Journal of Drug Metabolism and Toxicology

Sherkhane et al., J Drug Metab Toxicol 2014, 5:4
DOI: 10.4172/2157-7609.1000169

Research Article Open Access

Identification of MHC Class, Transports Antigenic Peptides from *Naja melanoleuca* Long Neurotoxin 1

Sherkhane AS[1], Changbhale SS[1] and Gomase VS[2]*

[1]*The Global Open University, Nagaland, India*
[2]*Dr Babasaheb Ambedkar Marathwada University, Aurangabad, India*

Abstract

Long neurotoxin 1 is a dangerous protein occurs in *N. melanoleuca*. It is highly dangerous snake due to the quantity of venom inject in a single bite. Peptide fragments of *N. Melanoleuca* Long neurotoxin 1 antigen protein having 71 amino acids, which shows 63 nanomers can be used to select nanomers for use in synthetic peptide vaccine design and to increase the understanding of roles of the immune system against snake bite. We use to apply computational intelligence algorithm PSSM (Position Specific Scoring Matrices) for the Prediction of MHC class-I binding peptides, we also predict antigenicity, Solvent accessibility, polar and nonpolar residue to analyses the membrane-spanning regions (hydrophobic) or regions that are likely exposed on the surface of proteins (hydrophilic domains) that are potentially antigenic that allows potential drug targets to identify active sites, for protection of host form Snake bites and to design synthetic peptide vaccine.

Keywords: *N. Melanoleuca*; Long neurotoxin 1; Antigenic peptides; MHC-Binders; Tap Pred; PSSM; Vaccine; Nanomers

Introduction

Naja melanoleuca (forest cobra, black cobra) is the largest in all the true cobra (Naja) species in the world [1,2]. It is highly dangerous snake due to the quantity of venom inject in a single bite and death can occur within 30 to 120 minutes of envenomation [3]. *N. melanoleuca* venom contains Long neurotoxin 1, which binds to muscular and neuronal nicotinic acetylcholine receptors and produces peripheral paralysis by blocking neuromuscular transmission at the postsynaptic site [4]. *N. melanoleuca* Long neurotoxin 1 antigenic peptides are most suitable for antigenic peptide vaccine development because an ample immune response can be generated even with single epitope. Major histocompatibility complex (MHC) molecules are cell surface proteins that binds to the peptides derived from host or antigenic proteins, and present them at the cell surface for recognition by T-cells [5,6]. T cell recognition is a fundamental mechanism of the adaptive immune system by which the host identifies and responds to foreign antigens [7,8]. There are two types of MHC molecule and are extremely polymorphic [9]. MHC class I molecules present peptides from intracellular proteins, which are targeted by proteasome, cleaved them into short peptides of 8-11 amino acids in length. These peptides are bound by the transmembrane peptide transporter (TAP) and translocate them from cytoplasm to endoplasmic reticulum, where they are bound by MHC molecule. The second and the C-terminal position of the peptide are the most important for binding [10,11]. These amino acids at each position contribute a certain binding energy [12]. These predicted MHC-binding peptides and T-cell epitopes helps improve our understanding of specificity of immune responses [13-15].

Materials and Methods

Database searching

The antigenic protein sequence of *N. melanoleuca* long neurotoxin 1 was retrieved from www.ncbi.nlm.nih.gov, Uni Prot databases are initially the most important [14-16].

Prediction of antigenicity

Prediction of antigenicity program predicts those segments from *N. melanoleuca* long neurotoxin 1 that are likely to be antigenic by eliciting an antibody response. Antigenic epitopes of *N. melanoleuca* long neurotoxin 1 are determined by using the Hopp and Woods, Welling, Parker, Bepipred, Kolaskar and Tongaonkar antigenicity methods [16-20].

Prediction of MHC binding peptide

The major histocompatibility complex (MHC) peptide binding of *N. melanoleuca* long neurotoxin 1 is predicted using neural networks trained on C terminals of known epitopes. Rankpep toll predicts peptide binders to MHC-I ligands whose C-terminal end is likely to be the result of proteosomal cleavage using Position Specific Scoring Matrices (PSSMs). Support Vector Machine (SVM) based method for prediction of promiscuous MHC class II binding peptides from protein sequence; SVM has been trained on the binary input of single amino acid sequence [21-28].

Prediction of antigenic peptides by cascade SVM based TAP Pred method

In the present study, we predict cascade SVM based several TAP binders which was based on the sequence and the features of amino acids [29]. We found the MHCI binding regions, the binding affinity of *N. melanoleuca* long neurotoxin 1.

Solvent accessible regions

We also predict solvent accessible regions of proteins having highest probability that a given protein region lies on the surface of a protein Surface Accessibility, backbone or chain flexibility by Emini

***Corresponding author:** Virendra S Gomase, Dr Babasaheb Ambedkar Marathwada University, Aurangabad, India, Tel: +91-9987770696; E-mail: gomase.viren@gmail.com

Received May 30, 2014; **Accepted** July 23, 2014; **Published** July 30, 2014

Citation: Sherkhane AS, Changbhale SS, Gomase VS (2014) Identification of MHC Class, Transports Antigenic Peptides from *Naja melanoleuca* Long Neurotoxin 1. J Drug Metab Toxicol 5: 169. doi:10.4172/2157-7609.1000169

et al. [30] and Karplus and Schulz [31]. By using different scale we predict the hydrophobic and hydrophilic characteristics of amino acids that are rich in charged and polar residues i.e. Sweet et al., Kyte and Doolittle, Abraham and Leo, Bull and Breese, Miyazawa et al., Roseman, Wolfenden et al., Wilson et al., Cowan, Chothia [32-41].

Results and Discussion

A antigenic sequence of *N. melanoleuca* Long neurotoxin 1 is 71 residues long as-

KRCYRTPDLKSQTCPPGEDLCYTKKWCADWCTSRGKVI ELGCVATCPKVKPYEQITCCSTDNCNPHPKMKP

Prediction of antigenic peptides

We predicted the antigenic determinants by finding the area of greatest local hydrophilicity. The Hopp-Woods scale Hydrophilicity Prediction Result Data was found to be high in position 4-5,8(1.014),9-10,16-18 in a protein, assuming that the antigenic determinants would be exposed on the surface of the protein and thus would be located in hydrophilic regions (Figure 1). 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 and Prediction Result Data found high in position 7-8, 21-23(0.571) (Figure 2). We also study Hydrophobicity plot of HPLC/Parker Hydrophilicity Prediction Result Data found 10-KSQTCPP-16(4.143),11-SQTCPPG-17(4.143),12-QTCPPGE-18(4.329),13-TCPPGED-19(4.900),56-TCCSTDN-62(5.243),57-CCSTDNC-63(4.700),58-CSTDNCN-64(5.500), 59-STDNCNP-65(5.600) (maximum), (Figure 3), Bepi Pred predicts the location of linear B-cell epitopes Result found that 4-Y-4,6-TPDLKSQTCPPGEDLC-21,47-PKVKPY-52, (Figure 4 and Table 1), Kolaskar and Tongaonkar [20] antigenicity methods Predicted peptides result found i.e. 9-LKSQTCPPGEDLCYTKKW-26, 34-RGKVIELGCVATCPKVKPYEQITCCSTD-61 (Figure 5 and Table 2). The maximal hydrophilicity region is assumed to be an antigenic site, having hydrophobic characteristics, because terminal regions of antigen protein are solvent accessible and unstructured; antibodies against those regions are also likely to recognize the native protein.

Solvent accessible regions

We also predict solvent accessible regions in proteins; different measurement was performed for the prediction of antigenic activity, surface region of peptides. Emini et al. [30] (Figure 6) predicts the highest probability i.e. found4-YRTPDL-9(2.103),5-RTPDLK-10(2.684),47-PKVKPY-52(2.479),48-KVKPYE-53(2.777),49-VKPYEQ-54(2.405),64-NPHPKM-69(2.308),65-PHPKMK-70(2.871) (maximum), 66-HPKMKP-71(2.871), that a given protein region lies on the surface of a protein and are used to identify antigenic determinants on the surface of proteins. Karplus and Schulz [31] (Figure 7) High score is found in residue i.e. 7-PDLKSQT-13(1.084), 8-DLKSQTC-14(1.094 (maximum), 9-LKSQTCP-15(1.091), Predict backbone or chain flexibility on the basis of the known temperature B factors of the a-carbons. The hydrophobicity and hydrophilic characteristics of amino acids is determined by using different scales that are rich in charged and polar residues i.e. Sweet et al. [32] hydrophobicity prediction Result Data found high in position 23-24,39-41(0.367), Kyte

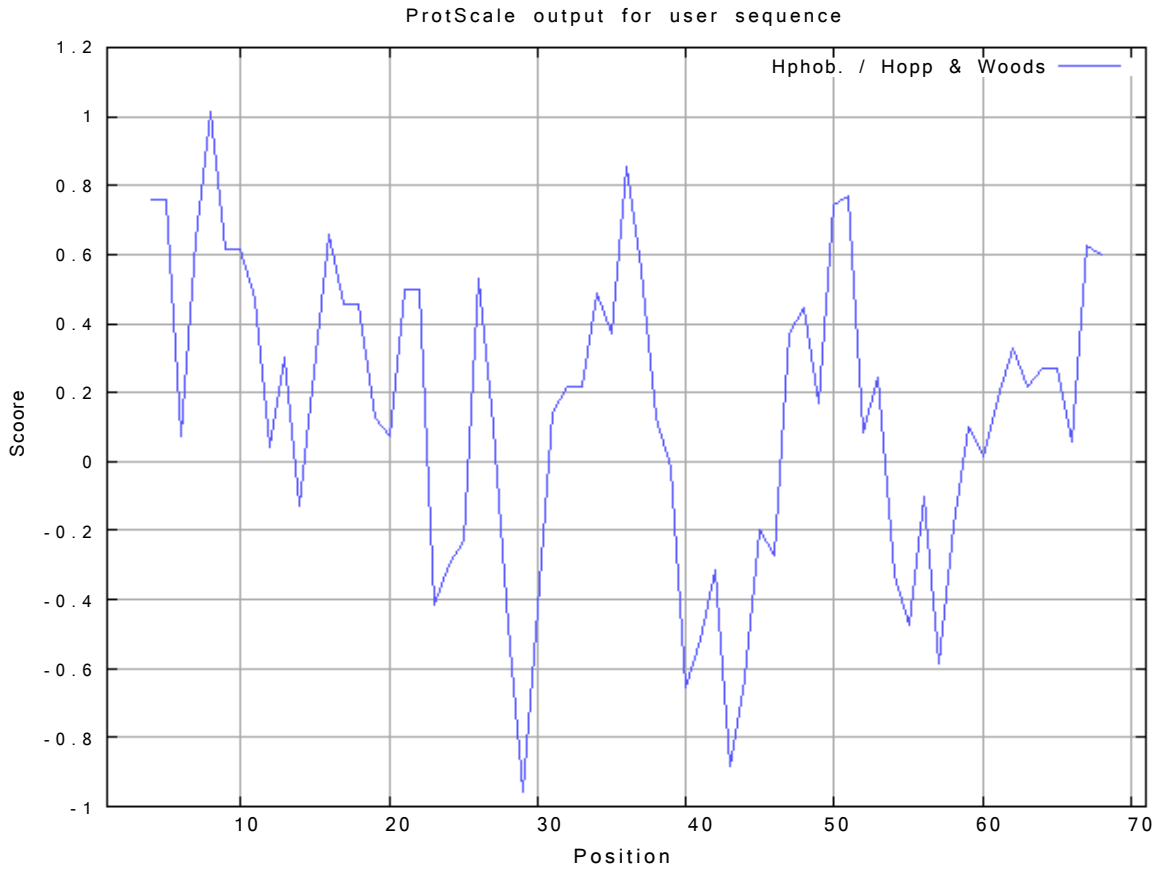

Figure 1: Hydrophobicity plot of Hopp and Woods [16] of *N. melanoleuca* Long neurotoxin 1.

