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Quantitative Immunoproteomics Approach for the Development of MHC Class I Associated Peptide Antigens of Alpha-Cobra Toxin from *Naja kaouthia*

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Abstract

Alpha-Cobratoxin from *N. kaouthia* binds to acetylcholine receptors which are located in neuromuscular junctions, once activated; they cause contract muscles and block their actions thereby bringing on muscle paralysis. Peptide fragments of Alpha-Cobratoxin from *N. kaouthia* having 71 amino acids, which shows 63 nonamers and are used synthetic peptide vaccine design and to increase the understanding of roles of the immune system against snake bite. For the immune responses against a protein antigen, it is clear that the whole protein is not necessary for raising the immune response, but small segments (15-PNGHVCYTKT-24, 26-CDAFCSIRG-34 and 36-RVDLGCAATCPTVKTGVDIQCCSTD-60) of Alpha-Cobratoxin protein from *N. kaouthia* called the antigenic determinants or the epitopes are sufficient for eliciting the desired immune response. The identification of specific peptides that binds to MHC class I molecules is important to recognize T-cell epitopes. In this research work, we predict antigenicity, Solvent accessibility to identify the membrane-spanning regions (hydrophobic) or regions that are likely exposed on the surface of proteins (hydrophilic domains) which are potentially antigenic that are used to design synthetic peptide vaccine.

Keywords: Alpha-cobratoxin; *N. kaouthia*; Antigenic peptides; MHC-binders; TapPred; PSSM

Introduction

The *N. kaouthia* is also called monocled cobra, is widespread across central and southern Asia regions [1]. It can adapt habitats from natural to anthropogenically impacted environments and are most active at dusk. Alpha-Cobratoxin from *N. kaouthia* venoms are postsynaptic neurotoxins, that have high affinity to muscular, Torpedo and neuronal alpha-7 nicotinic acetylcholine receptors which block the nerve transmission by binding specifically to the nicotinic acetylcholine receptor, leading paralysis and even death occurred due to respiratory failure [2-4]. Alpha-Cobratoxin *N. kaouthia* antigenic peptides are most suitable for 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 of antigenic proteins, present them at the cell surface and are recognized by T-cells [5,6]. T cell recognition is a fundamental mechanism by which the host identifies and responds to foreign antigens [7,8]. The MHC molecule is extremely polymorphic [9]. MHC class I molecules are expressed on most nucleated cells, generally present peptides from intracellular proteins that 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 to elicit an immune response via T-cell. T cells also recognize self-peptides but are eliminated during the thymic selection; therefore, the primary targets of T cell to recognize [10] foreign peptides and kill host target cells. The second and the C-terminal position of the peptide are the most important for binding [11,12] and the amino acids at each position contribute a certain binding energy [13]. Therefore, the identification of MHC-binding peptides and T-cell epitopes and study of antigenic properties helps to improve our understanding of specificity of immune responses are important for the development of new vaccines. However, this theme is implemented in designing synthetic peptide vaccines.

Materials and Methods

Antigenic epitopes of Alpha-Cobratoxin from *N. kaouthia* are determined by using the Hopp and Woods, Welling, Parker, Bepipred, Kolaskar and Tongaonkar antigenicity methods to predicts those segments which are likely to be antigenic by eliciting an immune response. [16-20]. The MHC peptide binding of antigen protein is predicted by using neural networks trained on C terminals of known epitopes. Rankpep 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) [21-28]. We predict cascade SVM based several TAP binders which was based on the sequence and the features of amino acids [29]. 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 Emani et al. [30] and Karplus and Schulz to identify active site of functionally important residues in membrane proteins. [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 Interpretation

A antigenic sequence of Alpha-Cobratoxin from *N. kaouthia* is 71 residues long as- >gi|128930|sp|P01391.1|NXL1_KAIRCFITP-

