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Immunoinformatic Approach for Development of Synthetic Peptide Vaccine from Translationally Controlled Tumor Protein of *Brugia malayi*

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Abstract:
Lymphatic filariasis affects over 120 million people in 72 countries throughout the tropics and sub-tropics of Asia and other parts of the world. *Brugiamalayi* is a gonochoristic (male-female) filarial parasite of medical attention and is phylogenetically representative of other infectious nematodes. One of the appealing and important drug targets against the controlling of filarial population is Translationally controlled tumor protein of *Brugia malayi* as these proteins play important role in various developmental stages of nematode. The MHC molecules are cell surface proteins, which contributes significantly in host immune responses and contribution of MHC class in response to nearly all antigens and it give effects on precise sites. Projected MHC binding areasturns like red flags which are antigen specific and produce immune response against the parent antigen. Therefore, a minor fragment of antigen can make immune response against the entire antigen. This theme is applied in designing a subunit and synthetic peptide vaccines against filarial nematode *Brugia malayi*. In current study, we analysed translationally controlled tumor protein of *Brugia malayi* and these predicted antigenic epitopes shows a very potential drug targets to identify active sites of proteins, which form antibodies against infestation.

Keywords: Translationally controlled tumor protein, *Brugia malayi,* MHC, Antigenicity, Vaccine.

INTRODUCTION

It is estimated that more than 120 million peoples are infected with *Brugia malayi* infestation and an additionally one billion peoples at the risk of developing this dreadful disease of lymphatic filariasis caused by *Brugia malayi* which is widely prevalent in the tropical countries and causing significant morbidity in humans and other vertebrates throughout the tropics[22].

Mosquitos from the genus *Mansonia*and *Aedes* acts as intermediate host for filarial worm *Brugia malayi*. Filarial parasite harbours in the stomach, thorax muscles, and the proboscis of intermediate host i.e. in mosquito. When the mosquito bites to definitive hosts like human, monkey, domestic cats, or other carnivores then parasite enters through the wound from where it migrates to the lymphatic

system through the blood stream where it remains throughout its adult life[16]. The filarial nematode *B. malayi* causes different types of physical and mental disabilities, the physical disabilities include specifically located from the waist and below, due to the blockage of the lymphatic circulation results in the inflammation of the lymph nodes. The extreme inflammation and increase in the size of appendages is known as elephantiasis. Because of the possible mutilations, it can disturb a person's quality of life and damage their ability to work. Due tophysical deformity society out casting them, the mental disabilities primarily come in the form of depression[26].

Translationally-controlled tumor proteins (TCTPs) are expressed by a wide range of organisms, including yeast, protozoa, nematodes and mammals. TCTPs are highly expressed in nematode eggs both free living nematodes such as *Caenorhabditis elegans* and parasitic nematodes such as *Ostertagiaostertagi, Teladorsagiacircumcincta* and *Haemonchuscontortus etc.,* where as they appear to play an important role in egg production[21]. The calcium binding and to release histamine invitro is the accepted, proved and reported functions of the translationally controlled tumor protein (TCTP) from lymphatic filarial parasites[10]. In the recent studies it is also observed that the parasite derived TCTPs mayplay significant role in the heat stress adaptation[20,19]. Interestingly, expression of TCTPs is upregulated by a combination of stress conditions such as oxidative stress, heat shock, and exposure to heavy metals[30, 3, 19.]

Above discoveries suggests us that TCTPs may have an antioxidant function and have possible target candidate to design the synthetic drug against the *B. malayi.* Therefore, in the present study, we have analysed immune informatic properties of *B. malayi* derived TCTPs as a drug target.

METHODOLOGY

Thetranslationally controlled tumor protein sequence of *Brugia malayi* was retrieved from National Centre for Biotechnology Information (NCBI) for its further analysis and study. The retrieved sequence was computed to analyse its physical and chemical parameters with the help of the ProtPram and its different parameters were recorded.

Prediction and analysis of Physico-chemical properties of TCT proteins

The targeted protein sequence of Translationally controlled tumor protein from *Brugia malayi* were retrieve successfully from the NCBI database (Table 1) and the same protein was analysed for its different Physico-chemical properties like the molecular weight of protein, theoretical pI, amino acid composition of protein, atomic composition, extinction coefficient of protein[9;7;24] along with estimated half-life[5;31], instability index of protein[14], aliphatic index[15] and grand average of hydropathicity of TCT Protein (GRAVY)[18] were analysed by the ProtParam[8]. ProtParam computes various Physico-chemical properties that can be deduced from a protein sequence, these predicted Physico-chemical properties plays a very crucial role and taken into consideration while in the process of drug designing or drug development process.

