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In Silico Prediction of Malayan Krait (*Bungarus candidus*) PLA_2 Epitope

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ABSTRACT

Malayan krait or *Bungarus candidus* is one of the medically important venomous snake species category I in Indonesia but there is no antivenom to cover *Bungarus candidus* envenomation. PLA_2 is one of dominant toxin protein in *Bungarus candidus* venom, it is a multifunctional toxin which has several pathological effects. One alternative way to solve this problem is by generating an epitope-based vaccine, toward the epitope of PLA_2 protein. This research aims to identify the epitope region of *Bungarus candidus* PLA_2 and evaluate the potency of inducing the immune response. PLA_2 amino acid sequence retrieved from NCBI (accession number: BAD06270.1), then the T cell epitope (MHC-I and MHC-II) are predicted from this sequence using the IEDB tools. We selected the prediction results with IC50 value below 200 nM. The prediction result was then analyzed by epitope conservancy, immunogenicity, and population coverage analysis. The three most potential epitopes are "FAKAPYNEE", "LSYFRYTEM", and "RTAALCFAK". The docking simulation is conducted between three epitope prediction results and four MHC molecules (PDB ID: 6EI2, 3LKO, 1DLH, 6DIG). The docking simulation result showed that the epitopes are interacting with MHC molecules inside the binding grooves, the binding energies are relatively low (epitope "FAKAPYNEE" with 3LKO, -151.37 kcal/mol; "LSYFRYTEM" with 6DIG, -188.06 kcal/mol; and "RTAALCFAK" with 3LKO, -104.5 kcal/mol). The docking simulation result showed that the epitopes are forming a complex with MHC molecules inside the binding grooves and the binding energies are relatively low, thus it indicates that three predicted epitopes are potent to induce the immune response.

Keywords: *Bungarus candidus*, docking, epitope, PLA_2

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INTRODUCTION

Envenomation was defined by WHO [1] as the delivery of snake venom by snakebite in the human body and followed by several pathological effects. WHO has categorized the envenomation cases into Neglected Tropical Disease (NTD). The distribution of medically important venomous snake species was mapped due to the antivenom priority production. The species are separated into 2 categories, based on venom level and the common contact with the human.

Malayan krait (*Bungarus candidus*) is one of the medically important venomous snake species category 1 in Indonesia, it is highly venomous and commonly contact with the human. Yet, there is no specific antivenom to cover the *Bungarus candidus* envenomation. Malayan kraits venom are known to be neurotoxic, the venom composition is dominated by *three-finger toxin* (30.1%), *phospholipase* A_2 (25.2%), and *Kunitz-type protease inhibitor* (12.6%) [2]. It is known that *phospholipase* A_2 (PLA_2) is a multifunctional toxin, because of several effects such as neurotoxic, myotoxic, induce the inflammatory response, etc. [3].

Due to the absence of *Bungarus candidus* antivenom, one alternative way to solve this problem is by generating an epitope-based vaccine, which can be designed by the specific antigen epitope. Several advantages of epitope-based vaccine are the possibility of the more specific and effective immune response, no unimportant component to stimulate the immune response, no unexpected immune response due to the epitope design specificity, the composition is adjustable for epitopes from several different antigens, more safety, easier production, high effectivity in production time and cost [4-8].

MATERIALS AND METHODS

Protein Sequence

The protein sequence of phospholipase A_2 (PLA_2) from Malayan krait (*Bungarus candidus*) was retrieved from the *National Center for Biotechnology Information* (NCBI) database. The accession number of the protein sequence is BAD06270.1. All the sequential experimental procedures of this study were approved by Research Ethics Committe, Universitas Brawijaya.

Secondary Structure Prediction

Physico-chemical properties of the PLA_2 secondary structure were predicted with the ProtParam tool on the ExPASy web server (https://web.expasy.org/protparam/). This tool predicts several parameters that describe the nature of the protein secondary structure.

T Cell Epitope Prediction (MHC-I & MHC-II binding prediction)

T cell epitope prediction was based on the binding interaction between the peptide sequence and the MHC-I and MHC-II molecules. This prediction was conducted using tools on Immune Epitope Database And Analysis Resource (IEDB). Interaction of peptide with MHC-I was predicted using the MHC-I Binding Prediction tool (http://tools.iedb.org/mhci/), we choose Artificial Neuronal Network (ANN 4.0) prediction method [9-11]. The peptide and MHC-II interaction was predicted using the MHC-II Binding Prediction tool (http://tools.iedb.org/mhcii/) with NN-align 2.3 prediction method [12]. Prediction result with the IC50 value under 200 nM will be chosen for further analysis.

Analysis of Epitope Conservancy, Immunogenicity, and Population Coverage

Epitope conservancy was analyzed to understand the similarity of the antigen peptide sequence compared to the given sequences. This analysis was conducted using the tool on IEDB (http://tools.iedb.org/conservancy/). Peptide sequences with the similarity percentage above 70% will be selected to analyze the immunogenicity. Immunogenicity is the antigen's ability to induce an immune response. Immunogenicity tool on IEDB (http://tools.iedb.org/immunogenicity/) assess the prediction of peptide-MHC complex (pMHC complex) to elicit an immune response. The positive immunogenicity score indicates that the pMHC complex has a high potential to stimulate the immune response, thus peptides with positive scores will be selected to further analysis. Population coverage analysis (http://tools.iedb.org/population/) was conducted to analyze the effectivity of vaccine used by any given population, this analysis is based on the coverage of HLA allele genotype frequency of population which could interact with antigen epitope.

Docking Simulation

The immunogenicity of the PLA_2 epitope was evaluated by docking the potential epitope peptides with four MHC molecules. We use two MHC-I molecules (PDB ID: 6EI2 and 3LKO) and two MHC-II molecules (PDB ID: 1DLH and 6DIG). 6EI2 represents the structure of HLA-A*68; 3LKO represents the structure of HLA-B*3501; 1DLH represents the structure of HLA-DR1; and 6DIG represents the structure of HLA-DQA1. The secondary structure of the epitope peptide was firstly constructed in the HHPred web server (https://toolkit.tuebingen.mpg.de/tools/hhpred) [13]. MHC molecule structure was retrieved from Protein Data Bank (https://www.rcsb.org/). MHC structures were prepared by deleting water molecules and small ligand molecules using Discovery Studio Visualizer v16.1.0.15350. Docking simulation was conducted using PatchDock (https://bioinfo3d.cs.tau.ac.il/PatchDock/) [14]. Docking results were visualized using Discovery Studio Visualizer v16.1.0.15350 and LigPlot+ v.1.4.5. Each of the three selected potential epitopes has interacted with four MHC molecules. The docking interaction of 3LKO with influenza epitope peptide "LPFERATVM" was performed as control interaction.

RESULTS

Secondary Structure Analysis

In this study, we use *the Bungarus candidus* PLA_2 protein sequence with accession number BAD06270.1. The sequence is consists of 152 amino acids. Analysis by the ProtParam tool described several physico-chemical properties of the secondary structure summarized in Table 1. This PLA_2 protein has a relatively low molecular weight (16855.05 Da), the isoelectric point (pI) is 4.68. PLA_2 protein is hydrophilic due to the low score of GRAVY.