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DITSKDCPNGHVCYTKTWCDAFCSIRGKRVDLGCAATCPTVKT-GVDIQCCSTDNCNPFPTRKRP

Prediction of antigenic peptides

Antigenicity is predicted by identifying antigenic determinants by finding the area of greatest local hydrophilicity. The Hopp-Woods scale Hydrophilicity Prediction Result Data found high in position 9-11, 13-15, 33-37 (1.243) 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 (Figures 1 and 2). 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 20-21 (0.440), 36-38 (Figure 3). We also study Hydrophobicity plot of HPLC/Parker Hydrophilicity Prediction Result Data found 7-PDITSKD-13 (4.500), 8-DITSKDC-14 (4.400), 10-TSKDCPN-16 (5.414), 11-SKDCPNG-17 (5.486), 55-QCCSTDN-61(5.357),57-CSTDNCN-63(5.500),58-STDNCNP-64(5.600) (maximum) (Figure 4), BepiPred predicts the location of linear B-cell epitopes Result found that 9-ITSKDCPNG-17,45-CPTVKT-50, (Figure 5) (Table 3), Kolaskar and Tongaonkara semi-empirical method used

Figure 1: Biological assembly image for Alpha-Cobratoxin protein. X-ray structure (4AEA) of Alpha-Cobratoxin from *N. kaouthia* showing the location of disulfides and possible mode of binding to nicotinic acetylcholine receptors.

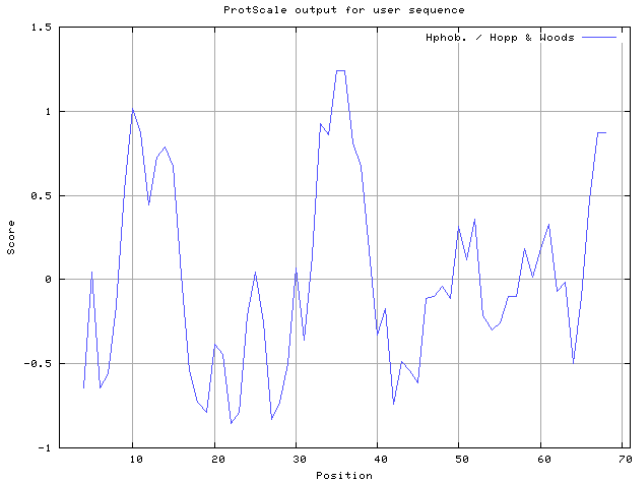

Figure 2: Hydrophobicity plot of Hopp and Woods (1981) of Alpha-Cobratoxin from *N. kaouthia*.

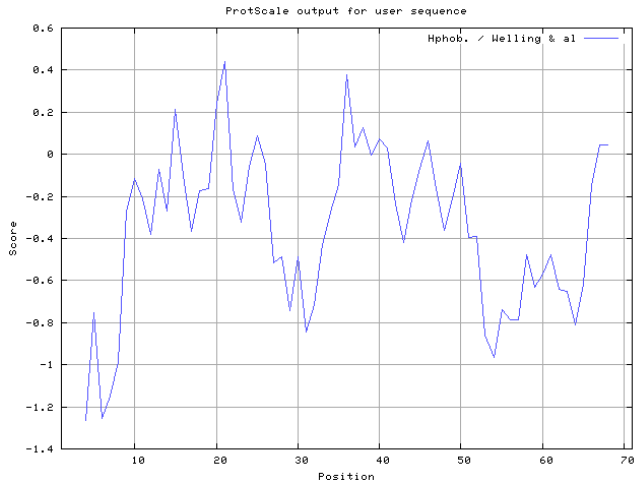

Figure 3: Hydrophobicity plot of Welling et al. (1985) of Alpha-Cobratoxin from *N. kaouthia*.

