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pHLA3D: An online database of predicted three-dimensional structures of HLA molecules

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ABSTRACT

HLA epitope analysis emerged as a strategy to determine alloimmune risk in solid organ transplantation. However, it requires not only knowledge on HLA amino acids sequences, but also on HLA three-dimensional structures. Unfortunately, the number of structures available is still unsatisfactory. This work reports the modelling of 106 heterotrimeric (alpha chain + β_2M + peptide) HLA class I molecules. The models were generated by homology modelling using Modeller, refined using GalaxyRefine server, heterodimerized with Swiss-PDB Viewer and, finally, assessed as to their structural quality through Dali server. The final structures were made available through a free online database, pHLA3D (www.phla3d.com.br), developed in Ruby language using the Ruby on Rails web framework. Structural parameters were similar between refined molecules and their templates. The new database may improve HLA epitope analysis and better guide risk assessment in solid organ transplantation setting.

1. Introduction

The old assumption that anti-HLA (Human Leukocyte Antigen) antibodies recognize a wide set of complete HLA molecules in transplantation setting is giving place to the idea that such antibodies in fact bind to specific epitopes located on HLA molecules [1,2]. Theoretically, any mismatched amino acid located on the surface of the donor's HLA molecule can be the target of the recipient's antibodies [3,4]. This notion has allowed immunologists to more safely identify the allelic targets of the recipient's antibodies that are carried by the potential donor, expanding the number of acceptable donors and increasing transplant rates without compromise to the success of transplants [5].

Transplantation based upon epitope matching was made possible after the development of structure-based tools [6,7], which are valuable in predicting epitope structure and antibody reactivity. The use of these tools, however, is possible if at least minimal information on molecule structures is available. In this sense, HLA molecules are poorly represented. Consequently, the knowledge on HLA epitope accessibility is still scarse, despite: (i) the frequent description and publication of HLA epitope composition and structure [8] and (ii) the progress in the epitope reactivity analysis through the development of specialized software such as HLAMatchmaker [9], EpHLA [10,11], EpViX [12,13], Cambrigde HLA Immunogenicity Algorithm [14], EpAssistant [15] and, more recently, EMS-3D [4].

Molecular targets of anti HLA antibodies are mostly based on their spatial conformations, and thus, their exposure on the molecular

Abbreviations: HLA, Human Leukocyte Antigen; IMGT, International ImMunoGeneTics Database; 3D, Three Dimensional; PDB, Protein Data Bank; DOPE, Discrete Optimized Protein Energy; SAVES, Structure Analysis and Verification Server; β_2M, Beta-2 Microglobulin; RMSD, Root-mean-square deviation of atomic positions

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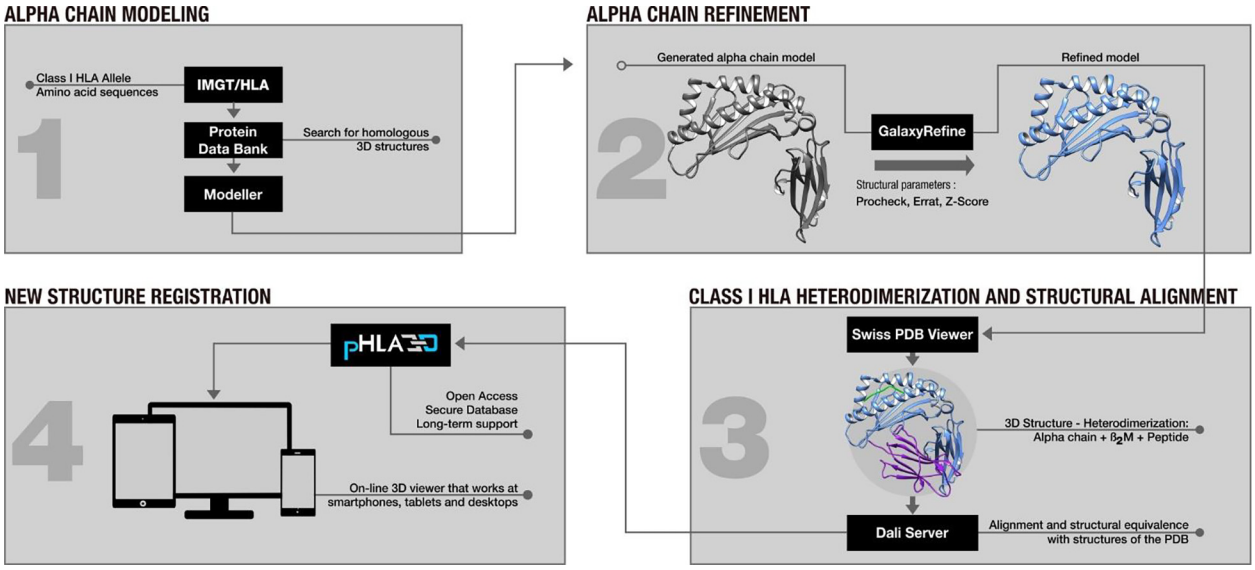