Table 1. Physico-chemical properties estimation of *B. candidus* PLA_2 by ProtParam

Parameter	Score
Number of amino acid	152
Molecular weight	16855.05
Isoelectric pH	4.68
Positively charged residues	11
Negatively charged residues	18
Formula	$C_{724}H_{1108}N_{192}O_{234}S_{19}$
Number of atoms	2277
Extinction coefficient	18755
	17880
Instability index	39.07
Aliphatic index	64.28
Grand average of hydropathicity (GRAVY)	-0.226

Prediction of T Cell Epitope (MHC-I & MHC-II binding prediction)

Tools on IEDB predicted the interaction between MHC-I and MHC-II molecules with binding peptides. We choose the Artificial Neuronal Network for MHC-I binding peptide prediction, and the NN-align method for the MHC-II binding peptide prediction. The interactions are measured by IC50 value, which could indicate the binding affinity. IC50 is the concentration when a substrate able to inhibit 50% of biological activity, it is rather known as half maximal inhibitory concentration [15]. We selected the prediction results with IC50 value below 200 nM since the lower value of IC50 means a higher binding affinity of the interaction. MHC Class I binding peptide prediction showed 48 interactions between MHC alleles and binding peptides, and 121 interactions for MHC Class II binding peptide prediction.

Epitope Conservancy, Immunogenicity, and Population Coverage Analysis

The conserved sequence area is likely to be the functional area of a protein [16]. Adhikari et. al. [17] and Ashraf et. al. [18] stated that epitope with high conservancy is correlated with vaccine effectivity. Thus, we selected results with the identity percentage above 70%. Epitope conservancy analysis showed that 46 epitope sequences out of 50 epitopes associated with the MHC-I and MHC-II binding peptide prediction have more than 70% sequence similarity. These results are then continued to immunogenicity analysis.

Immunogenicity prediction showed 11 epitope sequences with positive scores. A positive score indicates the ability of the T cell receptor to recognize the peptide sequence when it is bound to an MHC molecule. According to Fleri et. al. [19], immunogenicity prediction scoring relies on the propensity scale calculation of residue 3-8. Some amino acid residue such as tryptophan (W), phenylalanine (F), and isoleucine (I) are more likely to be found in the immunogenic peptide. Calis et. al. [20] stated that peptide with aromatic side-chain residue–such as tryptophan (W),

phenylalanine (F), and tyrosine (Y), is easier to be recognized by T cell receptor.

Population coverage is a tool to analyze the effectiveness of the vaccine to any given population. It is based on the HLA allele genotypic frequencies on different populations, analyzing whether the population will respond effectively to the given epitope or not. MHC is the most polymorphic trait in humans, considering the variations in population, and the individual only bears a small part of the variants. The polymorphic part of MHC is peptide binding cleft, it has limited ability to bind with certain antigenic peptides [21]. Thus, it restricts the immune response of the population to different antigens. Designed T cell peptide epitope could be recognized effectively by some population, but not effectively by other populations [22]. Malayan krait is distributed across Southeast Asia, thus in this analysis, we selected populations in the Southeast Asia region (Table 2). The population with the highest coverage is the Thailand population (83.13%), the vaccine is mostly applicable for this population, and the lowest coverage is the Borneo population (45.54%). Borneo is an island that is parted by 3 different countries–Indonesia (73%), Malaysia (26%), and Brunei Darussalam (1%). The Borneo in population coverage result is known to be the population from Sarawak, Malaysia.

Table 2. Population coverage of epitope prediction

Population/area	MHC class I & II		
	coverage[a]	Average hit[b]	PC90[c]
Thailand	83.13%	2.13	0.59
Singapura	81.78%	2.04	0.55
Malaysia	80.6%	2.03	0.52
Taiwan	78.0%	2.08	0.45
Filipina	76.59%	2.03	0.43
Vietnam	75.75%	1.81	0.41
Indonesia	72.41%	1.52	0.36
Borneo	45.54%	0.73	0.18
Average	74.2	1.8	0.44
Standard deviation	11.32	0.44	0.12

[a]Projected population coverage. [b]Average number of epitope hits/HLA combinations recognized by the population. [c]Minimum number of epitope hits/HLA combinations recognized by 90% of the population.

T Cell Epitope Candidate Selection

Predicted T cell epitopes with positive immunogenicity scores were evaluated for the selection of epitope candidates for docking simulation. We choose epitopes which mostly interact with MHC-II molecules, considering the ability to elicit CD4 T cell immune response. Interactions of epitopes with MHC-II molecules are summarized in Table 3. The three most potential epitopes are "FAKAPYNEE", "LSYFRYTEM", and "RTAALCFAK" (Table 3).

Table 3. Conservancy, immunogenicity, and interaction of T-cell epitope candidate peptide with MHC-II alleles

Core Peptide	Conservancy	Immunogenicity	Peptide (15-mer)	Allele
133-FAKAPYNEE-141	90%	0.00813	129-AALCFAKAPYNEENK-143	HLA-DPA1*01:03/DPB1*04:01
			128-TAALCFAKAPYNEEN-142	HLA-DPA1*01:03/DPB1*04:01
			130-ALCFAKAPYNEENKE-144	HLA-DPA1*01:03/DPB1*04:01
			127-RTAALCFAKAPYNEE-141	HLA-DPA1*01:03/DPB1*04:01
27-LSFYRYTEM-35	90%	0.17246	21-NIPPQPLSFYRYTEM-35	HLA-DPA1*01:03/DPB1*02:01
			23-PPQPLSFYRYTEMIQ-37	HLA-DPA1*01:03/DPB1*04:01
			22-IPPQPLSFYRYTEMI-36	HLA-DPA1*01:03/DPB1*04:01
			24-PQPLSFYRYTEMIQC-38	HLA-DPA1*01:03/DPB1*02:01
			23-PPQPLSFYRYTEMIQ-37	HLA-DPA1*01:03/DPB1*02:01
			25-QPLSFYRYTEMIQCT-39	HLA-DPA1*01:03/DPB1*02:01
			27-LSFYRYTEMIQCTIR-41	HLA-DQA1*01:01/DQB1*05:01
			22-IPPQPLSFYRYTEMI-36	HLA-DPA1*01:03/DPB1*02:01
127-RTAALCFAK-135	90%	0.11218	123-CNCDRTAALCFAKAP-137	HLA-DQA1*01:02/DQB1*06:02
			122-ICNCDRTAALCFAKA-136	HLA-DQA1*01:02/DQB1*06:02

Docking Simulation

Interaction of 3LKO with influenza epitope "LPFERATVM" (Figure 1A, 2A, 3A) was performed as a control docking scheme. The binding energy of this interaction is -75.92 kcal/mol. 3LKO and "LPFERATVM" were interacted by 4 electrostatic, 3 hydrophobic, and 3 hydrogen bonds. Epitope amino acid residues of Arg-5, Ala-6, and Glu-4 are involved in electrostatic interaction; Ala-6 and Arg-5 are involved in hydrogen bond; Leu-1 and Pro-2 are involved in the hydrophobic bonds of 3LKO and "LPFERATVM" interaction (Table 4A). "LPFERATVM" is represented by a green-colored peptide structure.

Three epitope peptide candidates–"FAKAPYNEE", "LSYFRYTEM", and "RTAALCFAK", were interacted by docking with four MHC molecules (6EI2, 3LKO, 1DLH, and 6DIG). The result of the docking simulation showed that the three peptides can bind on the MHC binding groove.

