

Insilico Peptide-Based Vaccine Against *Mycobacterium Tuberculosis*

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Abstract

Tuberculosis is a major global health issue over centuries despite a huge improvement in drug development. It is the leading cause of death worldwide, and it is an infectious disease. The range of this disease increased to this extent because of neglect for proper drug therapy and development, lack of facilities and resources. After huge advances in pharmaceutical and medical technology still, there is no proper protocol for the treatment of multiple drug resistance which occurs due to existing multiple drug therapy for tuberculosis treatment. The current treatment regimen consists of a class of drugs from different groups having a different mechanism of action, and the treatment duration is lengthy up to 24 months. This study emphasizes prevention rather than treatment by providing a vaccine candidate against mycobacterium tuberculosis which could be taken to wet lab for vaccine development.

Introduction

Vaccine designing and in silico tools are complementary, which means databases collect experimentally verified vaccine information with components and in silico tools help in computational analysis to design new vaccines. Some important vaccine components include vaccine preservatives, vaccine antigens, vaccine adjuvants, and vaccine vectors. The vaccine antigen can be a complete protein or an epitope. For the computational analysis, various online tools are available for analysis and the licensed databases are maintained by the FDA for human vaccines, and also US agricultural department keeps the licensed animal vaccine database. Epitope based vaccines are developed by the identification of B-cell epitopes,

diagnostic tools and therapeutic antibodies. In the field of pharmaceuticals, the epitope-based vaccines are now considered as the most promising class of biopharmaceuticals. Various tools and algorithms have been developed for in silico analysis and classification of experimentally identified epitopes. The prediction and identification of B-cell epitopes still face some major challenges, but due to advancement in techniques and tools required for mapping and computational analysis has enhanced biorecognition and formation of an antigen-antibody complex. The interaction between antigen and antibody is the key to the humoral immune response to pathogens. Antibodies are specific and recognize antigen at some specific regions by binding which is termed as antigenic determinants or B-cell epitopes. While as B-cell epitopes are surface accessible amino acids and were identified by specific antibodies and B-cell receptors can elicit cellular or humoral response [1]. Sometimes the overall surface of antigen becomes part of epitope after being recognized by antibody and why only some regions become B-cell epitopes. The mechanism and the process behind are not well understood yet [2]. The accurate analysis and identification of B-cell epitopes constitute the base for the development of antibody therapeutics, peptide vaccines [3, 4], and immunodiagnostic tools [5]. Multiple drug resistance for tuberculosis is resistant to isoniazid and rifampicin. These are the frontline drugs for the treatment of tuberculosis. In 2015 it was found in a survey that 480,000 fresh cases of multiple drug-resistant patients [6].

There are different types of vaccines some are preventive, and some are therapeutic and have designed for a particular disease. In tuberculosis priming, vaccines have developed for newborn babies against tuberculosis. Some are under trials example MTBVAC. The boosting vaccine is used to enhance the immune response. It is used for latent infection in adults. These vaccines can be a whole cell or viral vectored, and some examples are M72, ID93 are in the pipeline. New designing of vaccines have involved the use of adjuvants in vaccine development. TB vaccine having adjuvant IC31 having TLR9 ligand showing immune stimulatory response. Some of the antimicrobial peptides have also been used in designing peptide vaccines [15].

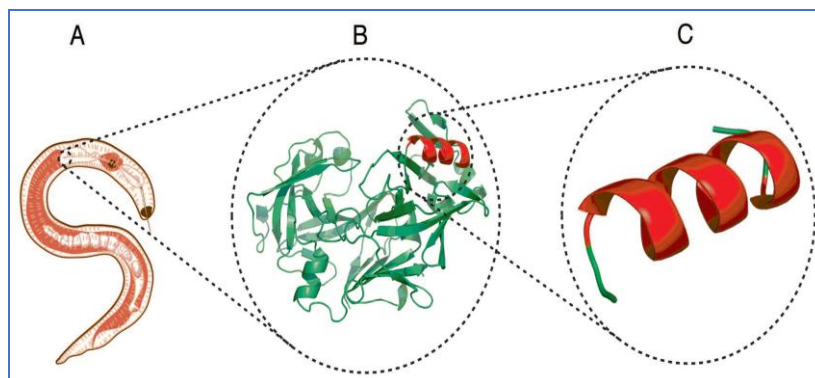


Figure 1. The evolution of vaccine (A) Traditional vaccine development whole-cell used for development. (B) A vaccine based on protein subunits. (C) Vaccines based on peptide subunits.

