 cases a pose with a RMSD value better than 1.0 Å. For all cross-docking scenarios scoring with the differential $IRECS_{score}$ resulted in best-scored peptide poses with RMSD values smaller than 2.0 Å. Except for one cross-docking run or three cross-docking runs, respectively, the same could be observed for scoring with the OPLS force field based peptide interaction energy score and the pepscore. For all cross-MHCII dockings scoring with the differential $IRECS_{score}$ yielded best-scored poses with RMSD values smaller than 2.0 Å. For 5 cross-MHCII dockings even best-scoring poses with RMSD values better than 1.0 Å could be obtained. The OPLS force field based peptide interaction energy score and the pepscore performed equally well in predicting poses better than 2.0 Å for all but one structures of the cross-MHCII dockings, with two best-scoring peptide poses featuring RMSD values better than 1.0 Å.

Additionally, we calculated the Pearson's correlation coefficient between the RMSD and scoring values. In about 60% of the docking runs coefficients higher than 0.5 could be found. A more detailed analysis of the correlation values revealed two reasons for the low performance of the remaining cases:

First, we observed a strong correlation between low correlation coefficients and the fact that in the corresponding X-ray structures the peptide was held in place during crystallization by a linker connecting the peptide ligand with the MHCII (Figure 4). This can lead to artificial ligand poses depending on the length of the linker, which cannot be reproduced by our linker-free docking procedure. In addition, most linked peptides were linked due to their moderate binding affinity, which can also affect the performance of our scoring results. The data in Figures 2 and 4 show that although we are able to reproduce the linked peptide poses within our assembly of final peptide conformations, the scoring does not perform well anymore. This indicates that the linked pose is a physical pose, but might not coincide with the energetically most favorable peptide pose without linker.

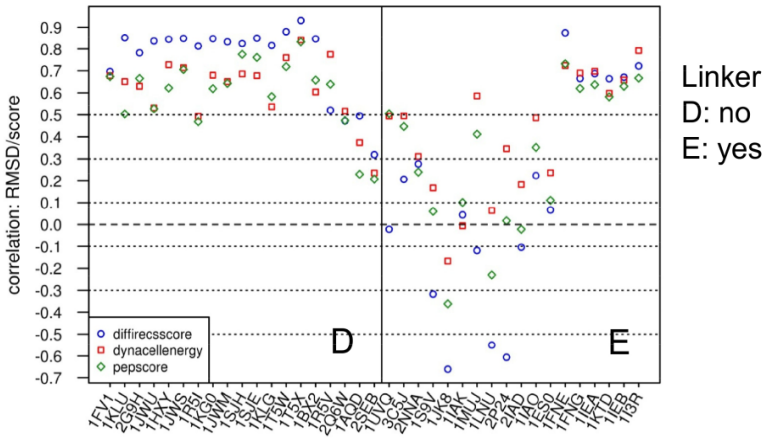

Figure 4: *Correlation between RMSD and score (Pearson's correlation coefficient) for all re-docking experiments (blue: differential $IRECS_{score;}$ red: OPLS all-atom force field-based peptide interaction score; green: pepscore).*

Considering only the poses without linker, correlation coefficients above 0.5 were obtained for about 80% of the systems and the best performance was found for the differential $IRECS_{score}$.

In contrast to peptide binders to MHC class I receptors, peptides binding to MHC class II receptors extend beyond the binding site and are normally 15-17 amino acids long. Therefore one critical topic

for the successful prediction of peptidic binders to MHC class II receptors is the correct identification of the central nonameric amino acid binding sequence within the 15mer peptide binding sequences. For this purpose, we performed so called "register docking" to predict these binding motifs for the 19 systems without linker. This was done by screening all possible nonameric amino acid sequences of the peptides using the differential $IRECS_{score}$. For 78% of the systems without linker the correct register could be identified by the best differential $IRECS_{score}$ value.

4 Conclusions

We have established a new p-MHCII docking approach based on our in house homology modeling tool IRECS and the knowledge-based scoring function ROTA, which is capable of modeling p-MHC complex conformations with high accuracy. Its performance in cross-MHCII docking experiments suggests that with this procedure it is possible to obtain high quality models even for MHCII proteins with unknown structure. In general, the presented theoretical approach is capable of docking peptides into p-MCHII complexes with peptide RMSD values of less than 2.0 Å for all 37 systems in the test set and for all experiments performed (re-, cross-, and cross-MHCII docking). In this context three scoring approaches were tested, from which the differential $IRECS_{score}$ performed best for all experiments with Pearson's correlation coefficients above 0.5 for most of the complexes with non-linked peptides. Overall, our docking procedure allows the docking of peptidic ligands into MHC class II receptors with very high accuracy, even if no experimental structure of the receptor is available. Register docking experiments demonstrated that the presented docking pipeline can also be applied to predict binding core motifs of MHCII-binding peptides. Evaluations concerning the prediction capability of MHCII binding peptides are currently in progress using different selected sets of binders and non-binders. Furthermore, it is planned to examine the influence of the peptides' flanking residues by incorporating longer peptide backbones to the library.

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