Interaction between "FAKAPYNEE" and 6EI2 (Figure 1D-F) required binding energy of -71.28 kcal/mol, this energy is higher than control docking binding energy. The interaction was formed by 5 hydrogen bonds and 10 hydrophobic bonds. Pro-5, Lys-3, Glu-8, and Asn-7 residues are involved in the hydrogen bonds. Tyr-6, Lys-3, Ala-2, Pro-5, and Ala-4 residues involved in hydrophobic bonds. "FAKAPYNEE" peptide is represented by purple-colored structure.

Interaction of "FAKAPYNEE" peptide with 3LKO showed in Figure 1G-I, it required -151.37 kcal/mol binding energy. The binding energy of this interaction is lower than the control docking interaction. This interaction is consists of 3 hydrogen bonds, 3 electrostatic interactions, and 10 hydrophobic bonds. Peptide residues involved in hydrogen bonds are Lys-3, Glu-8, Asn-7, and Tyr-6. Electrostatic interactions involved residues are Glu-8, Phe-1, and Lys-3. And the residues involved in hydrophobic bonds are Tyr-6, Lys-3, Ala-4, Pro-5, and Ala-2.

The binding energy of "FAKAPYNEE" and 1DLH interaction is -47.95 kcal/mol, this is higher than control docking binding energy. Part of the peptide binding position is a little bit out of the binding groove (Figure 1J-L). The formed interaction consists of 9 hydrogen bonds, 6 hydrophobic bonds, and 1 electrostatic. The amino acid residue of Glu-8, Glu-9, Ala4, Lys-3, and Asn-7 are involved in hydrogen bonds. Tyr-6, Pro-5, Lys-3, Ala-2, and Phe-1 residues are involved in hydrophobic bonds. And residue involved in electrostatic interaction is Phe-1.

The binding energy required by the interaction of "FAKAPYNEE" with 6DIG (Figure 1 M-O) is 35.30 kcal/mol. This is higher than control docking binding energy. Several bonds formed in this interaction are 4 hydrogen bonds, 1 electrostatic interaction, and 6 hydrophobic bonds. Amino acid residue Lys-3, Asn-7, and Glu-8 of the peptides are involved in hydrogen bonds. The electrostatic interaction involved Lys3 residue. While hydrophobic bonds involved Phe-1, Pro-5, Ala-2, Ala-4, and Lys-3 residues. Binding interactions of "FAKAPYNEE" epitope peptide with four MHC molecules are completely summarized in Table 4B.

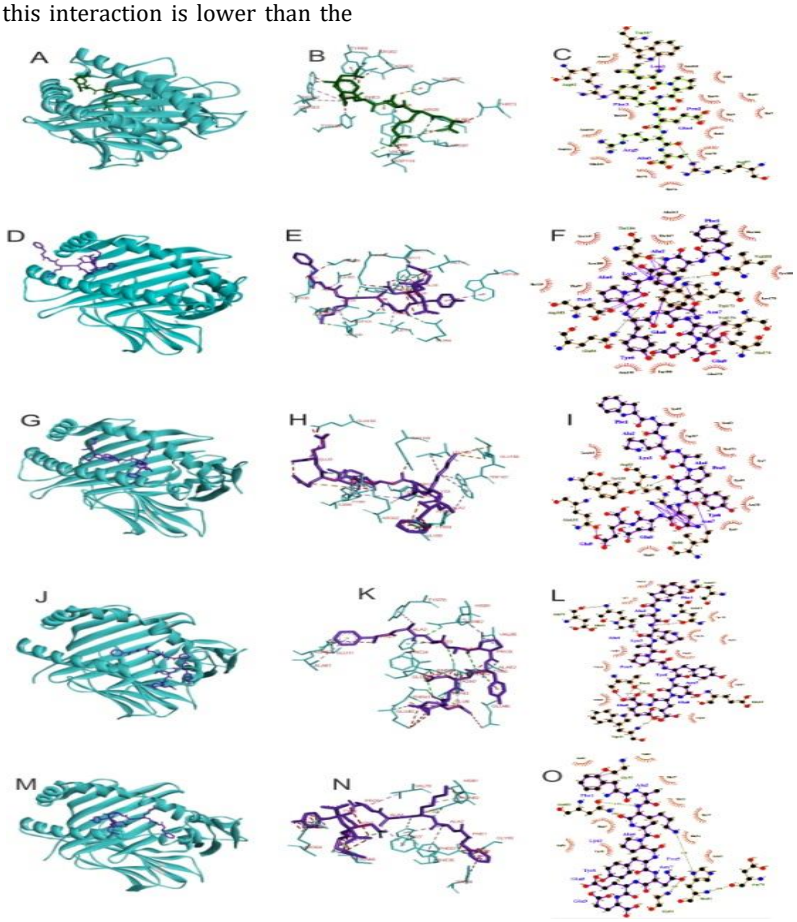

Figure 1. Interaction of 3LKO with influenza epitope "LPFERATVM" as a control docking scheme A.) 3D diagram, B.) Interaction of the residues, C.) 2D diagram; Interaction between "FAKAPYNEE" and 6EI2 D.) 3D diagram, E.) Interaction of the residues, F.) 2D diagram; Interaction between "FAKAPYNEE" and 3LKO G.) 3D diagram, H.) Interaction of the residues, I.) 2D diagram; Interaction between "FAKAPYNEE" and 1DLH J.) 3D diagram, K.) Interaction of the residues, L.) 2D diagram; Interaction between "FAKAPYNEE" and 6DIG M.) 3D diagram, N.) Interaction of the residues, O.) 2D diagram.

Interaction of "LSYFRYTEM" peptide with 6EI2 (Figure 2D-F) required 54.25 kcal/mol binding energy, it is higher than control docking interaction binding energy. Several bonds formed in this interaction are 10 hydrogen bonds, 3 electrostatic, and 7 hydrophobic interactions. Peptide residues involved in the hydrogen bonds are Tyr-6, Ser-2, Leu-1, Arg-5, and Phe-3. Electrostatic interactions involved Arg-5 and Tyr-6 residue. Hydrophobic bonds involved residues are Leu-1 and Arg-5. The brown-colored structure represents the "LSYFRYTEM" peptide.

"LSYFRYTEM" peptide and 3LKO interaction is showed in Figure 2G-I. The binding energy of this interaction is lower than control docking binding energy, -104.62 kcal/mol. About 5 electrostatic, 7 hydrogen bonds, and 5 hydrophobic bonds are formed in this interaction. Tyr-6, Leu-1, Arg-5, Ser-2, and Phe-3 residues are involved in hydrogen bonds. Arg-5 and Tyr-6 residues are involved in electrostatic interactions. Leu-1 and Tyr-6 residues are involved in hydrophobic bonds.

Interaction between "LSYFRYTEM" and 1DLH showed in Figure 2J-L. The binding energy of this interaction is -83.04 kcal/mol (lower than control docking). This interaction consists of 7 hydrogen bonds, 3 electrostatic interactions, and 5 hydrophobic bonds. The amino acid residue of Tyr-4, Arg-5, Ser-2, Tyr-6, and Phe-3 are involved in hydrogen bonds. Arg-5, Phe-3, and Tyr-6 residues are involved in electrostatic interactions. Amino acid residues involved in hydrophobic bonds are Tyr-4, Leu-1, and Arg-5.