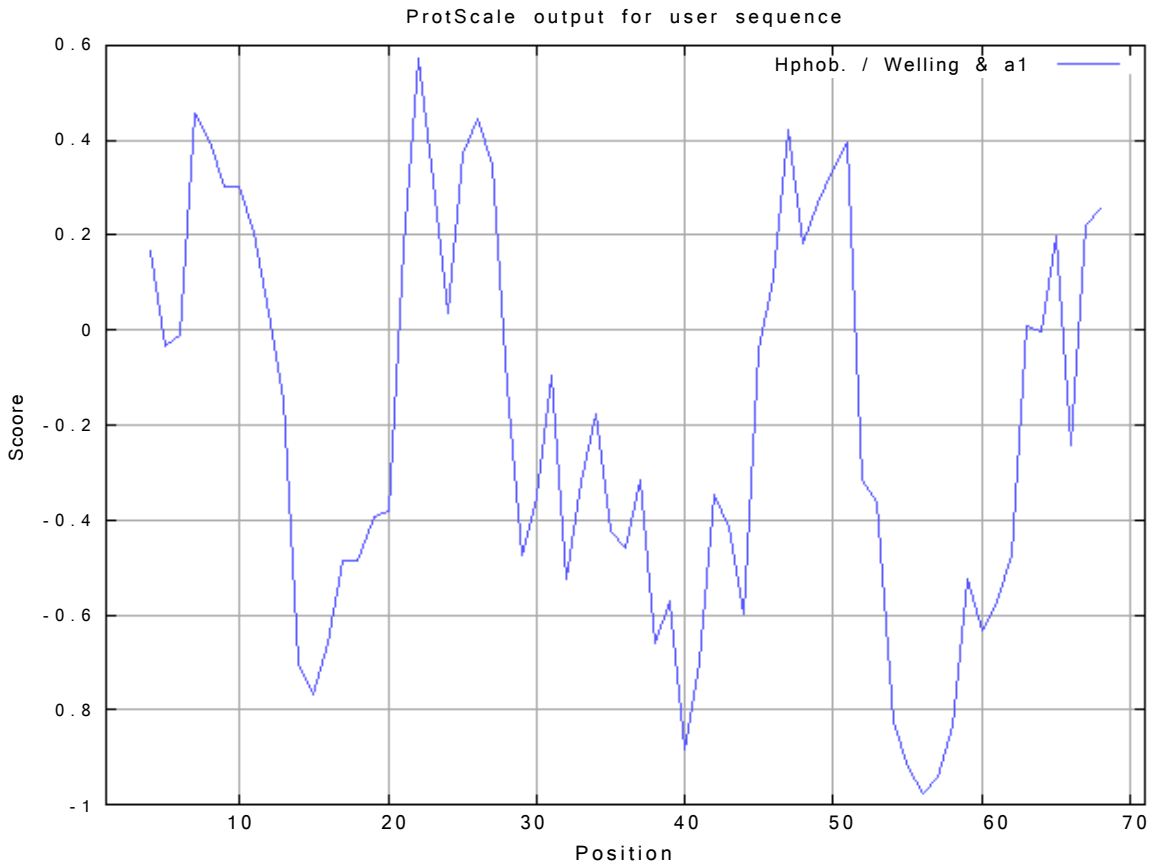

Figure 2: Hydrophobicity plot of Welling et al. [17] of *N. melanoleuca* Long neurotoxin 1.

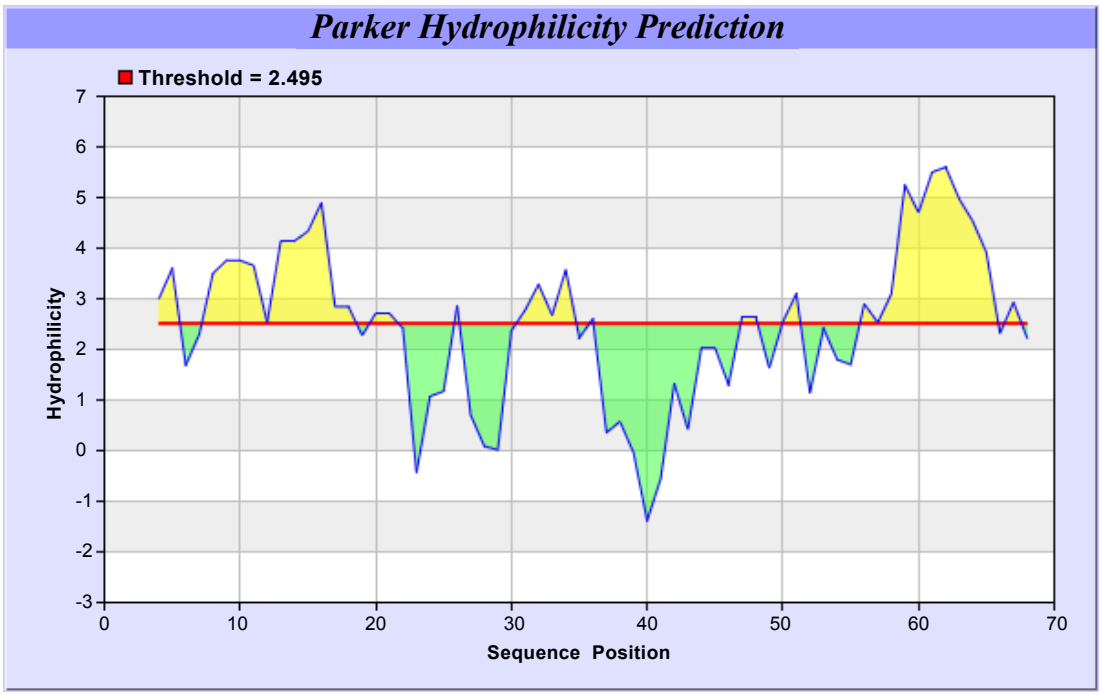

Figure 3: Hydrophobicity plot of HPLC by Parker et al. [18] of *N. melanoleuca* Long neurotoxin 1.

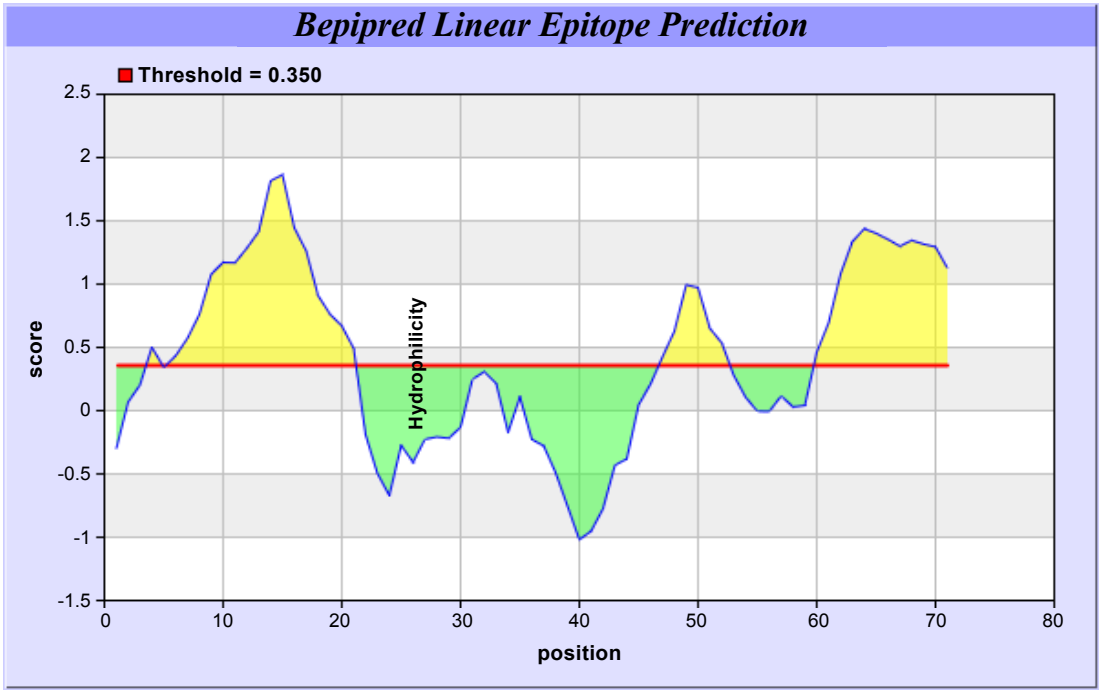

Figure 4: Bepipred Linear Epitope Prediction plot showing antibody recognized B-cell epitopes of *N. melanoleuca* Long neurotoxin 1.

No.	Start Position	End Position	Peptide	Peptide Length
1	4	4	Y	1
2	6	21	TPDLKSQTCPPGEDLC	16
3	47	52	PKVKPY	6

Table 1: Predicted Antigenic epitopes of *N. melanoleuca* Long neurotoxin 1 Bepipred.

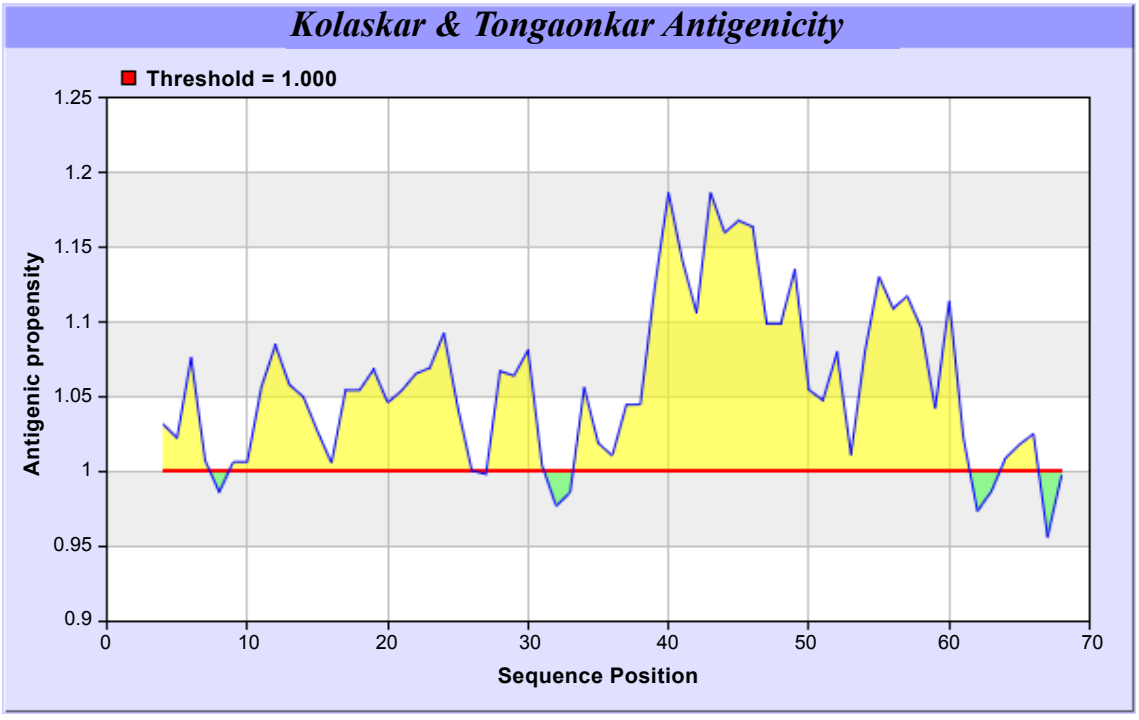

Figure 5: Kolaskar and Tongaonkar [20] antigenicity plot for the *N. melanoleuca* Long neurotoxin 1.