ISCOM are the structures which stimulate the immune system are antigen like containing saponins, cholesterol. For the production of ISCOM vaccines, the antigen remains inside incorporated during designing. ISCOMATRIX antigen helps in delivery. It shows adjuvant properties derived from APC which later on induces CD8 for cross-presentation of antigens.

Vaccines prevention from *Mycobacterium tuberculosis* infection

Improvisation in BCG vaccine created rBCG produced by one of the company in Germany, new product VPM1002, rBCG express lysine and the urease gene subunit showed in activation this will allow the escape of mycobacterium from phagosome to cytoplasm promoting T cell response and cross antigen presentation [7]. Availability of BCG having the ability to induce T cell response which acts as memory cells is not known in humans. Different approaches have been carried out to boost the memory without knowing the exact parameters. In some cases, it was observed that boosting induces T cell which leads to contraction. It takes ten weeks to develop memory by BCG vaccination which leads to an increase in longevity [8]. Synthetic long peptides have shown great results in combination with some adjuvant and have given some highly efficient vaccines for the treatment and control of mycobacterium tuberculosis. This strategy has not yet been implemented. The antigen presentation by T or B cell leads to the abrupt death of responsive T cell [9]. Short peptides of length 8-11 amino acids can be injected to HLA cell line. The presentation will be taken by unspecified cells which can be t or b cell and may lead to enhance immunological response due to absence of some signalling molecules. The use of SLP will lead to antigen processing by specific DCs, and particular epitope can get cross represented to T-cells. SLP

having both T helper and CTL epitope leads to epitope presentation to CD4⁺ and CD8⁺ simultaneously [10]. Although there has been a immunogen since the Nineteen thirties (BCG), it provides variable protection on the far side infancy age with restricted impact on TB transmission. Many immunogen candidates are presently within the pipeline, however their development and analysis hampered by the shortage of valid correlates of protection. As a result, immunogen candidates that appear immunogenic in animals and humans might not prove effective in humans once it involves protective against TB malady. Consequently, massive costly trials with longer follow-up presently required to ascertain protecting efficaciousness.

In Silico B-cell epitope prediction:

The relationship between different physicochemical properties of a B-cell has been demonstrated in various studies, but the prediction process was nonparametric i.e. based on single residue property which yields poor outcomes in practice. For modifying the prediction of B-cell epitopes and minimizing the errors leads to involvement of machine learning methods for the prediction of continuous and discontinuous epitopes. The methods developed on the basis of small datasets and a random set of peptides were used instead of experimentally verified non-epitopes as training set [11].

Initially, the method involved in prediction was a recurrent neural network, ABCPred using data of B-cell epitopes available in BciPep and non-epitopic data from swiss-Prot. ABCPred helps in the prediction of continuous epitopes by considering a fixed length pattern. It contains data of epitopes of different organisms like fungi, bacteria, viruses, parasites having prediction accuracy of 65.9%. Earlier methods like BayesB, ABCPred, BCPred, and AAp help in predicting shorter sequences of peptide fragments. For example, B-cell epitopes of EMYL162 protein was predicted by using BCPred and ABCPred [12-13]. ABCPred helps in combining amino acid pair composition, and it supports methods like SVM (support vector machine) and increases prediction accuracy. It has achieved a significant area under the curve of 0.794 [14].

