Vaccine Design Against Leptospirosis Using an Immunoinformatic Approach

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Chapter 7

Vaccine Design Against Leptospirosis Using an Immunoinformatic Approach

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Abstract

Vaccination is the best way to prevent the spread of emerging or reemerging infectious disease. Current research for vaccine development is mainly focused on recombinant-, subunit-, and peptide-based vaccine. At this point, immunoinformatics has been proven as a powerful method for identification of potential vaccine candidates, by analyzing immunodominat B- and T-cell epitopes. This method can reduce the time and cost of experiment to a great extent, by reducing the number of vaccine candidates for experimental testing for their efficacy. This chapter describes the use of immunoinformatics and molecular docking methods to screen potential vaccine candidates by taking *Leptospira* as a model.

Key words Immunoinformatics, Immunogenicity, Leptospirosis, Outer membrane protein, Epitopes, Vaccine candidate, Molecular docking, Simulation, Binding interaction

1 Introduction

Leptospirosis is a widespread zoonotic disease, caused by infection of pathogenic species of *Leptospira* [1–3]. Pathogenic species comprises more than 250 antigenically distinct serovars of *Leptospira* [3, 4]. This antigenic diversity makes up a challenge for the researcher to eradicate this disease. The global burden of this disease is increasing year by year [3]. Vaccination is usually considered to be the most feasible way to eradicate infectious diseases. Generally, a vaccine is made from whole cell inactivated or killed form of disease-causing microorganism and its toxins or immunogenic proteins. In some cases, vaccines derived from whole pathogen may cause adverse effects and provide short-term immunity and are insufficient in providing broad spectrum of protection across multiple strains of pathogen [3, 5, 6]. The advancement in the molecular understanding of antigen presentation has led to the

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development of peptide- and epitope-based vaccine. Usually, peptide-based vaccine is based on identification of immunodominant B- and T-cell epitopes, from immunogenic proteins, which can induce specific immune response. Epitope, also called as antigenic determinant, is a portion of an antigen that is recognized by particular receptors present on the surface of immune cells, mainly B and/or T cells. The epitopes recognized by B celles are known as B-cell epitopes, whereas epitopes recognized by T cells are known as T-cell epitopes. B cells recognize the epitopes on antigen are known as B-cell epitope and epitopes recognized by T cells are refers as T-cell epitopes.

The current research for developing peptide vaccines are mostly focused on outer membrane proteins (OMPs) because these proteins have been recognized as playing major role in the interaction of pathogens with host cells and probably associated with pathogenesis [3, 7]. The availability of genomics, proteomics, and immunological data and advances in the computational algorithms have improved the efficacy of identification of immunodominant outer membrane proteins and thereby potential vaccine candidates; this field of study is known as immunoinformatics [8–11]. Immunoinformatics is now becoming fascinating in the field of vaccine development which uses genome- and proteome-based information and offers high level of confidence for the prediction of potential vaccine candidates [3, 12, 13]. Recently, the approach has been broadly accepted for screening the potential immunogens for vaccine design of infectious diseases. Identification of epitopes in an antigen has become key for subunit- and peptide-based vaccine development against pathogens, which confer long-lasting effects. In silico approach may prove as a beneficial and directive approach for mapping of potential epitopes on antigens, whereas conventional methods focus more on pathogen cultivation and protein extraction, where testing of these proteins on a large scale is expensive and time-consuming [3]. Nowadays, several in silico tools are available for the prediction of epitopes on target antigen, which reduce the time and cost by reducing the list of potential epitopes for experimental validation. Several in silico vaccine candidates have been reported by researchers which were known to produce promising preclinical and clinical trial results [14, 15]. This chapter outlines the systematic methods to screen potential vaccine candidates using immunoinformatic approach. The methods described below are generalized and can be used for any of the targeted disease.

2 Materials and Methods

A pictorial representation of workflow is shown in Fig. 1.