No.	Start Position	End Position	Peptide	Peptide Length
1	9	26	LKSQTCPPGEDLCYTKKW	18
2	34	61	RGKVIELGCVATCPKVKPYEQITCCSTD	28

Table 2: Predicted Antigenic epitopes of *N. melanoleuca* Long neurotoxin 1.

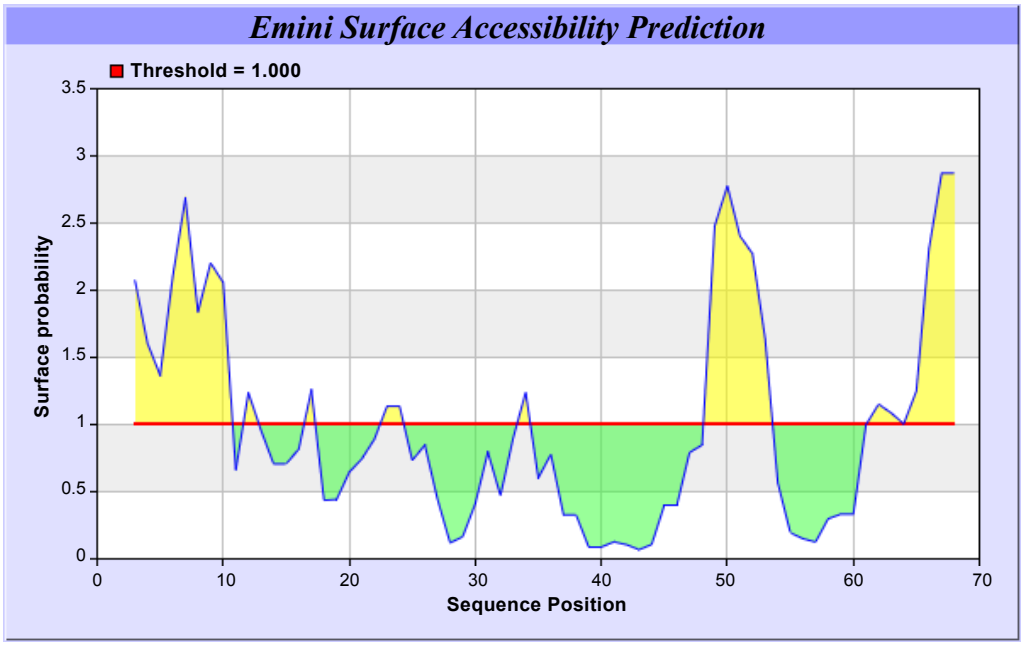

Figure 6: Emini et al. [30] surface accessibility prediction plot of *N. melanoleuca* Long neurotoxin 1.

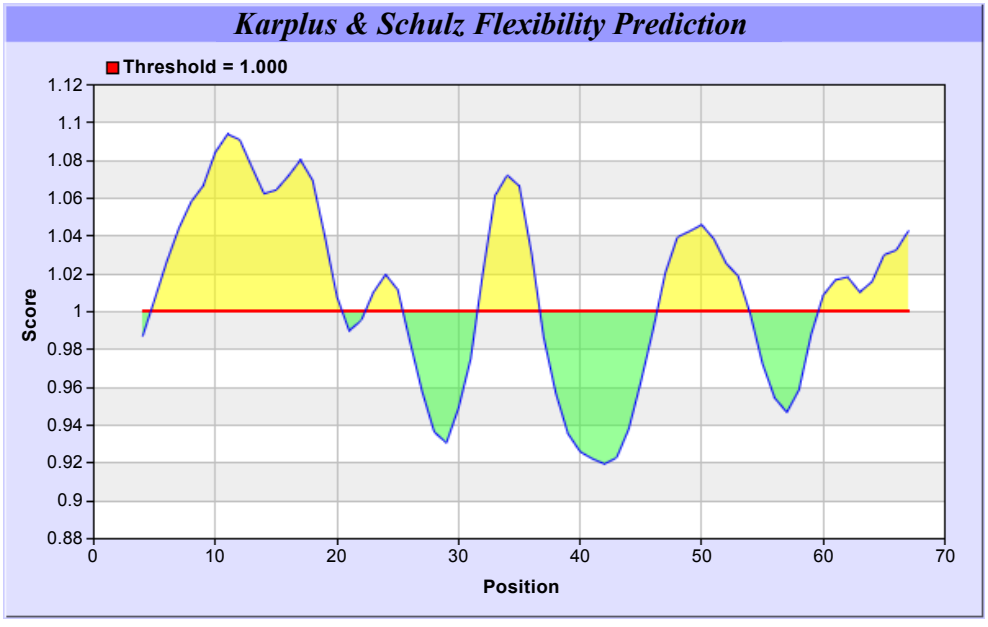

Figure 7: Karplus and Schulz [31] flexibility prediction of *N. melanoleuca* Long neurotoxin 1.

and Doolittle result high in position 39-41(2.186),42-44, Abraham and Leo [34] result high in position 17-19,28-29,39-43(1.231),Bull and Breese [35]result high in position 13-15,59-65(0.546), Miyazawa et al. [36] result high in position 27-30,39-42(6.946),54-58, Roseman [37] result high in position 40-41(0.511),43-44, Wolfenden et al. [38] result high in position 43(0.177), Wilson et al. [39] 18-20, 28-30, 38-46(4.314), Cowan and Whittaker [40] 39-44(0.966), Chothia [41] 37-46 (0.453) (Figures 8-18).

Prediction of MHC binding peptide

We found binding of peptides to a number of different alleles using

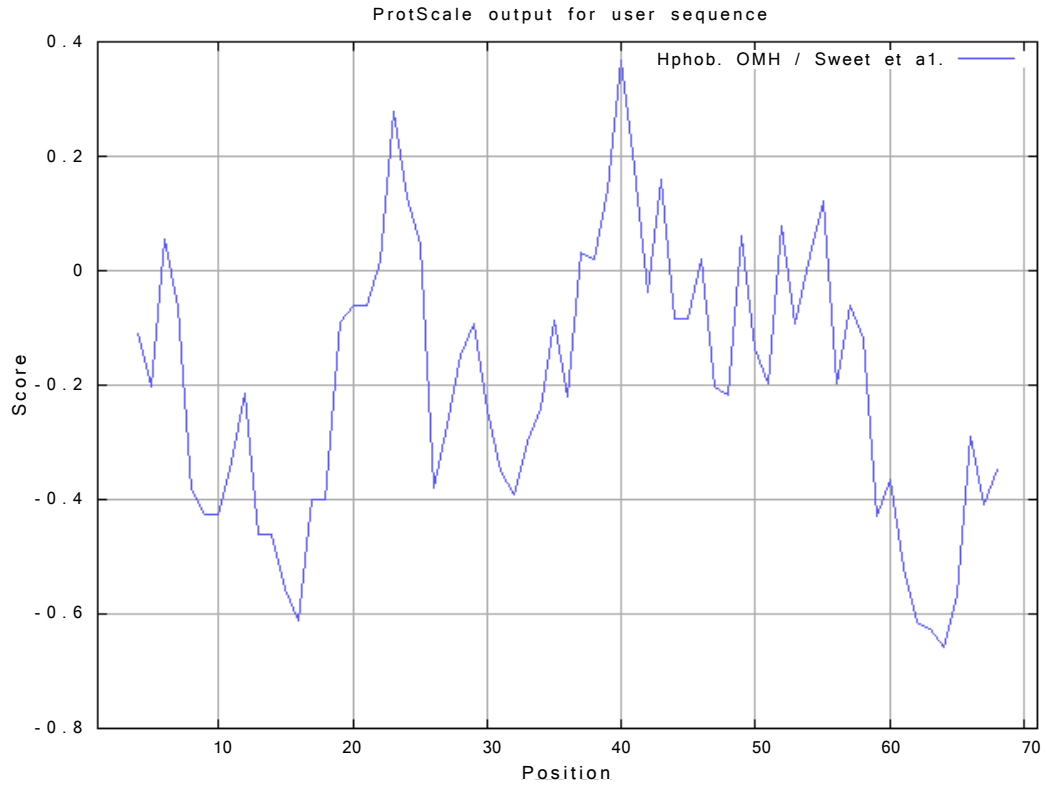

Figure 8: Hydrophobicity plot of Sweet et al. [32] of *N. melanoleuca* Long neurotoxin 1.

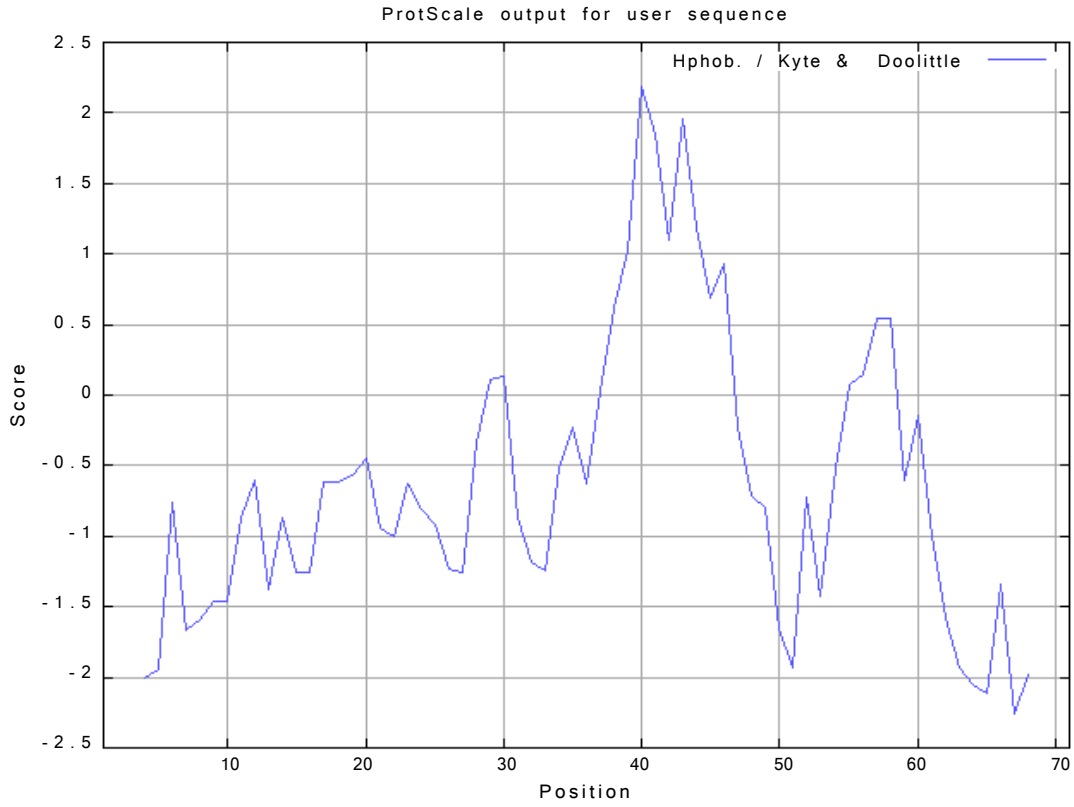

Figure 9: Kyte & Doolittle [33] hydrophobicity plot of *N. melanoleuca* Long neurotoxin 1.

