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Research Article

Multi Epitope Vaccine Candidate against *Mycobacterium Tuberculosis*

Mostafa Norizadeh Tazehkand[1]* and Orkideh Hajipour[2]

[1]*Department of pharmaceutical Biotechnology, Faculty of Pharmacy, Zonguldak Bulent Ecevit University, Turkey*

[2]*Department of Biology, Pamukkale University, Turkey*

***Corresponding author:** Mostafa Norizadeh Tazehkand, Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Zonguldak Bulent Ecevit University, Turkey

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Abstract

Tuberculosis or TB is a disease caused by a type of bacteria called *Mycobacterium tuberculosis* that mainly affects the lungs, even though other tissues and organs may be involved. The present study aims to choose and analyze different epitopes of Apa, PstS3, SecA2, and RD1 using bioinformatics analysis and software. After that molecular docking assay was used to recognize the binding energy and affinity of designed epitopes to HLA-A0201. The vaxiJen score of these sequences were 0.5233, 0.8066, 0.5008, and 0.5512 respectively. B-cell epitopes were predicted using IEDB and Vaxign webserver was used to prediction of binding of peptides to MHC class-I. Physicochemical analysis of vaccine showed that the molecular weight of candidate vaccine is 54.331kDa with the half-life of 30 hours in mammalian reticulocytes, greater than 20 hours for yeast, and greater than 10 hours for *Escherichia coli*. The chemical formula of vaccine was $C_{2410}H_{3750}N_{648}O_{747}S_{18}$ and theoretical pI of candidate vaccine was 5.85. There are 496 amino acids in the protein structure of which 67 residues with negative charge (Asp + Glu) and 63 (Arg + Lys) residues are negative charge. The instability index, aliphatic index, and grand average of hydropathicity of candidate vaccine were 27.44, 67.46, and -0.521 respectively. According to the result of protparam and pepcalc analysis, the vaccine is soluble in water. Parabi analysis showed that the membrane helices value of vaccine was 25.2%. The vaccine does not have more transmembrane helix, therefore no expression difficulties are predicted in the production of vaccine. 3Drefine was used to minimize and correct the hypothetical structure. Ramachandran plot analysis by procheck showed that 96.3% of residues are in most favored regions, 3.7% of residues are additional allowed regions and 0% of residues is in disallowed regions. Molecular docking result of this research revealed that our designed vaccine can stimulate HLA0210. The vaccine may activate humoral and cellular immune responses against of *Mycobacterium tuberculosis*. However, the vaccine could be cloned and expressed in different host cells.

Keywords: *Mycobacterium tuberculosis*; vaccine; HLA0210; B-cell

Introduction

Tuberculosis or TB is a disease caused by a type of bacteria called *Mycobacterium tuberculosis* that mainly affects the lungs, even though other tissues and organs may be involved. Tuberculosis is spread from a person to another by the air. Approximately one-third worldwide population has latent tuberculosis, which means people have been infected by *Mycobacterium tuberculosis*, but not sick. If *Mycobacterium tuberculosis* becomes active in the body and multiply, the person will go from having latent TB infection to being sick with TB disease [1]. Researchers have showed that greater than 90% of human reported to have clinically apparent disease are those who have harbored tuberculosis infection for at least a year or more; the remaining 10% have an immediate progression of a recently acquired infection. The number of persons with latent infection in the United States is estimated to range from 10 million to 15 million [2]. As of 2018 one-quarter of the world's population is mind to be infected to Tuberculosis (World Health Organization, 2018). About 1% of world population infected to TB each year (WHO, 2013). In 2017, there were about 10 million infection in the world that resulted in 1.6 million deaths. More than 95% of infection and deaths occurred in developing countries such as Pakistan, India, Indonesia, and Philippine [3]. The symptoms of TB such as a bad cough that lasts 3 weeks or longer, weight loss, Loss of appetite, coughing up blood or mucus, weakness or fatigue, fever, and night sweats may be mild for many months. This can lead to delays in seeking care, and results in transmission of the bacteria to others [1]. *Mycobacterium tuberculosis* is an aerobic, nonmotile bacillus with