Figure 2M-O showed the interaction between "LSYFRYTEM" and 6DIG. The binding energy of this interaction is -188.06 kcal/mol, it is lower than control docking interaction. About 10 hydrogen bonds, 5 hydrophobic bonds, and 2 electrostatic interactions formed in this interaction. Peptide residues involved in hydrogen bonds are Leu-1, Arg-5, Ser-2, Tyr-6, Phe-3, and Tyr-4. Tyr-4, Arg-5, leu-1, and Phe-3 residues are involved in hydrophobic bonds. Residues involved in electrostatic interactions are Tyr-6 and Arg-5. Binding interactions of "LSYFRYTEM" with four MHC molecules are completely summarized in Table 4C.

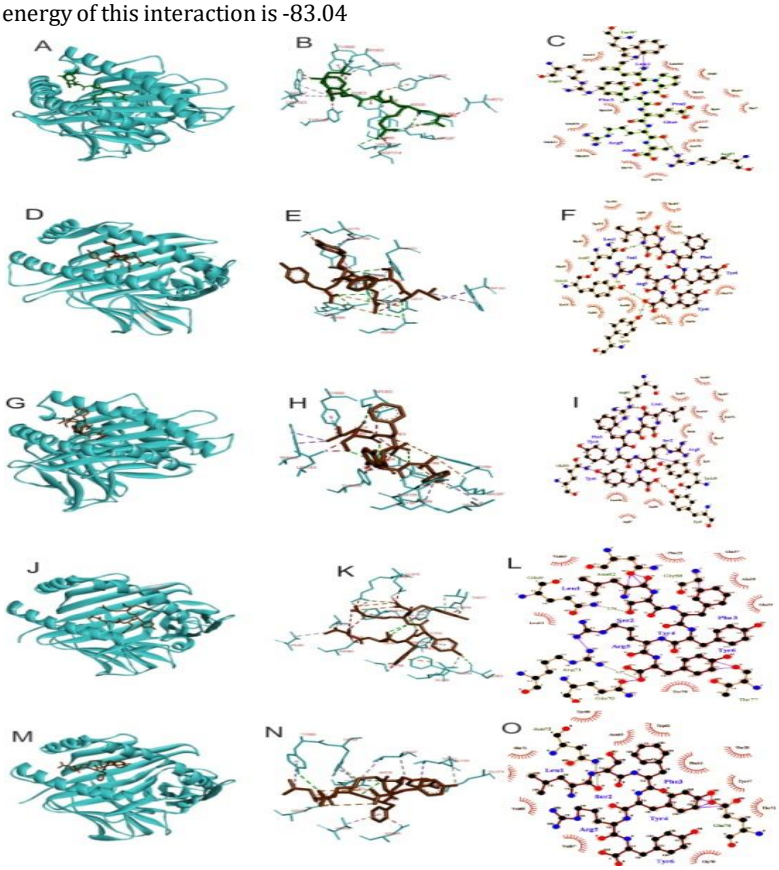

Figure 2. Interaction of 3LKO with influenza epitope "LPFERATVM" as a control docking scheme A.) 3D diagram, B.) Interaction of the residues, C.) 2D diagram; Interaction between "LSYFRYTEM" and 6EI2 D.) 3D diagram, E.) Interaction of the residues, F.) 2D diagram; Interaction between "LSYFRYTEM" and 3LKO G.) 3D diagram, H.) Interaction of the residues, I.) 2D diagram; Interaction between "LSYFRYTEM" and 1DLH J.) 3D diagram, K.) Interaction of the residues, L.) 2D diagram; Interaction between "LSYFRYTEM" and 6DIG M.) 3D diagram, N.) Interaction of the residues, O.) 2D diagram.

Interaction of "RTAALCFAK" with 6EI2 showed in Figure 3D-F required binding energy of -88.55 kcal/mol. This is lower than the binding energy of control docking. This interaction consists of 14 hydrogen bonds, 2 electrostatic interactions, and 8 hydrophobic bonds. Hydrogen bonds involved peptide residue of Arg-1, Lys-9, Ala-4, Leu-5, Thr-2, Cys-6, and Ala-3. Arg-1 is the only residue involved in electrostatic interactions. While Arg-1, Thr-2, Phe-7, Ala-4, Leu-5. Ala-8 and Lys-9 residues are involved in hydrophobic bonds. "RTAALCFAK" peptide is represented by navy colored structure.

"RTAALCFAK" and 3LKO interaction (Figure 3G-I) is formed by 9 hydrogen bonds and 10 hydrophobic bonds. All residues of "RTAALCFAK" peptide are involved in hydrogen bonds. Peptide residue of Phe-7, Lys-9, Leu-5, Arg-1, Ala-4, and Cys-6 are involved in hydrophobic bonds. The binding energy of "RTAALCFAK" and 3LKO interaction is -104.15 kcal/mol, this is lower than control docking.

Interaction between "RTAALCFAK" and 1DLH showed in Figure 3J-L. This interaction requires -24.05 kcal/mol binding energy (higher than control docking). About 8 hydrophobic bonds, 13 hydrogen bonds, 2 electrostatic

interactions, and 1 Pi-Sulfur formed the interaction between "RTAALCFAK" and 1DLH. Amino acid residues involved in hydrogen bonds are Cys-6, Lys-9, Thr-2, Ala-4, Arg-1, Leu-5, and Ala-3. Residues involved in hydrophobic bonds are Ala-4, Cys-6, Ala-8, Lys-9, Ala-3, and Phe-7. Electrostatic interaction is formed with Arg-1 and Lys-9 residues. Phe-7 is the residue involved in the Pi-Sulfur bond.

Figure 3M-O showed the interaction between "RTAALCFAK" and 6DIG. The required binding energy is -65.61 kcal/mol, this is higher than control docking binding energy. About 1 Pi-Sulfur, 12 hydrogen bonds, and 8 hydrophobic bonds are formed in this interaction. Ala-4, Thr-2, Cys-6, Arg-1, Leu-5, Ala-3, and Lys-9 residues are involved in hydrogen bonds. Lys-9, Ala-4, Leu-5, Arg-1, and Ala-3 are involved in hydrophobic bonds. And Cys-6 is the residue involved in the Pi-Sulfur bond. The summary of binding interactions between "LSYFRYTEM" with four MHC molecules are showed in Table 4D.

