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RESEARCH ARTICLE

In-silico Studies Reveal Potential Epitope based Vaccine against *M.leprae* Phosphoglycerate Mutase Protein

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Abstract Leprosy is an infectious disease caused by *Mycobacterium leprae* that mainly affects the skin, peripheral nerve, mucosa of the upper respiratory tract, and eyes. There is no vaccine designed specifically to prevent leprosy. The most common vaccine strategy is Bacille Calmette-Guérin (BCG), however its efficacy is highly variable between studies. Current study utilized a computational method to predict antigenic epitopes from *Mycobacterium leprae* for peptide vaccine development. Molecular docking of top predicted peptides from 6 antigenic B-cell and 3 CTL epitopes were analyzed. These predicted antigenic epitopes might potentially be target peptides for future leprosy vaccines.

Keywords: computational, leprosy, phosphoglycerate mutase, vaccine, epitopes, CTL, MHC.

Introduction

Leprosy is an infectious disease caused by *Mycobacterium leprae* that mainly affects the skin, peripheral nerve, mucosa of the upper respiratory tract, and eyes. In Indonesia, leprosy was a serious issue. There were a total of 126,221 incidents found in 1985. The prevalence was decreasing by 86% after 15 years, and the Indonesian government declared the elimination of leprosy was achieved in 2000 [1]. However, in 2018, 14,397 new cases were detected. By 2020, Indonesia was still ranked 3rd with leprosy [2].

There is no vaccine designed specifically to prevent leprosy. The most common vaccine strategy is Bacille Calmette-Guérin (BCG), which has been used to prevent both leprosy and tuberculosis [3]. The BCG vaccine is the live attenuated vaccine form of *Mycobacterium bovis*. This vaccine was designed as a tuberculosis vaccine but was speculated to give protection against leprosy due to the similar antigenic makeup of the causing agents [3][4].

According to Duthie, Gillis, and Reed [5], the effect of the BCG vaccine on leprosy is highly variable between studies; the efficacy was shown to be 26-41% in experimental studies and 61% in observational studies. Besides that, leprosy is still endemic in countries where BCG vaccination is common.

There are many methods to identify novel vaccine and drug targets. One example is a previous study by Jaiswal et al. [6], who screened *M.leprae*'s homologous conserved proteome for secreted, membrane and putative surface exposed proteins, in which the identified genes were analyzed further as potential vaccine candidates and docked.

This study screens for potential epitopes of a specific gene of *M.leprae*. The study determines potential B-cell and T-cell epitopes of *M.leprae*'s GPM1 that have strong affinity to major histocompatibility complexes (MHC). The specific gene is chosen because it encodes phosphoglycerate mutase (PGM), an important protein in the glycolysis metabolic pathway [7]. The study aims to find the potential peptide vaccine that inhibits *M.leprae*'s phosphoglycerate mutase.
Prediction of B-cell and cytotoxic T lymphocytes (CTL) epitopes are essential processes in vaccine

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development. By employing the in-silico method, current research will analyze potential B-cell and T-cell epitopes to obtain effective vaccine candidates. Moreover, the selection of peptides from the pathogen proteome that has strong affinity to major histocompatibility complexes (MHC) is also crucial. Numerous bioinformatics tools are utilized to model and analyze the peptide-MHC complexes for effective vaccine development