high lipid content of this pathogen accounts for many of its unique clinical characteristics [4]. The bacteria have extremely slow rate of dividing 16 to 20 hours [5]. *Mycobacterium tuberculosis* has mycolic acid content of its cell wall [6]. After phagocytosis, *Mycobacterium tuberculosis* bacteria reside in the endosomes of the macrophages [7]. Throughout infection, the bacteria synthesis and relies its lipases for hydrolyzing of host lipids to release Fatty acid synthetize via catalyzing the hydrolysis of ester bonds in long chain acyl glycerols [8]. Genomic sequencing of different *Mycobacterium tuberculosis* strains showed that *Mycobacterium tuberculosis* possesses more than 250 genes involved in lipid and fatty acid metabolism [9]. Some of proteins that contributed in lipid synthesis are also virulence related, and mutations of them lead to attenuate phenotypes in human and animal infections. These genes include mycolic acids metabolisms, polyketide synthase, FA -CoA synthase, isocitrate lyase, phospholipases, acyl -CoA dehydrogenases, lipid carriers, trehalose synthase, and lipid transporters [10]. Region of difference 1 or RD1 with 9.5 kb length encoding the secretory proteins ESAT-6 and CFP-10. RD1 is a critical region for virulence of *Mycobacterium tuberculosis* [11]. In many bacterial pathogens containing SecA2 systems, such as *Mycobacterium tuberculosis* SecA2 system that is not important but is required for virulence. Some SecA2 systems are common as SecA2-SecY2 systems because they involve an extra SecY, which is supposed to function as a specific channel. SecA2-SecY2 systems appear dedicated to transporting proteins that are glycosylated in the cytoplasm prior to export. Other SecA2 systems, that is found in *Mycobacterium tuberculosis* is SecA2 systems which lack an extra SecY or any obvious alternative membrane channels [12]. Phosphate-binding protein PstS 3 is the main carrier for phosphate uptake in *Mycobacterium tuberculosis*, it is the most highly expressed of the PstS proteins under phosphate starvation. PstS3 plays a role in host phagosome maturation arrest. Apa (Alanine and Proline-rich) is a secreted protein from *Mycobacterium tuberculosis*. Apa is A potent antigen in animals immunized with live bacteria, it induces a strong delayed-type hypersensitivity in immunized animals.

Elicits a mostly Th1 type of T-cell response in healthy humans; induces IFN-gamma production from CD4(+) and CD8(+) cells. Apa has some functions as an adhesion, binds to macrophages via mannose residues (https://www.uniprot.org/uniprot/P9WIR7). Apa, PstS3, SecA2, and RD1 have important roles in the survival and pathogenesis of *Mycobacterium tuberculosis*. Consequently, in the present research, we designed a multi epitope vaccine against *Mycobacterium tuberculosis* infections. Our present research was aimed to choose and analyze different epitopes of Apa, PstS3, SecA2, and RD1 using bioinformatics analysis and software. After that molecular docking assay was used to recognize the binding energy and affinity of designed epitopes to HLA-A0201.

Material and Methods

Selection of candidate proteins

Different protein sequences of Apa, PstS3, SecA2, and RD1 were obtained from National Center for Biotechnology Information (NCBI) (NCBI Gene ID, Apa: 885896, PstS3: 885366, SecA2: 887222, RD1: 885777).

B-Cell epitope prediction: B-cell epitopes were predicted using IEDB (Immune Epitope Database) webserver (https://www.iedb.org/). IEDB is a free available tool that funded by NIAID. It catalogs experimental data on antibody and T cell (MHC-I Binding, MHC-II Binding, MHC-I Processing (Proteasome, TAP), and MHC-I Immunogenicity) epitopes predicted in humans and animals in the background of infections, allergy, autoimmunity and transplantation. The IEDB also hosts tools to assist in the prediction and analysis of epitopes. In this research the epitopes higher than 0.35 threshold were subjected to B-cell epitope prediction [13].

MHC-I binding epitopes (CTL) prediction: Vaxign webserver was used to prediction of binding of peptides to MHC class I. The epitopes were evaluated for their binding affinity with predominant HLA-1 alleles (P-values<0.05 were considered significant) [14].