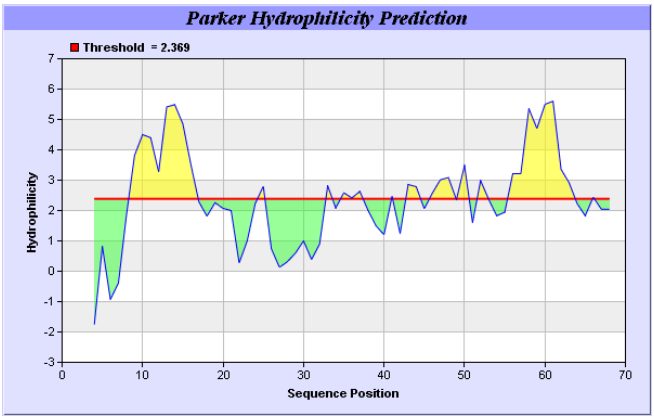

Figure 4: Hydrophobicity plot of HPLC / Parker et al. (1986) of Alpha-Cobratoxin from *N. kaouthia*.

MHC-I Allele	POS	N	SEQUENCE	C	MW (Da)	SCORE	% OPT.
8mer_H2_Db	41	DLG	CAATCPTV	KTG	746.89	21.518	40.99%
8mer_H2_Db	61	STD	NCNPFPTR	KRP	930.05	10.742	20.46%
8mer_H2_Db	58	QCC	STDNCNPF	PTR	878.91	3.74	7.12%
8mer_H2_Db	2	I	RCFITPDI	TSK	946.14	0.021	0.04%
8mer_H2_Db	14	SKD	CPNGHVCY	TKT	874.0	-0.041	-0.08%
8mer_H2_Db	23	CYT	KTWCDAFC	SIR	932.11	-0.625	-1.19%
8mer_H2_Db	18	PNG	HVCYTKTW	CDA	996.17	-4.872	-9.28%
8mer_H2_Db	12	ITS	KDCPNGHV	CYT	850.94	-5.222	-9.95%
8mer_H2_Db	13	TSK	DCPNGHVC	YTK	825.91	-5.524	-10.52%
8mer_H2_Db	50	TVK	TGVDIQCC	STD	819.94	-6.316	-12.03%
8mer_H2_Db	32	FCS	IRGKRVDL	GCA	938.14	-8.761	-16.69%
8mer_H2_Db	22	VCY	TKTWCDAF	CSI	930.07	-9.103	-17.34%
8mer_H2_Db	49	PTV	KTGVDIQC	CST	844.97	-9.479	-18.06%
8mer_H2_Db	26	KTW	CDAFCSIR	GKR	896.06	-9.479	-18.06%
8mer_H2_Db	62	TDN	CNPFPTRK	RP	944.12	-15.508	-29.54%
8mer_H2_Db	28	WCD	AFCSIRGK	RVD	863.05	-18.667	-35.56%
8mer_H2_Db	29	CDA	FCSIRGKR	VDL	948.16	-28.837	-54.93%

Table 1a: Peptide binders of Alpha-Cobratoxin from N. *kaouthia* to MHC-I molecules, 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	SCORE	% OPT.
9mer_H2_Db	57	IQC	CSTDNCNPF	PTR	982.05	14.379	28.55%
9mer_H2_Db	12	ITS	KDCPNGHVC	YTK	954.08	7.581	15.05%
9mer_H2_Db	13	TSK	DCPNGHVCY	TKT	989.09	5.283	10.49%
9mer_H2_Db	40	VDL	GCAATCPTV	KTG	803.94	2.756	5.47%
9mer_H2_Db	61	STD	NCNPFPTRK	RP	1058.22	1.882	3.74%
9mer_H2_Db	21	HVC	YTKTWCDAF	CSI	1093.25	-0.651	-1.29%
9mer_H2_Db	22	VCY	TKTWCDAFC	SIR	1033.21	-2.115	-4.20%
9mer_H2_Db	25	TKT	WCDAFCSIR	GKR	1059.27	-2.581	-5.12%
9mer_H2_Db	49	PTV	KTGVDIQCC	STD	948.11	-3.287	-6.53%
9mer_H2_Db	31	AFC	SIRGKRVDL	GCA	1025.22	-6.633	-13.17%
9mer_H2_Db	17	CPN	GHVCYTKTW	CDA	1053.22	-6.897	-13.69%
9mer_H2_Db	60	CST	DNCNPFPTR	KRP	1045.14	-9.799	-19.46%
9mer_H2_Db	11	DIT	SKDCPNGHV	CYT	938.02	-10.788	-21.42%
9mer_H2_Db	27	TWC	DAFCSIRGK	RVD	978.14	-14.271	-28.34%
9mer_H2_Db	1	-	IRCFITPDI	TSK	1059.3	-14.47	-28.73%
9mer_H2_Db	48	CPT	VKTGVDIQC	CST	944.1	-20.758	-41.22%
9mer_H2_Db	28	WCD	AFCSIRGKR	VDL	1019.24	-25.564	-50.76%

