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Prediction of *Wuchereria Bancrofti* Troponin Antigenic Peptides: Application in Synthetic Vaccine Design to Counter Lymphatic Filariasis

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Abstract

Wuchereria bancrofti is a threadlike nematode one of the causative worm of lymphatic filariasis in which the lymphatic and genital organs get disabled either temporarily or permanently; till date no effective drug or vaccine has been discovered to treat lymphatic filariasis. In this analysis we have predicted antigenic peptides from *Wuchereria bancrofti* troponin *protein* for synthetic peptide vaccine design against lymphatic filariasis because with a single protein subunit immune response can be generated in large population. Analysis shows predicted epitopes of *Wuchereria bancrofti troponin protein* are important determinant for protection against lymphatic filariasis. In this assay we have analysed the binding affinity of *Wuchereria bancrofti troponin protein* having 136 amino acids, which shows 128 nonamers. In this research work, we predicted CTL-epitopes by two different methods namely SVM (Support Vector Machine) and ANN (Artificial Neural Network), SVM based prediction shown sixteen valid epitopes having optimal score of 1.129 at cut off 0.36 whereas ANN based prediction shown thirty-one valid epitopes having optimal score of 1.000 at cut off 0.51. We also predicted cascade SVM based TAP binders and four potential antigenic epitopes as, 31-LRKLIRK-37, 49-DEFCALVYTVANT-61, 87-SRPTLKALLKE-97, 108-EAAVDE-113 (optimal propensity 1.223) predicted on the basis of highest local hydrophilicity; in addition to this we also have experimentally predicted tertiary structure of the two longest potential epitopes those can aid our understanding in sequence-structure-function relationship of *Wuchereria bancrofti troponin protein towards synthetic peptide vaccine design*. Thus a small fragment of troponin protein can produce immune response against activity of *Wuchereria bancrofti*. This approach can be applied for designing subunit and synthetic peptide vaccines.

Keywords: Lymphatic filariasis; Parasitic disease; Antigenic peptides; MHC; SVM; ANN; CTL; Nonamers; Synthetic peptide vaccines

Abbrevations: MHC: Major Histocompatibility Complex; CTL: Cytotoxic T lymphocytes; TAP: Transporter associated with Antigen Processing; SVM: Support Vector Machine; ANN: Artificial Neural Network

Introduction

Lymphatic filariasis

Lymphatic filariasis also known Elephantiasis is a parasitic infection caused by filarial roundworm, *Wuchereria bancrofti*. The infection is usually acquired in childhood, its indication of the existence occur later in life, causing temporary or permanent disability of an infected organ, causes severe damage and painful swelling, disfiguring swelling of the legs and genital organs is a classic symptom of late disease stage. In many tropical countries, lymphatic filariasis has a major social and economic impact, though World Health Organization is trying to eliminate it completely by the year 2020 yet there is no effective drug or vaccine has been invented to treat/prevent Elephantiasis (WHO, lymphatic Filariasis).

Pathogen transmission

An Infected mosquito deposits larvae on the individual's skin while biting and larvae enter Wound. The Larvae migrates into lymphatic system where they grow, Adult male worms are about 3-4 cm long while female worms are 8-10 cm long. When male and female worms mate they form nests; these nests cause blockages in the human lymphatic system resulting symptoms like swelling and fever. Female worm produces microscopic worms called microfilaria. When mosquito bites to an infected individual, ingests microfilaria with blood and infects to healthy individual, Microfilaria develops into adult worm over a week and the cycle continues [WHO, lymphatic Filariasis].

Strategy

This approach is based on the phenomenon of cross-protection [1] hereby an individual infected with a mild strain of pathogen possess immunity against more severe strain of the same pathogen. Body proteins are necessary for its production in or on all food commodities. An exemption from the requirement of a tolerance is established for residues of the drugs or chemicals. The lymphatic system is an important component of the body's immune system, it include several centres of initial infection so the immune response can be generated in the lymph organ even with single antigen subunit.

MHC class binding peptides

The new paradigm in vaccine design is emerging; following essential discoveries in immunology and development of new CTL binding peptides prediction tools [2]. MHC molecules are cell surface glycoproteins, which take active part in host immune reactions. The

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involvement of MHC class-I in response to almost all antigens and considering its binding affinity with peptides of 9 amino acids, we have predicted the 9 amino acid long epitopes from extracted data MHCBN Comprehensive data base of MHC-I and MHC-II binding as well as non-binding epitopes. MHC molecules have been well characterized in terms of their role in immune reactions. They bind to several peptide fragments generated after proteolytic cleavage of antigen [3]. This binding act like red flags for specific antigen and generates immune response against the parent antigen. So an antigen from *Wuchereria bancrofti* troponin subunit can induce immune response against *Wuchereria bancrofti* activity. CTL epitopes are most suitable for subunit vaccine development because with single epitope, the immune response can be generated in large population. MHC peptide complexes will be translocated on the surface of antigen presenting cells (APCs). This theme is implemented in developing subunit and synthetic peptide vaccines [4-7]. One of the important problems in subunit vaccine design is to search for antigenic regions in an antigen protein [8] that can stimulate T-cells called T-cell epitopes. In literature, fortunately, a large amount of data about such peptides is available. Previously and presently, a number of databases have been developed to provide comprehensive information related to T-cell epitopes [9-13].

Materials and Methods

Protein sequence analysis

Wuchereria bancrofti troponin protein is a diagnostic antigen against lymphatic filariasis. The antigenic protein sequence of *W. bancrofti* troponin antigen protein was analyzed to study the antigenicity solvent accessible regions and MHC class binding peptide, which allows potential drug targets to predict active sites against Lymphatic filariasis.

A *Wuchereria bancrofti* troponin (gi- 373938659) protein sequence is 136 amino acids long as-MFDRGKQGYIMATQIGQIMHAMEQDF DEKQLRKLIRKFDADGSGKLEFDEFCALVYTVANTVDKETLQKEL REAFRLFDKEGNGYISRPTLKALLKEIADDLSDEQLEAAVDEIDED GSGKIEFEEFWELMAGDAD

Antigenicity prediction

Antigenicity Prediction program results those segments from *Wuchereria bancrofti* troponin antigen protein that are likely to be antigenic by eliciting an antibody response. Antigenic epitopes are determined using the Gomase, (2007), Hopp and Woods, Welling, Parker, B-EpiPred Server and Kolaskar and Tongaonkar antigenicity methods [14-18].

Protein secondary structure prediction

The important concepts in secondary structure prediction are identified as: residue conformational propensities, sequence edge effects, hydrophobicity moments, insertions and Deletions positions in aligned homologous sequence, moments of conservation, auto-correlation, residue ratios, secondary structure feedback effects and filtering [19,20].

Finding the location in solvent accessible regions

Finding the location in solvent accessible regions in protein, type of plot determines the hydrophobic and hydrophilic scales and it is utilized for prediction. This may be useful in predicting membrane-spanning domains, potential antigenic sites and regions that are likely exposed on the protein surface [21-41].

CTL epitope prediction

The CTL Epitopes of *Wuchereria bancrofti* troponin are obtained from MHCBN comprehensive database of MHC binding and non-binding peptides using two different methods firstly with Support Vector Machine based method (Cut off is 0.36) and then by Artificial Neural Network based method (Cut off is 0.51). In this work predicted MHC-Peptide binding is a log-transformed value related to the IC50 values in nM units.

The average accuracy of Support Vector Machine (SVM) based epitope prediction method is ~76% at cut off 0.36. SVM has been trained on the binary input of single amino acid sequence. In Case of Artificial Neural Network ANN based epitope prediction method the average accuracy is ~74% at the cut off score 0.51 [2].

TAP binding prediction

TAP (Transporter associated with Antigen Peptide) play an important role in transportation of MHC-Peptide complexes, which elicits the immune response for clearing various intracellular infections. The prediction of TAP binding peptides is crucial in identifying the MHC class-I restricted T cell epitopes. The Prediction is based on cascade SVM, using properties of amino acid sequence at correlation coefficient of 0.88 as per Jack-Knife validation test [42].

Peptide structure prediction

Peptide Structure prediction is a challenge, which would aid our understanding of sequence–structure–function relationships towards synthetic peptide vaccine design. Peptide Structure prediction depends on the complexity and the accuracy of the models used to represent them. We have used Hidden Markov Model derived structural alphabet (SA) based tool that discretizes peptide experimental conformation as series of overlapping fragments of four residues length. The predicted structure can be considered to have an average accuracy of ~1.1A root-mean-square deviation (RMSd) [43-46].