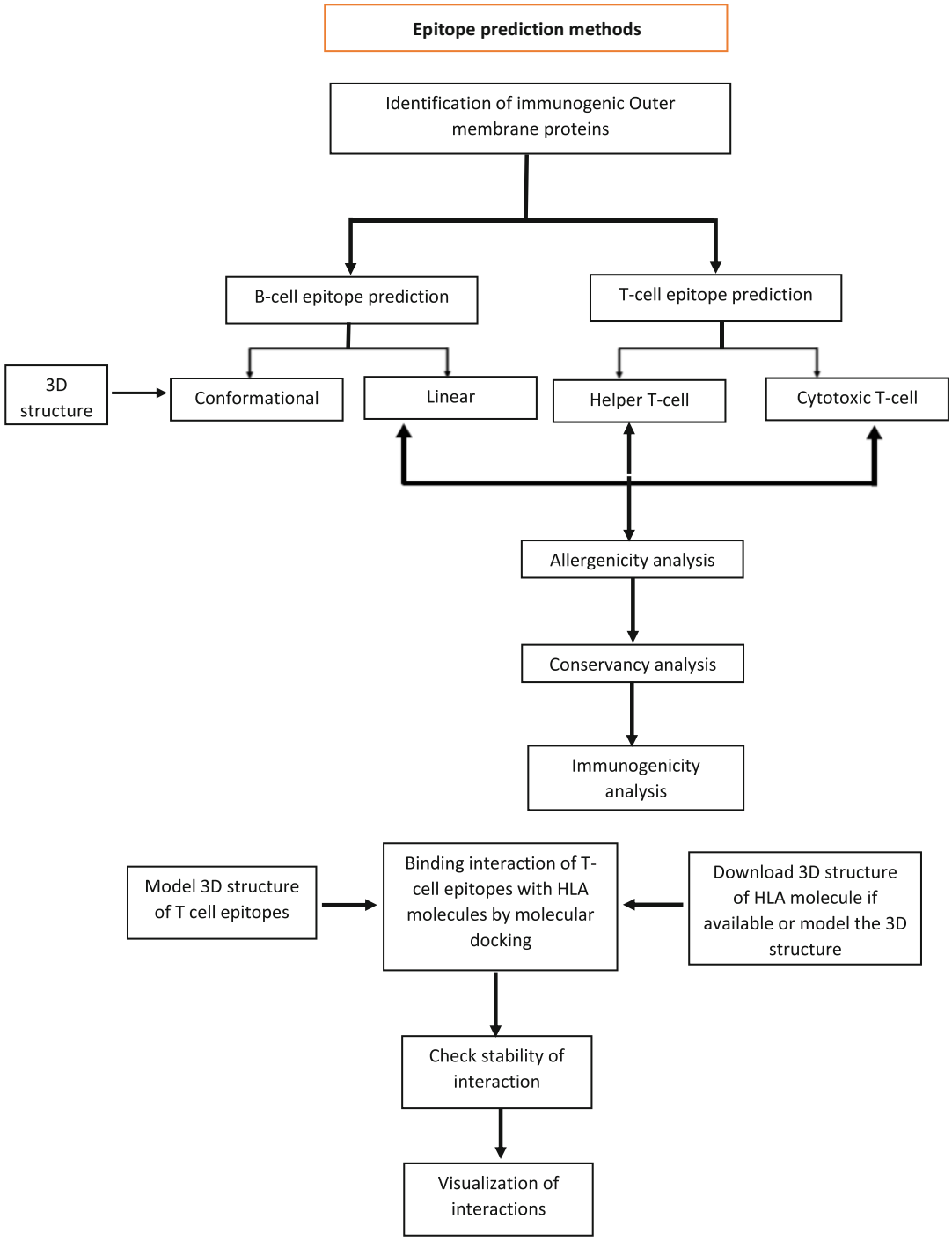

Fig. 1 Workflow representing the key steps in screening of potential B- and T-cell epitopes. The conformational B-cell epitope depicted in this flowchart was predicted from 3D structure (PDB ID: 2ZZ8) of LipL32 protein of *Leptospira*. Structure shown here for visualization of binding interaction was downloaded from RCSB PDB (PDB ID: 1B0G)

2.1 Retrieval of the Target Sequences

The first step in immunoinformatic approach for vaccine design is to retrieve protein sequences or whole proteome in FASTA format. We can extract protein sequences from UniProtKB or NCBI database and whole proteome sequence can be retrieved from UniProt Proteomes database. Once protein sequences are obtained, we will screen these sequences for their immunogenicity.