Table 1b: Peptide binders of Alpha-Cobratoxin from N. *kaouthia* to MHC-I molecules, 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.

MHC-I Allele	POS.	N	SEQUENCE	C	MW	SCORE	% OPT.
10mer_H2_Db	60	CST	DNCNPFPTRK	RP	1173.31	12.485	21.21%
10mer_H2_Db	12	ITS	KDCPNGHVCY	TKT	1117.26	7.127	12.11%
10mer_H2_Db	59	CCS	TDNCNPFPTR	KRP	1146.24	6.054	10.29%
10mer_H2_Db	39	RVD	LGCAATCPTV	KTG	917.1	1.576	2.68%
10mer_H2_Db	16	DCP	NGHVCYTKTW	CDA	1167.32	-2.258	-3.84%
10mer_H2_Db	27	TWC	DAFCSIRGKR	VDL	1134.33	-5.536	-9.41%
10mer_H2_Db	30	DAF	CSIRGKRVDL	GCA	1128.36	-6.587	-11.19%
10mer_H2_Db	24	YTK	TWCDAFCSIR	GKR	1160.37	-6.936	-11.78%
10mer_H2_Db	11	DIT	SKDCPNGHVC	YTK	1041.16	-9.087	-15.44%
10mer_H2_Db	48	CPT	VKTGVDIQCC	STD	1047.24	-11.614	-19.73%
10mer_H2_Db	10	PDI	TSKDCPNGHV	CYT	1039.12	-11.992	-20.37%
10mer_H2_Db	47	TCP	TVKTGVDIQC	CST	1045.2	-18.063	-30.69%
10mer_H2_Db	20	GHV	CYTKTWCDAF	CSI	1196.39	-18.172	-30.87%
10mer_H2_Db	56	DIQ	CCSTDNCNPF	PTR	1085.19	-21.639	-36.76%
10mer_H2_Db	21	HVC	YTKTWCDAFC	SIR	1196.39	-25.354	-43.08%
10mer_H2_Db	26	KTW	CDAFCSIRGK	RVD	1081.28	-27.786	-47.21%

Table 1c: Peptide binders of Alpha-Cobratoxin from N. *kaouthia* to MHC-I molecules, 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	SCORE	% OPT
11mer_H2_Db	11	DIT	SKDCPNGHVCY	TKT	1204.34	14.334	18.03%
11mer_H2_Db	47	TCP	TVKTGVDIQCC	STD	1148.34	-8.952	-11.26%
11mer_H2_Db	26	KTW	CDAFCSIRGKR	VDL	1237.47	-9.5	-11.95%
11mer_H2_Db	15	KDC	PNGHVCYTKTW	CDA	1264.44	-9.844	-12.38%
11mer_H2_Db	29	CDA	FCSIRGKRVDL	GCA	1275.54	-14.187	-17.85%
11mer_H2_Db	59	CCS	TDNCNPFPTRK	RP	1274.41	-16.292	-20.49%
11mer_H2_Db	20	GHV	CYTKTWCDAFC	SIR	1299.53	-17.308	-21.77%
11mer_H2_Db	19	NGH	VCYTKTWCDAF	CSI	1295.52	-18.359	-23.09%
11mer_H2_Db	10	PDI	TSKDCPNGHVC	YTK	1142.26	-18.948	-23.84%
11mer_H2_Db	23	CYT	KTWCDAFCSIR	GKR	1288.54	-20.282	-25.51%
11mer_H2_Db	46	ATC	PTVKTGVDIQC	CST	1142.32	-20.512	-25.80%
11mer_H2_Db	9	TPD	ITSKDCPNGHV	CYT	1152.28	-23.253	-29.25%
11mer_H2_Db	55	VDI	QCCSTDNCNPF	PTR	1213.32	-24.834	-31.24%
11mer_H2_Db	58	QCC	STDNCNPFPTR	KRP	1233.32	-24.917	-31.34%
11mer_H2_Db	38	KRV	DLGCAATCPTV	KTG	1032.19	-25.364	-31.91%
11mer_H2_Db	4	IRC	FITPDITSKDC	PNG	1221.39	-28.415	-35.74%
11mer_H2_Db	25	TKT	WCDAFCSIRGK	RVD	1244.49	-30.115	-37.88%