Result and Interpretation

Antigenic peptides prediction

In this assay we predicted the antigenic determinants by finding the area of highest local hydrophilicity. The Hopp & Woods scale was designed to predict the locations of antigenic determinants in linear

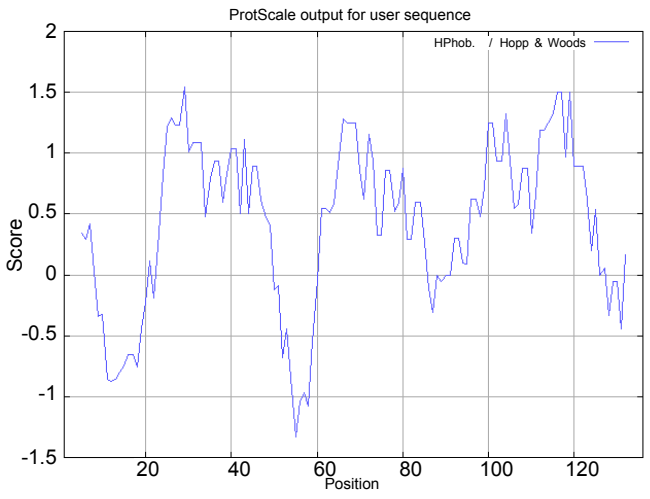


Figure 1: Hydrophobicity plot of Hopp and Woods (1981) of *Wuchereria bancrofti* troponin.

antigen protein sequence, assuming that the antigenic determinants would be exposed on the protein surface and thus would be located in hydrophilic regions (Figure 1). Its values are derived from the transfer-free energies for amino acid side chains between ethanol and water. Welling antigenicity plot gives value as the log of the quotient between percentage in a sample of known antigenic regions and percentage in average proteins (Figure 2). We also studied B-EpiPred Server, Parker, Kolaskar and Tongaonkar antigenicity methods and the predicted antigenic fragments can bind to MHC molecule is the first bottlenecks in vaccine design (Figures 3-6).

Secondary alignment

The Robson and Garnier method applied for secondary structure prediction of the *Wuchereria bancrofti troponin protein*. Each residue have specific assigned value for alpha helix (Shown in Red), beta sheet (Shown in Blue) and coil (Shown in Pink) using a window of 7 residues (Figure 7). Using these information parameters, the likelihood of a given residue assuming each of the four possible conformations alpha, beta, reverse turn or coils calculated and the conformation with the largest likelihood is assigned to the residue.

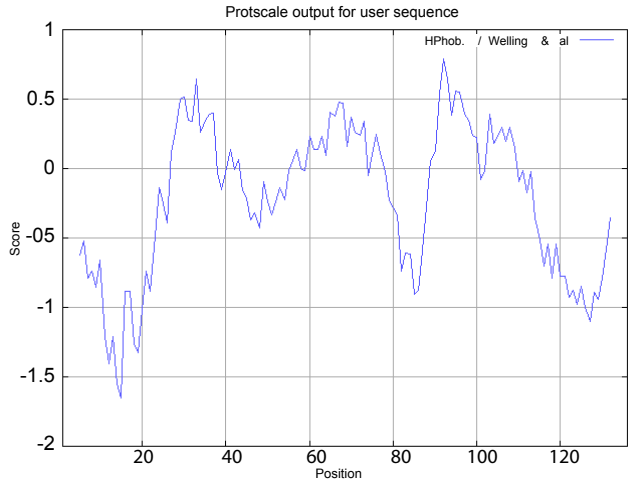


Figure 2: Hydrophobicity plot of Welling et al. (1985) of *Wuchereria bancrofti* troponin.

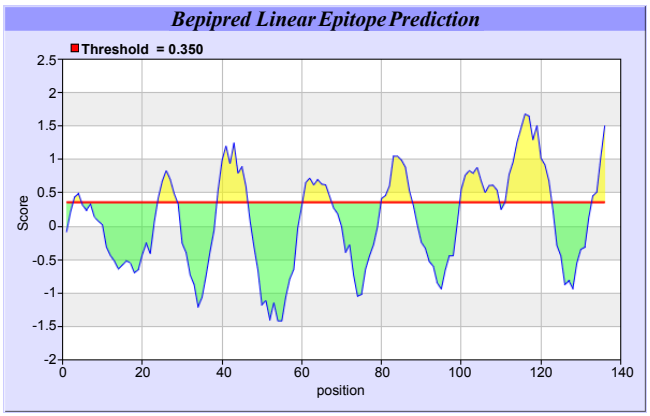


Figure 3: B-cell epitopes are the sites of molecules that are recognized by antibodies of the immune system of the *Wuchereria bancrofti* troponin.

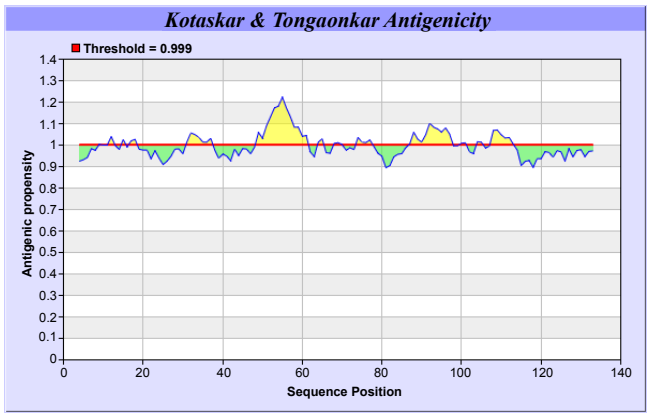


Figure 4: Kolaskar and Tongaonkar antigenicity are the sites of molecules that are recognized by antibodies of the immune system for the *Wuchereria bancrofti* troponin.

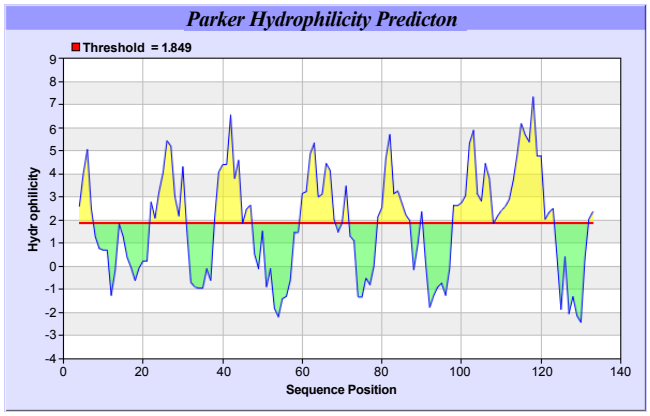


Figure 5: Hydrophobicity plot of HPLC / Parker et al. (1986) of *Wuchereria bancrofti* troponin.

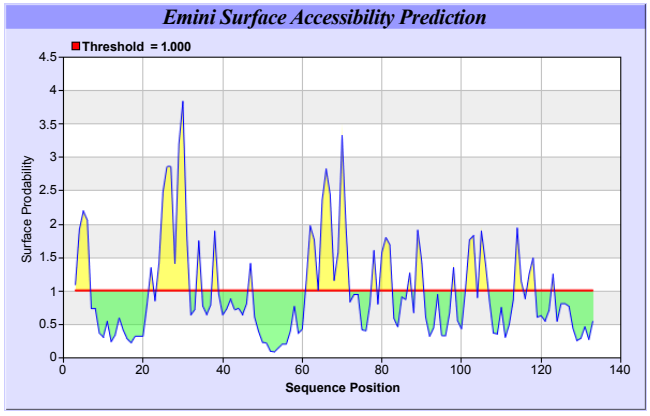


Figure 6: Hydrophobicity plot of Emini Surface Accessibility Prediction (1990) of *Wuchereria bancrofti* troponin.

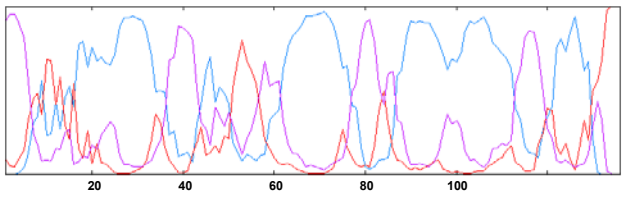
Solvent accessible regions

Solvent accessible scales for delineating hydrophobic and hydrophilic characteristics of amino acids and scales are developed for predicting potential antigenic sites of globular proteins, which

are likely to be rich in charged and polar residues. It was shown that *Wuchereria bancrofti troponin protein* is hydrophobic in nature and contains segments of low complexity and high-predicted flexibility (Figures 8-28).