Fig. 1. Workflow used to model, refine, assemble and register HLA class I molecules in pHLA3D.

surface depends upon the interaction with surrounding amino acids [16]. It is likely that HLA molecules sharing a certain epitope may carry it in many configurations varying from exposed to cryptic. The most reliable way to determine the arrangement and, therefore, the accessibility of epitopes to antibodies in HLA molecules is through direct analysis of the three-dimensional structure of such molecules [2]. Unfortunately, the number of completely solved HLA structures is quite small [17], as the current laboratory methods for obtaining such structures are expensive and laborious. Concurrently, homology modelling is one of the most successful (*in silico*) methods in predicting protein structures and a versatile, fast and low-cost alternative for generating reliable models [18]. In this work, the authors present pHLA3D, which primarily aims to be an online database of predicted three-dimensional structures of HLA molecules.

2. Methods

2.1. Repertoire of HLA molecules modelled

A set of 106 HLA class I molecules present in solid phase assays (Lifecodes classes I single antigen, Immucor-Lifecodes, Stamford, CT; and Single Antigen Beads, One Lambda, Canoga Park, CA), commonly used for screening of anti-HLA antibodies, were chosen to be part of the initial version of pHLA3D.

2.2. Protein sequence retrieval

Complete amino acid sequences of HLA class I alpha chains were retrieved from a global reference in immunogenetics and immunoinformatics, IPD-IMGT/HLA database (International ImMunoGeneTics Database, version 3.32) [19].

2.3. Homology modelling for structure prediction

HLA class I alpha chains were generated by homology modelling by using Modeller, version 9.20 [20,21]. In this process, the amino acid sequences, previously retrieved from IPD-IMGT/HLA database, were used to search for homologous sequences available in in the Protein Data Bank (PDB) [22]. Similarity between tatrget-template pairs was quantified using sequence identity and resolution, as well as the statistical measure of E-value. One hundred models were independently generated for each target, but only the model presenting the most negative values for the Discrete Optimized Proteins Energy (DOPE) [23] was chosen.

2.4. Refinement and assessment of predicted structures

All the generated models were further refined by using the GalaxyRefine module of the GalaxyWeb server [24]. In order to determine the reliability of predicted structures, the evaluation of each model was performed. The strategy was the calculation of important parameters of modelled structures using specific tools. Hence, Ramachandran plots of modelled structures were done and validated in PROCHECK [25]. Besides, stereo-chemical excellence and overall quality of each refined structure were analyzed by Z-scores [26] and ERRAT [27], respectively. Additional assessment of predicted structures was done by evaluation of superimposition of target-template structures using the matchmaker function of UCSF Chimera [28].

2.5. HLA molecules heterodimerization

Refined high resolution models of HLA class I alpha chains were hereterodimerized by using Swiss-PDB Viewer, version 4.1 [29]. In this process, the monomorphic β_2M chain and peptide from the template molecule were used, including their original coordinates. Quality analysis of the final structures was conducted by comparison of both structural alignment and equivalence between the heterodimerized and template structures by using the Dali server [30].

2.6. Development of the online database

An online database containing HLA class I predicted three-dimensional structures was developed in Ruby (www.ruby-lang.org), version 2.3.3, by using the Ruby on Rails web framework (www.rubyonrails.org), version 5.1.4. Its administrative pages were developed with the ActiveAdmin (www.activeadmin.info) library, version 1.3.0. All public pages were developed with Bootstrap framework (www.getbootstrap.com), version 4.1.1. A three-dimensional structure online viewer was developed by using 3Dmol (3dmol.csb.pitt.edu) library, version 1.3.7. The database management system was Postgres SQL (www.postgresql.org), version 9.5.15, and the application server was Apache (www.apache.org), version 2.4.37. Fig. 1 summarizes the workflow followed in the present study.

Table 1
Quality comparison between the template, original and refined models.