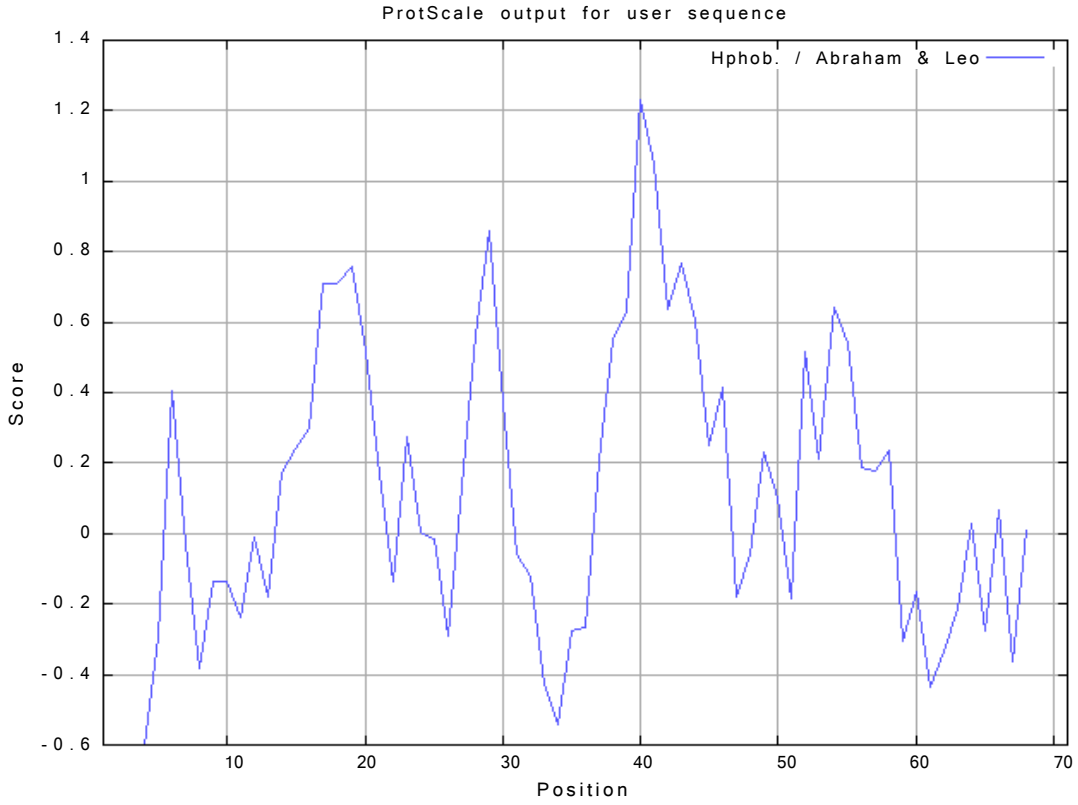

Figure 10: Abraham & Leo [34] hydrophobicity plot of *N. melanoleuca* Long neurotoxin 1.

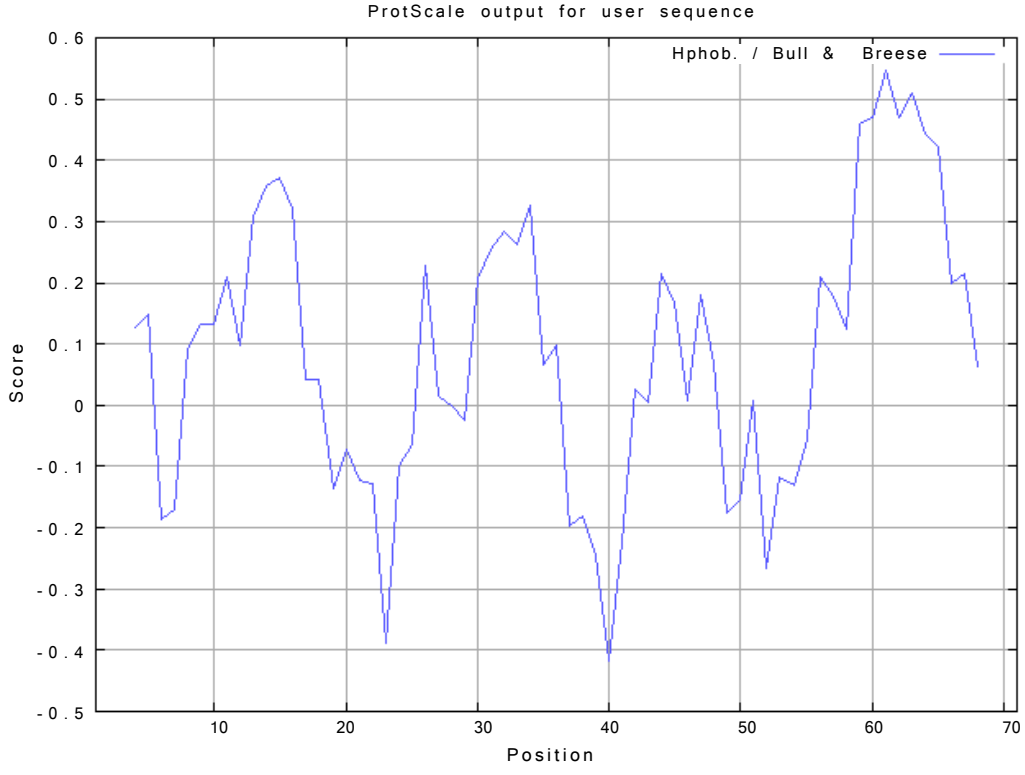

Figure 11: Bull & Breese [35] use surface tension to measure hydrophobicity and also uses negative values to describe the hydrophobicity of *N. melanoleuca* Long neurotoxin 1.

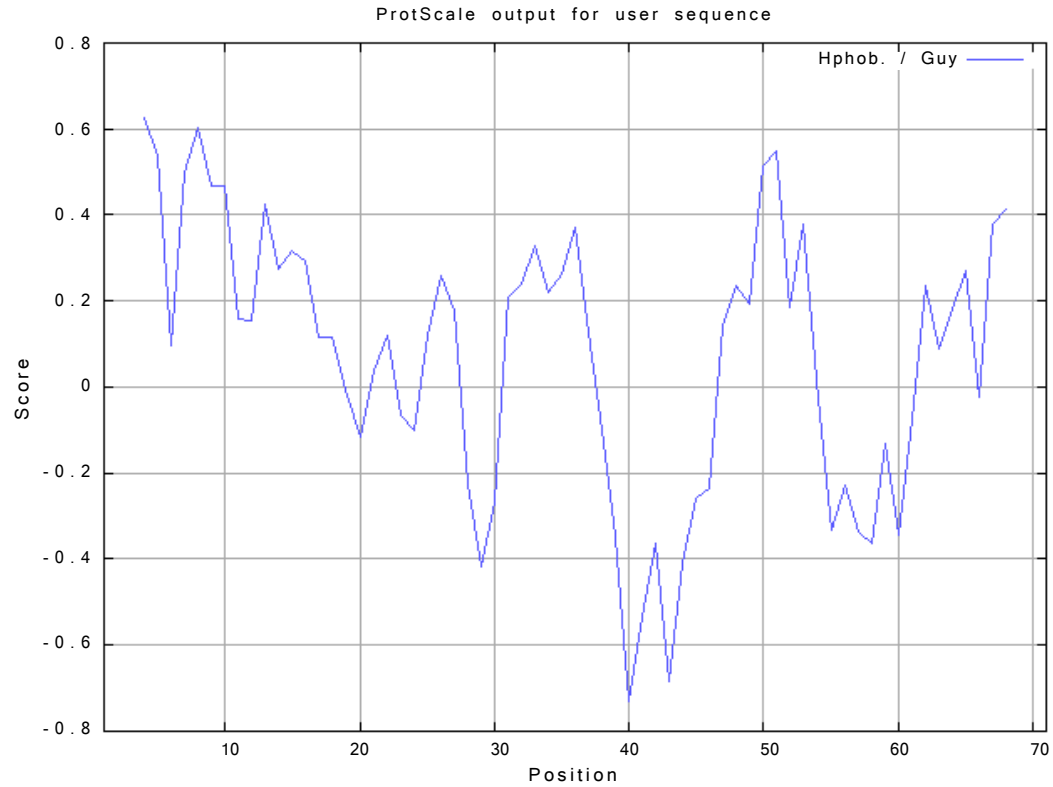

Figure 12: Hydrophobicity plot of Guy of *N. melanoleuca* Long neurotoxin 1.