Prediction of MHC binding peptides

MHC molecules are cell surface glycoproteins, which take active part in host immune reactions and involvement of MHC class-I and



**Red: helix, Blue: Sheet, Pink: Coil*

Figure 7: Secondary structure plot of the *Wuchereria bancrofti* troponin.

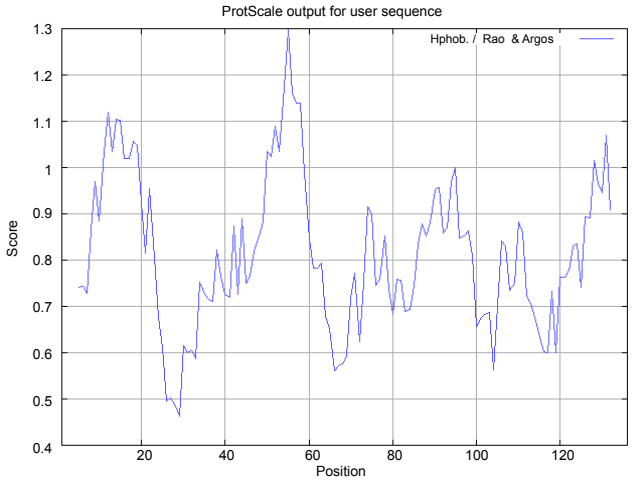


Figure 8: Hydrophobicity plot of Rao and Argos (1986) for the *Wuchereria bancrofti* troponin.

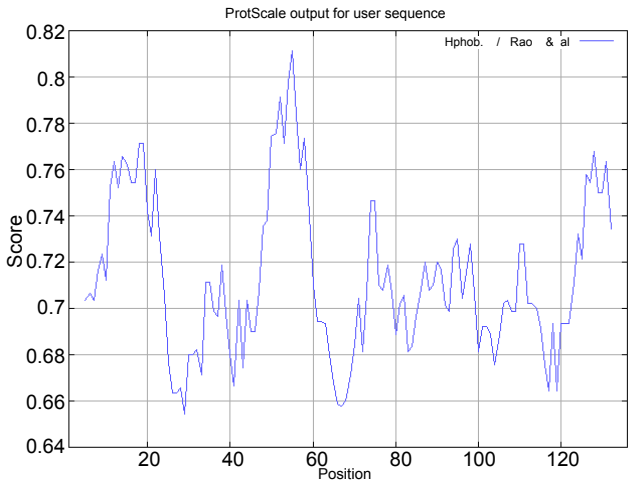


Figure 9: Hydrophobicity plot of Rose et al. (1985) for the *Wuchereria bancrofti* troponin.

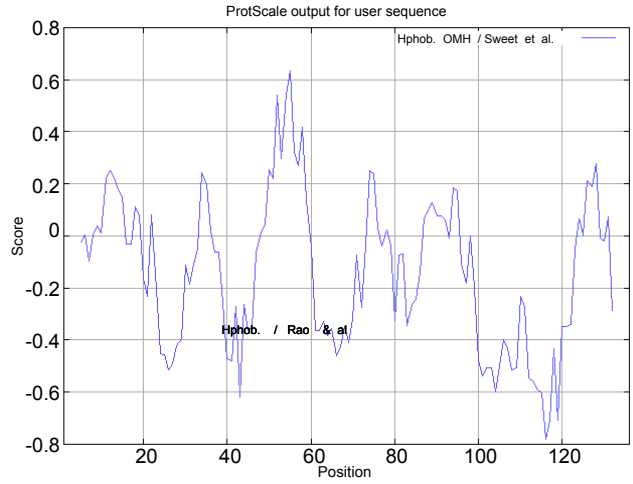


Figure 10: Hydrophobicity Sweet plot of OMH for the *Wuchereria bancrofti* troponin.

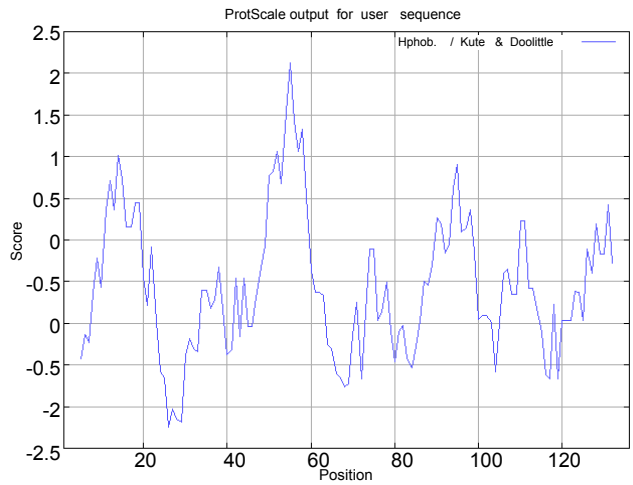


Figure 11: Hydrophobicity plot of Kyte and Doolittle (1982) for the *Wuchereria bancrofti* troponin.

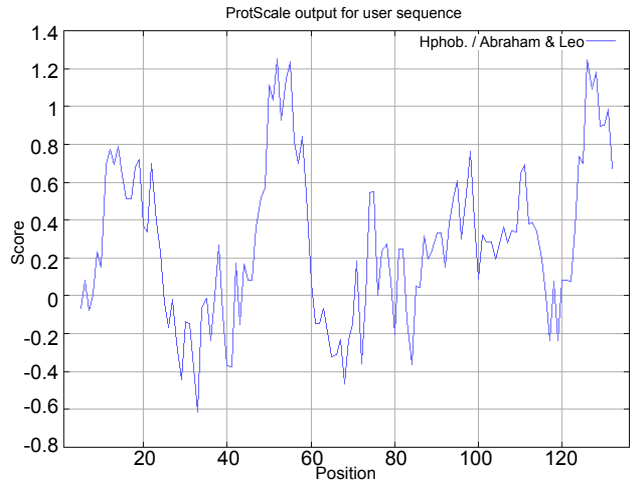


Figure 12: Hydrophobicity plot of Abraham and Leo (1987) for the *Wuchereria bancrofti* troponin.

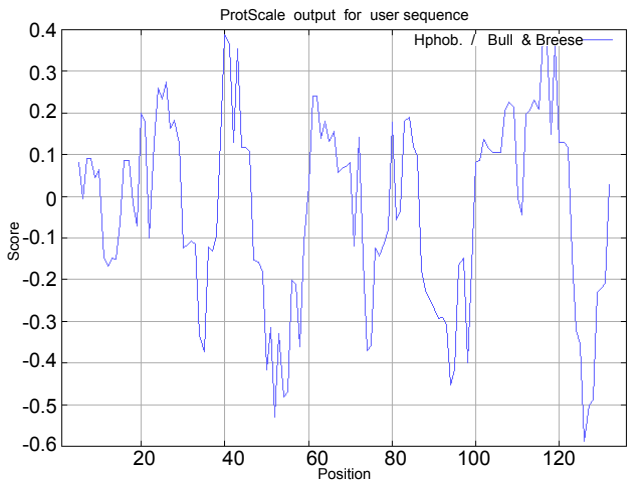


Figure 13: Hydrophobicity plot of Bull and Breese (1974) for the *Wuchereria bancrofti* troponin.

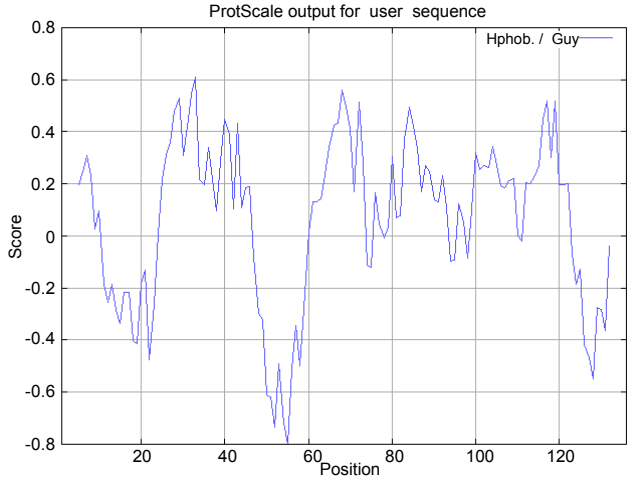


Figure 14: Hydrophobicity plot of Guy (1985) for the *Wuchereria bancrofti* troponin.