Model	Procheck					Errat	Z-Score
	Core[a]	Allow[b]	Gener[c]	Disall[d]	G-factor[e]		
3BO8:A (PDB)	92.4%	7.2%	0.4%	0.0%	0.10	94.67	−9.00
A*01*01:A(original)	97.0%	2.5%	0.4%	0.0%	0.01	84.21	−9.01
A*01*01:A (refined)	96.2%	3.4%	0.4%	0.0%	0.15	96.15	−9.06
3RL1:A (PDB)	92.5%	7.1%	0.4%	0.0%	0.18	95.09	−8.86
A*29:02:A (original)	94.6%	4.5%	0.4%	0.4%	0.02	86.84	−8.52
A*29:02:A (refined)	95.0%	3.7%	0.4%	0.8%	0.15	95.76	−8.72
3UTQ:A (PDB)	93.4%	6.1%	0.4%	0.0%	0.13	98.50	−9.43
A*02:01:A (original)	95.9%	3.7%	0.4%	0.0%	0.01	83.58	−8.52
A*02:01:A (refined)	95.5%	4.1%	0.4%	0.0%	0.12	91.60	−9.40
41UH:A (PDB)	91.7%	7.5%	0.8%	0.0%	−0.04	96.64	−8.91
B*07:02:A (original)	95.9%	3.3%	0.8%	0.0%	0.01	89.21	−8.93
B*07:02:A (refined)	95.9%	3.7%	0.0%	0.4%	0.13	97.35	−8.94
1XR9:A (PDB)	91.3%	7.9%	0.8%	0.0%	0.62	97.76	−8.96
B*15:12:A (original)	95.0%	4.2%	0.8%	0.0%	0.00	87.31	−8.95
B*15:12:A (refined)	95.4%	3.8%	0.8%	0.0%	0.11	97.77	−8.95
4NT6:A (PDB)	93.3%	6.3%	0.4%	0.0%	0.04	90.97	−9.12
C*01:02:A (original)	96.2%	3.4%	0.4%	0.0%	−0.02	88.34	−9.06
C*01:02:A (refined)	95.4%	4.2%	0.4%	0.0%	0.11	91.01	−9.06

[a] Percentage of residues in most favored regions.
[b] Percentage of residues in permitted areas.
[c] Percentage of residues in regions generally allowed.
[d] Percentage of residues in disallowed areas.
[e] Dihedral G-factor [31].

3. Results

3.1. Modeled HLA molecules

Alpha chains (mature proteins) for 106 alleles belonging to the three major HLA class I loci were modeled (33, 54 and 19 alleles for loci A, B and C, respectively). The amino acid sequences of the HLA-A and HLA-C showed an average size of 274 amino acids, whereas the amino acid sequences of HLA-B alleles showed 276 amino acids. The average identity of amino acid sequences between templates from the PDB and target HLA structures was 96% for loci HLA-A and HLA-C and 98% for locus HLA-B.

3.2. Quality analysis

Table 1 shows structural parameters of six randomly selected modeled molecules before (original) and after (refined) refinement as well as for X-ray solved structures used as a template. With respect to the Procheck parameter it is expected that the models have the highest percentage of residues in the favorable and allowed regions (columns Core and Allow, respectively) and G-factor with positive values. Models are expected to have a higher value with respect to the Errat parameter and a lower value with respect to Z-Score. High quality models present parameter values close to those of the template structure. Table 2 shows additional data obtained from the GalaxyRefine server for the same modeled molecules of the table one. Refined models with values less than or equal to the Poor rotamers parameter and greater or equal to the Rama favored parameter were selected.

Fig. 2A and B show, respectively, the structural alignments of the alpha and beta chains of the predicted three-dimensional structure of HLA-A*01:01, submitted to the Dali server. This predicted structure has 100% sequence identity and RMSD (Root-mean-square Deviation of atomic positions) equal to 0.4 with the template PDB ID: 3BO8. Fig. 2C and D show the overlap of the predicted structure with their template structure in the ribbon and surface representations, respectively. This procedure was important to show the strength of the modelling in cases where the predicted structure already had an identical equivalent for the same HLA allele [22].

Fig. 3 shows the structure comparison of two HLA molecules that did not present 100% sequence identity with their respective templates. Section 3A shows the structural alignment of the HLA-A*29:02 modelled alpha chain with its PDB template (ID: 3RL1, 94% sequence identity and RMSD equal to 0.4). The region between positions 61 and 80 shows some mismatched amino acids. Sections 3C and 3E highlights such a region in red in the representations of ribbons and surface, respectively. As observed in sections 3A, 3C and 3E, this region corresponds to an alpha helix for both target and template. Section 3B shows the structural alignment of the HLA-C*01:02 predicted alpha chain with its PDB template (ID: 4NT6, 95% sequence identity and RMSD equal to 0.5). The region between positions 5 and 12 shows mismatched amino acids. Sections 3D and 3F present the same region highlighted in red and confirm it corresponds to a beta sheet structure in the tertiary protein structure in both ribbons (3D) and surface (3F) representations.