The antigenic epitopes of Translationally controlled tumor protein of *Brugia malayi* is determined using different hydropathy and antigenicity plots like the, Hopp and Woods, Welling[32], Kyte-Doolittle Hydropathy[18], Kolaskar & Tongaonkar antigenicity[17] and Parker Hydrophilicity Prediction[25] etc.

By applying neural networks trained on C terminals of identified epitopes the Major Histocompatibility Complex (MHC) peptide binding of antigen protein is predicted. In the current immune informatics study predicted MHC/peptide binding of antigen protein is a log transformed value related to the IC50 values in nM units. The RANKPEP prediction of MHC-restricted ligands forecast the peptide binders to MHCI and MHCII molecules from protein sequences or sequence alignments using Position Specific Scoring Matrices (PSSMs). The Support Vector Machine (SVM) based method applied for prediction of promiscuous MHC class II binding peptides. The SVM has been proficient on the binary input of single amino acid sequence[11,12,13,27,4,23]. In addition, we expect those MHC ligands from whose C-terminal end is likely to be the result of proteosomal cleavage[2].

RESULTS AND DISCUSSION

Because of its presumed function, *B. malayi* TCTP appears to be a crucial and important protein for the existence of the parasite in the host. After retriving the sequence from NCBI its different Physico-chemical properties will be analysed (Table 2).

Table 1: **Translationally controlled tumor protein sequence of *Brugia malayi* retrived from the NCBI database.**

MLIFKDAFTDDELASDSFPMKLVDGLIWEFKGRQVVRREGEIQLAGANPSAEGEDGDEGSEECVER
GIDF
VLNHRLQEMNCYEDLATFKSYCKSFMKKVVELMQKNGKSEAEISEFKRKIQAWVVSLLSKDRFKQ
LQFFI
GERMAEGQGEGQVAVVEYRDEEDGEVPYLMLVKEALIEEKQ

Table 2: Predicted Physico-chemical properties of Translationally controlled tumor protein sequence of *Brugia malayi*.

Name of the Protein	Translationally controlled tumor protein
NCBI Reference Sequence	XP_001897741.1
Molecular weight:	20766.54
Theoretical pI:	4.62
Number of amino acids	181
Total number of negatively charged residues (Asp + Glu):	38
Total number of positively charged residues (Arg + Lys):	24
Estimated half-life:	The N-terminal of the sequence considered is M (Met).The estimated half-life is: 30 hours (mammalian reticulocytes, in vitro). >20 hours (yeast, in vivo). >10 hours (Escherichia coli, in vivo).
Instability index:	The instability index (II) is computed to be 30.37 This classifies the protein as stable
Aliphatic index:	78.07
Grand average of hydropathicity (GRAVY):	-0.485

The antigenic epitopes of Translationally controlled tumor protein of *Brugia malayi* is predicted by using different hydropathy and antigenicity tools[29,28].

Welling antigenicity prediction method

The Welling track computes antigenicity of protein by applying the technique of Welling et al., 1985. Previous methods assumed that antigenic regions were primarily hydrophilic regions on the facade of a protein. In contrast, the Welling method is established on the percentage of each amino acid create in known antigenic determinants compared to the percentage of the amino acids in the average composition of a protein (Fig. 1)[32].