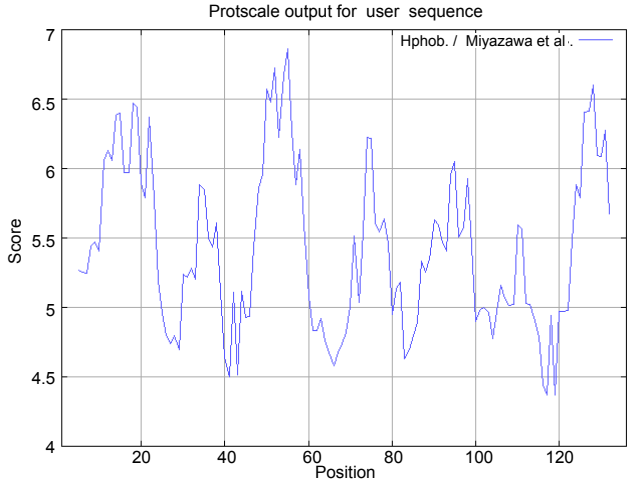


Figure 15: Hydrophobicity plot of Miyazawa and Jernigen (1985) for the *Wuchereria bancrofti* troponin.

MHC II in response to almost all antigens so these MHC binding peptides are sufficient for eliciting the desired immune response. In analysis we determined the CTL binding regions, TAP binding regions and several potential antigenic epitopes. In this study we predicted the binding affinity of *Wuchereria bancrofti* troponin protein having 136 amino acids, which shows number of peptides. The CTL epitope prediction is based on an elegant machine learning technique Support Vector Machine (SVM) and Artificial Neural Network (ANN). SVM has been trained on the binary input of single amino acid sequence. In this assay we have predicted the binding affinity of *Wuchereria bancrofti* troponin protein sequence having 136 amino acids, which shows 128 nonamers.The SVM based CTL epitopes, 86-ISRPTLKAL, 46-LEFDEFCAL, 84-GYISRPTLK, 122-IEFEEFWEL, 47-EFDEFCALV, 29-KQLRKLIRK, 80-KEGNGYISR, 87-SRPTLKALL, 69-KELREAFRL, 50-EFCALVYTV, 72-REAFRLFDK, 2-FDRGKQGYI, 11-MATQIGQIM, 4-RGKQGYIMA, 90-TLKALLKEI, 123-EFEEFWELM (optimal score is 1.129); the ANN based CTL epitopes recorded are, 60-NTVDKETLQ, 12-ATQIGQIMH, 36-RKFDADGSG, 85-YISRPTLKA, 109-AAVDEIDED, 76-RLFDKEGNG, 20-HAMEQDFDE, 69-KELREAFRL, 94-LLKEIADDL, 32-RKLIRKF-

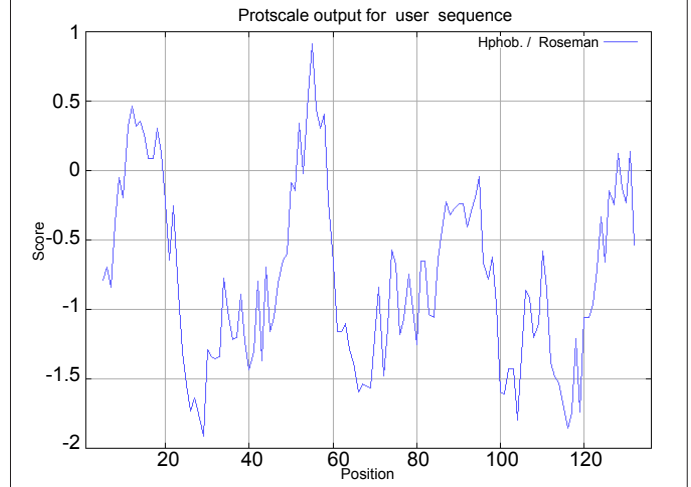


Figure 16: Hydrophobicity plot of Roseman (1988) for the *Wuchereria bancrofti* troponin.

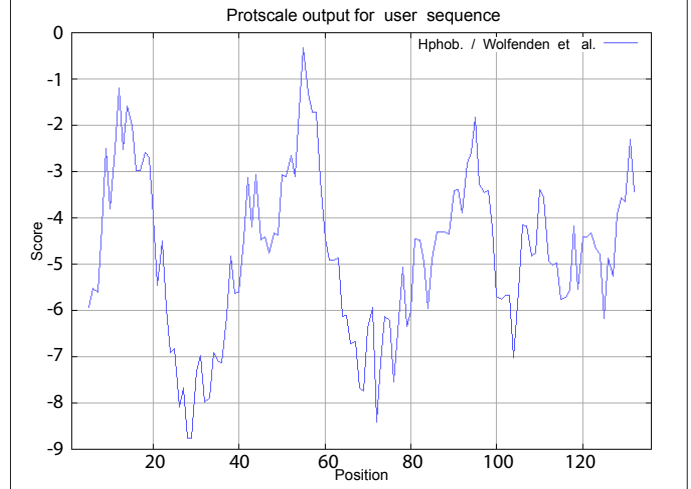


Figure 17: Hydrophobicity plot of Wolfenden et al. (1981) for the *Wuchereria bancrofti* troponin.

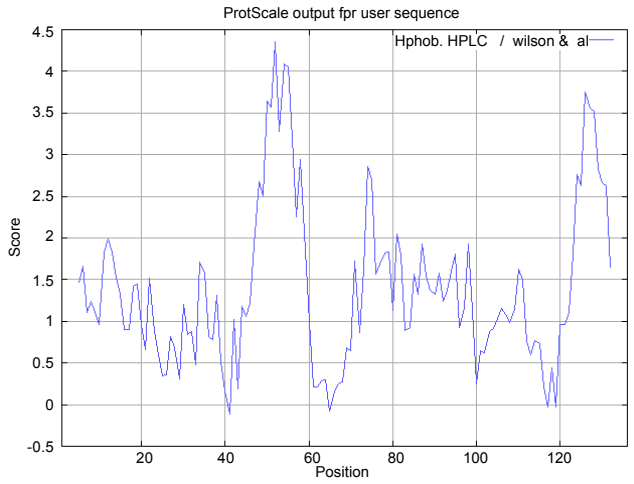


Figure 18: Hydrophobicity Wilson et al. (1981) plot of HPLC for the *Wuchereria bancrofti* troponin.

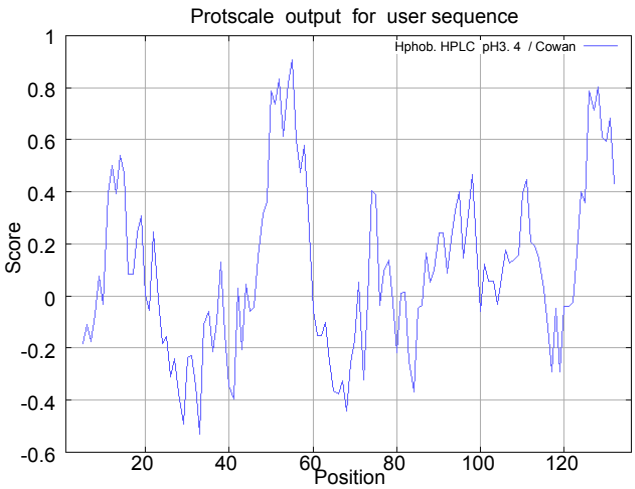


Figure 19: Hydrophobicity Cowan and Whittaker (1990) plot of HPLC pH 3.4 for the *Wuchereria bancrofti* troponin.

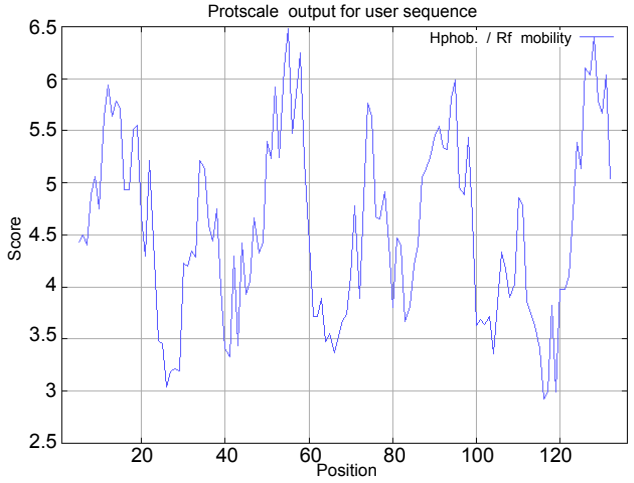


Figure 20: Hydrophobicity plot of RF mobility for the *Wuchereria bancrofti* troponin.