3.3. Online database

An online database consisting of a web application was developed to make the predicted three-dimensional structures ready for use. It is freely available at www.phla3d.com.br and does not require any personal information from the user. Fig. 4 shows the main page of pHLA3D containing three tabs, the HLA-A tab with 33 structures, the HLA-B tab with 54 structures and the HLA-C tab with 19 structures. For each tab the user can search for structures by the name of the allele. The first column of the page contains information about the alleles that were used as targets for homology modeling. The second and third columns contain information about their PDB templates (ID and resolution). Sequence identity and e-value are presented in the fourth and fifth columns, respectively. It is also possible to download the predicted structures in PDB or C3D format (Options menu) for offline visualization.

The database allows the user to visualize predicted structures through an online visualizer (Fig. 4, Options column > 3D View), where users may select and view specific amino acids, enabling them to visually identify whether an amino acid is exposed or not at the structure's surface (Fig. 5). In this picture, two amino acids are showed: the first is a partially exposed glycine in position 1 (A:GLY:1) and the second is a cryptic cystein in position 101 (A:CYS:101). The online viewer allows the user to toggle the chains (alpha – blue, β_2M – purple

Table 2
Additional quality comparison between the original and refined models.

Model	GDT-HA[a]	RMSD[b]	MolProbity[c]	Clash score[d]	Poor rotamers[e]	Rama favored[f]
A*01*01:A (original)	1.0000	0.000	2.032	35.6	0.9	98.5
A*01*01:A (refined)	0.9854	0.318	1.383	7.0	0.4	98.5
A*29:02:A (original)	1.0000	0.000	2.079	39.8	0.0	98.2
A*29:02:A (refined)	0.9863	0.322	1.611	12.6	0.0	98.2
A*02:01:A (original)	1.0000	0.000	2.082	40.1	0.4	98.5
A*02:01:A (refined)	0.9946	0.276	1.601	12.3	0.4	98.9
B*07:02:A (original)	1.0000	0.000	2.069	32.0	1.3	98.2
B*07:02:A (refined)	0.9874	0.316	1.377	6.8	0.4	98.5
B*15:12:A (original)	1.0000	0.000	2.340	43.6	1.7	97.8
B*15:12:A (refined)	0.9891	0.315	1.542	8.5	1.3	98.2
C*01:02:A (original)	1.0000	0.000	2.198	43.2	1.3	98.2
C*01:02:A (refined)	0.9690	0.362	1.601	12.3	0.4	98.9

[a] Global Distance Test – High Accuracy.
[b] root-mean-square deviation
[c,d,e] MolProbity, clashscore and poor rotamers [32].
[f] Ramachandran favored.