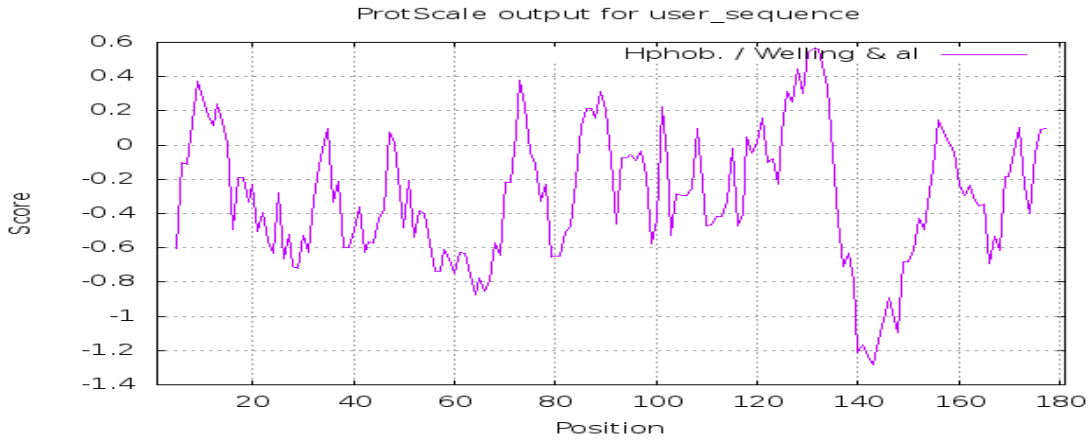

Figure 1: Hydrophobicity plot of TCTPs by Hphob/Wellinget al. scale. The Min score At Position: 143 with Score: -1.283 and Max score Score: 0.563 (max) at Position: 131 and 132 with Window size 09.

Kyte-Doolittle method to predicts regional hydropathy of proteins

The Hydropathy – Kyte-Doolittle track predicts regional hydropathy of proteins from their amino acid sequences, using the approach of Kyte[18]. Hydropathy values are assigned for all amino acids and are then averaged over a user-defined window. The average is plotted at the centre of the window. Residue hydropathy assignments are derived from water-vapor transfer free energies and the interior-exterior distribution of residue side-chains. (Fig. 2)

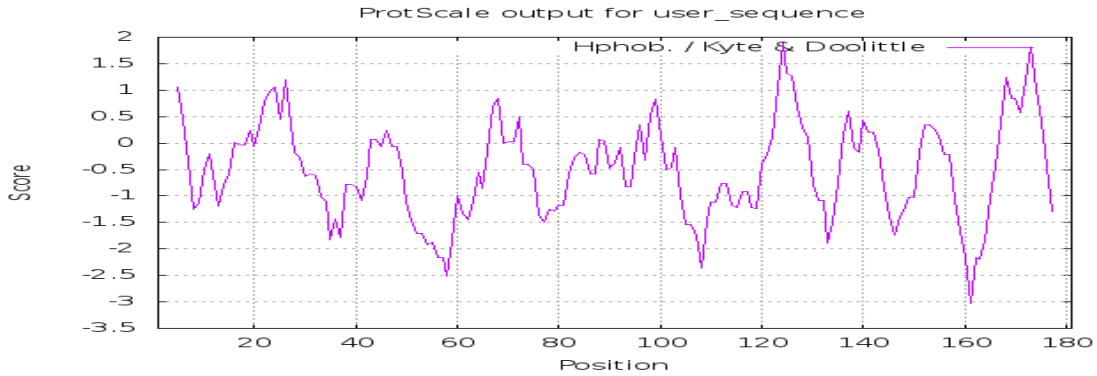

Figure 2: Hydrophobicity plot of TCTPs byHphob. / Kyte & Doolittle. The Min score At Position: 161with Score: -3.022 and Max score Score: 1.900 (max) at Position: 124 with Window size 09.

Parker hydrophilicity prediction

The Parker track practices the method of Parker et al., 1986, which is a hydrophilicity scale created on high-performance liquid chromatography (HPLC) withholding times of model synthetic peptides. Hydrophilicity arrangements have been applied significantly in the prediction of antigenic amino acid deposits. The Parker method applies an altered Hopp-Woods algorithm jointly with a different set of hydrophilicity possibilities (Fig. 3)[25].