DA, 50-EFCALVYTV, 67-LQKELREAF, 112-DEIDEDGSG, 54-LVYTVANTV, 98-IADDLSDEQ, 41-DGSGKLEFD, 89-PTLKALLKE, 79-DKEGNGYIS, 82-GNGYISRPT, 104-DEQLEAAVD, 100-DDLSDEQLE, 40-ADGSGKLEF, 62-VDKETLQKE, 27-DEKQLRKLI, 90-TLKALLKEI, 108-EAAVDEIDE, 42-GSGKLEFDE, 52-CALVYTVAN, 43-SGKLEFDEF, 61-TVDKETLQK, 53-ALVYTVANT, which represented predicted binders from *Wuchereria bancrofti* troponin protein (Tables 1 and 2). In addition to the CTL epitopes we also have predicted several high Affinity TAP binders as 102-LSDEQLEAA, 23-EQDFDEKQL, 26-FDEKQLRKL, 12-ATQIGQIMH, 92-KALLKEIAD, 73-EAFRLFDKE, 98-IADDLSDEQ, 57-TVANTVDKE, 82-GNGYISRPT,125-EEFWELMAG, 35-IRKFDADGS, 9-YIMATQIGQ, 21-AMEQDFDEK, 74-AFRLFDKEG, 95-LKEIADDLS, 44-GKLEFDEFC, 11-MATQIGQIM, 36-RKFDADGSG, 94-LLKEIADDL, 66-TLQKELREA, 51-FCALVYTVA, 48-FDEFCALVY, 124-FEEFWELMA, 33-KLIRKFDAD, 37-KFDADGSGK, 45-KLEFDEFCA, 22-MEQDFDEKQ, 99-ADDLSDEQL, 53-ALVYTVANT, 96-KEIADDLSD, 63-DKETLQKEL, 70-ELREAFRLF, 90-TLKALLKEI, 10-IMATQIGQI, 88-RPTLKALLK, 24-QDFDEKQLR, 123-EFEEFWELM, 52-CALVYTVAN, 120-GKIEFEEFW, 14-QIGQ-

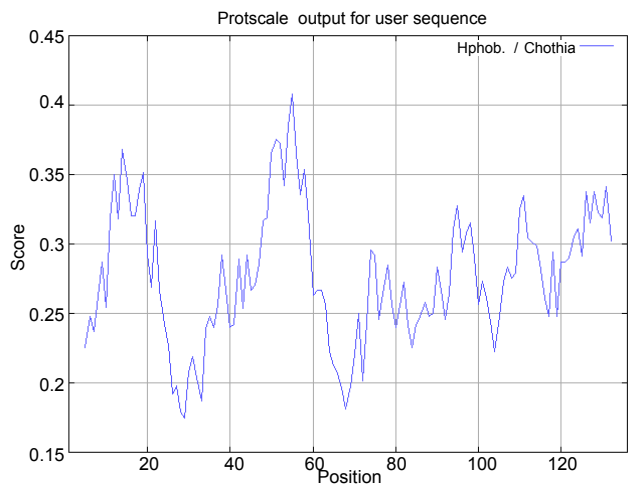


Figure 21: Hydrophobicity plot of Chothia (1976) for the *Wuchereria bancrofti* troponin.

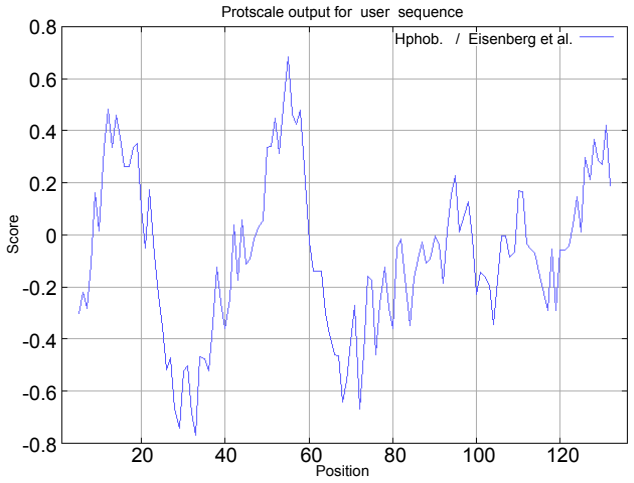


Figure 22: Hydrophobicity plot of Eisenberg et al. (1984) for the *Wuchereria bancrofti* troponin.

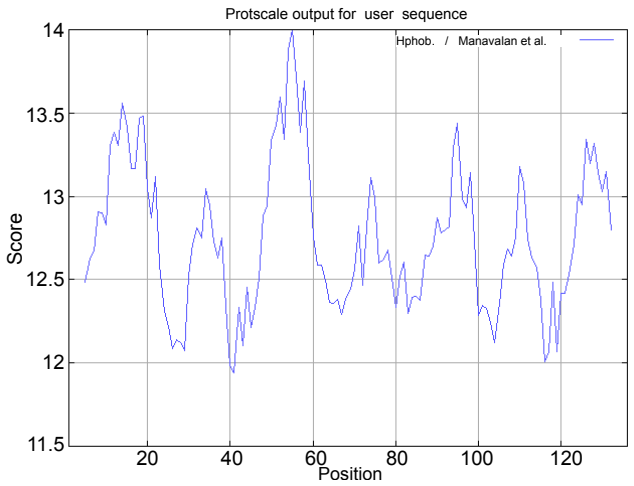


Figure 23: Hydrophobicity plot of Manavalan and Ponnuswamy (1978) for the *Wuchereria bancrofti* troponin.

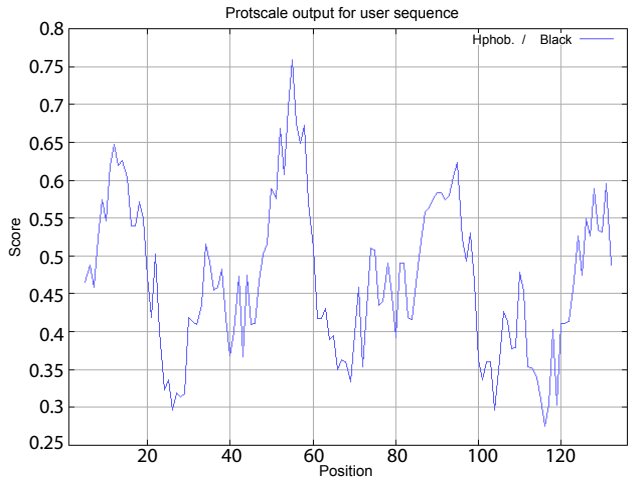


Figure 24: Hydrophobicity plot of Black and Mould (1991) for the *Wuchereria bancrofti* troponin.

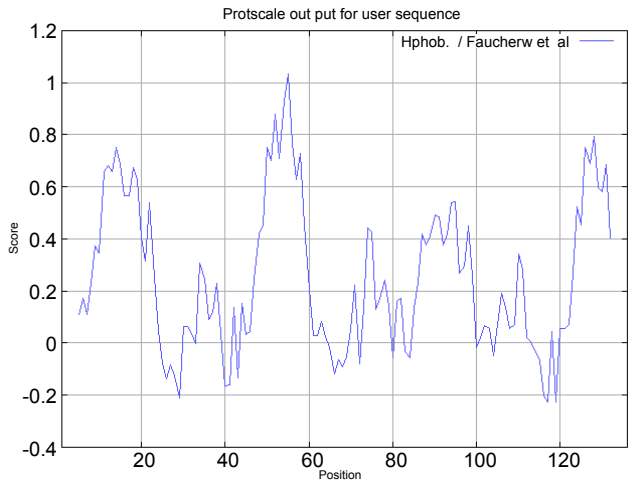


Figure 25: Hydrophobicity plot of Fauchere and Pliska (1983) for the *Wuchereria bancrofti* troponin.