Materials and Methods

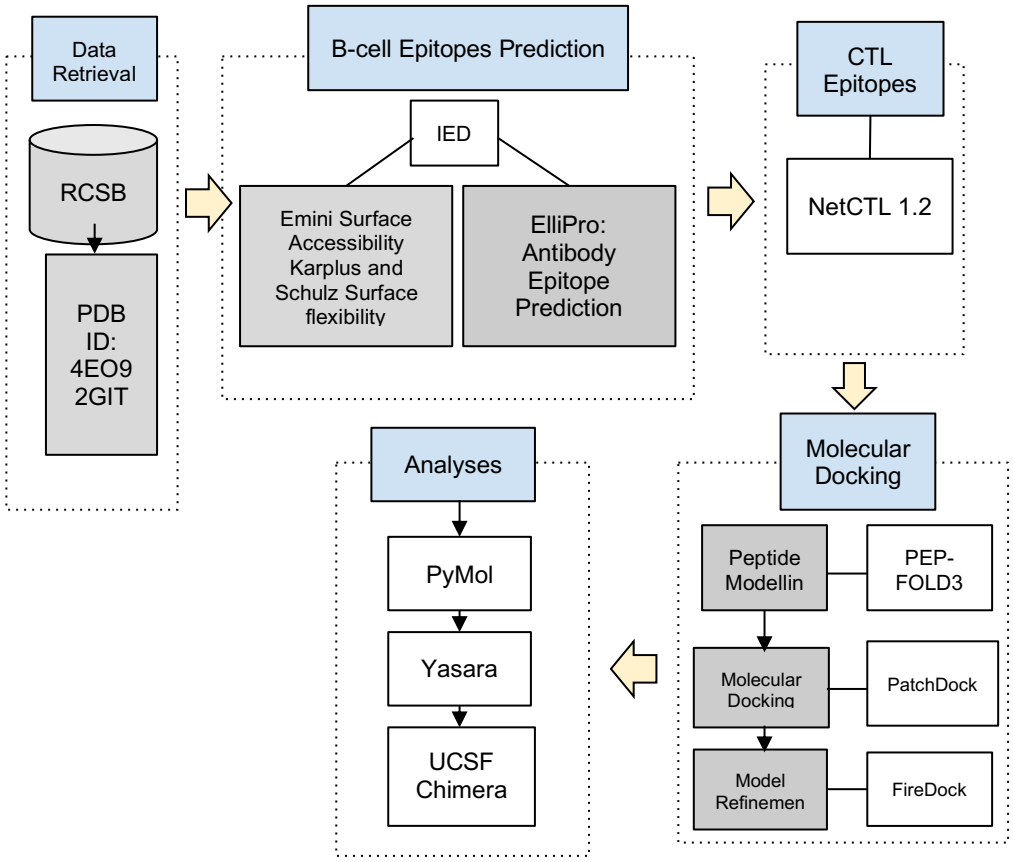


Figure 1. Overall Workflow

Data Retrieval

The crystal structures were obtained from Protein Data Bank. *M. leprae* phosphoglycerate mutase (PGM) GPM1 with PDB ID: 4EO9 and MHC Class I HLA-A2 with PDB ID: 2GIT. The amino acid sequence of PGM was also extracted from the crystal structure.

Prediction of linear and conformational B-cell epitopes

The the immune epitope database and analysis resource (IEDB) (http://www.iedb.org/) was utilized to predict surface accessibility based on Emini's method [8], predict flexibility based on Karplus and Schulz's method [9], predict hydrophilicity based on Parker's method [10], and predict antigenicity based on Kolaskar and Tongaonkar [11]. The conformational of B-cell epitopes was predicted with ElliPro (http://tools.iedb.org/ellipro/) [12]. The analysis uses three algorithms, which are approximation of protein shape, Protrusion Index (PI) of residues, and PI-based adjacent residues clustering [5].

Prediction of cytotoxic T-lymphocytes (CTL) epitopes

The NetCTL 1.2 server [13] was employed to predict CTL epitopes. To submit to the server, the sequence in Fasta format was inputted to the server, then peptide lengths and human leukocyte antigen (HLA) were chosen.

Molecular docking and analysis of peptide-MHC complex

Predicted antigenic CTL epitopes were selected to be 3D-modelled with PEP-FOLD3 server [13-15]. The higher scoring peptides were chosen for molecular docking with MHC I HLA-A2 by utilizing the PatchDock server with the complex type option antibody-antigen [16-17]. The model complexes were then refined with FireDock [18-20]. Firedock also predicted the docking scores. Molecular visualization was performed with PyMOL [21], Yasara [22], UCSF Chimera 1.15 [23].

Results and Discussion

The research identifies linear and conformational B-cell and T-cell epitopes to design peptide vaccines against leprosy. Computational analyses were carried out with molecular docking software to predict the conformational interactions between antigenic epitopes and MHC molecules as an effort to identify potential novel vaccines. *In-silico* methodologies to design vaccines have already been proven to be promising and vital as it helps reduce the load of vaccine candidates screening of *in-vitro* experiments [24].

The physicochemical of residues plays a major role in antigenic epitopes [11]. Kolaskar and Tongaonkar's antigenicity prediction method was utilized. It predicts antigenic determinants based on the psychochemical characteristics of residues and their occurrence frequencies and is proven to have 75% accuracy [11].