Table 1d: Peptide binders of Alpha-Cobratoxin from N. *kaouthia* to MHC-I molecules, 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.

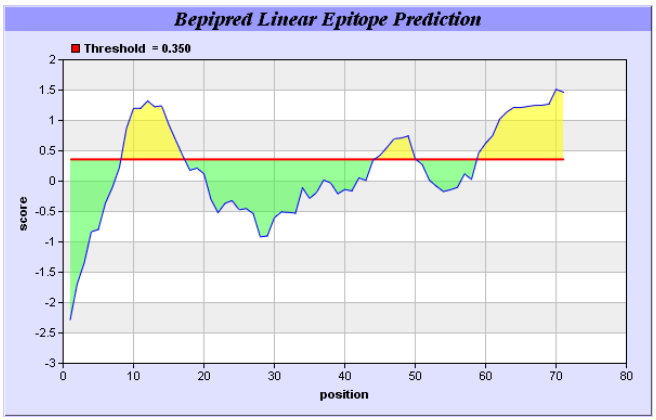

Figure 5: Bepipred Linear Epitope Prediction plot showing antibody recognized B-cell epitopes of Alpha-Cobratoxin from *N. kaouthia*.

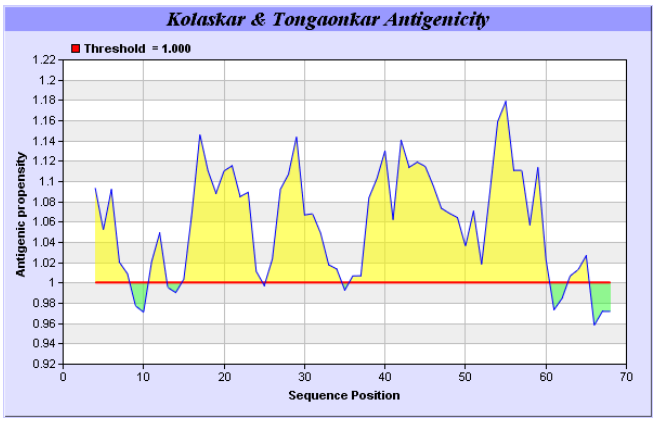

Figure 6: Kolaskar and Tongaonkar antigenicity plot for the Alpha-Cobratoxin from *N. kaouthia*.

Peptide Rank	Start Position	Sequence	Score	Predicted Affinity
1	41	CAATCPTVK	8.618	High
2	30	CSIRGKRVD	8.586	High
3	38	DLGCAATCP	8.549	High
4	20	CYTKTWCDA	8.487	High
5	50	TGVDIQCCS	8.385	High
6	58	STDNCNPFP	8.241	High
7	7	PDITSKDCP	8.071	High
8	40	GCAATCPTV	8.010	High
9	32	IRGKRVDLG	7.958	High
10	55	QCCSTDNCN	7.872	High
11	36	RVDLGCAAT	7.814	High
12	62	CNPFPTRKR	7.738	High
13	47	TVKTGVDIQ	7.576	High
14	46	PTVKTGVDI	7.561	High
15	22	TKTWCDAFC	7.380	High
16	48	VKTGVDIQC	7.319	High
17	51	GVDIQCCST	7.318	High
18	33	RGKRVDLGC	6.855	High
19	44	TCPTVKTGV	6.789	High
20	15	PNGHVCYTK	6.764	High
21	16	NGHVCYTKT	6.535	High
22	17	GHVCYTKTW	6.355	High
23	29	FCSIRGKRV	6.308	High
24	11	SKDCPNGHV	6.130	High
25	61	NCNPFPTRK	6.085	High