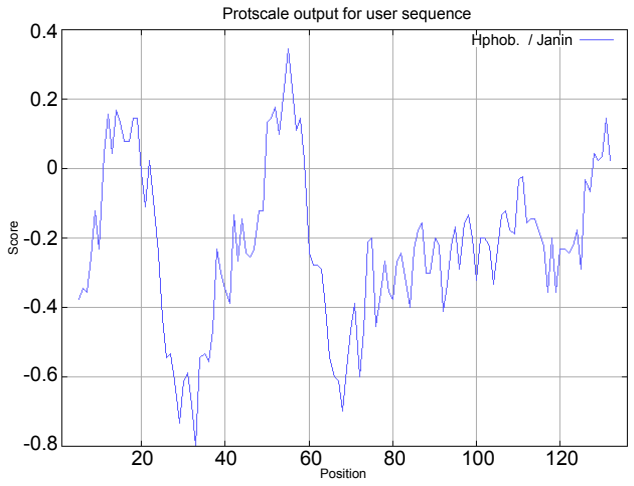


Figure 26: Hydrophobicity plot of Janin (1979) for the *Wuchereria bancrofti* troponin.

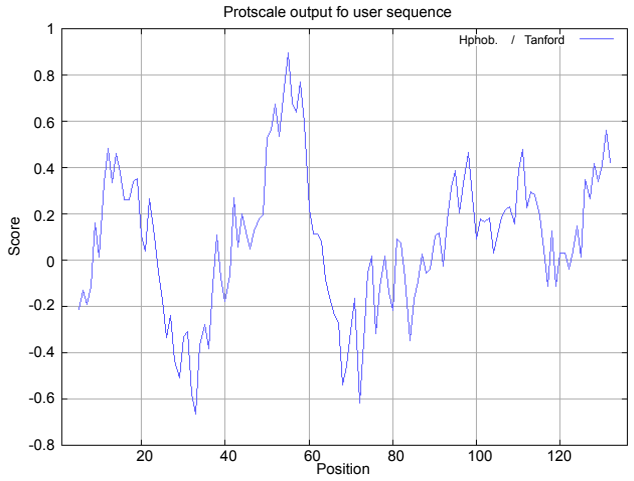


Figure 27: Hydrophobicity plot of Tanford (1962) for the *Wuchereria bancrofti* troponin.

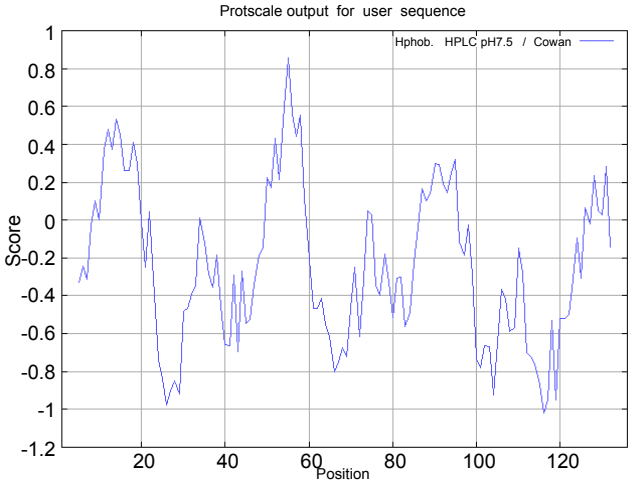


Figure 28: Hydrophobicity Cowan and Whittaker (1990) plot of HPLC pH 7.5 for the *Wuchereria bancrofti* troponin.

IMHAM, 116-EDGSGKIEF, these TAP binders are important in generating immune response (Table 3). The optimal propensity for the *Wuchereria bancrofti* troponin *protein* found is 1.223 (Figure 4). All residues having above 1.0 propensity are always potentially antigenic

Peptide rank	Start position	Sequence	Score
1	86	ISRPTLKAL	1.129
2	46	LEFDEFCAL	0.992
3	84	GYISRPTLK	0.971
4	122	IEFEEFWEL	0.912
5	47	EFDEFCALV	0.803
6	29	KQLRKLIRK	0.702
7	80	KEGNGYISR	0.679
8	87	SRPTLKALL	0.664
9	69	KELREAFRL	0.654
10	50	EFCALVYTV	0.594
11	72	REAFRLFDK	0.578
12	2	FDRGKQGYI	0.523
13	11	MATQIGQIM	0.405
14	4	RGKQGYIMA	0.383
15	90	TLKALLKEI	0.368
16	123	EFEEFWELM	0.363

Table 1: SVM based Predicted CTL-Epitopes of *Wuchereria bancrofti* troponin protein.

Peptide rank	Start position	Sequence	Score
1	60	NTVDKETLQ	1.000
2	12	ATQIGQIMH	0.990
3	36	RKFDADGSG	0.990
4	85	YISRPTLKA	0.990
5	109	AAVDEIDED	0.990
6	76	RLFDKEGNG	0.970
7	20	HAMEQDFDE	0.930
8	69	KELREAFRL	0.930
9	94	LLKEIADDL	0.930
10	32	RKLIRKFDA	0.910
11	50	EFCALVYTV	0.910
12	67	LQKELREAF	0.910
13	112	DEIDEDGSG	0.900
14	54	LVYTVANTV	0.870
15	98	IADDLSDEQ	0.860
16	41	DGSGKLEFD	0.830
17	89	PTLKALLKE	0.830
18	79	DKEGNGYIS	0.800
19	82	GNGYISRPT	0.800
20	104	DEQLEAAVD	0.800
21	100	DDLSDEQLE	0.760
22	40	ADGSGKLEF	0.720
23	62	VDKETLQKE	0.710
24	27	DEKQLRKLI	0.660
25	90	TLKALLKEI	0.630
26	108	EAAVDEIDE	0.630
27	42	GSGKLEFDE	0.570
28	52	CALVYTVAN	0.550
29	43	SGKLEFDEF	0.540
30	61	TVDKETLQK	0.540
31	53	ALVYTVANT	0.530

Table 2: ANN based Predicted CTL-Epitopes of *Wuchereria bancrofti* troponin protein.

Peptide rank	Start position	Sequence	Score
1	102	LSDEQLEAA	8.641
2	23	EQDFDEKQL	8.641
3	26	FDEKQLRKL	8.635
4	12	ATQIGQIMH	8.634
5	92	KALLKEIAD	8.633
6	73	EAFRLFDKE	8.602
7	98	IADDLSDEQ	8.598
8	57	TVANTVDKE	8.588
9	82	GNGYISRPT	8.563
10	125	EEFWELMAG	8.508
11	35	IRKFDADGS	8.315
12	9	YIMATQIGQ	8.266
13	21	AMEQDFDEK	8.266
14	74	AFRLFDKEG	8.251
15	95	LKEIADDLS	8.229
16	44	GKLEFDEFC	8.149
17	11	MATQIGQIM	8.148
18	36	RKFDADGSG	8.132
19	94	LLKEIADDL	8.113
20	66	TLQKELREA	8.112
21	51	FCALVYTVA	7.863
22	48	FDEFCALVY	7.805
23	124	FEEFWELMA	7.804
24	33	KLIRKFDAD	7.682
25	37	KFDADGSGK	7.669
26	45	KLEFDEFCA	7.521
27	22	MEQDFDEKQ	7.502
28	99	ADDLSDEQL	7.490
29	53	ALVYTVANT	7.335
30	96	KEIADDLSD	7.222
31	63	DKETLQKEL	7.175
32	70	ELREAFRLF	7.044
33	90	TLKALLKEI	6.969
34	10	IMATQIGQI	6.961
35	88	RPTLKALLK	6.933
36	24	QDFDEKQLR	6.612
37	123	EFEEFWELM	6.521
38	52	CALVYTVAN	6.408
39	120	GKIEFEEFW	6.349
40	14	QIGQIMHAM	6.301
41	116	EDGSGKIEF	6.046

Table 3: Cascade SVM based High affinity TAP epitopes of *Wuchereria bancrofti* troponin protein.

No.	Start position	End position	Peptide	Peptide length
1	31	37	LRKLIRK	7
2	49	61	DEFCALVYTVANT	13
3	87	97	SRPTLKALLKE	11
4	108	113	EAAVDE	6

Table 4: Potential Antigenic epitopes of *Wuchereria bancrofti* troponin protein.

(Table 4). The predicted segments in *Wuchereria bancrofti* troponin protein are 31-LRKLIRK-37, 49-DEFCALVYTVANT-61, 87-SRPTLKALLKE-97, 108-EAAVDE-113 Fragments identified through this approach tend to be high-efficiency binders, which is a larger percentage of their atoms are directly involved in binding as compared to larger molecules. These MHC binding peptides are sufficient for inducing the desired immune response. Predicted MHC binding regions in *Wuch-*

ereria bancrofti troponin sequence and these are actively taking part in immune reactions.