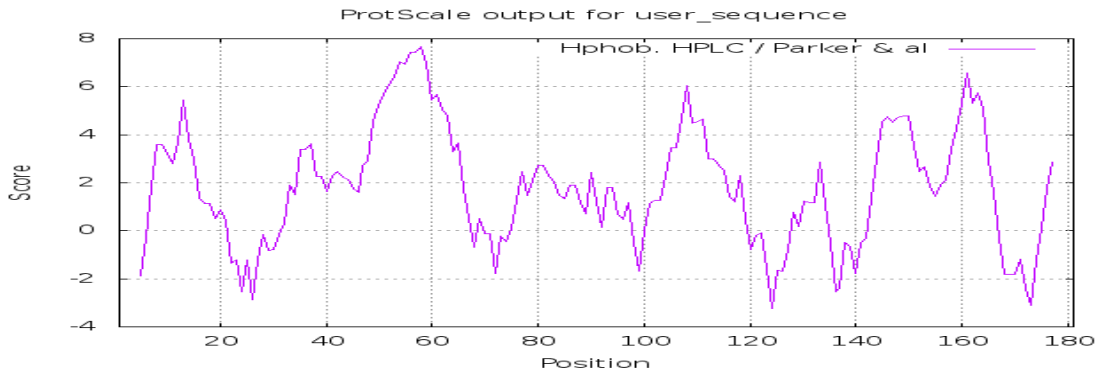

Figure 3: **Parker antigenecity plot of TCTPs from *B. Malayi*. The Min score At Position: 124 with Score: -3.244 and Max score Score: 7.678 (max) at Position: 58 with Window size 09.**

Kolaskar & Tongaonkar Antigenicity prediction method

This is a semi-empirical method which builds use of physicochemical properties of amino acid residues and their frequencies of existence in experimentally identified regions. Segmental epitopes were established to calculate antigenic determinants upon proteins. Use of this technique to a substantial number of proteins has revealed by the Kolaskar & Tongaonkar this method can predict antigenic determinants with about 75% accuracy which is improved than most of the established antigenicity prediction approaches (Fig.4).[17].

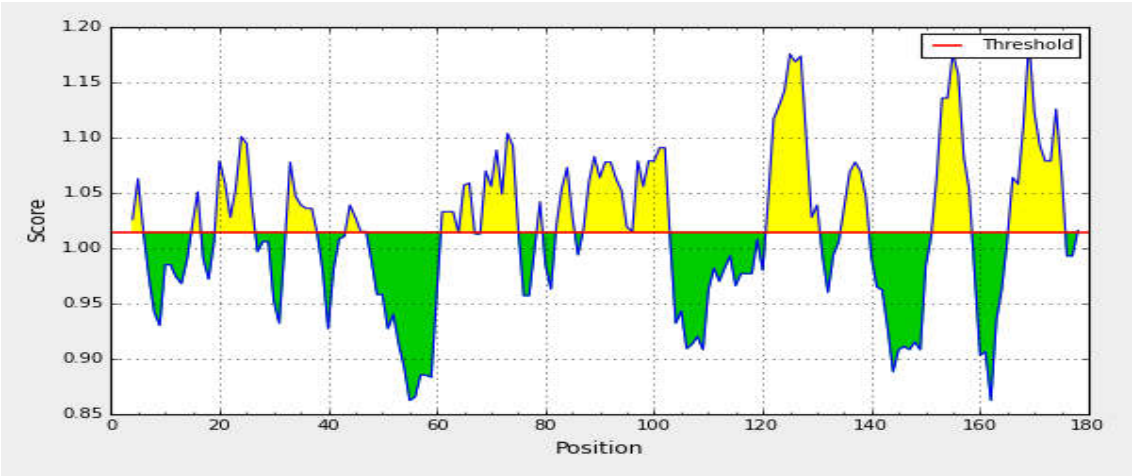

Figure 4: **Kolaskar & Tongaonkar Antigenicity prediction plot.**

Linear B-cell epitopes prediction by BepiPred

By using a combination of a hidden Markov model and a propensity scale method the Bepi Pred predicts the position of linear B-cell epitopes. The residues with scores beyond the threshold (default value is 0.35) are predicted to be part of an epitope and coloured in yellow on the graph and marked with "E" in the output table. The Evalues E of the scores are not changed by the selected threshold (Fig. 5)[16].