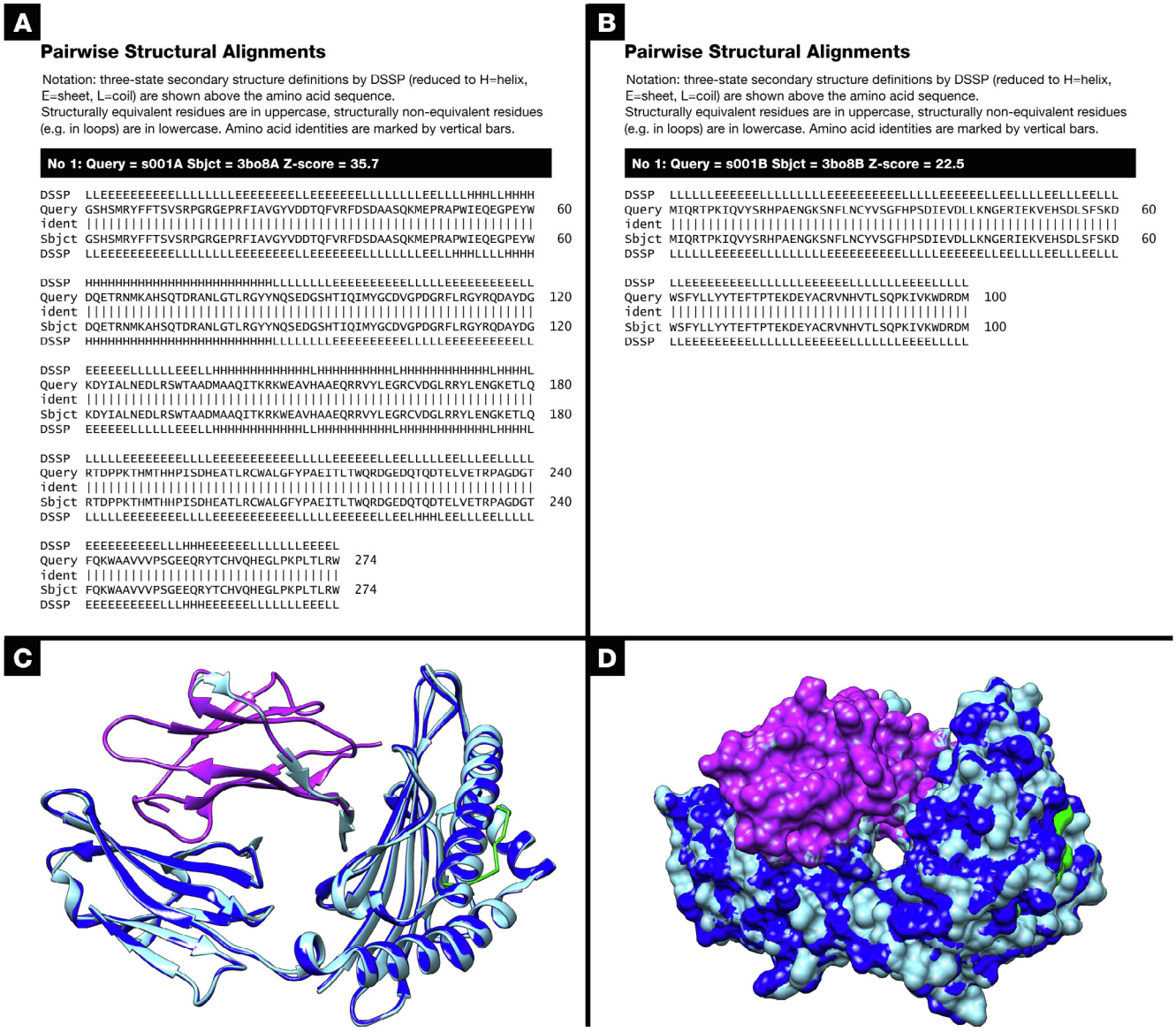

Fig. 2. Structural alignments of the alpha (A) and beta (B) chains between target (HLA-A*01:01) and template (PDB ID: 3BO8) structures with 100% sequence similarity. Sections C and D show the overlapping of the predicted and the template structures in the ribbon and surface shapes, respectively. Blue – alpha chain, purple – β_2M, green – peptide. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