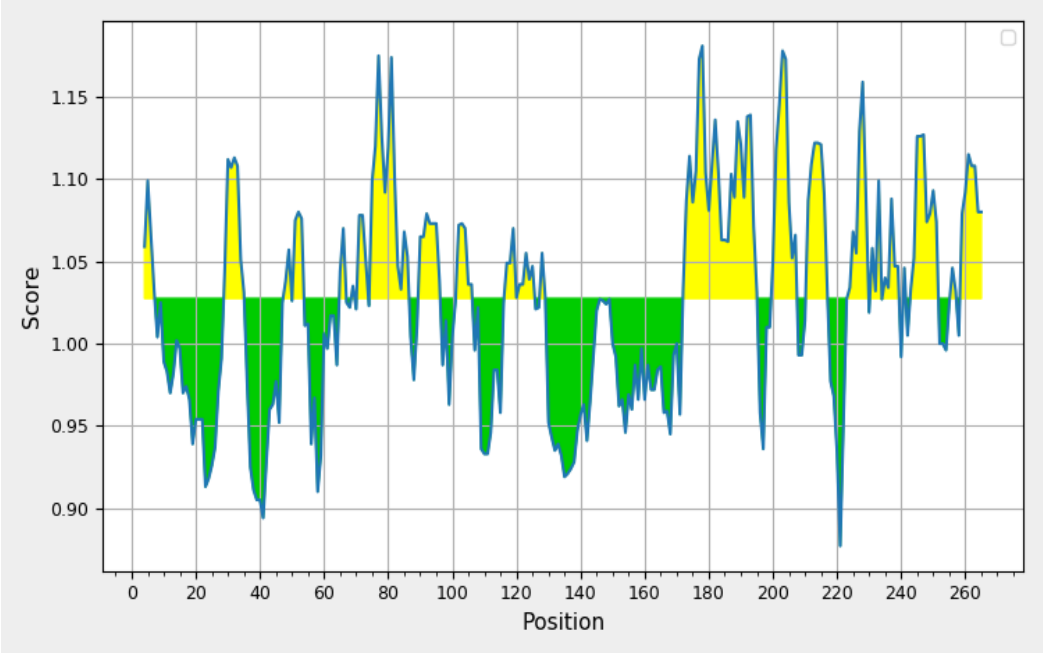


Figure 2. Antigenicity of *M.leprae* PGM with Kolaskar and Tongaonkar's scale. Residual score is represented along the X-axis while sequence position is represented along Y-axis.

A total of 6 antigenic epitopes have been predicted to occur within the range 8-42 amino acids from the complete sequence of M.leprae PGM (Table 1). By Kolaskar and Tongaonkar's calculation, there are 170 out of 265 residues exhibiting more than 1.000 residual score. This is shown graphically in figure 2 An observation also reveals that some of the predicted residues in B-cell epitopes are also found in CTL epitope prediction (Table 1 in bold). Thereby, the common residues at these positions are significant as potential peptide vaccine candidates.

Table 1. Predicted antigenic B-cell epitopes of *M.leprae* PGM and common residues (in bold) found in B-cell and Cytotoxic T lymphocytes.

Start	End	Peptide	Number of Residues	Starfish
100	141	GLDKAVTKARYGEERFMAWRRSYDTPPPPIEKGSE**FSQDADP**	42	0.74
75	85	D**TADWLWIPV**R	11	0.725
218	229	DLDADLRPVVPG	12	0.717
171	179	VPDLRTGRT	9	0.661
144	152	**TDIGGGPL**T	9	0.655
49	56	AEHNLLPD	8	0.638

Surface Accessibility of PGM

Emini surface accessibility calculation is based on a product's surface where a hexapeptide with surface probability more than 1.0 indicates high probability that it will be on the surface [8]. Figure 3 is the graphical representation of the predicted peptides of PGM based on sequence position along the X-axis and surface accessibility prediction score along the Y-axis. It has been observed that the maximum surface probability score is 4.489 and held by hexapeptide sequence RRSYDT within the range of 140 to 145, while the minimum surface score is 0.095 with hexapeptide sequence CLADVV within the range of 175-180 residues.

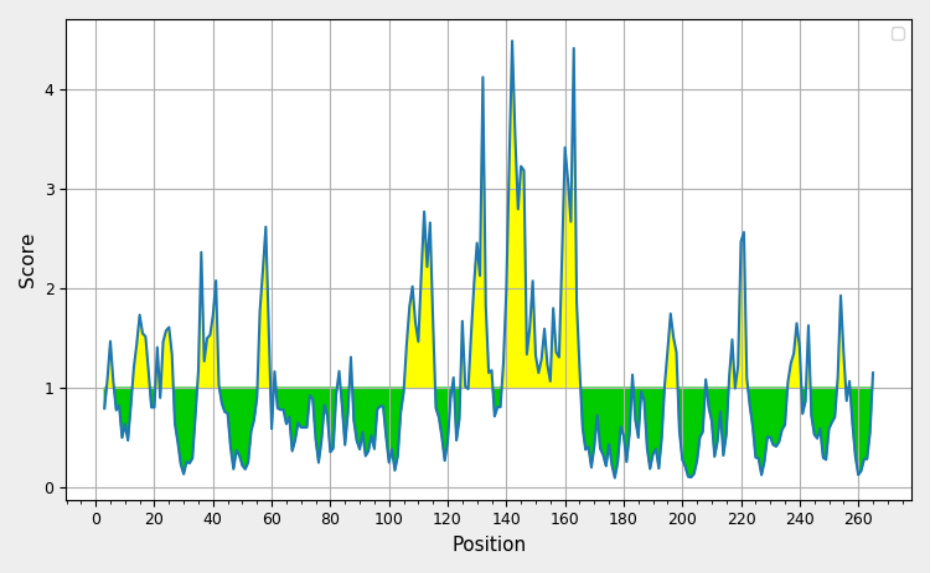


Figure 3. Emini Surface Accessibility Calculation for *M.leprae* PGM. Sequence position is along the X-axis, while surface access probability is along the Y-axis.

Surface flexibility of PGM

Karplus and Schulz' method calculates flexibility based on protein structure's atomic vibrational motions which are constructed through known temperature of B factors [9]. Lower B-factor values indicate that the model structure is well organized, while higher B-factor values indicate that the structure is unorganized and unordered [24]. The surface flexibility prediction was graphically represented in figure 4. The predicted maximum flexibility score is 1.108 at the range 15-21 with the heptapeptide sequence QTQGPGS, while the minimum flexibility score is 0.891 at the range 99-105 with DWLWIPV heptapeptide sequence.