2.2 Identification of Immunogenic Proteins

The immunogenicity refers to the ability of an antigen to elicit an immune response. The selection of optimal immunogen is the first step for vaccine design; hence, to identify the most probable immunogenic protein, the whole proteome of *Leptospira* was submitted to VaxiJen v2.0 server [16] (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html), which was developed for the prediction of potent antigen and subunit vaccines with accuracy of 70 to 89% (*see* **Note 1**). As an input, we can paste a protein sequence in a plain format or upload a list of protein sequences in FASTA format. A target organism can also be selected based on source of antigens. Here, in case of *Leptospira*, we have selected bacteria as a target organism. An overall score depicts the potentiality of each protein sequence to induce immune response. Proteins with higher score are predicted to be more immunogenic.

2.3 Identification of Outer Membrane Protein (OMP)

The localization of a protein plays a vital role in determining its functionality. A potential immunogen has to be easily recognized by the immune cells. Outer membrane proteins are surface-exposed which is easily recognized by the host immune system and involved in the interaction between bacterial cells and their host [17, 18]. In pathogenic bacteria, OMPs are proven to be the most promising vaccine candidates, due to its interaction with the host immune cells; and hence, identification of OMPs is crucial for a reliable and rapid vaccine development [3]. Therefore, protein sequences, predicted as antigenic in Vaxijen server, were subjected to CELLO v.2.5 server [19, 20] (http://cello.life.nctu.edu.tw/) to retrieve outer membrane protein (*see* **Note 1**). CELLO uses machine learning, support vector machine algorithm to predict localization of the proteins.

2.4 B-Cell Epitope Prediction

B-cell epitope is the main antigenic region of an antigen which are recognized by the B-cell receptors of the immune system and are able to stimulate humoral immune response, which cause the B-lymphocytes to differentiate into antibody-secreting plasma and memory cells [21]. After activation plasma cells secrete antigen-specific antibodies and circulated to the bone marrow where they can encounter the antigen. Memory B cells are distributed throughout the body and respond quickly to kill the antigen if it is encountered again [22]. B-cell epitopes can be categorized as linear (continuous) and conformational (discontinuous) based on their spatial structure.

2.4.1 Linear B-Cell Epitope

Linear B-cell epitope is a consecutive sequence of amino acids on an antigen. B-cell epitope can be predicted using IEDB analysis resource, where we have to paste protein sequence in plain format or provide a Swiss-Prot ID and select an appropriate prediction method. Linear B-cell epitope can be predicted using BepiPred prediction method [23]. Antigenic B-cell epitope were predicted by Kolaskar and Tongaonkar method at Immune Epitope Database (IEDB) analysis resource (http://tools.iedb.org/main/bcell/) [24]. This method predicts antigenic peptide by analyzing the physicochemical properties of amino acid residues and their abundance in experimentally determined antigenic epitopes [3, 24]. The accuracy of this method to predict epitope is about 75%. Surface accessibility, flexibility, and hydrophilic properties are also main characteristics of B-cell epitopes [25]; hence, to predict these properties, Emini surface accessibility [26], Karplus and Schulz flexibility [27], and Parker hydrophilicity [28] prediction methods were employed, respectively, with default parameters of IEDB analysis resource (*see* **Note 2**).

2.4.2 Conformational B-Cell Epitope

A conformational B-cell epitope consists of discontinuous residue of amino acids in a protein sequence and present in close proximity in 3D structure of protein (*see* **Note 3**). Conformational B-cell epitopes in 3D structure were predicted using Ellipro [29]. This tool predicts the epitopes based on the geometrical properties of the protein structure, and it discriminates predicted epitopes from non-epitopes on the basis of known protein antibody complex. The conformational B-cell epitopes with a protrusion index (PI) value above 0.7 were selected. The score (PI) reflects the percentage of protein atoms that extend beyond the molecular bulk and are responsible for antibody binding [29].

2.5 T-Cell Epitope

Unlike B cells, T cells do not recognize antigen directly, here antigen first processed by antigen-presenting cell (APCs), e.g., dendritic cells or B cells or macrophages, and then present to the T-cell receptor (TCR) by major histocompatibility complex [22, 30]. There are mainly two types of T-cell epitopes, CTL (cytotoxic T-lymphocytes) and HTC (Helper T cell). T cell expresses a cluster of differentiation (CD) receptor on its cell surface that recognizes the antigen presented on MHC molecule [8]. CTL expresses the CD8+ receptor on its surface and recognizes peptides presented by MHC class I molecules, while HTC expresses CD4+ receptor, which recognize MHC II antigen complex [8].