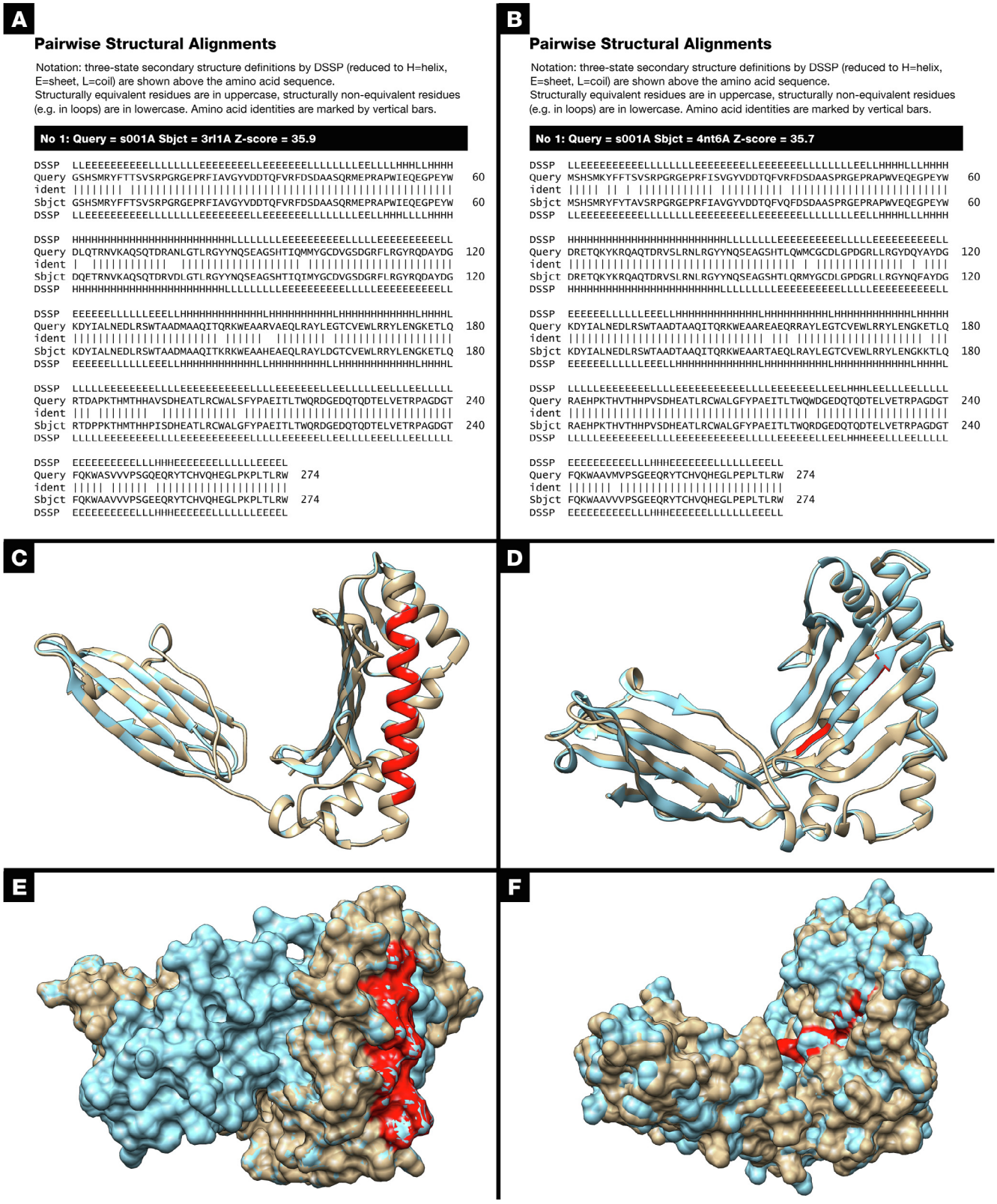

Fig. 3. Structural alignments of predicted and X-ray solved HLA class I molecules with sequence similarity lower than 100%. Sections A, C and E shows the overlay of predicted HLA-A*29:02 (gray) and X-ray solved HLA-A*03:01 (PDB ID: 3RL1, cyan) alpha chain. Sections B, D and F shows the overlay of predicted HLA-C*01:02 (grey) and X-ray solved HLA-C*08:01 (PDB ID: 4NT6, cyan) alpha chain. Highlighted (red) are selected regions containing mismatched amino acid. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