Table 1. List of epitopes (MHC-I) predicted and their properties using PyRx

Epitopes (MHC1)	MW	Pi	half-life (h)	Instability Index	Stability
AEMKTDAATL	1050.2	4.37	4.4	9	stable
AEMKTDAATLA	1121.3	4.37	4.4	9.09	stable

ALYLLDGLRA	1104.3	5.88	4.4	28.26	stable
ATYDKGILTV	1080.3	5.88	4.4	26.47	stable
AVHDTLFYCV	1167.3	5.08	4.4	19.03	stable
AVINTTCNYGQ	1183.3	5.56	4.4	-6.35	stable
FIYAGSLAL	1041.2	5.52	1.1	32.68	stable
FLLADERVRV	1217.4	6.07	1.1	0.51	stable
GIVSGILLSI	971.21	5.52	30	2.79	stable
GLAGTVLRFV	1032.3	9.75	30	19.77	stable
GLFLTTEAVV	1049.2	4	30	28.26	stable

Table 2. List of epitopes (MHC-II) predicted and their properties using PyRx

Epitope MHC2	MW	Pi	half-life (h)	Instability Index	Stability
ADLVPTATLLDTY	1392.57	3.56	4.4	5.95	stable
DRNLGIGNTGNW	1316.4	5.84	1.1	-30.85	stable
EGVVLLLVGALVL	1294.64	4	1	-3.83	stable
GIGNIGDRNLGI	1198.34	5.84	30	28.56	stable
GNPNRPDGGILT	1210.31	5.84	30	28.72	stable
IRQAGVQYSR	1177.33	10.84	20	39.98	stable
LSFTLTGNPNRP	1316.48	9.75	5.5	8.67	stable
RPGLDELSFTLT	1348.52	4.37	1	25.22	stable
STEGNVTGMFA	1113.21	4	1.9	3.45	stable
VNLVDTLNSGQYT	1423.54	3.8	100	-26.22	stable
GATPNTGPAPQGA	1138.2	5.52	30	27.55	stable

Table 3. Molecular properties of MHC-I epitopes

	Stability	Hydrophobicity (kJ/mol)	pKa	pKb	Surface accessibility	Polarity	MW
AEMKTDAATL	High	5.36	22.09	93.76	46.63	145.31	1050.19
AEMKTDAATL A	High	4.264	24.43	103.45	44.918	145.31	1121.27
ALYLLDGLRA	Normal	2.85	22.71	95.13	40.1	96.11	1104.31
ATYDKGILTV	Normal	2.17	22.16	93.97	43.52	95.86	1080.25
AVINTTCNYGQ	High	0.645	23.91	103.37	40.764	16.98	1183.3
GIVSGILLSI	High	-8.67	23.22	95.12	28.03	4.82	971.21
GLAGTVLRFV	High	-2.93	22.47	94.6	34.46	55.17	1032.25
GLFLTTEAVV	High	-4.73	22.24	94.19	33.86	54.74	1049.23

Table 4. Molecular properties of MHC-I epitopes

	Stability	Hydrophobicity (kj /mol)	pKa	pKb	Surface accessibility	Polarity	MW
ADLVPTATLLDTY	High	0.485	28.3	124.01	40.385	91.05	1392.57
GIGNIGDRNLGI	High	5.225	26.89	113.04	39.125	100.36	1198.34
GNPNRPDGGILT	Normal	7.458	25.9	114.54	45.617	104.88	1210.31
IRQAGVQYSR	High	10.3	22.45	94.19	52.28	114.62	1177.33
LSFTLTGNPNRP	High	3.142	25.47	113.12	46.258	68.16	1316.48
STEGNVTGMFA	High	-1.255	24.05	102.13	38.164	60.19	1113.21
VNLVDTLNSGQYT	High	2.838	28.38	121.37	43.369	58.75	1423.54
GATPNTGPAPQGA	High	-0.231	28.38	126.34	40.785	14.79	1138.2

Table 5. Binding Score of T-cell epitopes using patch dock against (MHC-I and MHC-II)

	Pi	Formula	Half-life (h)	MHC-I binding energy	binding score	(r)MHC-II/MHC-I PEP	Binding Score
AEMKTDAATL	4.37	C43H75N11O17S1	4.4	-161.86	8428	78.76	7920
AEMKTDAATLA	4.37	C46H80N12O18S1	4.4	23.49	7860	52.33	7848
ALYLLDGLRA	5.88	C51H85N13O14	4.4	70.95	7680	-462.15	7142
ATYDKGILTV	5.88	C49H81N11O16	4.4	55.53	8814	56.86	7998
AVINTTCNYGQ	5.56	C49H78N14O18S1	4.4	108.27	8398	67.2	7442
GIVSGILLSI	5.52	C45H82N10O13	30	-111.52	7430	-109.86	7088
GLAGTVLRFV	9.75	C48H81N13O12	30	-110.38	8182	98.25	7682
GLFLTTEAVV	4	C49H80N10O15	30	-96.47	8294	78.69	7994