2.5.1 Helper T-Cell (HTC) Epitope

Helper T cell is crucial for activating an efficient humoral and cell-mediated immune response, by stimulating the differentiation and proliferation of B and cytotoxic T cell [31]. The binding of epitopes, complexed with MHC class II, to the T-cell receptor can

result in the activation of HTC response. Hence, in order to predict MHC class II-restricted HTL epitopes, the protein sequences were submitted to NetMHCIIpan 3.1 server (http://www.cbs.dtu.dk/services/NetMHCIIpan/) with threshold value set as 0.5% and 2% for strong binding peptides (SB) and weak binding peptides (WB), respectively, to determine the binding affinities of epitopes and MHC-II allele [32]. NetMHCIIpan is one of the most accurate prediction tools that covers all human leukocyte antigen (HLA) class II molecules based on artificial neural network algorithm. In this tool we can upload or paste protein sequences in FASTA format and can select HLA loci from the drop-down option. It has been anticipated that the binding strength of HTL epitope to the HLA molecules is a key factor in immunogenicity of the T-cell epitope and a good T-cell epitope candidate should bind to the maximum number of HLA alleles to get more population coverage [33, 34]. Therefore, the strong binder epitopes, having IC50 value <50 nM, and epitopes binding with maximum number of HLA allele were considered as putative HTC epitope for vaccine candidates.

2.5.2 Cytotoxic T-Lymphocyte (CTL) Epitope

Consistent predictions of CTL epitopes are very important for the coherent vaccine design. Because sometimes humoral immunity is not far enough to completely clean the infection, cell-mediated immunity is required to induce cell death and completely destroy the bacterial habitat. Although pathogenic *Leptospira* is not considered as a typical intracellular pathogen, indeed some bacterial proteins may be able to escape from the phagolysosome and reach to the cytosol of host cells and are exposed to the host CD8+ T-cell response [3, 35]. Hence, the presence of CTL epitopes in OMP protein was predicted using NetCTL.1.2 server (http://www.cbs.dtu.dk/services/NetCTL), with default parameters [36]. This server predicts epitopes by integrating predictions of MHC class I binding, proteasomal C-terminal cleavage, and the TAP transport efficiency. The MHC class I binding and proteasomal C-terminal cleavage were predicted by the artificial neural network, while a weight matrix was used to predict the TAP transport efficiency. This tool has an option to select MHC-I supertype; we can select any one of the MHC-I supertypes at a time from the drop-down option. As an output, it generates 9-mer epitope sequence and their respective score for C-terminal cleavage, TAP transport efficiency, threshold of epitope identification, and overall combined score.

2.6 Immunogenicity Prediction of T-Cell Epitopes

The peptides with strong immunogenicity are more probable T-cell epitopes than those with weak immunogenicity. Therefore, the immunogenicity of putative T-cell epitopes was evaluated using IEDB immunogenicity prediction tool. CD4 T-cell immunogenicity prediction method at IEDB (http://tools.iedb.org/CD4episcore/)

was used for immunogenicity prediction of HTC epitope [37]. Here, we have to submit HTC epitope sequences and can select the prediction method and maximum percentile rank threshold. By default, there is IEDB combined method for prediction of immunogenicity. This method predicts the final score combined from seven-allele and immunogenicity prediction methods. On the other hand, immunogenicity of CTL epitopes was predicted using class I immunogenicity method with default parameter at IEDB T-cell analysis tool (http://tools.iedb.org/immunogenicity/) [38].

2.7 Allergenicity Prediction

We also predicted the allergenicity of predicted epitopes, because a potential vaccine candidate should be non-allergen. In order to predict allergenicity of epitopes, AllerHunter server (http://tiger.dbs.nus.edu.sg/AllerHunter) was used, which is based on support vector machine (SVM) and pair-wise sequence similarity [39] (*see* **Note 4**). AllerHunter predicts allergen in addition to non-allergen with high sensitivity and specificity and efficiently distinguishes allergens and non-allergens from allergen-like non-allergen sequences, which make AllerHunter a very constructive tool for allergen predictions.