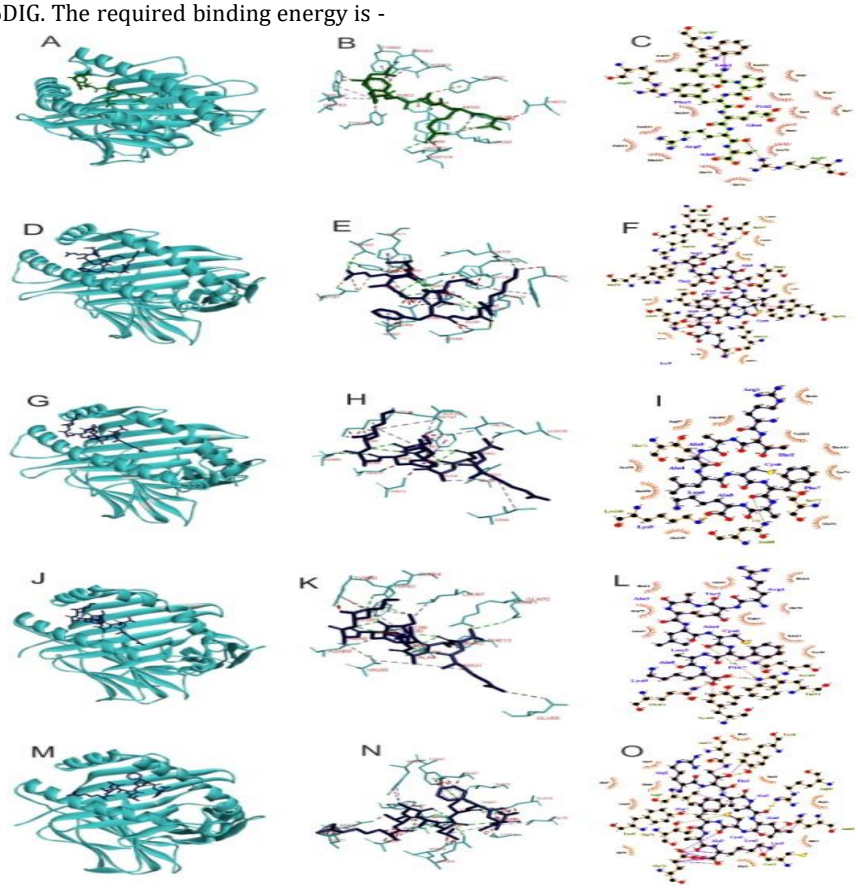

Figure 3. Interaction of 3LKO with influenza epitope "LPFERATVM" as a control docking scheme A.) 3D diagram, B.) Interaction of the residues, C.) 2D diagram; Interaction between "RTAALCFAK" and 6EI2 D.) 3D diagram, E.) Interaction of the residues, F.) 2D diagram; Interaction between "RTAALCFAK" and 3LKO G.) 3D diagram, H.) Interaction of the residues, I.) 2D diagram; Interaction between "RTAALCFAK" and 1DLH J.) 3D diagram, K.) Interaction of the residues, L.) 2D diagram; Interaction between "RTAALCFAK" and 6DIG M.) 3D diagram, N.) Interaction of the residues, O.) 2D diagram.

Table 4. Ligand interaction between each epitopes and MHC molecules

A. Binding interaction between "LPFERATVM" and 3LKO as the control docking scheme			
Interaction	Amino acid residue	Category	Binding energy (kcal/mol)
"LPFERATVM" and 3LKO	A:ARG97:NH1 - X:ALA6:OXT	Hydrogen Bond; Electrostatic (Salt Bridge)	-75.92
	X:ARG5:NH2 - A:ASP114:OD2	Electrostatic (Attractive Charge)	
	A:ARG97:NH1 - X:ARG5:O; X:ARG5:NH2 - A:VAL152:O	Hydrogen Bond (Conventional Hydrogen Bond)	
	X:GLU4:OE1 - A:PHE67; X:GLU4:OE2 - A:TYR7	Electrostatic (Pi-Anion)	
	X:PRO2 - A:LEU163	Hydrophobic (Alkyl)	
	A:TYR59 - X:LEU1; A:TRP167 - X:PRO2	Hydrophobic (Pi-Alkyl)	

	A:TYR9:OH - X:GLU4:CG; A:ARG62:CG - X:LEU1:CD2; A:ARG62:NH2 - X:PHE3:CD2; A:ASN63:ND2 - X:LEU1:CD1; A:THR73:CG2 - X:ALA6:CB; A:TYR99:OH - X:GLU4:CG; A:VAL152:CG1 - X:ARG5:NH2; A:TYR159:CE1 - X:PRO2:CB; A:TRP167:CE2 - X:LEU1:N;	Unfavorable (Unfavorable Bump)	
	A:ARG97:NH2 - X:ARG5:NH1	Unfavorable (Unfavorable Positive-Positive)	
B. Binding interaction between "FAKAPYNEE" and four MHC molecules			
Interaction	Amino acid residue	Category	Binding energy (kcal/mol)
"FAKAPYNEE" and 6EI2	A:GLN94:NE2 - X:PRO5:O; A:TYR147:OH - X:LYS3:O; X:LYS3:NZ - A:VAL100:O	Hydrogen Bond (Conventional Hydrogen Bond)	-71.28
	X:GLU8:N - A:TRP171	Hydrogen Bond (Pi-Donor Hydrogen Bond)	
	A:TRP180 - X:TYR6	Hydrophobic (Pi-Pi T-shaped)	
	A:VAL100 - X:LYS3; A:ALA163 - X:ALA2; X:ALA2 - A:LEU105; X:PRO5 - A:ILE119	Hydrophobic (Alkyl)	
	A:TYR108 - X:ALA2; A:TYR147 - X:ALA4; A:TYR147 - X:PRO5; A:TRP171 - X:ALA4	Hydrophobic (Pi-Alkyl)	
	A:ASP101:CG - X:ALA4:O; A:THR104:CB - X:LYS3:CG; A:THR166:CG2 - X:PHE1:CB; A:TRP171:NE1 - X:ASN7:OD1; A:TRP171:CZ2 - X:TYR6:C; A:ALA174:CB - X:GLU8:OE2; A:VAL176:CG2 - X:GLU8:CB; A:LYS170:NZ - X:ASN7:ND2	Unfavorable (Unfavorable Bump)	
"FAKAPYNEE" and 3LKO	X:LYS3:NZ - A:GLU166:OE2:B	Hydrogen Bond; Electrostatic (Salt Bridge)	-151.37
	A:ARG62:NH1 - X:GLU8:OE2; X:PHE1:N - A:GLU58:OE2; X:LYS3:NZ - A:GLU166:OE1	Electrostatic (Attractive Charge)	
	A:TYR9:OH - X:TYR6	Hydrogen Bond (Pi-Donor Hydrogen Bond)	
	A:TYR9 - X:TYR6	Hydrophobic (Pi-Pi T-Shaped)	
	X:LYS3 - A:LEU163; X:ALA4 - A:ARG62; X:ALA4 - A:ILE66	Hydrophobic (Alkyl)	
	A:TYR7 - X:PRO5; A:TYR59 - X:ALA2; A:TRP167 - X:LYS3; A:TRP167 - X:ALA2; X:TYR6 - A:ILE66	Hydrophobic (Pi-Alkyl)	
	A:GLU58:CB - X:PHE1:CB; A:ARG62:NE - X:ASN7:CG; A:ILE66:CD1 - X:TYR6:C; A:ILE66:CD1 - X:ASN7:CA; A:GLN155:CD - X:GLU9:OE2; A:TYR159:CE1 - X:PRO5:O	Unfavorable (Unfavorable Bump)	
"FAKAPYNEE" and 1DLH	X:PHE1:N - A:GLU11:OE2	Electrostatic (Attractive Charge)	-47.95
	A:THR41:OG1 - X:GLU8:O; A:TRP43:N - X:GLU9:OE1; A:SER53:N - X:ALA4:O; X:LYS3:N - B:ASN82:OD1; X:ALA4:N - A:SER53:O	Hydrogen Bond (Conventional Hydrogen Bond)	
	A:GLU40:CA - X:GLU8:OE2; A:TRP43:CD1 - X:GLU9:OE1; X:GLU9:CA - A:THR41:OG1	Hydrogen Bond (Carbon Hydrogen Bond)	
	A:ALA52:C,O;SER53:N - X:TYR6	Hydrophobic (Amide-Pi Stacked)	
	B:VAL85 - X:PRO5	Hydrophobic (Alkyl)	
	A:PHE24 - X:LYS3; B:TYR78 - X:ALA2; B:HIS81 - X:PRO5; X:PHE1 - A:ALA61	Hydrophobic (Pi-Alkyl)	
	A:PHE24:CD2 - X:LYS3:CE; A:THR41:N - X:GLU9:O; A:ALA52:CA - X:PRO5:O; A:SER53:CA - X:TYR6:C; A:SER53:CA - X:ASN7:N; A:PHE54:N - X:ASN7:CA; A:GLU55:N - X:ASN7:OD1; A:ASN62:OD1 - X:PHE1:CE2;	Unfavorable (Unfavorable Bump)	