Table 2: Cascade SVM based High affinity TAP Binders of Alpha-Cobratoxin from N. *kaouthia*.

No.	Start Position	End Position	Peptide	Peptide Length
1	9	17	ITSKDCPNG	9
2	45	50	CPTVKT	6

Table 3: Predicted Antigenic epitopes of Alpha-Cobratoxin from N. *kaouthia* Bepipred.

No.	Start Position	End Position	Peptide	Peptide Length
1	15	24	PNGHVCYTKT	10
2	26	34	CDAFCSIRG	9
3	36	60	RVDLGCAATCPTVKTGVDIQCCSTD	25

Table 4: Predicted Antigenic epitopes of Alpha-Cobratoxin from N. *kaouthia*.

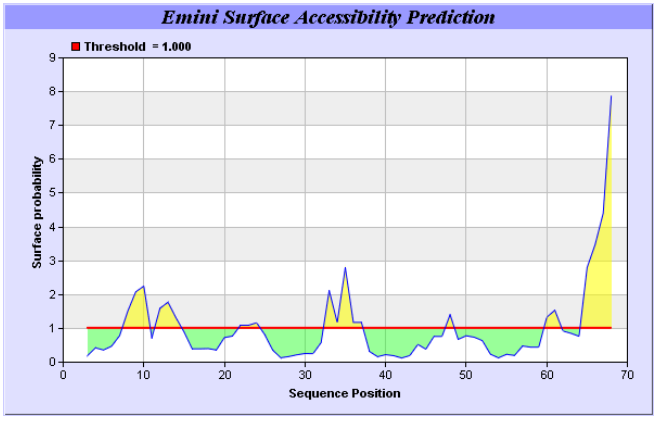

Figure 7: Emini Surface Accessibility Prediction plot of Alpha-Cobratoxin from *N. kaouthia*.

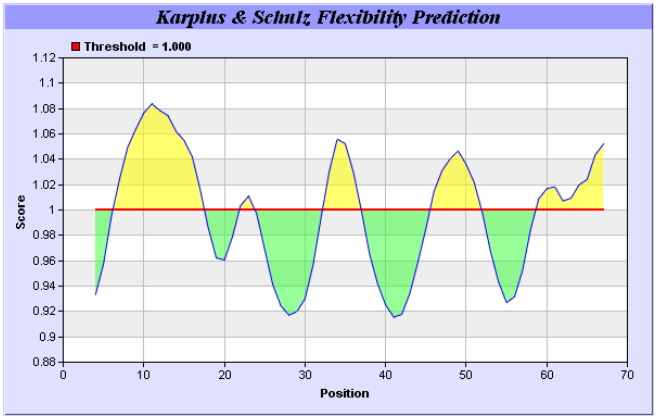

Figure 8: Karplus and Schulz Flexibility Prediction of Alpha-Cobratoxin from *N. kaouthia*.