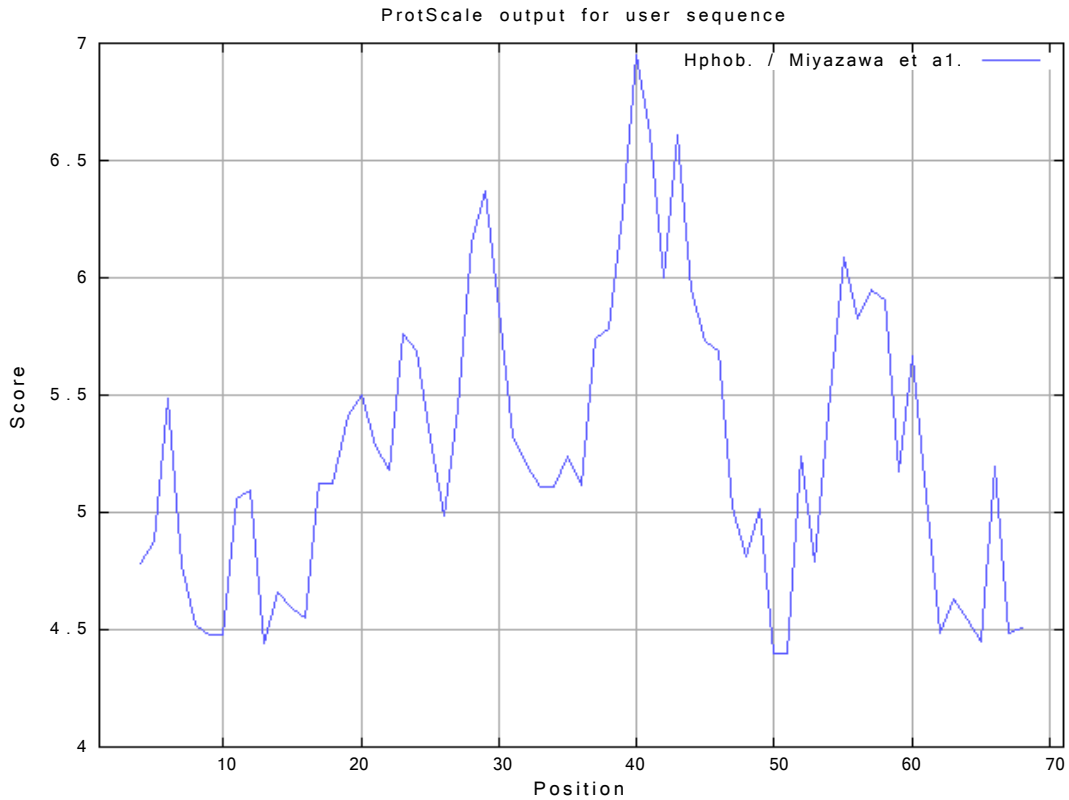

Figure 13: Hydrophobicity plot of Miyazawa et al. [36] of *N. melanoleuca* Long neurotoxin 1.

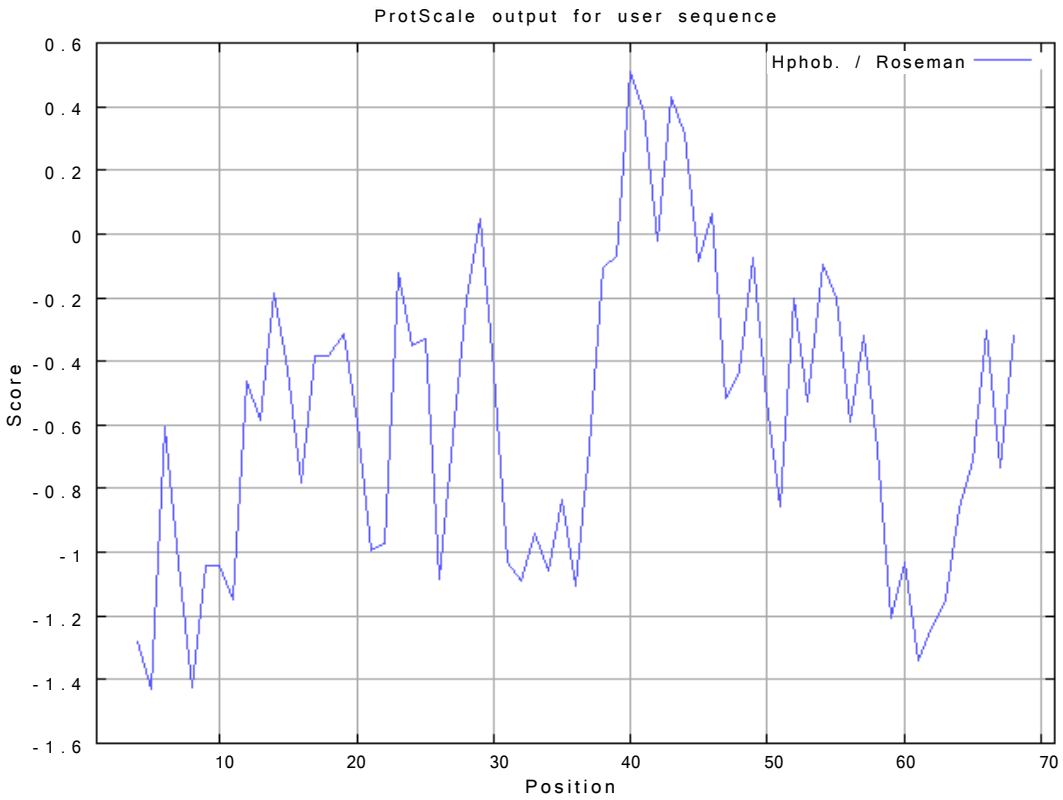

Figure 14: Hydrophobicity plot of Roseman [37] of *N. melanoleuca* Long neurotoxin 1.

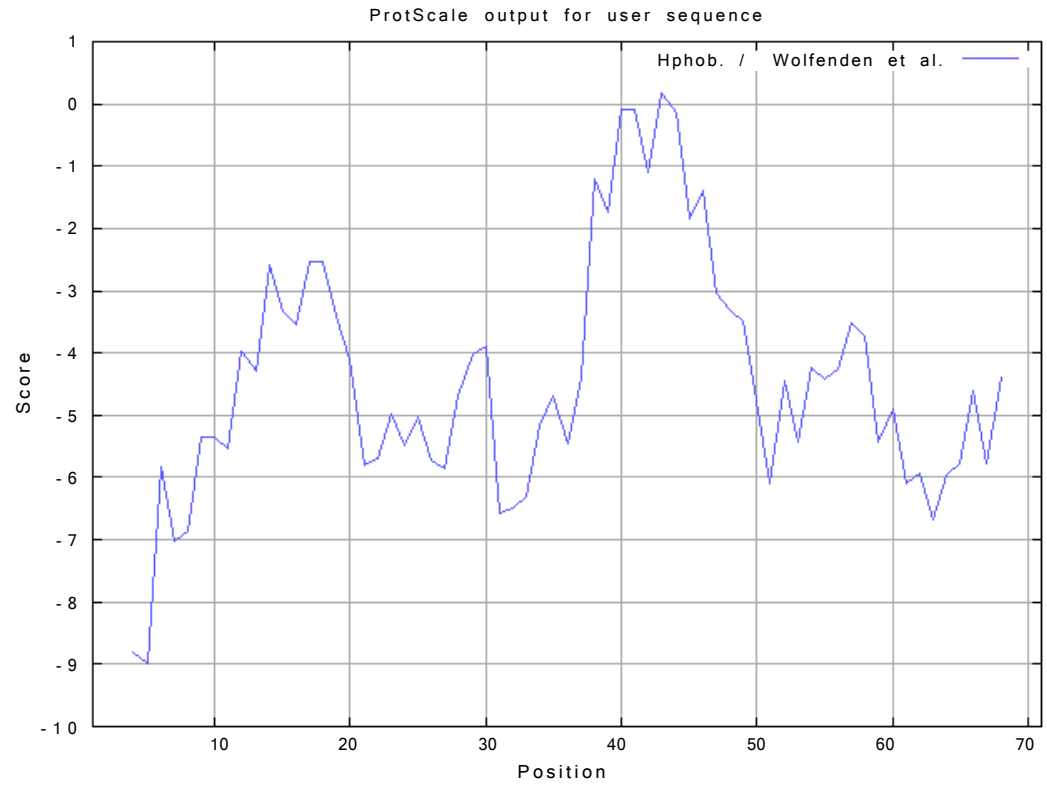

Figure 15: Hydrophobicity plot of Wolfenden et al. [38] of *N. melanoleuca* Long neurotoxin 1.

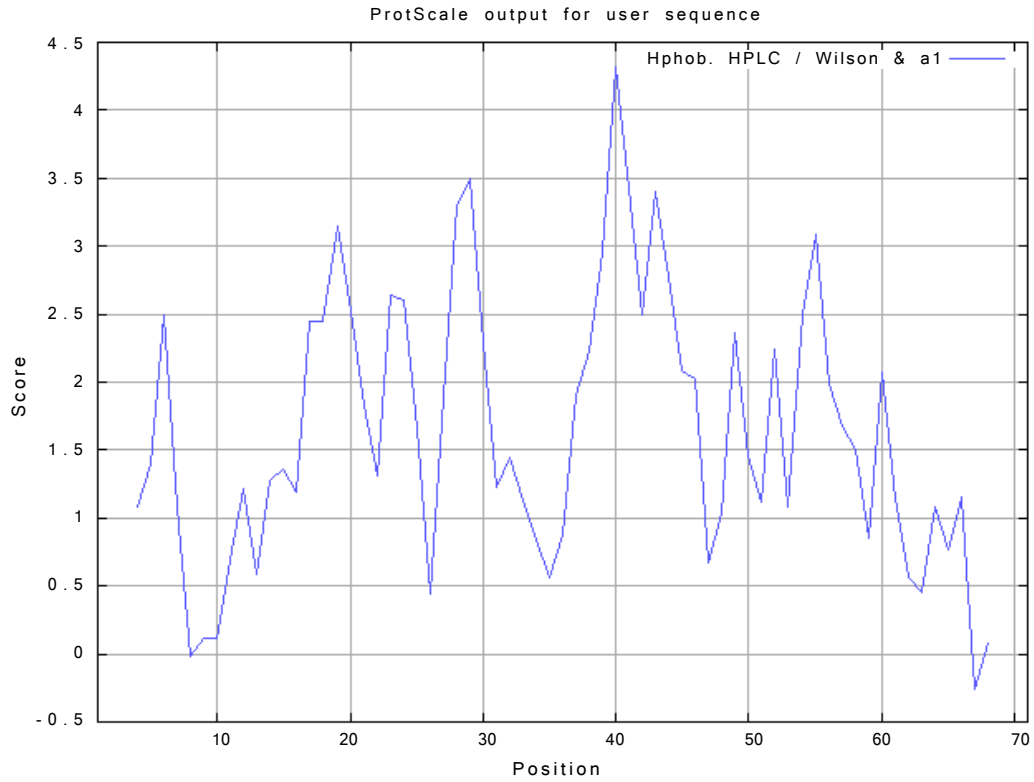

Figure 16: Hydrophobicity/HPLC plot of Wilson et al. [39] of *N. melanoleuca* Long neurotoxin 1.