Immunogenicity analysis of selected epitopes: The binding character (with high score) of MHC-I and B cell epitopes were taken into consideration of the selection of the suitable epitopes. The antigenicity of selected epitopes was checked in vaxijen prediction webserver [15].

Allergenicity analysis of selected epitopes: The allergenicity of epitopes were analyzed by Allertop and algpred webserver. This software allows allergenicity predication based on the similarity of known epitopes with any region of the protein [16, 17].

Toxicity analysis of selected epitopes: Toxicity analysis of selected B-cell and MHC-I epitopes were performed using toxinpred server. Toxinpred allows to recognize the highly toxic or nontoxic peptide sequences [18].

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        10         20         30         40         50         60
MARMAMGADD VVDSSKSFVM ENFSSYHGTK PGYVDSIQKG IQKPKSGTQG NYDDDWKGFY

        70         80         90        100        110        120
STDNKYDAAG YSVDNENPLS GKAGGVVKVT YPGLTKVLAL KVDNAETIKK ELGLSLTEPL

       130        140        150        160        170        180
MEQVGTEEFI KRFGDGASRV VLSLPFAEGS SSVEYINNWE QAKALSVELE INFETRGKRG

       190        200
QDAMYEYMAQ ACAGNRVRRI M
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Figure 1: The part (201 amino acid length) of diphtheria toxin (ACCESSION: AAT37555.1).

Construction of vaccine sequence: The selected B and MHC-I epitopes were linked by Lysine-Lysine (KK) amino acid linker together. To increase the antigenecity of designed vaccine, the amino acid sequence of diphtheria toxin (ACCESSION: AAT37555.1, 201 amino acid length) was linked to the N and C-terminal end of candidate vaccine. The sequence of diphtheria toxin that was used as adjuvant is shown in (Figure 1). Before of attaching, diphtheria toxin fragment sequence was checked on toxinpred server. Diphtheria toxin fragment was the nontoxic portion of *Corynebacterium diphtheriae* toxin. We used PAPAP amino acid linker sequence for joining of candidate vaccine to adjuvant. After joining of vaccine to adjuvant the antigenicity, allergenicity and toxicity of poly-epitope vaccine were tested by vaxijen, allertop and toxinpred software [19].

Physicochemical analysis of vaccine candidate

After that, half-life, molecular weight, isoelectric point in stability index, aliphatic index and stability of candidate vaccine were calculated by Paratparam and Papcolc software [20].

Secondary structure prediction: Parabi server was used to check the potential transmembrane helices of candidate vaccine structure.

3D Structure of vaccine and 3D structure refinement: Further analysis of candidate protein 3D structural stability was done by SWISS-MODEL webserver. The SWISS-MODEL server is an automated modeling software that develops a protein tertiary structure model of an unknown structure protein based on the sequence similarity with the known structured protein [21]. The 3D structure was refined using 3Drefine analysis. 3Drefine is a program for computationally efficient protein shape refinement with the ability to perform web based statistical and visual analysis. The 3Drefine refinement protocol utilizes iterative optimization of hydrogen bonding network combined with atomic-level energy minimization on the optimized model using a composite physics and knowledge-based force fields for efficient protein structure refinement. The webserver subjected the five model to candidate vaccine. The refined models were checked for 3D refine score, GDT-HA score, GTD-TS, RMSD score, and MolProbity, and the best model was chosen. The selected model was investigated by Ramachandran plot analysis with Procheck online software [22].

Discovery of disorder regions: For discovery of disorder regions of our designed vaccine, we used IUPred2A software. IUPred2A is a server which recognize disordered protein regions. It also has ability to identifying protein regions that do or do not adopt a stable structure depending on the redox state of their environment [23].