	A:GLU40:OE1 - X:GLU8:OE1; A:GLU40:OE1 - X:GLU9:O; A:GLU46:OE2 - X:GLU9:OE2	Unfavorable (Unfavorable Negative-Negative)	
"FAKAPYNEE" and 6DIG	B:ASN82:ND2 - X:LYS3:O; X:LYS3:N - B:ASN82:OD1	Hydrogen Bond (Conventional Hydrogen Bond)	-35.30
	A:HIS27:CE1 - X:LYS3:O;	Hydrogen Bond (Carbon Hydrogen Bond)	
	X:LYS3:NZ - B:HIS81	Electrostatic (Pi-Cation)	
	A:TRP46 - X:PHE1	Hydrophobic (Pi-Pi T-shaped)	
	B:VAL78 - X:PRO5	Hydrophobic (Alkyl)	
	A:HIS27 - X:ALA2; A:PHE35 - X:ALA2; A:PHE57 - X:ALA4; B:HIS81 - X:LYS3	Hydrophobic (Pi-Alkyl)	
	A:GLY55:C - X:PHE1:CG; A:GLY56:N - X:PHE1:CG; A:GLN60:CB - X:GLU8:CG; A:ARG64:CG - X:TYR6:CE2;	Unfavorable (Unfavorable Bump)	
C. Binding interaction between "LSYFRYTEM" and four MHC molecules			
Interaction	Amino acid residue	Category	Binding energy (kcal/mol)
"LSYFRYTEM" and 6EI2	A:TYR33:OH - X:TYR6:O; A:ASN90:ND2 - X:SER2:O; A:TYR123:OH - X:TYR6:O; X:LEU1:N - A:ASN87:OD1; X:ARG5:NH2 - A:ASN87:OD1	Hydrogen Bond (Conventional Hydrogen Bond)	-54.25
	X:ARG5:CD - A:TYR123:OH; X:ARG5:CD - A:TYR183:OH	Hydrogen Bond (Carbon Hydrogen Bond)	
	X:ARG5:NH1 - A:TYR31	Electrostatic (Pi-Cation)	
	X:LEU1:CD2 - A:TRP191	Hydrophobic (Pi-Sigma)	
	A:GLN179:C,O;TRP180:N - X:TYR4; X:ARG5:C,O;TYR6:N - A:TYR123	Hydrophobic (Amide-Pi Stacked)	
	A:TYR123 - X:ARG5; A:TRP180 - X:ARG5; A:TYR183 - X:ARG5; A:TRP191 - X:LEU1	Hydrophobic (Pi-Alkyl)	
	A:GLN179:CD - X:TYR4:CE2; A:TYR183:CD1 - X:SER2:OG	Unfavorable (Unfavorable Bump)	
	X:ARG5:NH1 - A:TYR123:OH	Unfavorable (Unfavorable Bump;Conventional Hydrogen Bond)	
"LSYFRYTEM" and 3LKO	A:ARG97:NH1 - X:TYR6:OXT	Electrostatic (Attractive Charge)	-104.62
	A:TYR9:OH - X:TYR6:OXT; A:ARG62:NE - X:LEU1:O; X:ARG5:NH1 - A:TYR99:OH	Hydrogen Bond (Conventional Hydrogen Bond)	
	X:ARG5:CD - A:TYR159:OH	Hydrogen Bond (Carbon Hydrogen Bond)	
	X:ARG5:NH1 - A:TYR7	Electrostatic (Pi-Cation)	
	X:LEU1:CD1 - A:TRP167	Hydrophobic (Pi-Sigma)	
	A:TYR59 - X:LEU1; X:TYR6 - A:VAL152; X:TYR6 - A:LEU156	Hydrophobic (Pi-Alkyl)	
	A:ARG62:NH1 - X:PHE3:CE2; A:GLN155:CB - X:TYR6:OH; A:TYR159:CD1 - X:ARG5:CA; A:LEU163:CD1 - X:SER2:CB	Unfavorable (Unfavorable Bump)	
	A:ARG62:NH2 - X:PHE3:N	Unfavorable (Unfavorable Donor-Donor)	
"LSYFRYTEM" and 1DLH	B:ARG71:NH1 - X:TYR6:O	Hydrogen Bond; Electrostatic (Salt Bridge)	-83.04

	X:TYR4:OH - A:SER53:O	Hydrogen Bond (Conventional Hydrogen Bond)	
	A:PHE54:CA - X:TYR4:OH	Hydrogen Bond (Carbon Hydrogen Bond)	
	A:GLU55:OE1 - X:PHE3	Electrostatic (Pi-Anion)	
	B:THR77:OG1 - X:TYR6	Hydrogen Bond (Pi-Donor Hydrogen Bond)	
	A:PHE24 - X:TYR4	Hydrophobic (Pi-Pi T-shaped)	
	A:VAL65 - X:LEU1; B:LEU11 - X:LEU1	Hydrophobic (Alkyl)	
	B:PHE13 - X:ARG5; B:TYR78 - X:ARG5	Hydrophobic (Pi-Alkyl)	
	A:GLY58:N - X:PHE3:CE1; A:ASN62:CG - X:LEU1:CA; B:GLN70:CD - X:TYR6:O; B:ARG71:CZ - X:ARG5:NH1; B:THR77:O - X:TYR6:CE2; B:TYR78:CD1 - X:TYR4:O	Unfavorable (Unfavorable Bump)	
	B:ARG71:NH1 - X:ARG5:NH2	Unfavorable (Unfavorable Positive-Positive)	
"LSYFRYTEM" and 6DIG	A:ASN72:ND2 - X:LEU1:O; X:ARG5:NH1 - B:TYR60:OH	Hydrogen Bond (Conventional Hydrogen Bond)	-188.06
	B:VAL67:CA - X:TYR4:O; B:GLY70:CA - X:TYR6:OH; X:SER2:CB - B:TYR30:OH	Hydrogen Bond (Carbon Hydrogen Bond)	
	X:SER2:OG - B:TRP61	Hydrogen Bond (Pi-Donor Hydrogen Bond)	
	B:THR71:CG2 - X:TYR4	Hydrophobic (Pi-Sigma)	
	X:ARG5 - B:VAL67	Hydrophobic (Alkyl)	
	B:TYR60 - X:LEU1; B:TRP61 - X:ARG5; X:PHE3 - A:VAL68	Hydrophobic (Pi-Alkyl)	
	A:ASN65:OD1 - X:PHE3:CD2; B:GLU74:CD - X:TYR4:CE2	Unfavorable (Unfavorable Bump)	