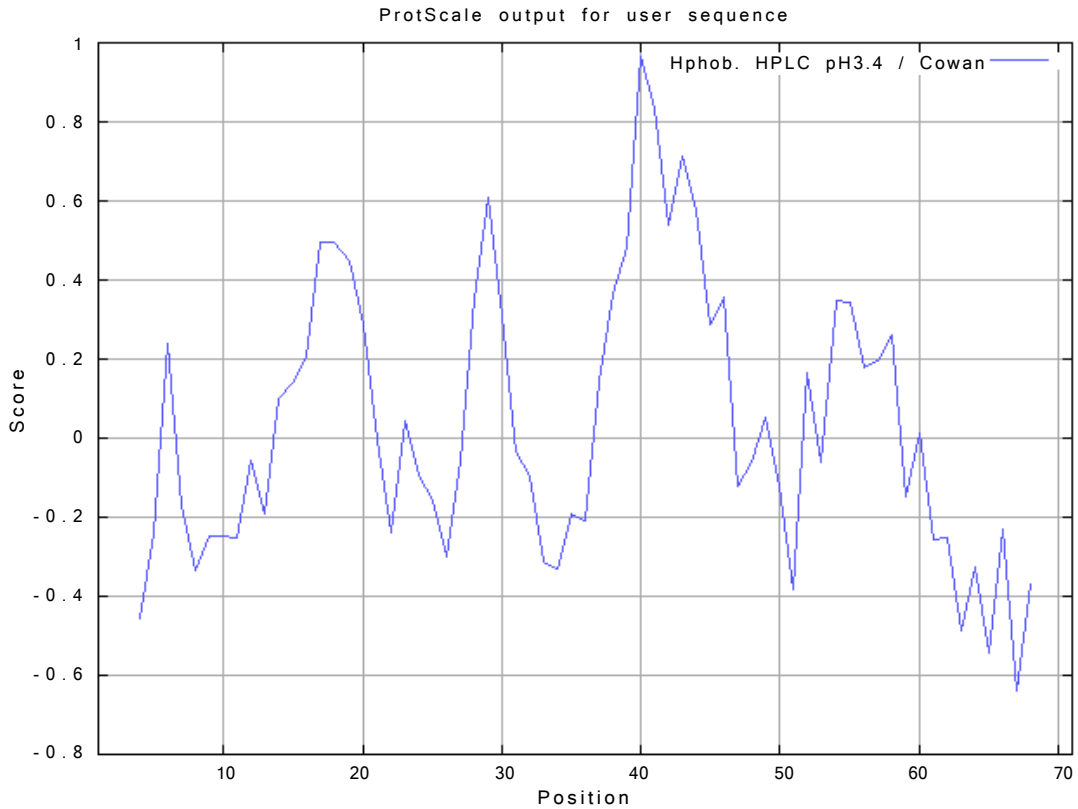

Figure 17: Hydrophobicity/HPLC pH 3.4/ plot of Cowan [40] of *N. melanoleuca* Long neurotoxin 1.

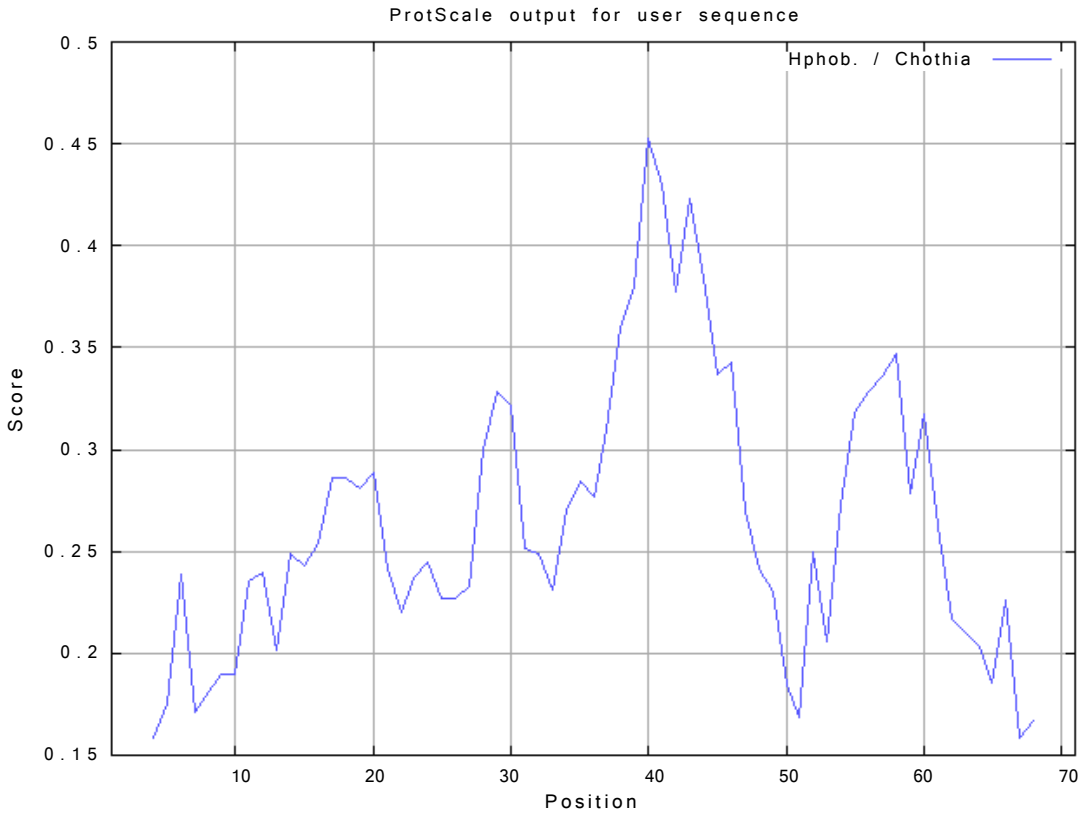

Figure 18: Hydrophobicity plot of Chothia [41] of *N. melanoleuca* Long neurotoxin 1.

Position Specific Scoring Matrix. *N. melanoleuca* Long neurotoxin 1 sequence is 71 residues long, having 63 nanomers. MHC molecules are cell surface proteins, which actively participate in host immune reactions and involvement of MHC-I in response to almost all antigens. We have predicted MHC-I peptide binders of *N. melanoleuca* Long neurotoxin 1 was tested with on a set of 4 different alleles i.e. H2-Db (mouse) 8mer, H2-Db (mouse) 9mer, H2-Db (mouse) 10mer, H2-Db (mouse) 11mer (Tables 3a-3d). Here RANKPEP report PSSM-specific binding threshold and is obtained by scoring all the antigenic peptide sequences included in the alignment from which a profile is derived, and is defined as the score value that includes 85% of the peptides within the set. Peptides whose score is above the binding threshold will appear highlighted in red and peptides produced by the cleavage prediction model are highlighted in violet. We also use a cascade SVM based TAP Pred method which found 20 High affinity TAP Transporter peptide regions which represents predicted TAP binders residues which occur at N and C termini from *N. melanoleuca* Long neurotoxin 1 (Table 4).

Prediction of protein secondary structure

The important concepts in secondary structure prediction are identified as: residue conformational propensities, sequence edge effects, moments of hydrophobicity, position of insertions and Deletions in aligned homologous sequence, moments of conservation, auto-correlation, residue ratios, secondary structure feedback effects, and filtering [42,43]. The Robson and Garnier method predicted the secondary structure of the *N. melanoleuca* Long neurotoxin 1. Each residue is assigned values for alpha helix, beta sheet, turns and coils using a window of 7 residues (Figure 19). 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.

Discussion

The antigenic determinants predicted by finding the area of greatest local hydrophilicity. Hopp and Woods hydrophilicity scale is used to identify of potentially antigenic sites in proteins by analyzing amino acid sequences in order to find the point of greatest hydrophilic. Hydrophilicity Prediction result data found high in sequence position at 4-5, 8(1.014), 9-10, 16-18 in a protein this scale is basically a hydrophilic index where apolar residues have been assigned negative values. The Window size of 5-7 is good for finding hydrophilic regions, greater than 0 values are consider as hydrophilic which is consider as antigenic. Welling scale is used information on the relative occurrence of amino acids in antigenic regions to make a scale which is useful for prediction of antigenic regions and the predicted result data found high in sequence position 7-8, 21-23(0.571). 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. We also study Hydrophobicity plot of HPLC/Parker Hydrophilicity Prediction Result Data found 10-KSQTCPP-16(4.143), 11-SQTCPPG-17(4.143),12-QTCPPGE-18(4.329),13-TCPPGED-19(4.900),56-TCCSTDN-62(5.243),57-CCSTDNC-63(4.700),58-CSTDNCN-64(5.500),59-STDNCNP-65(5.600) (maximum). BepiPred predicts the location of linear B-cell epitopes Result found that there are 3 predicted epitopes are found 4-Y-4,6-TPDLKSQTCPPGEDLC-21,47-PKVKPY-52 (Table 2).There are 2 antigenic determinant sequences is found by Kolaskar and Tongaonkar antigenicity scales the results

MHC-I Allele	POS.	N	Sequence	C	MW (Da)	Score	% OPT.
8mer_H2_Db	42	ELG	CVATCPKV	KPY	802.01	15.402	29.34 %
8mer_H2_Db	31	ADW	CTSRGKVI	ELG	845.02	7.006	13.35 %
8mer_H2_Db	15	QTC	PPGEDLCY	TKK	874.98	4.918	9.37 %
8mer_H2_Db	33	WCT	SRGKVIEL	GCV	883.06	3.157	6.01 %
8mer_H2_Db	17	CPP	GEDLCYTK	KWC	910.01	2.172	4.14 %
8mer_H2_Db	48	TCP	KVKPYEQI	TCC	986.18	2.149	4.09 %
8mer_H2_Db	50	PKV	KPYEQITC	CST	963.12	0.227	0.43 %
8mer_H2_Db	2	K	RCYRTPDL	KSQ	1005.17	0.027	0.05 %
8mer_H2_Db	61	CST	DNCNPHPK	MKP	905.98	-4.024	-7.67 %
8mer_H2_Db	13	KSQ	TCPPGEDL	CYT	812.9	-5.168	-9.84 %
8mer_H2_Db	36	SRG	KVIELGCV	ATC	842.06	-5.296	-10.09 %
8mer_H2_Db	63	TDN	CNPHPKMK	P	936.15	-9.81	-18.69 %
8mer_H2_Db	43	LGC	VATCPKVK	PYE	827.04	-10.044	-19.13 %
8mer_H2_Db	23	LCY	TKKWCADW	CTS	973.17	-10.171	-19.38 %
8mer_H2_Db	27	KKW	CADWCTSR	GKV	900.03	-13.229	-25.20 %
8mer_H2_Db	45	CVA	TCPKVKPY	EQI	917.13	-13.527	-25.77 %
8mer_H2_Db	3	KR	CYRTPDLK	SQT	977.15	-14.346	-27.33 %
8mer_H2_Db	51	KVK	PYEQITCC	STD	938.09	-14.429	-27.49 %
8mer_H2_Db	19	PGE	DLCYTKKW	CAD	1015.22	-17.864	-34.03 %
8mer_H2_Db	14	SQT	CPPGEDLC	YTK	814.94	-20.054	-38.20 %

Table 3a: Promiscuous MHC ligands, having C-terminal ends are proteosomal cleavage sites, Binding potential (score) of antigenic peptide to the MHC-1 Allele i.e. 8mer_H2_Db.