Tertiary structure prediction of the predicted epitopes

We have predicted the experimentally confirmed structures of the two potential epitopes 49-DEFCALVYTVANT-61, 87-SRPTLKALLKE-97, these are the longest epitopes predicted from the *Wuchereria bancrofti* troponin protein showing the highest pick. The peptide structures are validated by applying A Hidden Markov Model based approach which predicts the tertiary confirmation of the peptide sequence at accuracy ~1.1 RMSd. The validated structures are shown in Balls & Sticks model over solvent-accessible surface (VDW+1.4 angstrom) (Figures 29 and 30) [47,48].

Discussion and Conclusion

Gomase method (2007), B-EpiPred Server, Hopp and Woods, Welling, Parker, Kolaskar and Tongaonkar antigenicity scales were designed to predict the locations of antigenic determinants in *Wuchereria bancrofti* troponin protein sequence. It shows beta sheets regions, which have higher antigenic response than helical region of this peptide and shows high antigenicity (Figures 1-5) [49-52]. We also found the Sweet hydrophobicity, Kyte & Doolittle hydrophobicity, Abraham & Leo, Bull & Breese hydrophobicity, Guy, Miyazawa hydrophobicity, Roseman hydrophobicity, Cowan HPLC pH 7.5 hydrophobicity, Rose hydrophobicity, Eisenberg hydrophobicity, Manavalan hydrophobicity, Black hydrophobicity, Fauchere hydrophobicity, Janin hydrophobicity, Rao & Argos hydrophobicity, Wolfenden hydrophobicity, Wilson HPLC hydrophobicity, Cowan HPLC pH 3.4, Tanford hydrophobicity, RF mobility hydrophobicity and Chothia hydrophobicity scales, Theses scales are essentially a hydrophilic index, with a polar residues assigned negative values (Figures 8-28). In this assay we predicted the binding affinity of *Wuchereria bancrofti* troponin protein having 136 amino acids, which shows 128 nonamers. We predicted SVM and ANN based CTL epitopes (Tables 1 and 2) [53,54].

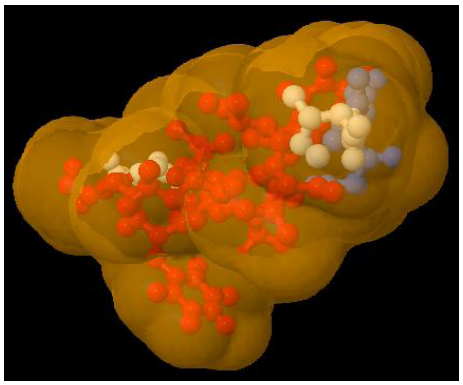


Figure 29: Experimentally Confirmed Structure of 49-DEFCALVYTVANT-61 epitope shown over Solvent-Accessible Surface.

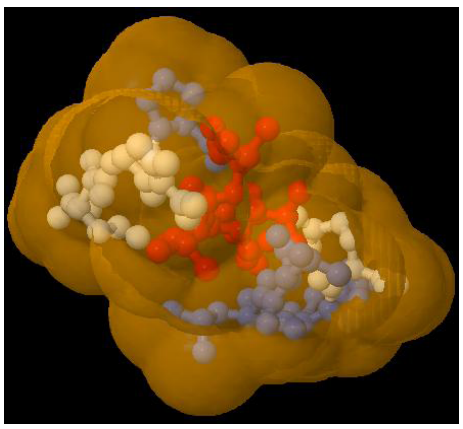


Figure 30: Experimentally Confirmed Structure of 87-SRPTLKALLKE-97 epitope shown over Solvent-Accessible Surface.

We have determined sixteen CTL predicted epitopes (Optimal score is 1.129) at cut off 0.36 using SVM based method (Table 1) and thirty one CTL predicted epitopes using ANN based method at cut off 0.51 (Optimal score is 1.000) (Table 2) which represents predicted peptide binders from *Wuchereria bancrofti* troponin protein. We also have predicted Cascade SVM based forty one High affinity TAP binding epitopes (Optimal score is 8.641) (Table 3) in addition to four potentially antigenic peptides recognized by antibodies of the immune system for troponin protein in the region of maximum local hydrophilicity (Table 4) [55]. Kolaskar and Tongaonkar antigenicity are the sites of molecules that are recognized by antibodies of the immune system for troponin protein, analysis shows epitopes present in the troponin protein eliciting desired immune response [18]. The region of maximal hydrophilicity is likely to be an antigenic site, having hydrophobic characteristics, because C- terminal regions of *Wuchereria bancrofti* troponin protein is solvent accessible and unstructured, antibodies against those regions are also likely to recognize the native protein. For the prediction of antigenic determinant site of *Wuchereria bancrofti* troponin protein, we predicted four antibody recognized antigenic determinant sites in the *Wuchereria bancrofti* troponin sequence. The highest pick is recorded between sequence of amino acid in the region are 49-DEFCALVYTVANT-61 and 87-SRPTLKALLKE-97 (Table 4) [56]. We also predicted the surface accessible tertiary structure for the peptide recorded with the highest pick; these are the longest peptides predicted in the antigenic sequence of the *Wuchereria bancrofti* troponin protein, the experimentally confirmed structures of the predicted peptides are considered to have an average accuracy of ~1.1A RMSd, the predicted peptide structures can be applied in synthetic peptide vaccine design approach to understand the sequence–structure–function relationship of the *Wuchereria bancrofti* troponin protein [57-59].

Future Perspectives

This method will be useful in cellular immunology, Vaccine design, immunodiagnostics, immunotherapeutics and molecular understanding of autoimmune susceptibility. *Wuchereria bancrofti* troponin protein sequence contains multiple antigenic components to direct and empower the immune system to protect an individual from lymphatic filariasis disease. MHC molecules are cell surface proteins, which actively participates in host immune reactions and involvement of MHC class in response to almost all antigens and it give effects on target sites. Predicted MHC binding regions acts like red flags for specific antigen and generate immune response against the whole antigen. So a small antigen fragment can generate immune response against entire antigen. The method integrates prediction of Peptide-MHC class binding; proteosomal C terminal cleavage and TAP transport efficiency. This approach is implemented in designing subunit and synthetic peptide vaccines.

References

1. Valkonen JP, Rajamaki ML, Kekarainen T (2002) Mapping of viral genomic regions important in cross-protection between strains of a potyvirus. Mol Plant Microbe Interact 15: 683-692.