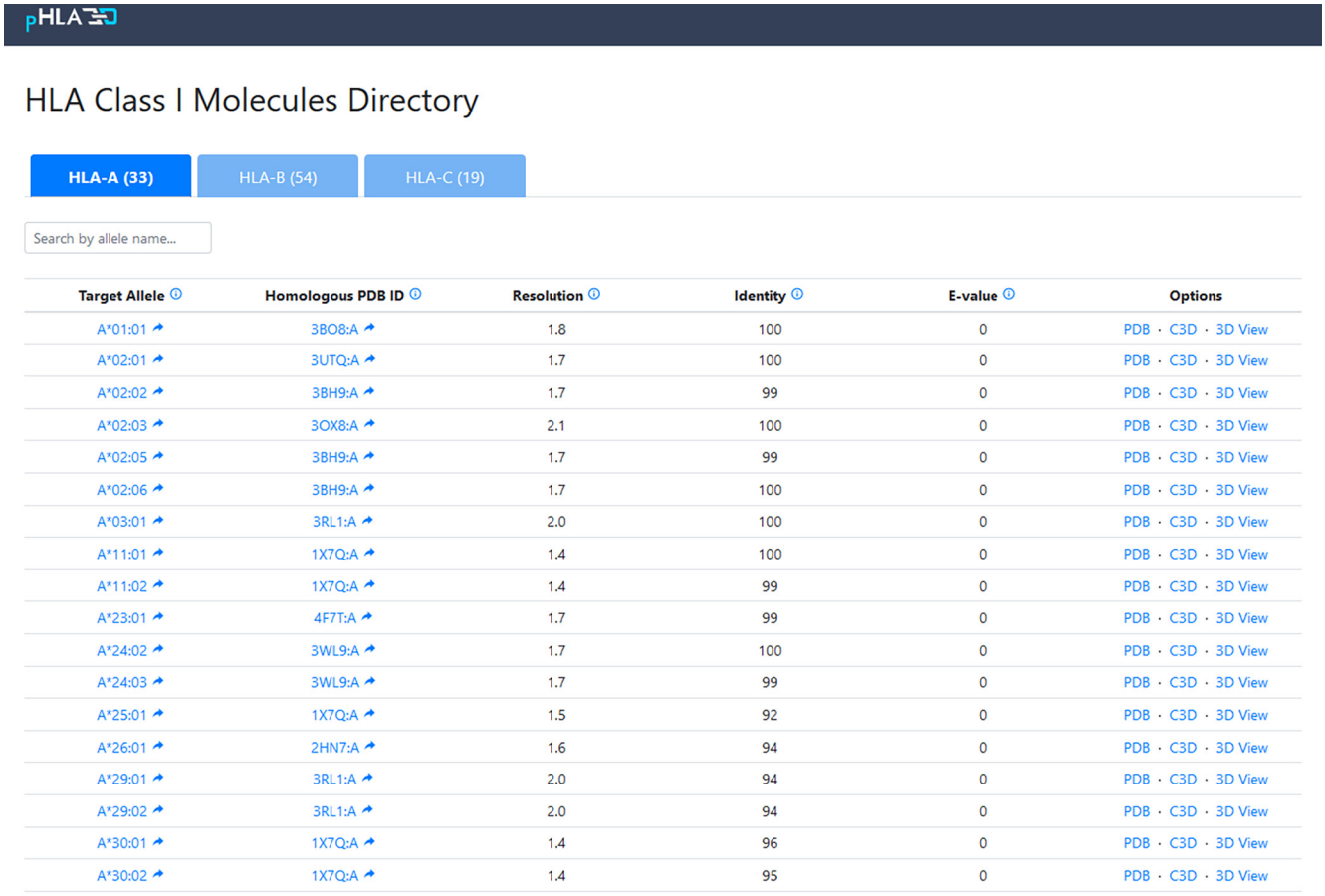

Fig. 4. pHLA3D page with information on HLA class I molecules.

and peptide – red) which can be visualized in surface (default) or spacefill representations.

4. Discussion

pHLA3D was built to provide the community of immunologists and immunogeneticists with three-dimensional structures of the class I HLA molecules currently tested in solid phase assay panels. For laboratory practice, this database represents a great breakthrough, as it provides means to visualize target epitopes recognized by anti-HLA antibodies, as determined by the epitope reactivity analysis using programs such as the HLAMatchmaker [9], EpHLA [10,11], EpViX [12,13], Cambrigde HLA Immunogenicity Algorithm [14], EpAssistant [15] and EMS-3D [4].

Currently, the limited number of structures available for HLA proteins forces epitope reactivity analyzers to infer the exposure degree (potential for antigenicity) of some epitopes in alleles from which three-dimensional structures have not been solved [17]. Such inferences are accomplished with similar alleles whose structures have been solved. In practice, this may result in misleading assumptions, as the exposure of a certain epitope depends not only on the charge and hydrophobicity of its residues but also on their interaction with neighboring amino acids [1,3,4,33]. In this sense, the use of pHLA3D may ease the work of epitope reactivity analyzers by increasing the number of available models and allowing the visualization of such epitopes directly in the molecule of interest, preventing the need for inference and decreasing the probability of misleading assumptions.

To provide a means to spatially locate any amino acids in a certain HLA molecule, pHLA3D offers epitope reactivity analyzers the ability to visualize any epitope, either antibody-verified or not. In this regard, pHLA3D is shown to be complementary to programs such as DiscoTope [34], NetSurfP [7,35], ElliPro [6] and BepiPred [36], which are tools built for inferring the exposure and/or antigenicity of amino acids based on their physicochemical properties. Notably, despite the innovation provided by these programs, all of them share the feature of making inferences about the exposure degree from the linear sequence of amino acids in the polypeptide; in this respect, pHLA3D differs from all above-mentioned programs.