2.8 Conservancy Analysis

In epitope-based vaccine design, the selection of conserved epitopes would be crucial for providing a broad spectrum of protection across several serovars, strains, or species of *Leptospira*. In order to evaluate conservancy of predicted epitopes among available strains of *Leptospira*, we first downloaded orthologues protein sequences of target protein, from NCBI, for all strains and saved all sequences in a file (in FASTA format). Furthermore, we performed conservancy of predicted epitopes, among orthologues sequences, by using epitope conservancy analysis tool at the IEDB analysis resource (http://tools.immuneepitope.org/tools/conservancy) [40]. This tool calculates the degree of conservancy of an epitope within a provided protein sequence set at different degree of identities. The degree of conservancy is defined as the portion of protein sequences that contain the epitope at a specified identity level.

2.9 Molecular Binding Interaction Analysis of Predicted Epitopes with HLA Molecules

A molecular docking study was performed to ensure the molecular binding interaction between HLA molecules and our predicted T-cell epitopes. For docking study, we need three-dimensional (3D) structure of predicted epitopes and HLA molecules. Since, HLA-A2 allele is one of the most frequent MHC class I alleles in most of the human populations; we downloaded the 3D structure of HLA-A2 allele from RCSB PDB Protein Data Bank (https://www.rcsb.org/) [41, 42].

2.9.1 3D Structure Prediction of T-Cell Epitopes

The 3D structures of all predicted T-cell epitopes excluding the allergen one were modeled with the PEP-FOLD3 server (http://mobyle.rpbs.univ-paris-diderot.fr/cgi-bin/portal.py#forms::PEP-FOLD3), using 200 simulation runs [43]. First the PEP-FOLD3 server clustered different conformational models of given epitope and then sorted them with the sOPEP energy value. The best ranked model was selected to analyze the interactions with selected HLA molecules.

2.9.2 Molecular Docking

Molecular docking between HLA molecule and predicted T-cell epitopes was performed using PatchDock rigid-body server (https://bioinfo3d.cs.tau.ac.il/PatchDock/php.php). In this tool, we have to upload 3D structure of receptor and ligand molecule; here, receptor will be HLA molecule and epitopes as a ligand. This tool computes complexes with good molecular shape complementarity based on geometry of the molecules. The output of PatchDock contains a list of predicted complex structures with their rank, based on score. The best ranked docked complex was further refined using FireDock (Fast Interaction Refinement in Molecular Docking) server82,83 (http://bioinfo3d.cs.tau.ac.il/FireDock/php.php) [44, 45] (*see* **Notes 1** and **5**). The output of FireDock result includes ten best solutions for final refinement complex, based on the binding score. This tool ranks the refined complex based on global energy, attractive and repulsive Van der Waals forces, atomic contact energy, and hydrogen binding interaction scores. The complex having lowest global energy was ranked first and considered the best suited confirmation for complex formation. A screenshot of FireDock result outputs, taken from help file, is shown in Fig. 2.

Receptor	Ligand	TransFile
2kaiAB.pdb	2kaiI.pdb	trans.txt

Rank	Solution Number	Global Energy ↓	Attractive VdW	Repulsive VdW	ACE	HB
1	10	-87.13	-41.96	7.52	-14.04	-5.71
2	9	-85.93	-41.29	6.12	-13.37	-4.85
3	8	-27.50	-42.96	14.48	5.35	-3.68
4	3	-26.41	-36.27	33.70	4.63	-9.06
5	5	-18.66	-34.26	21.32	-1.57	-4.92
6	1	-17.57	-42.02	35.06	4.83	-5.77
7	7	-15.08	-27.43	17.51	2.12	-5.86
8	6	-12.51	-28.48	15.19	7.33	-1.85
9	2	-10.10	-41.38	46.78	-9.21	-3.71
10	4	-6.47	-30.95	42.24	1.94	-2.82

Fig. 2 Screenshot of refined docking complex, created from help file of FireDock tool. The complexes are ranked based on their global energy

2.9.3 Molecular Dynamics Simulations

The binding stability of epitopes-HLA docked complex was checked by molecular dynamics simulations using GROMACS v2016.3 software [46]. For each of the docked epitope-HLA complexes, a production simulation of 5 ns at 300 K temperature and 1 bar pressure was obtained after carrying out stepwise energy minimization and equilibration protocol of the solvated systems with TIP3P water model. Further, trajectory analysis was performed to explore H-bonding and root mean square deviation (RMSD) [3].