for prediction of antigenic determinants on protein antigens. Predicted peptides result found i.e. 15-PNGHVCYTKT-24, 26-CDAFCSIRG-34, 36-RVDLGCAATCPTVKTGVDIQCCSTD-60, (Figure 6) (Table 4). 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 to identify antigenic activity, surface region of peptides. Emani et al., (Figure 7) predicts the highest probability i.e. found 7-PDITSK-12 (2.082), 8-DITSKD-13 (2.248), 31-SIRGKR-36 (2.121), 33-RGKRVD-38 (2.798), 63-NPFPTR-68 (2.798), 64-PFPTRK-69 (3.480), 65-FPTRKR-70 (4.408), 66-PTRKRP-71 (7.872) (maximum), 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 (Figure 8) method used for the Selection of Peptide Antigens. High score is found in residue i.e. 6-TPDITSK-12 (1.064), 7-PDITSKD-13 (1.077), 8-DITSKDC-14 (1.084) (maximum), 9-ITSKDCP-15 (1.078), 10-TSKDCPN-16 (1.074), 11-SKDCPNG-17 (1.062). Karplus and Schulz 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. hydrophobicity prediction Result Data found high in position 4 (0.461), 6-7, 21-23, Kyteand Doolittle result high in position 4,6-7, 29-31, 40-44 (1.614), Abraham and Leo result data shows high in position 6-7 (1.230), 27-29, 40-42, Bull and Breese result high in position 13-16, 43-44, 58-61 (0.557),Guy result high in position 9-10, 13-15, 33-37,

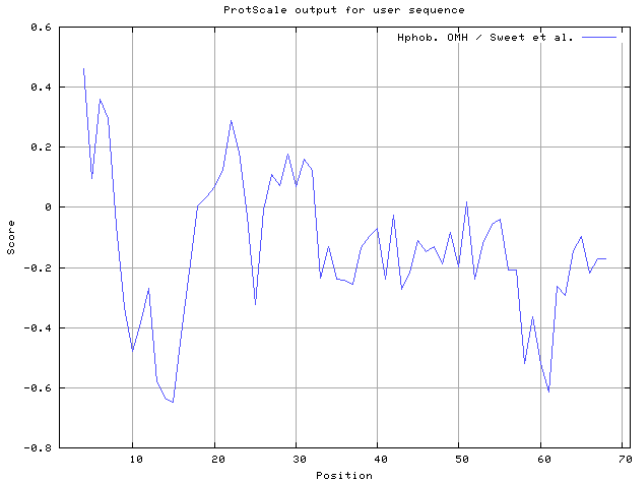

Figure 9: Hydrophobicity plot of Sweet et al. (1983) of Alpha-Cobratoxin from *N. kaouthia*.

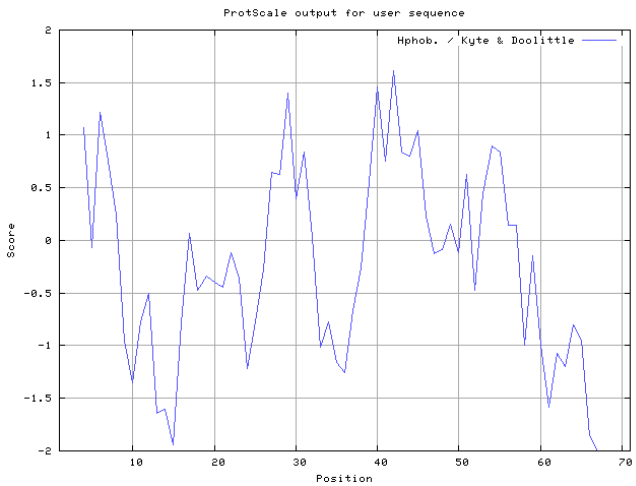

Figure 10: Kyte and Doolittle hydrophobicity plot of Alpha-Cobratoxin from *N. kaouthia*.

66-68 (0.661), Miyazawa result high in position 4-7 (6.737), 27-31, 41-42, 54-57, Roseman result high in position 6-7 (0.334), 17-18,42-45, Wolfenden result high in position 42 (0.170), Wilson et al. 4-6, 17-19, 22-23, 26-32 (3.671), 39-40, 54-55, Cowan 4-7 (0.899), 27-29, 40-42, Chothia4-8, 27-32 (0.407), 39-45, 53-57, (Figures 9-19).