2. Bhasin M, Raghava GPS (2004) Prediction of CTL epitopes using QM, SVM and ANN techniques. Vaccine 22: 3195-3204.
3. Kumar M, Gromiha MM, Raghava GP (2007) Identification of DNA-binding proteins using support vector machines and evolutionary profiles. BMC Bioinformatics 8: 463.
4. Gomase VS, Chitlange NR (2012) Sensitive Quantitative Predictions of MHC Binding Peptides and Fragment Based Peptide Vaccines from Trichinella spiralis. J Vaccines Vaccin 3: 1-4.
5. Gomase VS, Chitlange NR (2012b) Sensitive Quantitative Predictions of MHC Binding Peptides and Fragment Based Peptide Vaccines from Taenia crassiceps. J Vaccines Vaccin 3: 1.
6. Gomase VS, Chitlange NR (2011) Sensitive Quantitative Predictions of MHC Binding Peptide from Schistosoma Mansoni. Bioinfo Journal of Proteomics 1: 6-10.
7. Gomase VS, Kale KV, Chikhale NJ, Changbhale SS (2007) Prediction of MHC Binding Peptides and Epitopes from Alfalfa mosaic virus. Curr Drug Discov Technol 4: 117-215.
8. Schirle M, Weinschenk T, Stevanovic S (2001) Combining computer algorithms with experimental approaches permits the rapid and accurate identification of T cell epitopes from defined antigens. J Immunol Methods 257: 1-16.
9. Gomase VS, Changbhale SS, Kale KV (2008a) *insilico* analysis of Mesobuthus tamulus neurotoxin from groundnut ringspot virus. Advancements in Information Technology and Internet Security 370-378.
10. Rammensee H, Bachmann J, Emmerich NP, Bachor OA, Stevanovic S (1999) SYFPEITHI: database forMHC ligands and peptide motifs. Immunogenetics 50: 213-219.
11. Blythe MJ, Doytchinova IA, Flower DR (2002) JenPep: a database of quantitative functional peptide data for immunology. Bioinformatics 18: 434-439.
12. Schonbach C, Koh JL, Flower DR, Wong L, Brusic V (2002) FIMM, a database of functional molecular immunology: update 2002. Nucleic Acids Res 30: 226-229.
13. Korber TMB, Brander C, Haynes BF, Koup R, Kuiken C, et al. (2001) Los Alamos National Laboratory, Theoretical Biology and Biophysics, Los Alamos, New Mexico. LA-UR 02-4663.
14. Gomase VS (2006) Prediction of Antigenic Epitopes of Neurotoxin Bmbktx1 from Mesobuthus martensii. Curr Drug Discov Technol 3: 225-229.
15. Hopp TP, Woods KR (1981) Prediction of Protein Antigenic Determinants from Amino Acid Sequences. Proc Natl Acad Sci USA 78: 3824-3828.
16. Welling GW, Weijer WJ, van der Zee R, Welling-Wester S (1985) Prediction of sequential antigenic regions in proteins. FEBS Lett 188: 215-218.
17. Parker JMR, Guo D, Hodges RS (1986) New Hydrophilicity Scale Derived from High-Performance Liquid Chromatography Peptide Retention Data: Correlation of Predicted Surface Residues with Antigenicity and Xray- Derived Accessible Sites. Biochemistry 25: 5425-5432.
18. Kolaskar AS, Tongaonkar PC (1990) A semi-empirical method for prediction of antigenic determinants on protein antigens. FEBS Lett 276: 172-174.
19. Garnier J, Osguthorpe DJ, Robson B (1978) Analysis of the accuracy and implications of simple methods for predicting the secondary structure of globular proteins. J Mol Biol 120: 97-120.
20. Robson B, Garnier J (1993) Protein structure prediction. Nature 361: 506.
21. Sweet RM, Eisenberg D (1983) Correlation of sequence hydrophobicities measures similarity in three-dimensional protein structure. J Mol Biol 171: 479-488.
22. Abraham DJ, Leo AJ (1987) Extension of the fragment method to calculate amino acid zwitterions and side chain partition coefficients. Proteins 2: 130-152.
23. Bull HB, Breese K (1974) Surface tension of amino acid solutions: A hydrophobicity scale of the amino acid residues. Arch Biochem Biophys 161: 665-670.
24. Guy HR (1985) Amino acid side chain partition energies and distributions of residues in soluble proteins. Biophys J 47: 61-70.
25. Miyazawa S, Jernigen RL (1985) Estimation of Effective Interresidue Contact Energies from Protein Crystal Structures: Quasi-Chemical Approximation. Macromolecules 18: 534-552.
26. Roseman MA (1988) Hydrophilicity of polar amino acid side-chains is markedly reduced by flanking peptide bonds. J Mol Biol 200: 513-522.
27. Wolfenden RV, Andersson L, Cullis PM, Southgate CCF (1981) Affinities of amino-acid side-chains for solvent water. Biochemistry 20: 849-855.
28. Wilson KJ, Honegger A, Stotzel RP, Hughes GJ (1981) The behaviour of peptides on reverse-phase supports during high-pressure liquid chromatography. Biochem J 199: 31-41.
29. Chothia C (1976) The nature of accessible and buried surfaces in proteins. J Mol Biol 105: 1-12.
30. Eisenberg D, Schwarz E, Komaromy M, Wall R (1984) Analysis of membrane and surface protein sequences with the hydrophobic moment plot. J Mol Biol 179: 125-142.
31. Eisenberg D, Weiss RM, Terwilliger TC (1984) The hydrophobic moment detects periodicity in protein hydrophobicity. Proc Natl Acad Sci 81: 140-144.
32. Manavalan P, Ponnuswamy PK (1978) Hydrophobic character of amino acid residues in globular proteins. Nature 275: 673-674.
33. Black SD, Mould DR (1991) Development of Hydrophobicity Parameters to Analyze Proteins Which Bear Post- or Cotranslational Modifications. Anal Biochem 193: 72-82.
34. Fauchere JL, Pliska VE (1983) Hydrophobic parameters of amino-acid side-chains from the partitioning of N-acetyl-amino-acid amide. Eur J Med Chem 18: 369-375.
35. Janin J (1979) Surface and inside volumes in globular proteins. Nature 277: 491-492.
36. Rao MJK, Argos P (1986) A conformational preference parameter to predict helices in integral membrane proteins. Biochem Biophys Acta 869: 197-214.
37. Tanford C (1962) Amino Acid scale: Hydrophobicity scale (Contribution of hydrophobic interactions to the stability of the globular conformation of proteins). J Am Chem Soc 84: 4240- 4274.
38. Cowan R, Whittaker RG (1990) Hydrophobicity indices at pH 3.4 determined by HPLC. Pept Res 3: 75-80.
39. Cowan R, Whittaker RG (1990) Hydrophobicity indices for amino acid residues as determined by HPLC. Pept Res 3: 75-80.
40. Rose GD, Geselowitz AR, Lesser GJ, Lee RH, Zehfus MH (1985) Hydrophobicity of amino acid residues in globular proteins. Science 229: 834-838.
41. Wilkins MR, Gasteiger E, Bairoch A, Sanchez JC, Williams KL, et al. (1999) Protein identification and analysis tools in the ExPASy server. Methods Mol Biol 112: 531-552.
42. Bhasin M, Raghava GPS (2004) Analysis and prediction of affinity of TAP binding peptides using cascade SVM. Protein Sci 13: 596-607.
43. Camproux AC, Gautier R, Tuffery P (2004) A hidden markov model derived structural alphabet for proteins. J Mol Biol 339: 591-605.
44. Maupetit J, Derreumaux P, Tuffery P (2009) PEP-FOLD: an online resource for de novo peptide structure prediction. Nucleic Acids Res 37: 498-503.
45. Maupetit J, Derreumaux P, Tuffery P (2010) A fast method for large-scale de novo peptide and miniprotein structure prediction.J Comput Chem 31: 726-738.
46. Maupetit J, Tuffery P, Derreumaux P (2007) A coarse-grained protein force field for folding and structure prediction.Proteins 69: 394-408.
47. Tim Wallace (2011) Using Java applets to enhance online teaching and assessment in Blackboard. Symposium on the Benefits of eLearning Technologies. University of Manchester, USA.
48. Brusic V, Rudy G, Honeyman G, Hammer J, Harrison L (1998) Prediction of MHC class II-binding peptides using an evolutionary algorithm and artificial neural network. Bioinformatics 14: 121-130.
49. Gomase VS, Chitlange NR (2010) Antigen protein from Schistosoma mansoni: New paradigm of synthetic vaccine development. International Journal of Pharma and Bio Sciences 1: 1-7.
50. Gomase VS, Chitlange NR (2009) Binding ability prediction of antigen peptides to major histocompatibility complex for development of synthetic peptide

vaccine from Plasmodium falciparum. Int'l Journal of Immunology Research 1: 1-6.

51. Gomase VS, Chitlange NR (2010) Microbial Proteomics Approach for Synthetic Vaccine Development form Bacillus Weihenstephanensis. International Journal of Drug Design Discovery 1: 310-313.

52. Gomase VS, Changbhale SS, Patil SA, Kale KV (2008) Metabolomics, Curr Drug Metab 9: 88-98.

53. Larsen JE, Lund O, Nielsen M (2006) Improved method for predicting linear B-cell epitopes. Immunome Res 2: 2.

54. David JT, William PA, Edward KM, Voge M (2006) Markell and Voge's Medical Parasitology. (9thedn) St. Louis: Saunders Elsevier.

55. Larry S. Roberts, Schmidt Gerald D (2005) Foundations of Parasitology (7thedn). 247–261. ISBN 978-0-8016-4345-3. OCLC 2645424

56. Ponomarenko JV, Bourne PE (2007) Antibody-protein interactions: benchmark datasets and prediction tools evaluation. BMC Struct Biol 7: 64.

57. Singh H, Raghava GPS (2001) ProPred: prediction of HLA-DR binding sites. Bioinformatics 17: 1236-1237.

58. Waghmare S, Gomase V, Dhole J, Chavan R, Kale KV (2012) Immunoproteomics Approach for Development of Synthetic Peptide Vaccine from Thioredoxin Glutathione Reductase. Metabolomics 2:111.

59. (2012) lymphatic Filariasis, WHO.