Table 6. Binding Score of B-cell epitopes using patch dock against (MHC-I and MHC-II)

	pi	Formula	Half-life (h)	MHC-II binding energy	Binding Score	(r)MHC-I/MHC-2 pep	Binding Score
ADLVPTATLLDTY	3.56	C63H101N13O22	4.4	-345.32	7780	36.12	7476
GIGNIGDRNLGI	5.84	C50H87N17O17	30	30.33	8038	84.48	7928
GNPNRPDGGILT	5.84	C50H83N17O18	30	-56.48	7928	101.84	8490
IRQAGVQYSR	10.84	C50H84N18O15	20	88.78	7350	236.1	7666

LSFTLTGNPNRP	9.75	C58H93N17O18	5.5	178.63	8014	35.69	7844
STEGNVTGMFA	4	C46H72N12O18S1	1.9	-10.9	7848	-106.65	7420
VNLVDTLNSGQYT	3.8	C61H98N16O23	100	-97.82	7800	99.23	9390
GATPNTGPAPQGA	5.52	C47H75N15O18	30	12.16	7556	60.62	8650

Structure Prediction of Ligand Molecules (PDB 3D view):

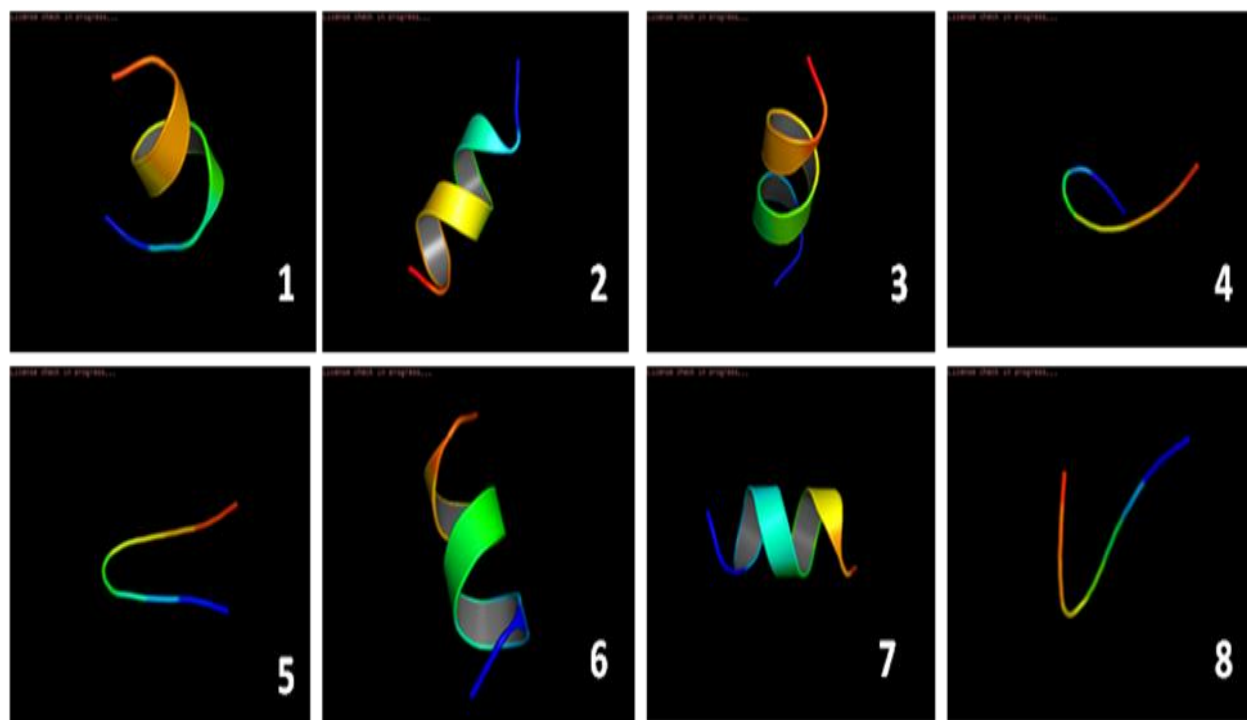


Figure 2. 3D structure of T cell epitopes predicted against MHC-1 (1-8 as per table-5)