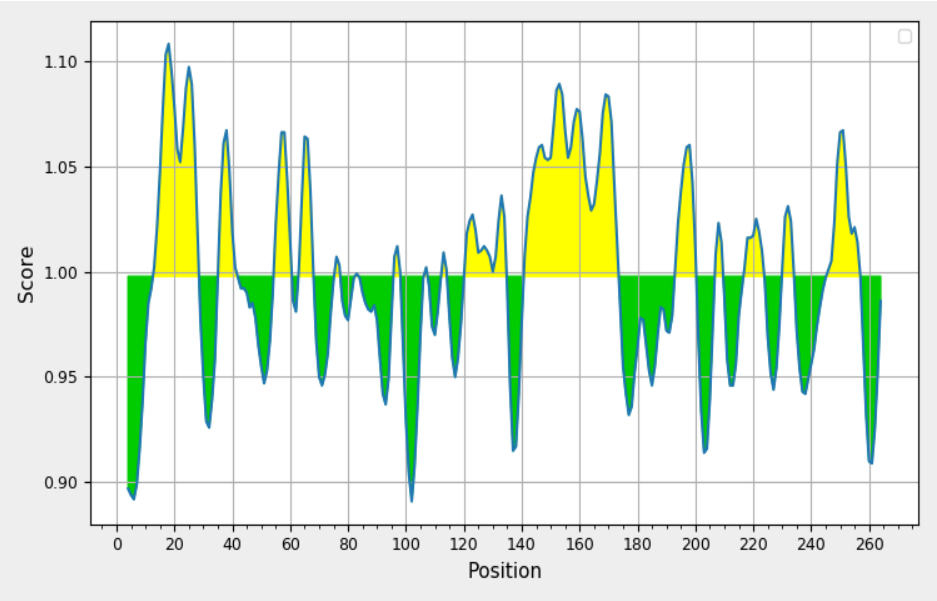


Figure 4. Surface flexibility prediction of *M.leprae* PGM. X-axis represents sequence position, while Y-axis represents surface flexibility scores.

Hydrophilicity Prediction of PGM

Parker's calculation was utilized to predict the hydrophilicity based on retention times of peptide through high-performance liquid chromatography (HPLC) on a reversed phase column [10]. Antigenicity is associated with hydrophilicity as an antigen should be accessible on the surface [24]. The hydrophilicity of PGM is represented graphically in Figure 5 where hydrophilicity is plotted based on sequence position (X-axis) against hydrophilicity prediction score (Y-axis).

By observation of Parker's hydrophilicity calculation result, it is shown that the maximum and minimum hydrophilicity predicted scores are 6.843 and -4.943 at the heptapeptide sequences 218DEMSDDE224 and 100WLWIPVR106 respectively.

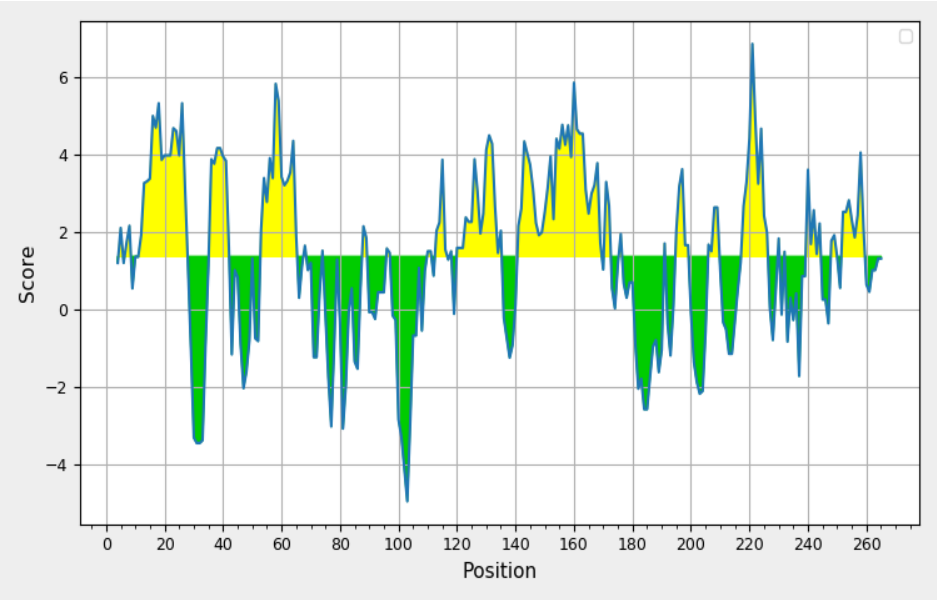


Figure 5. Parker hydrophilicity prediction for *M.leprae* PGM. X-axis represents the sequence position and Y-axis represents hydrophilicity predicted score.