MHC-I Allele	POS.	N	Sequence	C	MW (Da)	Score	% OPT.
9mer_H2_Db	13	KSQ	TCPPGEDLC	YTK	916.04	11.234	22.31 %
9mer_H2_Db	60	CCS	TDNCNPHPK	MKP	1007.08	5.919	11.75 %
9mer_H2_Db	42	ELG	CVATCPKVK	PYE	930.18	4.654	9.24 %
9mer_H2_Db	2	K	RCYRTPDLK	SQT	1133.34	4.316	8.57 %
9mer_H2_Db	41	IEL	GCVATCPKV	KPY	859.06	1.441	2.86 %
9mer_H2_Db	62	STD	NCNPHPKMK	P	1050.25	-0.266	-0.53 %
9mer_H2_Db	30	CAD	WCTSRGKVI	ELG	1008.23	-0.46	-0.91 %
9mer_H2_Db	44	GCV	ATCPKVKPY	EQI	988.21	-3.842	-7.63 %
9mer_H2_Db	35	TSR	GKVIELGCV	ATC	899.11	-4.856	-9.64 %
9mer_H2_Db	47	ATC	PKVKPYEQI	TCC	1083.3	-4.938	-9.80 %
9mer_H2_Db	12	LKS	QTCPPGEDL	CYT	941.03	-5.15	-10.23 %
9mer_H2_Db	49	CPK	VKPYEQITC	CST	1062.25	-6.286	-12.48 %
9mer_H2_Db	14	SQT	CPPGEDLCY	TKK	978.12	-7.991	-15.87 %
9mer_H2_Db	32	DWC	TSRGKVIEL	GCV	984.16	-8.745	-17.36 %
9mer_H2_Db	16	TCP	PGEDLCYTK	KWC	1007.13	-8.946	-17.76 %
9mer_H2_Db	26	TKK	WCADWCTSR	GKV	1063.24	-10.603	-21.05 %
9mer_H2_Db	1	-	KRCYRTPDL	KSQ	1133.34	-10.828	-21.50 %
9mer_H2_Db	50	PKV	KPYEQITCC	STD	1066.26	-12.095	-24.01 %
9mer_H2_Db	22	DLC	YTKKWCADW	CTS	1136.35	-17.825	-35.39 %
9mer_H2_Db	18	PPG	EDLCYTKKW	CAD	1144.34	-18.176	-36.09 %

Table 3b: Promiscuous MHC ligands, having C-terminal ends are proteosomal cleavage sites, the binding potential (score) of antigenic peptide to the MHC-1 Allele i.e. 9mer_H2_Db.

show highest pick at position 9-LKSQTCPPGEDLCYTKKW-26, 34-RGKVIELGCVATCPKVKPYEQITCCSTD-61 (Figure 4). Result of determined antigenic sites on proteins has revealed that the hydrophobic residues if they occur on the surface of a protein are more likely to be a part of antigenic sites. This method can predict antigenic determinants with about 75% accuracy and also gives the information of surface accessibility and flexibility. Further this region form beta sheet which show high antigenic response than helical region of this peptide and shows highly antigenicity.

We predict Solvent accessibility by using Emani et al. [30] the result found the highest probability i.e. found 4-YRTPDL-9(2.103),5-RTPDLK-10(2.684),47-PKVKPY-52(2.479),48-KVKPYE-53(2.777),49-VKPYEQ-54(2.405),64-NPHPKM-69(2.308),65-PHPKMK-70(2.871) (maximum),66-HPKMKP-71(2.871), that a given protein region lies on the surface of a protein and are used to identify antigenic determinants on the surface of proteins. This algorithm also used to identify the antigenic determinants on the surface of proteins and Karplus and Schulz predict backbone or chain flexibility on the basis of the known temperature B factors of the a-carbons here we found the result with High score is i.e. 7-PDLKSQT-13(1.084), 8-DLK**S**QTC-14(1.094 (maximum),9-LKS**Q**TCP-15(1.091). We predict Solvent accessibility of *N. melanoleuca* Long neurotoxin 1 for delineating hydrophobic and hydrophilic characteristics of amino acids. Solvent accessibility used to identify active site of functionally important residues in membrane proteins. Solvent-accessible surface areas and backbone angles are continuously varying because proteins

MHC-I Allele	POS.	N	Sequence	C	MW (Da)	Score	% OPT.
10mer_H2_Db	61	CST	DNCNPHPKMK	P	1165.34	2.595	4.41 %
10mer_H2_Db	49	CPK	VKPYEQITCC	STD	1165.39	2.44	4.15 %
10mer_H2_Db	11	DLK	SQTCPPGEDL	CYT	1028.11	0.818	1.39 %
10mer_H2_Db	1		KRCYRTPDLK	SQT	1261.51	0.264	0.45 %
10mer_H2_Db	34	CTS	RGKVIELGCV	ATC	1055.3	-2.273	-3.86 %
10mer_H2_Db	40	VIE	LGCVATCPKV	KPY	972.22	-3.357	-5.70 %
10mer_H2_Db	13	KSQ	TCPPGEDLCY	TKK	1079.22	-4.556	-7.74 %
10mer_H2_Db	12	LKS	QTCPPGEDLC	YTK	1044.17	-6.559	-11.14 %
10mer_H2_Db	41	IEL	GCVATCPKVK	PYE	987.23	-9.316	-15.83 %
10mer_H2_Db	25	YTK	KWCADWCTSR	GKV	1191.41	-11.111	-18.88 %
10mer_H2_Db	59	TCC	STDNCNPHPK	MKP	1094.16	-13.201	-22.43 %
10mer_H2_Db	43	LGC	VATCPKVKPY	EQI	1087.34	-13.538	-23.00 %
10mer_H2_Db	31	ADW	CTSRGKVIEL	GCV	1087.3	-15.615	-26.53 %
10mer_H2_Db	48	TCP	KVKPYEQITC	CST	1190.42	-16.503	-28.04 %
10mer_H2_Db	29	WCA	DWCTSRGKVI	ELG	1123.32	-18.559	-31.53 %
10mer_H2_Db	46	VAT	CPKVKPYEQI	TCC	1186.44	-19.76	-33.57 %
10mer_H2_Db	17	CPP	GEDLCYTKKW	CAD	1201.39	-19.941	-33.88 %
10mer_H2_Db	15	QTC	PPGEDLCYTK	KWC	1104.25	-20.854	-35.43 %
10mer_H2_Db	21	EDL	CYTKKWCADW	CTS	1239.49	-25.799	-43.83 %

Table 3c: Promiscuous MHC ligands, having C-terminal ends are proteosomal cleavage sites, the binding potential (score) of antigenic peptide to the MHC-1 Allele i.e. 10mer_H2_Db.

MHC-I Allele	POS.	N	Sequence	C	MW (Da)	Score	% OPT.
11mer_H2_Db	33	WCT	SRGKVIELGCV	ATC	1142.38	-0.276	-0.35 %
11mer_H2_Db	58	ITC	CSTDNCNPHPK	MKP	1197.3	-1.339	-1.68 %
11mer_H2_Db	12	LKS	QTCPPGEDLCY	TKK	1207.35	-2.425	-3.05 %
11mer_H2_Db	42	ELG	CVATCPKVKPY	EQI	1190.48	-7.703	-9.69 %
11mer_H2_Db	60	CCS	TDNCNPHPKMK	P	1266.44	-8.342	-10.49 %
11mer_H2_Db	47	ATC	PKVKPYEQITC	CST	1287.54	-8.838	-11.12 %
11mer_H2_Db	16	TCP	PGEDLCYTKKW	CAD	1298.51	-10.047	-12.64 %
11mer_H2_Db	45	CVA	TCPKVKPYEQI	TCC	1287.54	-11.861	-14.92 %
11mer_H2_Db	10	PDL	KSQTCPPGEDL	CYT	1156.28	-13.738	-17.28 %
11mer_H2_Db	28	KWC	ADWCTSRGKVI	ELG	1194.4	-14.642	-18.42 %
11mer_H2_Db	14	SQT	CPPGEDLCYTK	KWC	1207.39	-16.733	-21.05 %
11mer_H2_Db	48	TCP	KVKPYEQITCC	STD	1293.56	-16.815	-21.15 %
11mer_H2_Db	40	VIE	LGCVATCPKVK	PYE	1100.39	-18.496	-23.27 %
11mer_H2_Db	20	GED	LCYTKKWCADW	CTS	1352.65	-19.377	-24.38 %
11mer_H2_Db	39	KVI	ELGCVATCPKV	KPY	1101.34	-20.253	-25.48 %
11mer_H2_Db	24	CYT	KKWCADWCTSR	GKV	1319.58	-20.616	-25.93 %
11mer_H2_Db	30	CAD	WCTSRGKVIEL	GCV	1250.51	-21.4	-26.92 %
11mer_H2_Db	11	DLK	SQTCPPGEDLC	YTK	1131.25	-23.165	-29.14 %

Table 3d: Promiscuous MHC ligands, having C-terminal ends are proteosomal cleavage sites the binding potential (score) of antigenic peptide to the MHC-1 Allele i.e. 11mer_H2_Db.

can move freely in a three-dimensional space. The mobility of protein segments which are located on the surface of a protein due to an entropic energy potential and which seem to correlate well with known antigenic determinants. We also found the i.e. Sweet et al. hydrophobicity prediction result data found high in position 23-24, 39-41(0.367), Kyte and Doolittle result high in position 39-41(2.186),42-44, Abraham & Leo result high in position 17-19,28-29,39-43(1.231), Bull and Breese result high in position 13-15,59-65(0.546), Guyresult high in position 4-5(0.627),7-10,13-15,50-51, Miyazawa result high in position 27-30, 39-42(6.946), 54-58, Roseman result high in position 40-41(0.511), 43-44, Wolfenden result high in position 43(0.177), Wilson et al. 18-20,28-30,38-46(4.314), Cowan 39-44 (0.966),Chothia 37-46 (0.453) (Figures 7-17).These scales are a hydrophilic with a polar residues assigned negative value. Because the N- and C- terminal regions of proteins are usually solvent accessible and unstructured, antibodies against those regions recognize the antigenic protein. Predicted MHC-I peptide binders of toxin protein for 8mer_H2_Db alleles with the consensus sequence QNWNCCTI that yields the maximum score i.e. 52.494, 9mer_H2_Db with, the consensus sequence FCIHNCDYM that yields the maximum score i.e. 50.365, 10mer_H2_Db with, the consensus sequence SGYYNFFWCL that yields the maximum score i.e. 58.858, 11mer_H2_Db with, the consensus sequence CGVYNFYYCCY that yields the maximum score i.e. 79.495. We also use a cascade SVM based TAP Pred method which found 20 High affinity TAP Transporter peptide regions which represents predicted TAP binders residues which occur at N and C termini from *N. melanoleuca* Long neurotoxin 1. TAP is an important transporter that transports antigenic peptides from cytosol to ER. TAP binds and translocate selective antigenic peptides for binding to specific MHC molecules. The efficiency of TAP-mediated translocation of antigenic peptides is directly proportional to its TAP binding affinity. Thus, by understanding the nature of peptides, that bind to TAP with high affinity, is important

Peptide Rank	Start Position	Sequence	Score	Predicted Affinity
1	42	CVATCPKVK	8.641	High
2	12	QTCPPGEDL	8.621	High
3	48	KVKPYEQIT	8.559	High
4	51	PYEQITCCS	8.444	High
5	4	YRTPDLKSQ	8.416	High
6	49	VKPYEQITC	8.411	High
7	41	GCVATCPKV	8.398	High
8	17	GEDLCYTKK	8.293	High
9	29	DWCTSRGKV	8.257	High
10	8	DLKSQTCPP	8.116	High
11	9	LKSQTCPPG	8.008	High
12	63	CNPHPKMKP	7.991	High
13	30	WCTSRGKVI	7.869	High
14	5	RTPDLKSQT	7.854	High
15	34	RGKVIELGC	7.813	High
16	25	KWCADWCTS	7.561	High
17	21	CYTKKWCAD	7.280	High
18	14	CPPGEDLCY	7.241	High
19	52	YEQITCCST	7.110	High
20	16	PGEDLCYTK	6.841	High

Table 4: Cascade SVM based High affinity TAP Binders of *N. melanoleuca* Long neurotoxin 1.