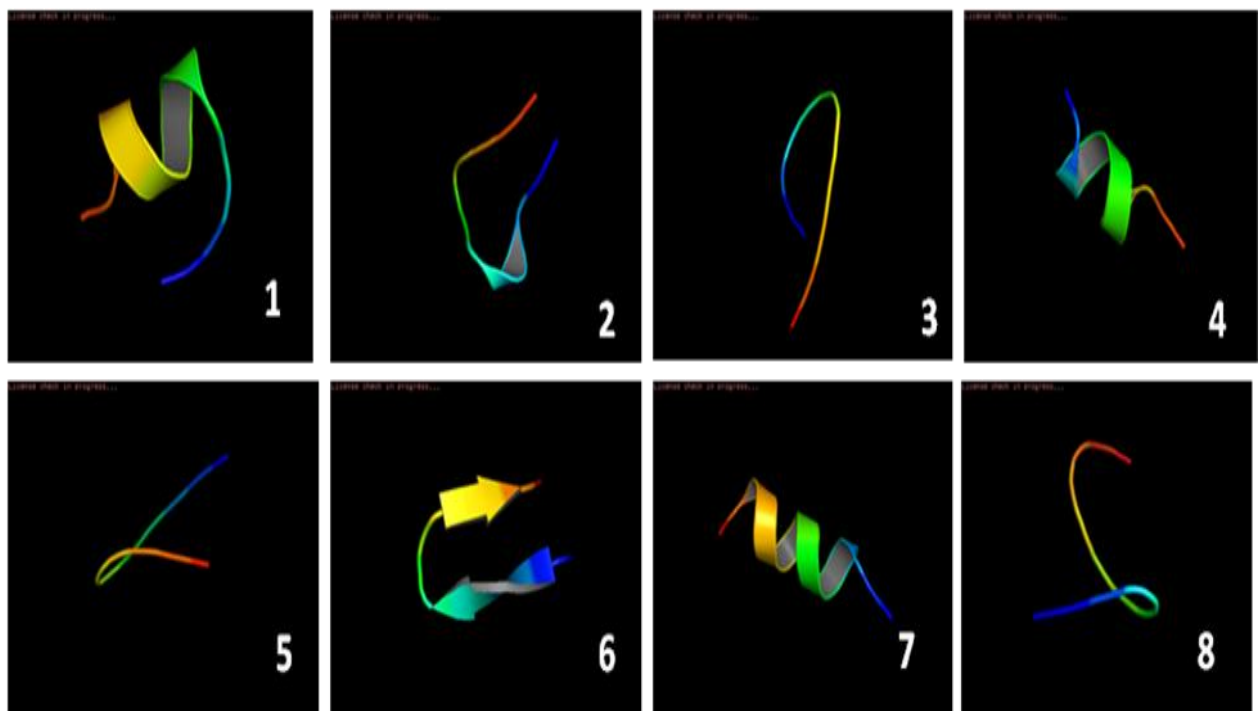


Figure 3. 3D structure of B cell epitopes predicted against MHC-II (1-8 as per table-6)

MHC-I and MHC-II receptor (docking)