D. Binding interaction between "RTAALCFAK" and four MHC molecules

Interaction	Amino acid residue	Category	Binding energy (kcal/mol)
"RTAALCFAK" and 6EI2	X:ARG1:N - A:ASP140:OD2	Hydrogen Bond;Electrostatic (Salt Bridge)	-88.55
	X:ARG1:NH1 - A:ASP101:OD1	Electrostatic (Attractive Charge)	
	A:ASN90:ND2 - X:LYS9:OXT; X:ARG1:NH2 - A:TYR147:OH; X:ARG1:NH2 - A:THR167:OG1; X:LYS9:NZ - A:GLN179:O	Hydrogen Bond (Conventional Hydrogen Bond)	
	X:ARG1:CD - A:ASP101:OD2	Hydrogen Bond (Carbon Hydrogen Bond)	
	X:THR2:N - A:TRP171	Hydrogen Bond (Pi-Donor Hydrogen Bond)	
	X:THR2:CG2 - A:TRP171	Hydrophobic (Pi-Sigma)	
	A:ALA93:C,O;GLN94:N - X:PHE7	Hydrophobic (Amide-Pi Stacked)	
	X:ALA4 - A:MET121	Hydrophobic (Alkyl)	
	A:TYR123 - X:ALA8; A:TRP171 - X:ARG1; A:TYR183 - X:LYS9	Hydrophobic (Pi-Alkyl)	
	A:ASN90:CB - X:PHE7:O; A:ALA93:CB - X:PHE7:CG; A:GLN94:CD - X:ALA4:CB;	Unfavorable (Unfavorable Bump)	

	A:TRP171:CZ2 - X:ARG1:CA; A:TRP180:CD2 - X:LEU5:CD2		
	A:ASN90:ND2 - X:ALA8:O	Unfavorable (Unfavorable Bump;Conventional Hydrogen Bond)	
	A:ARG138:NH2 - X:ARG1:N	Unfavorable (Unfavorable Bump;Unfavorable Donor-Donor)	
	A:TYR183:N - X:LYS9:NZ	Unfavorable (Unfavorable Donor-Donor)	
"RTAALCFAK" and 3LKO	A:ASN80:ND2 - X:PHE7:O; A:ASN80:ND2 - X:ALA8:O	Hydrogen Bond (Conventional Hydrogen Bond)	-104.15
	A:TRP147:CZ2 - X:PHE7	Hydrophobic (Pi-Sigma)	
	A:TRP147 - X:PHE7	Hydrophobic (Pi-Pi T-shaped)	
	A:LYS146 - X:LYS9; A:ALA150 - X:LEU5; A:ALA150 - X:LYS9; X:ARG1 - A:ILE66; X:CYS6 - A:VAL152	Hydrophobic (Alkyl)	
	A:THR73:CG2 - X:ALA4:O; A:SER77:OG - X:PHE7:CB; A:ARG97:NH1 - X:ALA3:CB; A:LYS146:CD - X:LYS9:C; A:TRP147:CG - X:CYS6:SG; A:GLN155:NE2 - X:THR2:CB	Unfavorable (Unfavorable Bump)	
	A:LYS146:NZ - X:LYS9:OXT	Unfavorable (Unfavorable Bump;Attractive Charge)	
"RTAALCFAK" and 1DLH	X:ARG1:NH2 - A:GLU55:OE1	Electrostatic (Attractive Charge)	-24.05
	B:TRP61:NE1 - X:CYS6:O; B:GLN64:NE2 - X:LYS9:O; B:ARG71:NH1 - X:THR2:OG1; X:THR2:OG1 - B:GLN70:OE1	Hydrogen Bond (Conventional Hydrogen Bond)	
	X:LYS9:O - B:TYR60	Electrostatic (Pi-Anion)	
	X:CYS6:SG - B:TRP61	Hydrogen Bond;Other (Pi-Donor Hydrogen Bond;Pi-Sulfur)	
	B:CYS30:SG - X:PHE7	Other (Pi-Sulfur)	
	X:ALA4 - A:VAL65; X:CYS6 - B:LEU67; X:ALA8 - A:VAL65	Hydrophobic (Alkyl)	
	B:PHE13 - X:ALA3; B:TYR60 - X:LYS9; X:PHE7 - B:LEU11	Hydrophobic (Pi-Alkyl)	
	A:ASN69:ND2 - X:PHE7:CB; B:TYR60:CE2 - X:LYS9:OXT; B:TYR60:CZ - X:LYS9:OXT; B:GLN64:OE1 - X:LYS9:CD; B:GLN64:OE1 - X:LYS9:CE	Unfavorable (Unfavorable Bump)	
"RTAALCFAK" and 6DIG	A:ARG64:NH2 - X:ALA4:O; B:TYR30:OH - X:THR2:O; B:TYR47:OH - X:CYS6:SG; B:THR71:OG1 - X:CYS6:SG; X:CYS6:SG - B:GLU74:OE1	Hydrogen Bond (Conventional Hydrogen Bond)	-65.61
	X:LEU5:CD1 - B:PHE11	Hydrophobic (Pi-Sigma)	
	X:CYS6:SG - B:TYR47	Other (Pi-Sulfur)	
	B:VAL78 - X:LYS9; X:ALA4 - A:VAL68	Hydrophobic (Alkyl)	
	A:HIS71 - X:ARG1; B:TRP61 - X:ALA3	Hydrophobic (Pi-Alkyl)	
	A:CYS11:C - X:LYS9:NZ; A:ASN65:CG - X:LEU5:CG; A:VAL68:CA - X:ARG1:N; A:VAL68:CG1 - X:THR2:N; A:HIS71:CG - X:ARG1:NE; A:ASN72:ND2 - X:THR2:CG2; B:TYR30:CE2 - X:THR2:OG1; B:VAL67:CA - X:PHE7:CE1; B:GLU74:CG - X:LYS9:C; B:GLU74:OE2 - X:CYS6:O	Unfavorable (Unfavorable Bump)	

DISCUSSION

The docking simulation result showed that three epitopes can bind with the four MHC molecules on the binding groove site, this is the site where antigenic peptide normally bind with MHC molecules and then forming peptide-MHC complex (pMHC complex). Between interaction with four MHC molecules, the "FAKAPYNEE" epitope is most easily bind to 3LKO or HLA-B*3501. This is indicated by the interaction has the lowest binding energy (-151.37 kcal/mol) compared to the other three interaction. By the same condition, the "LSYFRYTEM" epitope is most easily bind to 6DIG or HLA-DQA1 with the binding energy of -188.06 kcal/mol. "RTAALCFAK" epitope is also most easily bind to HLA-B*3501 (3LKO), which requires binding energy of -104.5 kcal/mol. The interactions of three epitopes with four different MHC by *in silico* simulation indicate the potential ability of predicted epitope peptides to bind with MHC molecules *in vivo*. pMHC complex formation is then followed by T cell receptor recognition and the production of the immune response. None the less, the MHC restriction is still needed to be counted. MHC restriction is a phenomenon where T cell recognition only occurs by the specific interaction of T cell receptor with p-MHC complex, which means the T cell receptor must bind with the peptide and the MHC molecule [21]. If the T cell receptor binds the antigenic peptide or MHC molecule only, there will be no T cell recognition.