Prediction of CTL epitopes

Activation of T-cells happens when the protein in the body is altered by an infectious agent and acts as an antigen. MHC molecules on the cell surface bind to the pathogenic peptide and display the infected cell for recognition by CTL, subsequently killing the cell [25]. MHC molecules exhibit high affinity to variety peptides to counteract pathogens' effort to mutate MHC epitopes [24]. Therefore, determination of T-cell epitope is a major part of computational vaccine design. This research utilized NetCTL 1.2 [26] to predict CTL epitopes from *M.leprae* PGM. Observed from table 2, only one CTL epitope (TADWLWIPV) has full antigenic site, while two (FSQDADPRY and YTDIGGGPL) have partial antigenic sites. These common residues inferred from both B-cell and T-cell epitopes prediction are considered as potential vaccine candidates.

Table 2. Identified CTL epitopes of *M.leprae* PGM and predicted antigenic residues (in bold). Predicted MHC binding affinity in nM units and rescale binding affinity is normalized binding affinity by first percentile score.

Residue Number	Peptide Sequence	Predicted MHC Binding Affinity	Rescale Binding Affinity	C-terminal Cleavage Affinity	Transport Affinity	Prediction Score
26	NTATLILLR	0.1862	0.7907	0.5587	1.4870	0.9488
94	ALDTADWLW	0.2477	1.0515	0.7839	0.9200	1.2151
97	**TADWLWIPV**	0.1557	0.6609	0.8126	0.0310	0.7844
156	**FSQDADP**RY	0.4082	1.7332	0.8713	2.6710	1.9974
164	Y**TDIGGGPL**	0.3579	1.5197	0.9575	0.8690	1.7067
172	LTECLADVV	0.2219	0.9422	0.0788	0.0080	0.9545
178	DVVTRFLPY	0.1347	0.5719	0.9570	2.9790	0.8644
187	FTDVIVPDL	0.2693	1.1436	0.8973	0.6100	1.3087
220	MSDDEVVGL	0.2220	0.9428	0.9618	0.6990	1.1220

Prediction of Structure-Based Epitopes for *M.leprae* PGM

ElliPro is a web-based tool with the ability to correlate antigenicity characteristics, structure flexibility, and solvent accessibility of protein to predict conformational epitopes [28]. The conformational epitopes are scored based on the percentage of atoms that protrude over the molecular bulk and considered liable for antibodies to bind [28]. Seen from table 3, top three of the predicted conformational epitopes score above 0.6. The 3D structures visualization is also presented in figure 6.

Table 3. Predicted conformational epitopes of *M.leprae* PGM.

No.	Residues	Number of Residues	Score
1	A:N5, A:T6, A:A7, A:A39, A:V42, A:R43, A:E46, A:A49, A:E50, A:H51, A:N52, A:L53, A:L54, A:P55, A:D56, A:H71, A:L72, A:D75, A:T76, A:A77, A:D78, A:W79, A:W81, A:I82, A:P83, A:T167, A:D168, A:V171, A:P172, A:D173, A:R175, A:T176, A:G177, A:R178, A:T179, A:D218, A:L219, A:D220, A:A221, A:D222, A:L223, A:R224, A:P225, A:V226, A:V227, A:P228, A:G229, A:G230, A:L233	49	0.685
2	A:E16, A:D18, A:A21, A:R22, A:N23, A:L24, A:F25, A:V29, A:G32, A:L33, A:T34, A:D35, A:K36, A:R38, A:A97, A:G100, A:L101, A:D102, A:K103, A:A104, A:V105, A:T106, A:K107, A:A108, A:R109, A:Y110, A:G111, A:E112, A:E113, A:R114, A:F115, A:M116, A:A117, A:W118, A:R120, A:S121, A:Y122, A:D123, A:T124, A:P125, A:P126, A:P127, A:P128, A:I129, A:E130, A:K131, A:G132, A:S133, A:E134, A:F135, A:S136, A:Q137, A:D138, A:A139, A:D140, A:P141, A:T144, A:D145, A:I146, A:G147, A:G148, A:G149, A:P150, A:L151, A:T152, A:M199, A:S200, A:D201, A:D202, A:E203	70	0.682
3	A:N208, A:P210, A:D234, A:P235, A:E236, A:A237, A:A238, A:A239, A:A240, A:V241, A:I242, A:S243, A:Q244	13	0.663