Protein-protein docking analysis: Protein-docking is a molecular modeling software which aims to predict, with computer science algorithms and techniques, the mutual orientation and position of two molecules forming a complex. Molecular docking analysis of designed vaccine was performed using HEX protein docking software by considering HLA0210 molecule as a ligand and candidate vaccine as receptor. Firstly, molecular structure of HLA0210 was downloaded in PDB format from Protein Data Bank (PDB) database. HexServer (http://hexserver.loria.fr/) is the First Fourier transform (FFT)-based protein docking server to be powered using graphics processors. Using two graphics processors simultaneously, a typical 6D docking run takes ~15 s, which is up to two instructions of magnitude faster than conventional FFT-based docking approaches using comparable resolution and scoring functions. The server requires two protein structures in PDB format to be uploaded, and start it produces a ranked list of up to 1000 docking predictions. In this study 3D structure of HLA0210 as a ligand, and the candidate vaccine structure as a receptor were uploaded to Hex software. In this study we used human serum albumin (protein structure in PDB format) as negative control and investigated the affinity of human serum albumin as receptor to HLA0210 as ligand in Hex software.

Result and Discussion

Different protein sequences of Apa, PstS3, SecA2, and RD1 were obtained from National Center for Biotechnology Information (NCBI). The antigenicity of these sequences was tested by VaxiJen server. The vaxiJen score of these sequences were 0.5233, 0.8066, 0.5008, and 0.5512 respectively. The score above the threshold (0.4) have good antigenicity, for this reason all sequences were suitable for our research. In this in silico research B cell epitopes were predicted using IEDB webserver. *The* B cell epitopes having a *score* above 0.8 were taken into consideration. These epitopes were tested to antigenicity with VaxiJen. The selected peptide epitopes must also possess antigen as assessed via VaxiJen (above the threshold 0.4). Then the selected epitopes were analyzed by allertop, algpred, and toxinpred server. The predicted B-cell epitopes from four protein sequences that produced the best IEDB and vaxijen score with negative toxicity and negative allergenicity were AATPDTGPDLD (with Vaxijn ecore of 0.9092), VDLWGADGAEGWTADP (with Vaxijn ecore of 0.8174) and KTVGETWGL (with Vaxijn ecore of 0.8174). The MHC class I epitopes were predicted by vaxign server. The epitopes were assessed for their binding affinity with predominant HLA I alleles (P-values < 0.05 were considered significant). The selected peptide epitopes must also possess antigen as evaluated by VaxiJen (above the threshold 0.4). Then the selected epitopes were analyzed by allertop, algpred, and toxinpred servers. The predicted MHC-I epitopes which produced the best vaxijen score, were FSFALPAGW (0.929), DVHANTWRY (1.686), WTADPIIGV (0.5996), and LPGERIHVV (0.8976). These selected epitopes have the strongest affinity to HLA0210. therefore, HLA0210 will be used as a model in Hex protein molecular docking. The sequence of candidate vaccine with 496 amino acid is shown in Figure 2. Physicochemical analysis of *Mycobacterium tuberculosis* vaccine was done by protparam webserver and the result showed that molecular weight of candidate vaccine is 54.331kDa. The estimated half-life of candidate vaccine was found to be greater than 30 hours (mammalian reticulocytes, in vitro), greater than 20 hours (yeast, in vivo), and greater than 10 hours (Escherichia coli, in vivo). The chemical formula of vaccine was $C_{2410}H_{3750}N_{648}O_{747}S_{18}$ and theoretical pI of candidate vaccine was 5.85. There are 496 amino acids in the protein structure of which 67 residues with negative charge (Asp + Glu) and 63 (Arg + Lys) residues are negative charge. The instability index is computed to be 27.44, consequently the vaccine was