Other studies associated with the epitope-based vaccine designed from snake venom protein were done by Ashraf et. al. [18] and Cao et. al. [23]. A study conducted by Ashraf et. al. [18] designed the epitope-based vaccine from the alpha-delta-bungarotoxin-4 protein of *Bungarus caeruleus* venom. Alam & Ashraf [24] has a similar study, but they used venom protein from Australian box jellyfish *Chironex fleckeri*. Meanwhile, on Cao et. al. [23], a *Deinagkistrodon acutus* antivenom based on continuous B cell epitope has been studied. Peptide synthesized from the predicted epitope were immunized to rat. This antivenom was proved to neutralize the hemorrhagic effect of *Deinagkistrodon acutus* venom effectively.

MHC molecules involved in this study are HLA-A*68 (6EI2), HLA-B*3501 (3LKO), HLA-DR1 (1DLH), and HLA-DQA1 (6DIG). The structure of 1DLH was also used in the study of Ashraf et. al. [18] and Alam & Ashraf [24]. On those studies, 1DLH has interacted with several different epitope peptides with low binding energies. Thus, HLA-DR1 may have high potency as the MHC molecule which able to bind venom protein epitope peptides. However, there is opportunity to validate this finding by in vitro or in vivo approach in the future. Epitope prediction is one of the crucial steps to generate the epitope-based vaccine, on this case, it is expected as an alternative way to overcome the snake envenomation problem.

CONCLUSIONS

The prediction of *Bungarus candidus* PLA_2 epitope and several following analysis has discovered the three potential epitope candidate: "FAKAPYNEE", "LSYFRYTEM", "RTAALCFAK". The docking simulation result showed that the epitopes are forming a complex with MHC molecules inside the binding grooves. "FAKAPYNEE" epitope is most easily bind to 3LKO (binding energy: -151.37 kcal/mol). "LSYFRYTEM" epitope is most easily bind to 6DIG (binding energy: -188.06 kcal/mol). And "RTAALCFAK" epitope is most easily bind to 3LKO, (binding energy: -104.5 kcal/mol). The binding energies are relatively low, thus the three epitopes are potent to induce the immune response.

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REFERENCES

1. World Health Organization. WHO Guidelines for the Production Control and Regulation of Snake Antivenom Immunoglobulins. Geneva: WHO Press; 2010.
2. Rusmili MRA, Yee TT, Mustafa MR, Hodgson WC, Othman I. Proteomic characterization and comparison of Malaysian *Bungarus candidus* and *Bungarus fasciatus* venoms. J Proteom. 2014;110:129-144.
3. Ferraz CR, Arrahman A, Xie C, Casewell NR, Lewis RJ, Kool J, et al. Multifunctional Toxins in Snake Venoms and Therapeutic Implications: From Pain to Hemorrhage and Necrosis. Front Ecol Evol. 2019;7(218):1-19.
4. Sette A, Fikes J. Epitope-based vaccines: an update on epitope identification, vaccine design, and delivery. Curr Opin Immunol. 2003;15:461-470.
5. Li W, Joshi MD, Singhania S, Ramsey KH, Murthy AK. Peptide Vaccine: Progress and Challenges. Vaccines. 2014;2:515-536.
6. Khan MA, Hossain MU, Rakib-us-Zaman SM, Morshed MN. Epitope-based vaccine design and target site depiction against Ebola viruses: an immunoinformatics study. Scand J Immunol. 2015;82:25-34.
7. Qamar MT, Saleem S, Ashfaq UA, Bari A, Anwar F, Alqahtani S. Epitope-based peptide vaccine design and target site depiction against Middle East Respiratory Syndrome Coronavirus: an immune-informatics study. J Transl Med. 2019;17(362):1-14.
8. Parvizpour S, Pourseif MM, Razmara J, Rafi MA, Omidi Y. Epitope-based vaccine design: a comprehensive overview of bioinformatics approaches. Drug Discov Today. 2020;25(6):1034-1042.
9. Nielsen M, Lundegaard C, Worning P, Lauemoller SL, Lamberth K, Buus S, et al. Reliable prediction of T-cell epitopes using neural networks with novel sequence representations. Protein Sci. 2003;12:1007-1017.
10. Lundegaard C, Lamberth K, Harndahl M, Buus S, Lund O, Nielsen M. NetMHC-3.0: Accurate web accessible predictions of Human, Mouse, Monkey MHC class I affinities for peptides of length 8-11. Nucleic Acid Res. 2008;36:W509-512.
11. Andreatta M, Nielsen M. Gapped sequence alignment using artificial neural networks: application to the MHC class I system. Bioinformatics. 2016;32:511-7.
12. Nielsen M, Lund O. NN-align, An artificial neural network-based alignment algorithm for MHC class II peptide binding prediction. BMC Bioinform. 2009;10(296):1-10.
13. Zimmermann L, Stephens A, Nam SZ, Rau D, Kubler J, Lozajic M, et al. A Completely Reimplemented MPI Bioinformatics Toolkit with a New Hhpred Server at its Core. BMJ. 2018;430(15):2237-2243.

14. Schneidman-Duhovny D, Inbar Y, Nussinov R, Wolfson HJ. PatchDock and SymmDock: servers for rigid and symmetric docking. Nucl Acid Res. 2005;33:W363-367.
15. He Y, Zhu Q, Chen M, Huang Q, Wang W, Li Q, et al. The changing 50% inhibitory concentration of cisplatin: a pilot study on the artifacts of the MTT assay and the precise measurement of density-dependent chemoresistance in ovarian cancer. Oncotarget. 2016;7(43):70803-70821.
16. Sruthi CK, Prakash M. Amino acid impact factor. PLoS One. 2018;13(6).
17. Adhikari UK, Tayebi M, Rahman MM. Immunoinformatics Approach for Epitope-Based Peptide Vaccine Design and Active Site Prediction against Polyprotein of Emerging Oropouche Virus. J Immunol Res. 2018;4:1-22.
18. Ashraf KUM, Barua P, Saha A, Mahammad N, Ferdoush J, Das D, et al. An immunoinformatics approach toward epitope-based vaccine design through computational tools from *Bungarus caeruleus*'s neurotoxin. JYP. 2014;6(2):35-43.
19. Fleri W, Paul S, Dhanda SK, Mahajan S, Xu X, Peters B, et al. The Immune Epitope Database and Analysis Resource in Epitope Discovery and Synthetic Vaccine Design. Front Immunol. 2017;8(278):1-16.
20. Calis JJA, Maybeno M, Greenbaum JA, Weiskopf D, de Silva AD. Properties of MHC Class I Presented Peptides That Enhance Immunogenicity. PLoS Comput Biol. 2013;9(10):1-13.
21. Murphy K, Weaver C. Janeway's Immuno Biology. 9th edition. New York: Garland Science, Taylor & Francis Group, LLC; 2017.
22. Misra N, Panda PK, Shah K, Sukla LB, Chaubey P. Population coverage analysis of T-Cell epitopes of *Neisseria meningitis* serogroup B from Iron acquisition proteins for vaccine design. Bioinformation. 2011;6(7):255-261.
23. Cao YL, Guo GN, Zhu GY, Tian Z, Gou YJ, Chen C, et al. Bioinformatics-based design of novel antigenic B cell linear epitopes of *Deinagkistrodon acutus* venom. Eur Rev Med Pharmacol Sci. 2016;20:781-7.
24. Alam MJ, Ashraf KUM. Prediction of an Epitope-based Computational Vaccine Strategy for Gaining Concurrent Immunization Against the Venom Proteins of Australian Box Jellyfish. Toxicol Int. 2013;20(3):235-253.