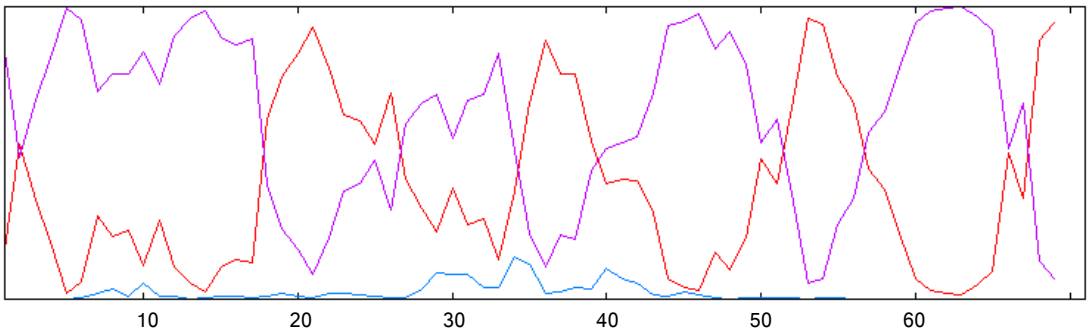

Figure 19: Secondary Structure of *N. melanoleuca* Long neurotoxin 1 showing helix (Red), Sheet (Blue), Coil (Pink) regions.

steps in endogenous antigen processing. The correlation coefficient of 0.88 was obtained by using jackknife validation test. T cell immune responses are derived by antigenic epitopes hence their identification is important for design synthetic peptide vaccine. T cell epitopes are recognized by MHCI molecules producing a strong defensive immune response against *N. melanoleuca* Long neurotoxin 1. Therefore, the prediction of peptide binding to MHCI molecules by appropriate processing of antigen peptides occurs by their binding to the relevant MHC molecules. Because, the C-terminus of MHCI-restricted epitopes results from cleavage by the proteasome and thus, proteasome specify is important for determine T-cell epitopes. Consequently, RANKPEP also focus on the prediction of conserved epitopes. C-terminus of MHCI-restricted peptides is generated by the proteasome, and thus RANKPEP also determines whether the C-terminus of the predicted MHCI-peptide binders is the result of proteasomal cleavage. Moreover, these sequences are highlighted in purple in the output results. Proteasomal cleavage predictions are carried out using three optional models obtained applying statistical language models to a set of known epitopes restricted by human MHCI molecules as indicated here.

Conclusion

From the above result and discussion it is concluded that the ability of RANKPEP to predict MHC binding peptides, and thereby potential T-cell epitopes. The specificity of transporter associated with antigen processing (TAP) plays an important role in the transport of the antigenic peptide fragments of the proteolysed to the endoplasmic reticulum where they associate with the major histocompatibility complex (MHC) class I molecules. Therefore, prediction of TAP-binding peptides is highly helpful in identifying the MHC class I-restricted T-cell epitopes and hence useful in the synthetic peptide vaccine designing. Antigenic peptides should be located in solvent accessible regions containing both hydrophobic and hydrophilic residues. High peaks in the surface accessibility plot predict regions that have a higher chance of producing antibodies that can bind to native protein. This means the increase in affinity of MHC binding peptides may result in enhancement of immunogenicity of *N. melanoleuca* Long neurotoxin 1 and are helpful in the designing of synthetic peptide vaccine. This approach can help reduce the time and cost of experimentation for determining functional properties of *N. melanoleuca* Long neurotoxin 1. Overall, the results are encouraging, both the 'sites of action' and 'physiological functions' can be predicted with very high accuracies helping minimize the number of validation experiments.

References

1. O'Shea M (2005) Venomous Snakes of the World. United Kingdom: New Holland Publishers.

2. Broadley DG (1983) Fitzsimons' Snakes of Southern Africa. Delta Books. Johannesburg, South Africa.
3. Spawls S, Branch B (1995) The Dangerous Snakes of Africa: Natural History, Species directory, Venoms, and Snakebite. Ralph Curtis-Books, Florida, USA.
4. Shipolini RA, Bailey GS, Banks BE (1974) The separation of neurotoxin from the venom of Naja melanoleuca and the primary sequence determination. Eur J Biochem 42: 203-211.
5. Betzel C Lange G, Pal GP, Wilson KS, Maelicke A, et al. (1991) The refined crystal structure of alpha-cobratoxin from Naja naja siamensis at 2.4-A resolution. J Biol Chem 266: 21530-21536.
6. Rammensee HG Falk K, Rötzschke O (1993) Peptides naturally presented by MHC class I molecules. Annu Rev Immunol 11: 213-244.
7. Cresswell P (1994) Assembly, transport, and function of MHC class II molecules. Annu Rev Immunol 12: 259-293.
8. Batalia MA Collins EJ (1997) Peptide binding by class I and class II MHC molecules. Biopolymers 43: 281-302.
9. Flower DR (2008) Vaccines: how they work, in Bioinformatics for Vaccinology. Wiley-Blackwell, Oxford, UK.
10. Williams TM (2001) Human leukocyte antigen gene polymorphism and the histocompatibility laboratory. J Mol Diagn 3: 98-104.
11. Rammensee H Bachmann J, Emmerich NP, Bachor OA, StevanoviÄ‡ S (1999) SYFPEITHI: database for MHC ligands and peptide motifs. Immunogenetics 50: 213-219.
12. Falk K Rötzschke O, Rammensee HG (1990) Cellular peptide composition governed by major histocompatibility complex class I molecules. Nature 348: 248-251.
13. Stryhn A, Pedersen LO, Romme T, Holm CB, Holm A, et al. (1996) Peptide binding specificity of major histocompatibility complex class I resolved into an array of apparently independent subspecificities: quantitation by peptide libraries and improved prediction of binding. Eur J Immunol 26: 1911-1918.
14. Dadley-Moore DL Lightowlers MW, Rothel JS, Jackson DC (1999) Synthetic peptide antigens induce antibodies to Taenia ovis oncospheres. Vaccine 17: 1506-1515.
15. McDonald D, Stockwin L, Matzow T, Blair Zajdel ME, Blair GE (1999) Coxsackie and adenovirus receptor (CAR)-dependent and major histocompatibility complex (MHC) class I-independent uptake of recombinant adenoviruses into human tumour cells. Gene Ther 6: 1512-1519.
16. Hopp TP, Woods KR (1981) Prediction of protein antigenic determinants from amino acid sequences. Proc Natl Acad Sci U S A 78: 3824-3828.
17. Welling GW, Weijer WJ, van der Zee R, Welling-Wester S (1985) Prediction of sequential antigenic regions in proteins. FEBS Lett 188: 215-218.
18. Parker JM, 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 X-ray-derived accessible sites. Biochemistry 25: 5425-5432.
19. Larsen JE Lund O, Nielsen M (2006) Improved method for predicting linear B-cell epitopes. Immunome Res 2: 2.
20. Kolaskar AS Tongaonkar PC (1990) A semi-empirical method for prediction of antigenic determinants on protein antigens. FEBS Lett 276: 172-174.
21. Reche PA Glutting JP, Reinherz EL (2002) Prediction of MHC class I binding peptides using profile motifs. Hum Immunol 63: 701-709.
22. Reche PA Reinherz EL (2003) Sequence variability analysis of human class I and class II MHC molecules: functional and structural correlates of amino acid polymorphisms. J Mol Biol 331: 623-641.
23. Craiu A Akopian T, Goldberg A, Rock KL (1997) Two distinct proteolytic processes in the generation of a major histocompatibility complex class I-presented peptide. Proc Natl Acad Sci U S A 94: 10850-10855.
24. Gomase VS (2006) Prediction of antigenic epitopes of neurotoxin Bmbktx1 from Mesobuthus martensii. Curr Drug Discov Technol 3: 225-229.
25. Sherkhane AS, Changbhale SS, Chitlange NR, Waghmare S, Gomase VS, et al. (2012) Prediction of Major Histocompatibility Complex Binding Peptides and Epitopes from Naja naja Cardiotoxin (CTX). Drug Invention Today 4: 435-438.
26. 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.
27. Gomase VS, Chitlange NR, Sherkhane AS, Changbhale SS, Kale KV (2013) Prediction of Wuchereria Bancrofti Troponin Antigenic Peptides: Application in Synthetic Vaccine Design to Counter Lymphatic Filariasis. J Vaccines Vaccin 4: 169.
28. Gomase VS, Shyamkumar K (2009) Prediction of antigenic epitopes and MHC binders of neurotoxin alpha-KTx 3.8 from Mesobuthus tamulus sindicus. African Journal of Biotechnology 8: 6658-6676.
29. Bhasin M Raghava GP (2004) Analysis and prediction of affinity of TAP binding peptides using cascade SVM. Protein Sci 13: 596-607.
30. Emini EA, Hughes JV, Perlow DS, Boger J (1985) Induction of hepatitis A virus-neutralizing antibody by a virus-specific synthetic peptide. J Virol 55: 836-839.
31. Karplus PA, Schulz GE (1985) Prediction of chain flexibility in proteins: a tool for the selection of peptide antigen. Natur wissen schaften 72: 212-213.
32. Sweet RM, Eisenberg D (1983) Correlation of sequence hydrophobicities measures similarity in three-dimensional protein structure. J Mol Biol 171: 479-488.
33. Kyte J, Doolittle RF (1982) A simple method for displaying the hydropathic character of a protein. J Mol Biol 157: 105-132.
34. Abraham DJ, Leo AJ (1987) Extension of the fragment method to calculate amino acid zwitterion and side chain partition coefficients. Proteins 2: 130-152.
35. 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.
36. Miyazawa S, Jernigen RL (1985) Estimation of Effective Interresidue Contact Energies from Protein Crystal Structures: Quasi-Chemical Approximation. Macromolecules 18: 534-552.
37. Roseman MA (1988) Hydrophilicity of polar amino acid side-chains is markedly reduced by flanking peptide bonds. J Mol Biol 200: 513-522.
38. Wolfenden R, Andersson L, Cullis PM, Southgate CC (1981) Affinities of amino acid side chains for solvent water. Biochemistry 20: 849-855.
39. Wilson KJ, Honegger A, Stötzel RP, Hughes GJ (1981) The behaviour of peptides on reverse-phase supports during high-pressure liquid chromatography. Biochem J 199: 31-41.
40. Cowan R, Whittaker RG (1990) Hydrophobicity indices for amino acid residues as determined by high-performance liquid chromatography. Pept Res 3: 75-80.
41. Chothia C (1976) The nature of the accessible and buried surfaces in proteins. J Mol Biol 105: 1-12.
42. 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.
43. Robson B, Garnier J (1993) Protein structure prediction. Nature 361: 506.