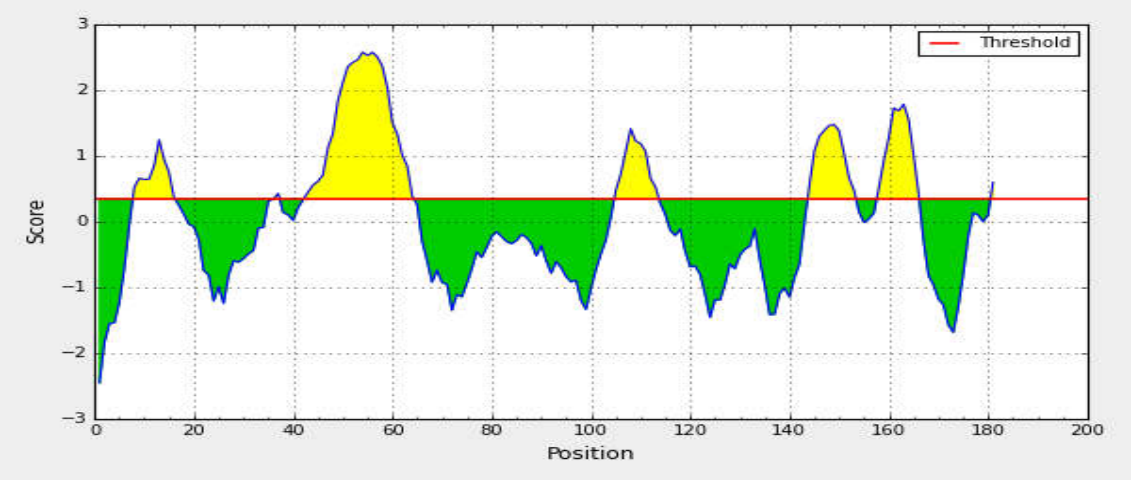

Figure 5: **Bepipred Linear Epitope Prediction of TCTPs from *B. Malayi.***

MHC class I and MHC class II binding peptides prediction.

By using Position Specific Scoring Matrix (PSSM) RANKPEP study of MHC class I and MHC class II binding peptides shows the binding of peptides to a number of different alleles. To almost all antigens MHC molecules are cell surface receptors proteins, which actively participate in host immune responses. From filarial nematode *B. malayi*we able to guess MHC-I peptide binders of translationally controlled tumor protein (TCTPs). We found predicted MHC-I peptide binders of protein for Matrix: 8mer_H2_Db.p.mtx, Consensus: QNWNCCTI, Optimal Score: 52.49 and binding threshold was 33.04;Matrix: 9mer_H2_Db.p.mtx, Consensus: YCVHNYDYM, Optimal Score: 43.648 and binding threshold 11.05; Matrix: 10mer_H2_Db.p.mtx, Consensus: SGYYNFFWCL, Optimal Score: 58.858, binding threshold 41.32; Matrix: 11mer_H2_Db.p.mtx, Consensus: CGVYNFYYCCY, Optimal Score: 79.495, Binding threshold 56.96 (Table 4) in addition the MHC-II peptide binders intended for Matrix: I_Ab.p.mtx, Consensus: YYAPWCNNA, Optimal Score: 35.632, Binding threshold 9.52; Matrix: I_Ad.p.mtx, Consensus: QMVHAAHAE, Optimal Score: 53.145, Binding threshold 7.10; Matrix: I_Ag7.p.mtx, Consensus: WYAHAFKYV, Optimal Score: 40.873, Binding threshold 7.54 for MHC II allele (Table) was confirmed and selected for the result.

Table 3: Promiscuous MHC ligands, having C-terminal ends are proteasomal cleavage sites of *B. malayi* (All rows highlighted in redrepresent predicted binders and A peptide highlighted in violet has a C-teminus predicted by the cleavage model used).

MHC-I Allele	Rank	Pos.	N	Sequence	C	Mw (Da)	Score	% Opt.
8mer_H2_Db.	1	77	HRL	QEMNCYED	LAT	1013.07	9.469	18.04 %
8mer_H2_Db.	2	147	MAE	GQGEGQVA	VVE	726.74	8.468	16.13 %
8mer_H2_Db.	3	151	QGE	GQVAVVEY	RDE	845.95	6.184	11.78 %
8mer_H2_Db.	4	59	GDE	GSEECVER	GID	889.95	5.986	11.40 %
8mer_H2_Db.	5	63	SEE	CVERGIDF	VLN	920.06	4.376	8.34 %
9mer_H2Db	1	95	CKS	FMKKVVELM	QKN	1106.44	9.463	21.68 %
9mer_H2Db	2	88	LAT	FKSYCKSFM	KKV	1122.37	6.615	15.16 %
9mer_H2Db	3	80	QEM	NCYEDLATF	KSY	1057.15	6.063	13.89 %
9mer_H2Db	4	71	IDF	VLNHRLQEM	NCY	1121.32	5.141	11.78 %
9mer_H2Db	5	91	FKS	YCKSFMKKV	VEL	1115.41	4.815	11.03 %
10mer_H2_Db	1	83	NCY	EDLATFKSYC	KSF	1158.3	11.86	20.15 %
10mer_H2_Db	2	102	VVE	LMQKNGKSEA	EIS	1087.25	10.385	17.64 %
10mer_H2_Db	3	111	KSE	AEISEFKRKI	QAW	1202.43	9.126	15.51 %
10mer_H2_Db	4	21	FPM	KLVDGLIWEF	KGR	1178.43	8.978	15.25 %
10mer_H2_Db	5	160	EYR	DEEDGEVPYL	MLV	1147.18	8.515	14.47 %