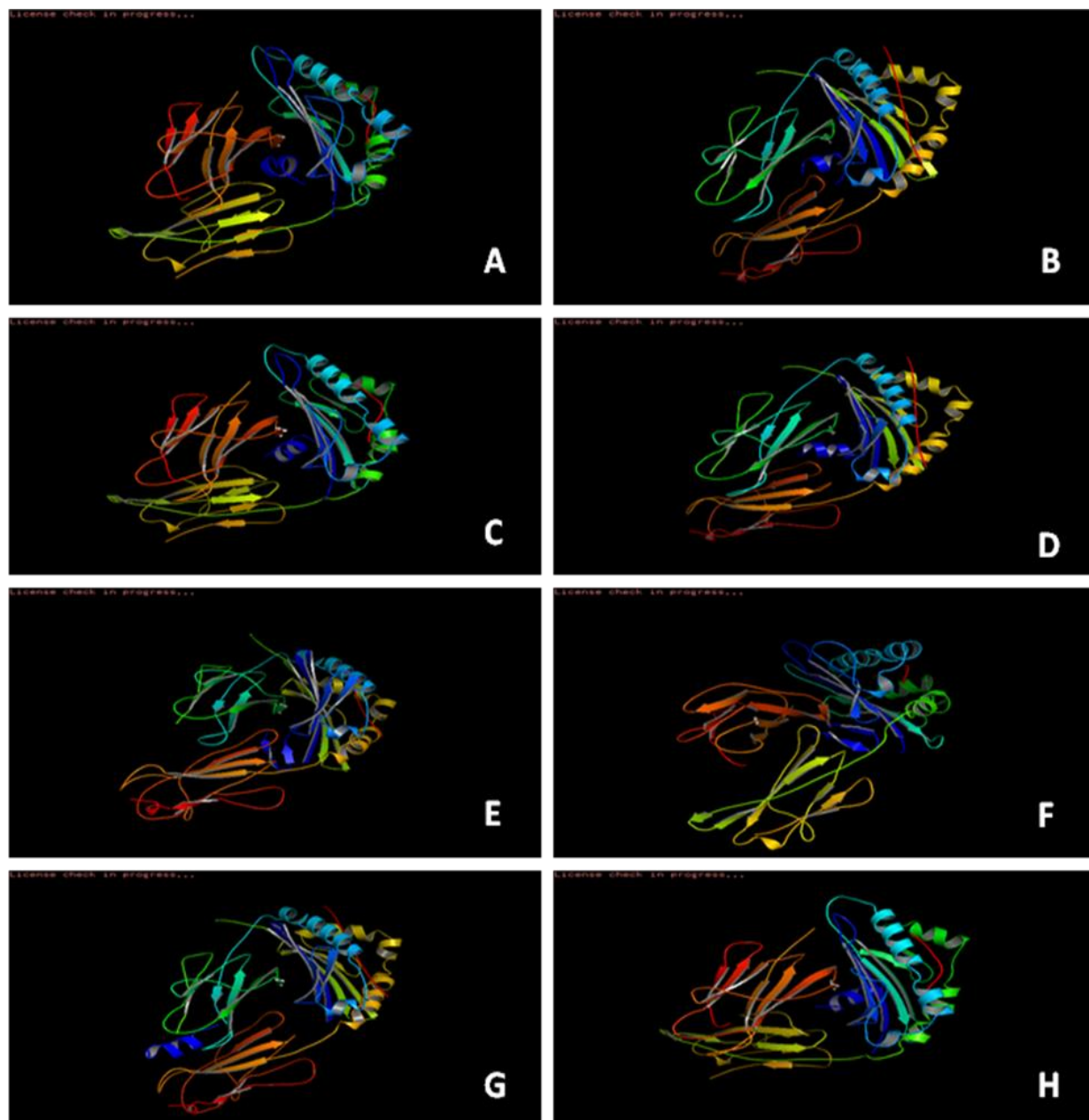


Figure 4. Docking results. A. Receptor MHC1-T cell epitope-1 B. Receptor MHC-II- T cell epitope-1 C. Receptor MHC I-T cell epitope-6 D. Receptor MHC-II- T cell epitope-6 E. Receptor MHC-II-B cell epitope 6 F. Receptor MHC-I- B cell epitope 6 G. Receptor MHC-II - B cell epitope 7. H. Receptor MHC-I - B cell epitope 7

Conclusion

A effective vaccine against Tb is the need of hour as patients are getting resistant to drugs. To solve the issue, in this study we have predicted 4 epitopes which could be used for designing of a potential vaccine against tuberculosis.

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