considered stable. The aliphatic index was found to be 67.46, so the designed antigen is probable to be thermostable. The grand average of hydropathicity of candidate vaccine is -0.521, therefore the vaccine is a hydrophilic protein and likely interact with molecules of water. According to the result of protparam and pepcalc analysis, the vaccine is soluble in water. Parabi analysis showed that the membrane helices value of vaccine was 25.2% (Figure 3). The vaccine does not have more transmembrane helix, so no expression difficulties are predicted in the production of vaccine. 3D structure of *Mycobacterium tuberculosis* vaccine was done using via SWISS-MODEL server (Figure 4). Afterward, 3Drefine was used to minimize and correct the hypothetical structure. All of five chosen templates showed good alignment as per their *3Drefine* values (ranging from 7517-9648 to 5.46). The five predicted models had GTD-HA score values ranging from 0.996 to 1.000. The RMSD score value of models was ranging from 0.245-0.127. The five predicted models had *GTD-HA score* values ranging from 0.996 to 1.000. The model with the highest RMSD score was selected for further refinement (Figure 5). The choose model was examined by Ramachandran plot analysis using Procheck server. Ramachandran plot analysis by procheck showed that 96.3% of residues are in most favored regions, 3.7% of residues are additional allowed regions and 0% of residues are in disallowed regions (Figure 6). The result of Ramachandran plot assayed supported the high-quality structure of the refined model. In this study we used IUPred2A webserver to detection of disorder regions. The result from IUPred2A revealed that the protein is stable and does not have important disorders (Figure 7). Molecular docking analysis of designed vaccine was performed by considering HLA0210 molecule as a ligand and candidate vaccine as receptor. The affinity of epitopes to HLA0210 investigated using HEX protein docking offline software. For this reason, 3D structure of HLA0210 downloaded from Protein Data Bank (PDB) and uploaded on software as ligand and the 3D structure of candidate vaccine uploaded as a receptor. In this study human serum albumin (3D structure) was used as negative control and checked the affinity of that human serum albumin as receptor to HLA0210 as ligand. The result of protein docking analysis showed that maximum affinity of candidate vaccine HLA0210 with the score of -547.81 while the affinity of human serum albumin to HLA0210 was -100.35. The result of this research revealed that our designed vaccine can stimulate HLA0210 (Figure 8 & 9). In the present study protein sequences of Apa, PstS3, SecA2, and RD1 from *Mycobacterium tuberculosis* were used for construct of new vaccine and bioinorganic analysis of candidate vaccine was done. Molecular docking of designed vaccine checked by HEX protein-protein docking. The peptide sequences having the antigenicity score above the threshold (0.4) can be candidate to vaccine. The Peptide having higher scores mean that it can be recognized by B-cell and T-cells. Cytotoxic T-lymphocyte T-Cell receptors recognize endogenous antigen accessible on MHC-I, but inflammatory T-Cell receptors and T- helper recognize exogenous antigen accessible on MHC-II. In this research we used MHC-I epitopes for candidate vaccine against *Mycobacterium tuberculosis*. These selected epitopes had affinity to HLA0210. A physicochemical analysis of *Mycobacterium tuberculosis* vaccine showed that the vaccine had a molecular weight of 54.331 kDa. [24-27]. reported that the proteins having higher 110 kDa molecular weight are suitable vaccine candidates [24, 28-31]. The estimated half-life of candidate vaccine was greater than 30 hours in mammalian reticulocytes, higher than 20 hours in yeast, and higher than 10 hours in *Escherichia coli*. The result showed that the candidate vaccine can be overexpress in *E. coli* and yeast, and the produced vaccine can be used in human. The Instability index of candidate vaccine was 27.44, this score is less than 40, so the designed peptide is considered as stable. The allergist analysis of candidate vaccine revealed that the non-allergenicity of our vaccine, thus the vaccine is not estimated to drop harmful allergic responses in humans. The result of this study showed that the vaccine having the affinity to HLA0210 [32,33].

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        10         20         30         40         50         60
MARMAMGADD VVDSSKSFVM ENFSSYHGTK PGYVDSIQKG IQKPKSGTQG NYDDDWKGFY

        70         80         90        100        110        120
STDNKYDAAG YSVDNENPLS GKAGGVVKVT YPGLTKVLAL KVDNAETIKK ELGLSLTEPL

       130        140        150        160        170        180
MEQVGTEEFI KRFGDGASRV VLSLPFAEGS SSVEYINNWE QAKALSVELE INFETRGKRG

       190        200        210        220        230        240
QDAMYEYMAQ ACAGNRVRRI MPAPAPAATP DTGPDLDKKV DLWGADGAEG WTADPKKFSF

       250        260        270        280        290        300
ALPAGWKKDV HANTWRYKKK TVGETWGLKK WTADPIIGVK KLPGERIHVV PAPAPMARMA

       310        320        330        340        350        360
MGADDVVDSS KSFVMENFSS YHGTKPGYVD SIQKGIQKPK SGTQGNYDDD WKGFYSTDNK