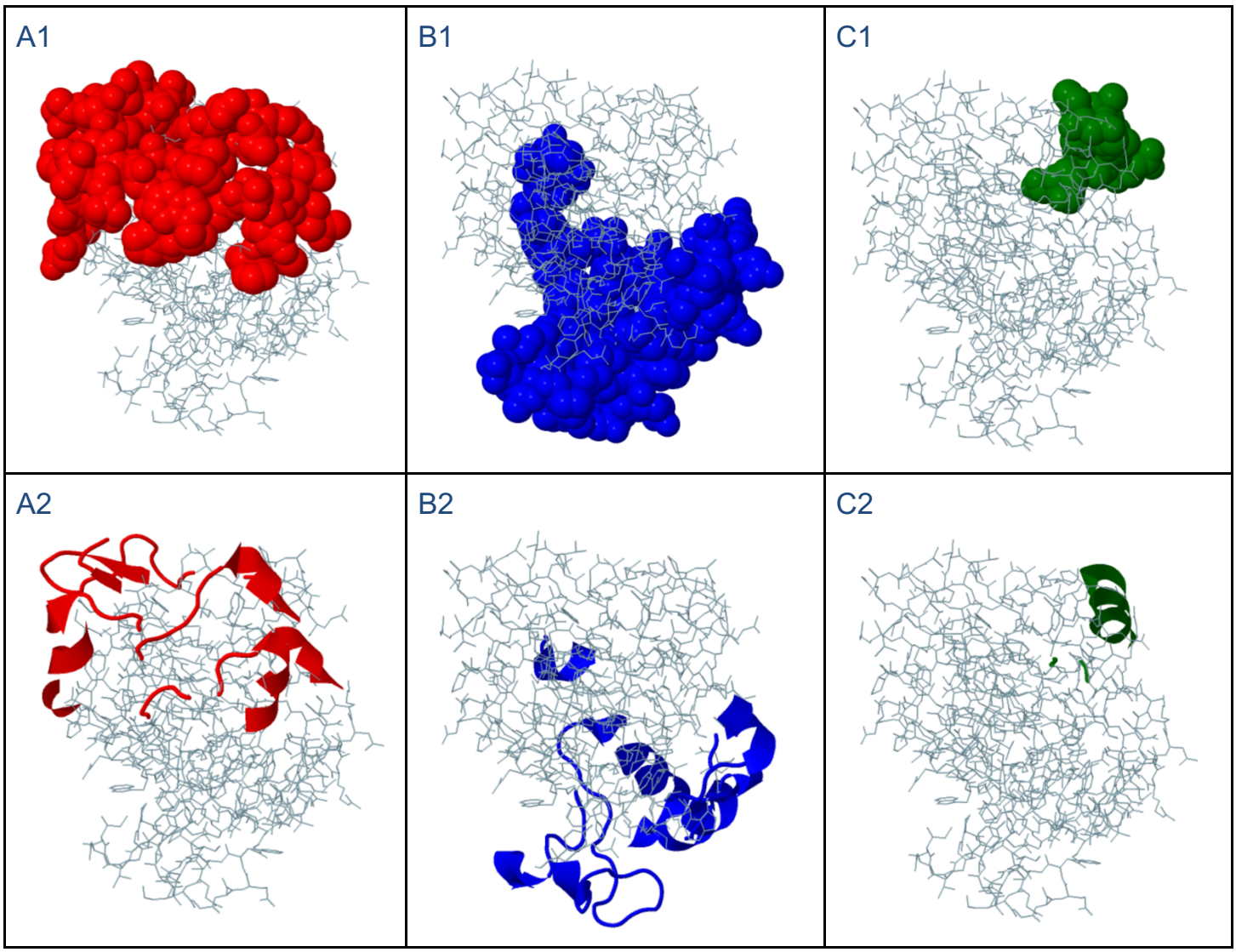


Figure 6. 3D structure of the predicted conformational epitopes of *M.leprae* PGM in two styles (style 1 and 2). Score of A is 0.685; score of B is 0.682; and score of C is 0.663.