11mer_H2_Db	1	92	KSY	CKSFMKKVVEL	MQK	1293.64	6.802	8.56 %
11mer_H2_Db	2	44	EIQ	LAGANPSAEGE	DGD	997.04	5.335	6.71 %
11mer_H2_Db	3	69	RGI	DFVLNHRLQEM	NCY	1383.59	3.975	5.00 %
11mer_H2_Db	4	83	NCY	EDLATFKSYCK	SFM	1286.47	2.852	3.59 %
11mer_H2_Db	5	84	CYE	DLATFKSYCKS	FMK	1244.43	2.047	2.58 %

Table 4: Prediction of MHC-II ligands all rows highlighted in red represent predicted binders to the MHC-II Allele i.e., MHC-III_Ad, MHC-II I_Ag7, I_Ak.

MHC-II Allele	Rank	Pos.	N	Sequence	C	Mw (Da)	Score	% Opt.
MHC-II I_Ad	1	104	ELM	QKNGKSEAE	ISE	972.02	12.503	23.53 %
MHC-II I_Ad	2	154	GQV	AVVEYRDEE	DGE	1091.16	10.931	20.57 %
MHC-II I_Ad	3	150	GQG	EGQVAVVEY	RDE	975.07	10.482	19.72 %
MHC-II I_Ad	4	78	RLQ	EMNCYEDLA	TFK	1069.18	8.814	16.58 %
MHC-II I_Ad	5	98	FMK	KVVELMQKN	GKS	1070.3	7.921	14.90 %
MHC-II _Ag7	1	80	QEM	NCYEDLATF	KSY	1057.15	14.792	36.19 %
MHC-II _Ag7	2	63	SEE	CVERGIDFV	LNH	1019.19	8.605	21.05 %
MHC-II _Ag7	3	154	GQV	AVVEYRDEE	DGE	1091.16	7.65	18.72 %
MHC-II _Ag7	4	21	FPM	KLVDGLIWE	FKG	1031.25	7.506	18.36 %
MHC-II _Ag7	5	31	WEF	KGRQVVRRE	GEI	1109.3	7.323	17.92 %
MHC-II _Ak 1	1	24	KLV	DGLIWEFKG	RQV	1023.19	15.993	40.08 %
MHC-II _Ak 1	2	137	KQL	QFFIGERMA	EGQ	1080.28	13.719	34.38 %
MHC-II _Ak 1	3	55	EGE	DGDEGSEEC	VER	921.86	11.694	29.31 %
MHC-II _Ak 1	4	57	EDG	DEGSEECVE	RGI	977.97	10.805	27.08 %
MHC-II _Ak 1	5	163	DEE	DGEVPYLML	VKE	1018.2	10.651	26.69 %

CONCLUSION

Translationally controlled tumor protein of *Brugia malayi* peptides nonamers are also involved in some antigenic components too direct and empower the immune system. Translationally controlled tumor proteins are from a set of aligned peptides known to bind to a given MHC molecule as they are the predictor of MHC peptide binding molecules. The MHC class Iand class II molecules precisely bind to their specific epitopes. In host immune responses these surface proteins (MHC) dynamically join against nearly all the antigen and deliver protection to the host from infections. To understand the immune response against an antigen it is widely known that the whole protein is not essential toproduce the immune responses, but only the modest fragment of antigen is also sufficient to stimulate the immune responses against whole antigen. This phenomenon confirms that by increasing in the affinity of MHC binding peptides may result in improved immunogenicity of Translationally controlled tumor protein of *Brugia malayi*, hence may projected in silico drug designing might be supportive in the improvement of the more advanced highly analytical computational tools for the improved identification of the T cell epitopes. Accurate prediction antigenic proteins are also essential and remains very significant for the future synthetic peptide drug vaccine designing and development.

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