The simplified navigation of pHLA3D fulfils our desire to develop an intuitive and easy-to-use tool. The presence of relevant information, such as PDB ID of the template structures, sequence identity and e-value of the alignments, contribute to the reliability of the tool. As pHLA3D is intuitive and reliable, we consider that it may also be useful for teaching histocompatibility and immunology in basic and advanced courses on this subject. In this respect, the tool was made freely available via the web and has not only the option to visualize the structures online, through smartphones and tablets, but also the option to download the predicted structures to facilitate offline visualization in tools such as Chimera and Swiss-PDB Viewer, which allow users to explore these structures more deeply. As future directions we intend to build and validate three-dimensional structures for class II HLA-DR, DQ and DP. Besides, the repertoire of HLA class I molecules is supposed to be expanded to include not only common and well-documented alleles, but only well-documented, too.

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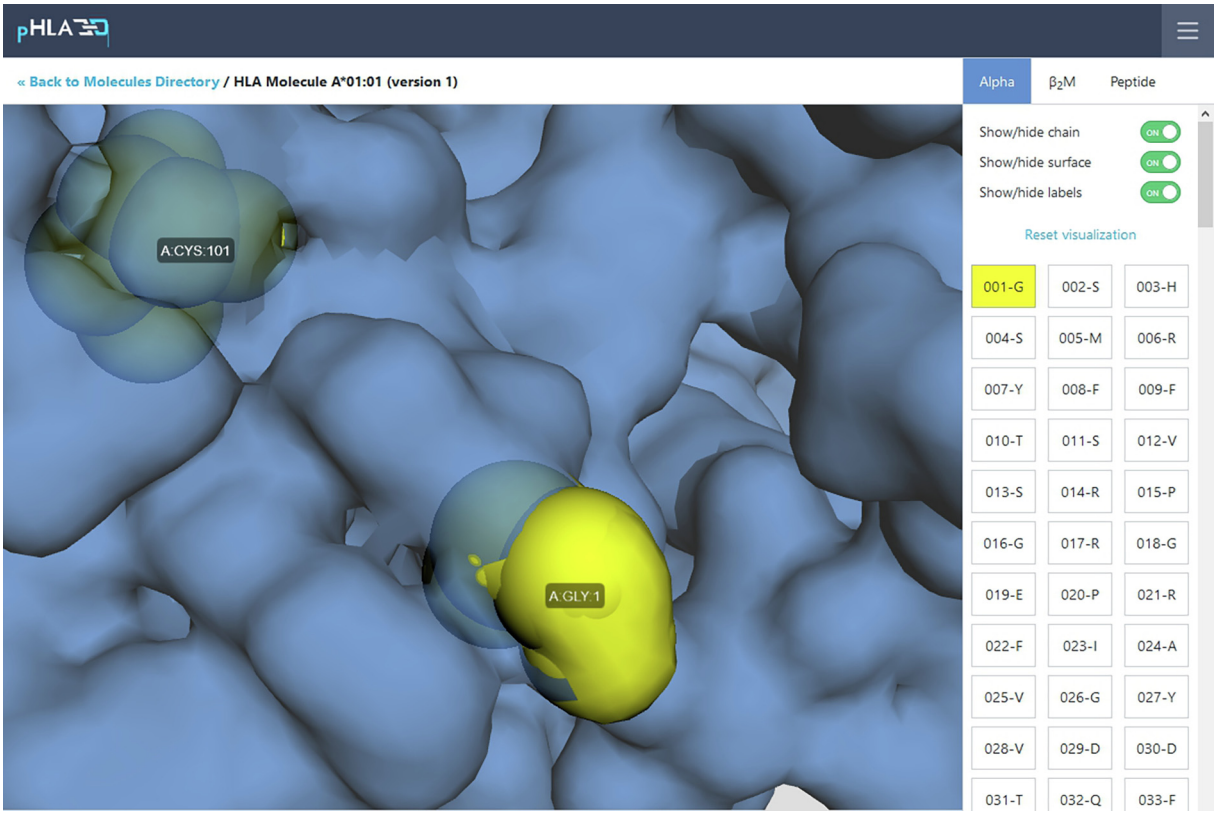

Fig. 5. A closer view of HLA-A*01:01 allele in the pHLA3D online visualizer. On the right side, the table from where each HLA composing chain (alpha – blue, β_2M – purple and peptide – red), can be selected, is showed. In this table, amino acids (position and one letter code) of alpha chain are displayed. Selection of amino acids on the table shows the selected such amino acid in yellow. On the left side, the selected amino acids are easily identified as exposed or cryptic since the structure's surface is semitransparent. Labels in such selected amino acids show the following codes: the chain (A = alpha, B = β_2M and P = peptide), the three-letter amino acid name (for example, GLY) and the position occupied by the amino acid (for example, 1). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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Declaration of Competing Interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humimm.2019.06.009.

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