2.9.4 Visualization of Interaction

Hydrogen bond interaction of the docked-complex can be analyzed with the molecular visualization tool UCSF Chimera 1.11.2 or PyMOL [47, 48]. We used Chimera to visualize the hydrogen bond interaction between HLA and epitope (*see* **Note 6**).

3 Notes

1. Users are suggested to use the most recent versions of any tools or servers, as these tools are continuously being updated with improved prediction algorithms and datasets.
2. As an alternative to the tools mentioned above for B-cell epitope, you can use BcePred Prediction Server (http://crdd.osdd.net/raghava/bcepred/). This tool gives scores for hydrophilicity, turns, the surface exposed, flexibility, polar, accessibility, and antigenicity propensity score for each residue of the protein.
3. For predicting conformational B-cell epitopes, you will require a 3D structure of target protein. 3D structure of target protein can be downloaded from RCSB PDB database (https://www.rcsb.org/), if available. Otherwise, you can model the 3D structure of target protein using Modeller (https://salilab.org/modeller/) or I-TASSER tool (https://zhanglab.ccmb.med.umich.edu/I-TASSER/). Modeller is a command line tool, whereas I-TASSER is a web server.
4. As an alternative, allergenicity of epitopes can be predicted using AllergenFP (http://ddg-pharmfac.net/AllergenFP/) and AlgPred (http://crdd.osdd.net/raghava/algpred/submission.html) tool.
5. Before using any web server or tools, users are requested to go through the help/FAQ section of tools, so that you can easily understand the stepwise procedure, the basic principles, and the usage of parameters.

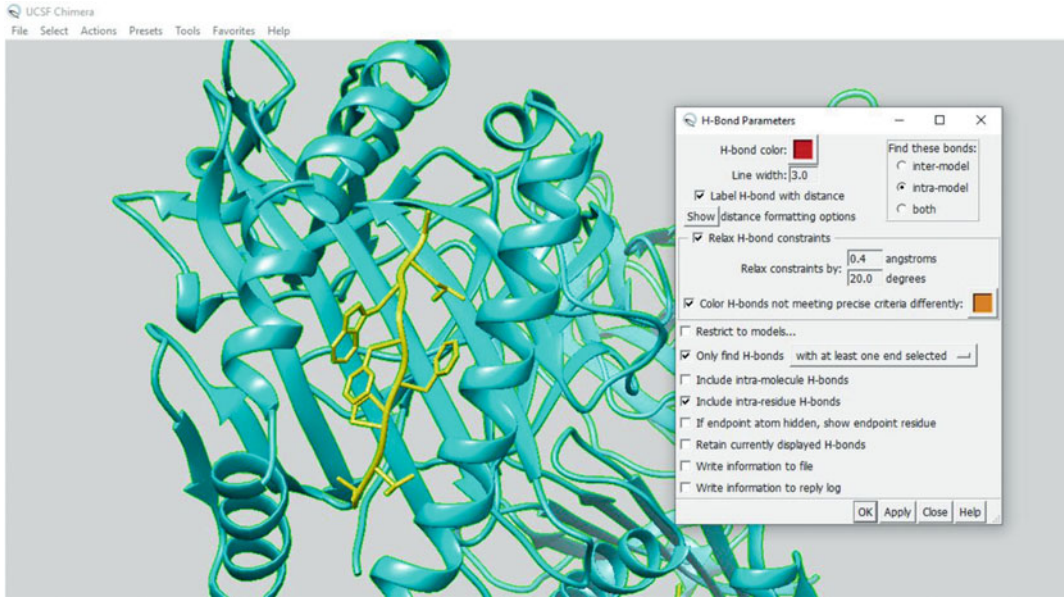

Fig. 3 Parameters for visualization of H-bond between epitope and HLA molecule. The 3D structure of HLA-A2-peptide complex (visualized in this image) was downloaded from RCSB PDB (PDB ID: 1B0G)

6. In Chimera, first open a PDB file of docked complex by browsing file location. Then, in select menu select the structure → protein. Go to tool menu → Structure analysis FindHBond. For H-bond parameter, *see* Fig. 3.

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