       370        380        390        400        410        420
YDAAGYSVDN ENPLSGKAGG VVKVTYPGLT KVLALKVDNA ETIKKELGLS LTEPLMEQVG

       430        440        450        460        470        480
TEEFIKRFGD GASRVVLSLP FAEGSSSVEY INNWEQAKAL SVELEINFET RGKRGQDAMY

       490
EYMAQACAGN RVRRIM
```

Figure 2: The sequence of designed vaccine.

Figure 3: Secondary structure of *Mycobacterium tuberculosis* vaccine.

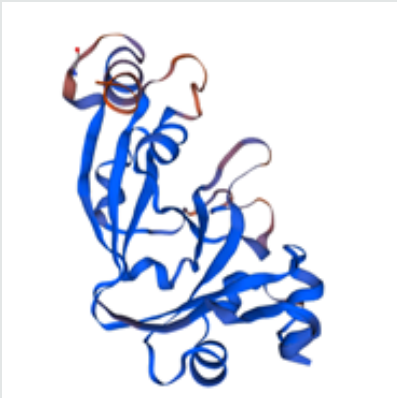

Figure 4: 3D structure of *Mycobacterium tuberculosis* vaccine.

Model #	3D^refine Score	GDT-TS	GDT-HA	RMSD (Å)	MolProbity	RWPlus
5	7517.80	1.0000	0.9960	0.245	1.733	-35066.966561
4	7490.03	1.0000	1.0000	0.224	1.737	-35039.495201
3	7628.85	1.0000	1.0000	0.203	1.638	-34982.821856
2	7918.65	1.0000	1.0000	0.172	1.630	-34890.306502
1	9648.80	1.0000	1.0000	0.127	1.579	-34740.396375