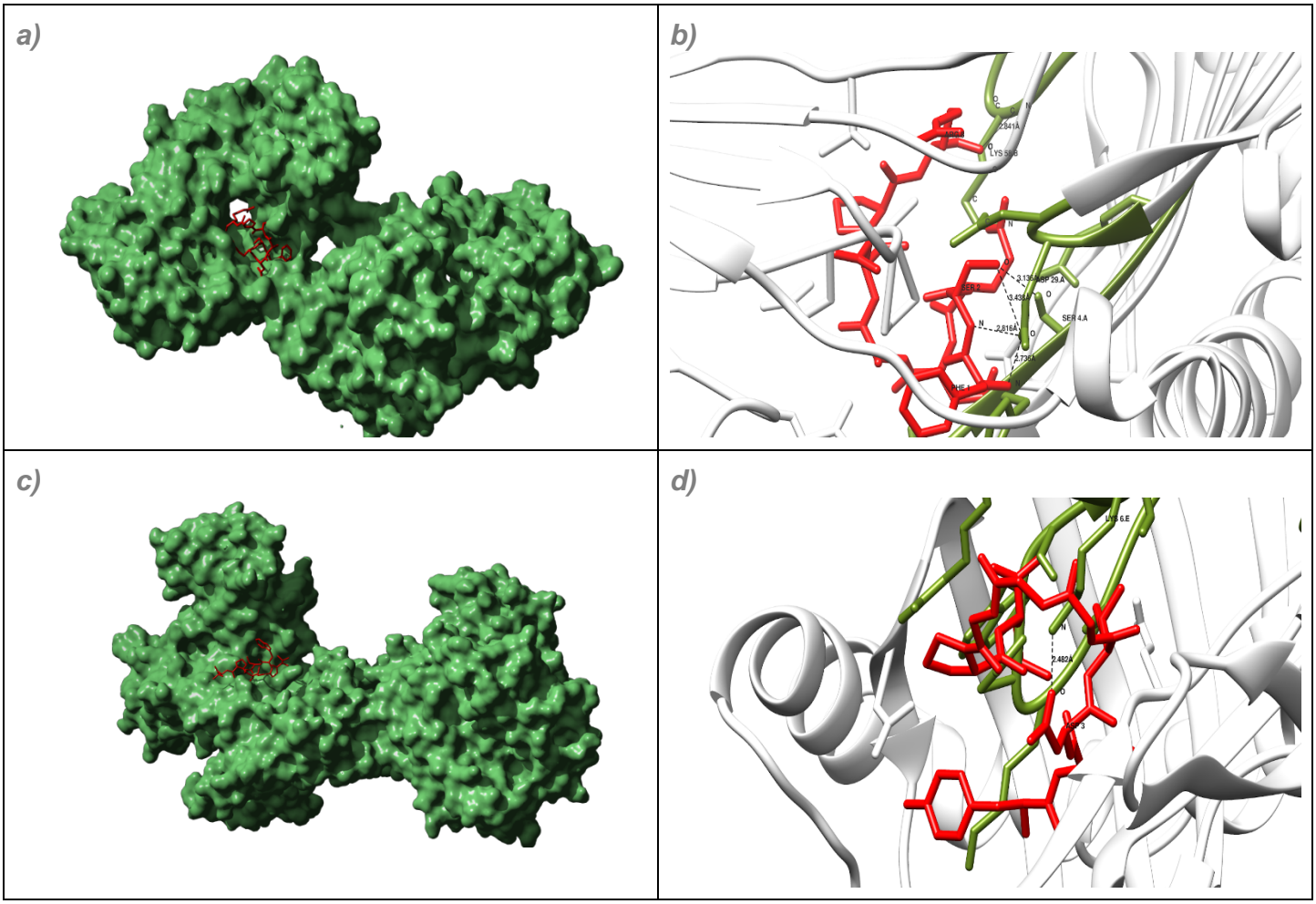
Molecular Docking Analyzation of *M.leprae* PGM with HLA-A2

Molecular docking analyses were performed with all three of the predicted CTL epitopes for *M.leprae* PGM and MHC class I HLA-A2. The binding affinities of the selected CTL based on attractive van der Waals (vdW) energy range from -16.26 to -10.29; repulsive vdW range from 5.81 to 3.34; atomic contact energy range from 7.75 to 2.56; hydrogen bonding range from -1.13 to 0; and overall global energy range from -3.31 to -0.96 kcal/mol (Table 4). Post docking analyses were executed by using PyMol, Yasara, and UCSF Chimera and the result is the effective binding affinities of the peptide with HLA-A2, which are visualized and can be observed in figure 7.

FSQDADPRY-MHC 1 HLA-A2 is predicted to have a stable complex because of 5 in total of hydrogen bonds with the interactions range from 2.7-3.4 Å. On the other hand, YTDIGGGPL-MHC 1 HLA-A2 and TADWLWIPV-MHC 1 HLA-A2 complexes only have one and two hydrogen bonds respectively. These suggest that the two might have unstable complexes.

Table 4. Statistical detail of *M.leprae* PGM-MHC class I HLA-A2 binding interactions. ACE: atomic contact energy. HB: hydrogen bonding energy

Peptide	Global Energy (kcal/mol)	Attractive vdW energy	Repulsive vdW	ACE	HB	H. Bond Interaction		
						Complex Pair		Bond Distance (Å)
						Peptide	MHC	
FSQDADPRY	-16.86	-16.26	5.81	7.75	-1.13	SER2 OG	ASP29 OD1	3.438
						Ser2 OG	Ser4 OG	3.136
						ARG 8 O	Lys58 N	2.841
						SER2 N	ASP29 OD1	2.816
						PHE1 N	ASP29 OD1	2.736
YTDIGGGPL	-22.28	-24.02	20.61	-0.70	-2.55	ASP3 OD1	LYS6 NZ	2.482
TADWLWIPV	-9.27	-10.29	3.34	2.56	0	THR1 OG1	GLU212 OE1	3.508
						PRO8 O	ARG3 NH1	1.675