Figure 5: Refinement of a protein model by 3D refine software.

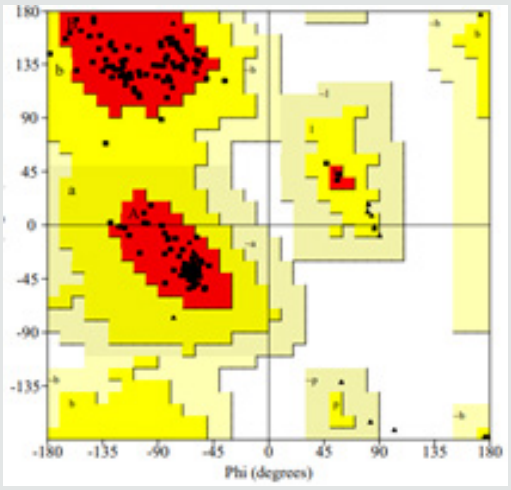

Figure 6: Ramachandran plot of *Mycobacterium tuberculosis* vaccine.

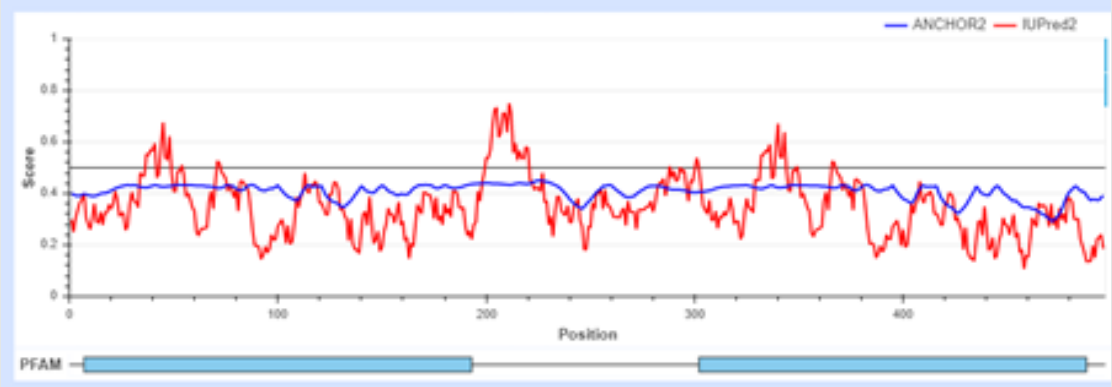

Figure 7: Prediction of protein disorder by IUPred2a.

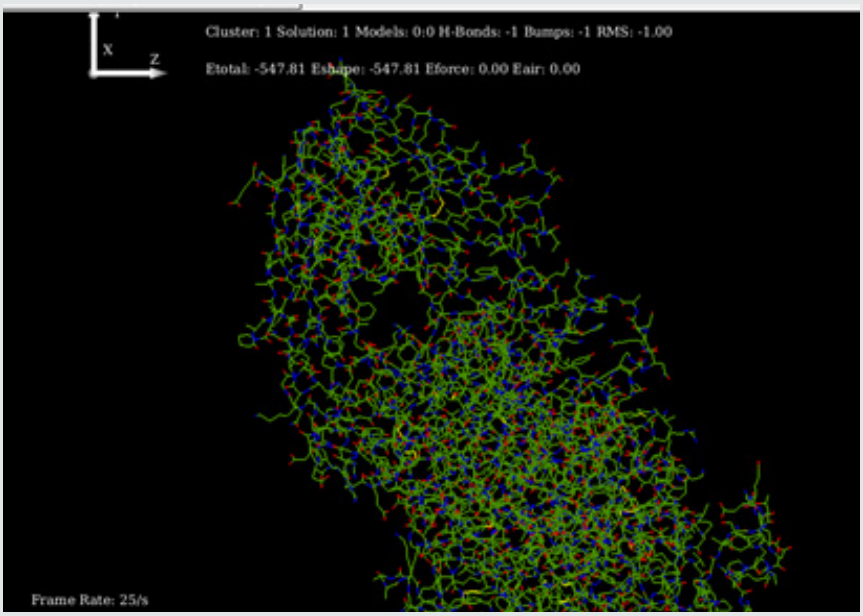

Figure 8: Molecular docking of HLA0210 with candidate vaccine.

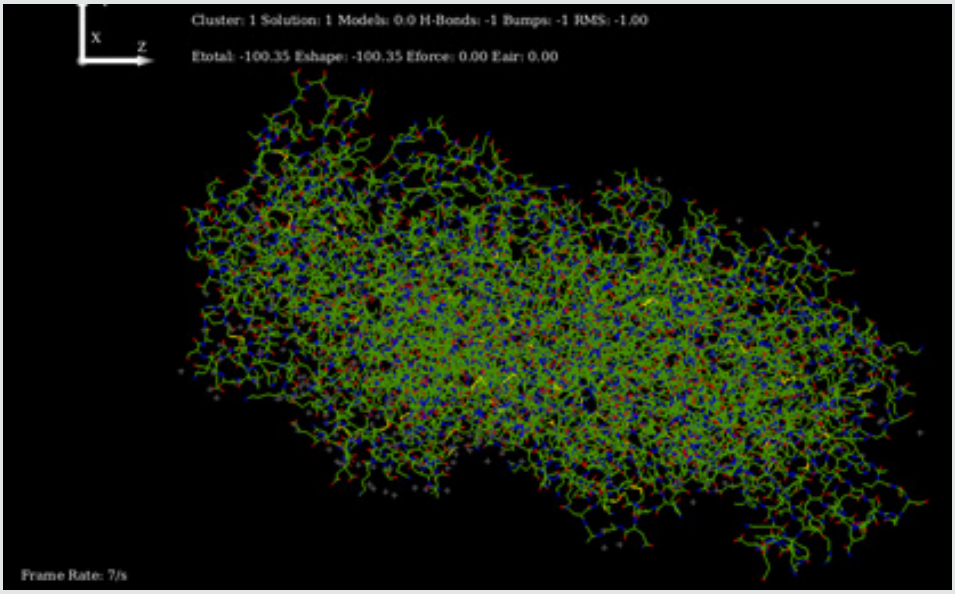

Figure 8: Molecular docking of HLA0210 with Human serum albumin.

Conclusion

Our designed vaccine may activate humoral and cellular immune responses against of *Mycobacterium tuberculosis*. The vaccine had suitable structural, physiochemical, and immunological properties. However, the vaccine could be cloned and expressed in different host cells. The candidate vaccine may be produced and need to experiment on animals.

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