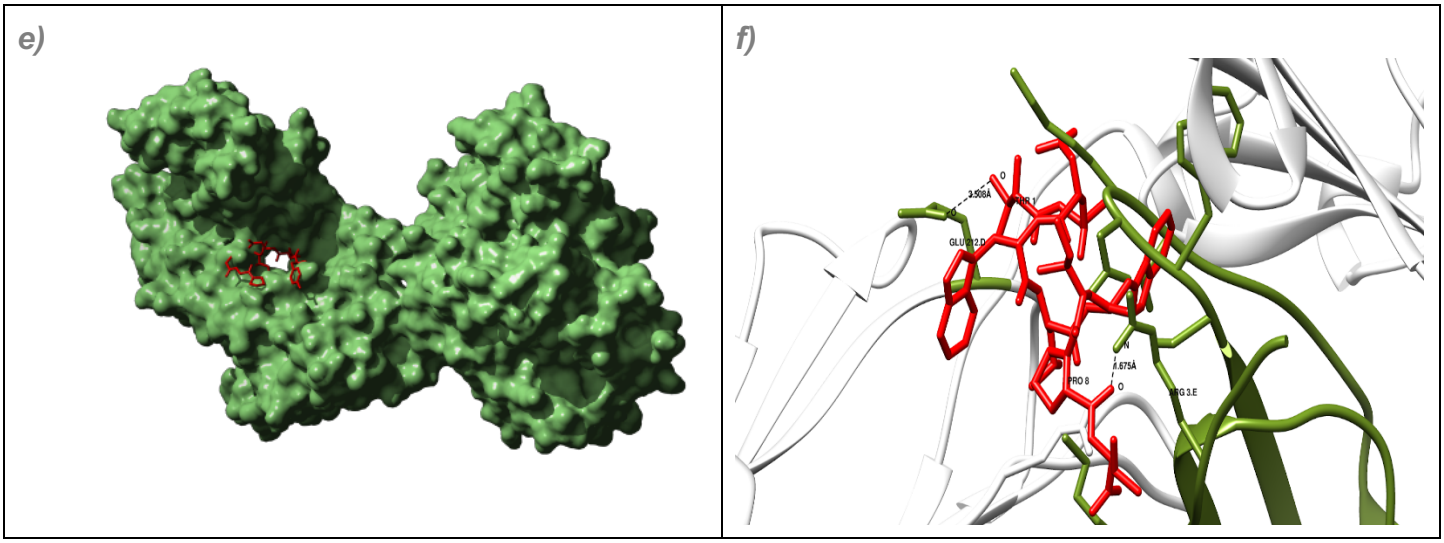


Figure 7. Binding interactions of peptide and MHC class I HLA-A2 complexes. From the top is a) hydrophobic map and b) hydrogen bond of FSQDADPRY-MHC 1 HLA-A2; c) hydrophobic map and d) hydrogen bond of YTDIGGGPL-MHC 1 HLA-A2; and e) hydrophobic map and f) hydrogen bond of TADWLWIPV-MHC 1 HLA-A2 complexes, respectively.

With new cases of leprosy increasing in India, Brazil, and Indonesia, a solution is needed. The ability to remain undetected and its asymptomatic character in the early stage, make a vaccine much more vital to eradicate the disease. *In-silico* methods have proven to reduce the time and resources needed to screen vaccine candidates for *in-vitro* experiments in vaccine design. This research predicts antigenic epitopes from *M.leprae* PGM, an important catalyzation that is involved in glycolysis which if inhibited will stop ATP production and impede the metabolic pathway [7]. Therefore, a vaccine that acts as a peptide inhibitor is of great interest, especially considering the rapid action and having no toxic metabolites to human health. The research also includes molecular docking as an effort to analyze the binding affinities of candidate peptides to MHC-1 protein. Previous studies have validated peptide-MHC I complex binding affinity and the utilization of computational approaches, such as Tahir et al., [24] who identify potential epitope against DENV-NS3 protein, and Mirza et al. [29], who utilized the method against Zika virus. Gupta et al., [30] has also utilized a computational method to identify vaccine or drug targets by screening the whole bacteria genome.

In this study, three peptides are predicted to show strong interaction with the MHC I HLA-A2 protein based on the global energy scores. The antigenic sites observed from the linear epitopes, which is also in the CTL epitopes prediction, are present in the selected peptides. And the antigenicity increases the chance of being a promising target for vaccine. Surface accessibility, flexibility, and hydrophilicity are also predicted to validate further with IEDB. The three predicted peptides were modeled with PEP-FOLD3, followed by docking to the MHC class I HLA-A2 with PatchDock. The top models were refined by using FireDock to have stable protein structures. The resulting complexes were visualized by using PyMol, Yasara, and UCSF Chimera 1.15 to analyze the docked complexes' bonding interactions.

The three selected peptides show promise due to their antigenicity, but only one (FSQDADPRY) shows a possibility of having a stable docked complex with MHC protein HLA-A2. However, the predicted epitope does not show interactions with the binding residues of MHC I HLA A-2 (THR4, ARG97, ASP30 and HIS70) [24]. Therefore, the current study needs to be researched further to be of help in developing effective peptide vaccines in *in-vitro* experiment.

Conclusions

The aim is to identify potential peptides as vaccine targets to inhibit *M.leprae*-PGM protein. To achieve this, selected epitopes from PGM GPM1 were modeled and docked against MHC class I to analyze their interactions and binding affinities. The method was concluded with one antigenic CTL epitope that had possibility as vaccine target candidates. Further research should be conducted to validate the *in-silico* study

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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