Prediction of MHC binding peptide

We predict the peptide binders of Alpha-Cobratoxin from *N. kaouthia* to MHC-I molecules to a number of different alleles using Position Specific Scoring Matrix. Alpha-Cobratoxin from *N. kaouthia* sequence is 71 residues long, having 63 nonamers. 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 Alpha-Cobratoxin from *N. kaouthia* 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 1a-1d). Here RANKPEP report PSSM-specific binding threshold and is obtained by scoring all the antigenic peptide

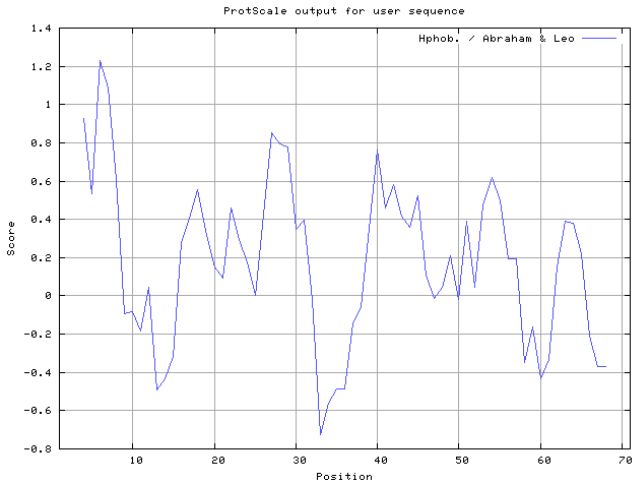

Figure 11: Abraham & Leo hydrophobicity plot of Alpha-Cobratoxin from *N. kaouthia.*

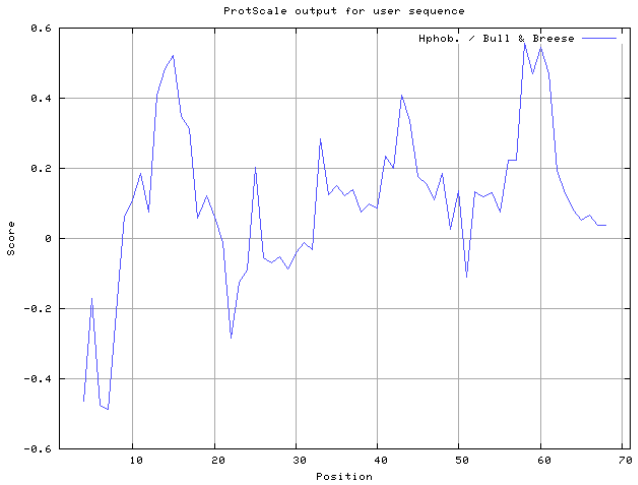

Figure 12: Bull & Breese use surface tension to measure hydrophobicity of Alpha-Cobratoxin from *N. kaouthia.*

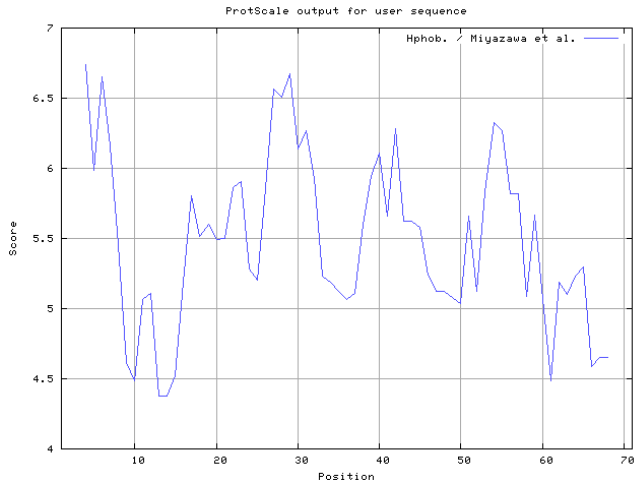

Figure 13: Hydrophobicity plot of Miyazawa et al. (1985) of Alpha-Cobratoxin from *N. kaouthia.*

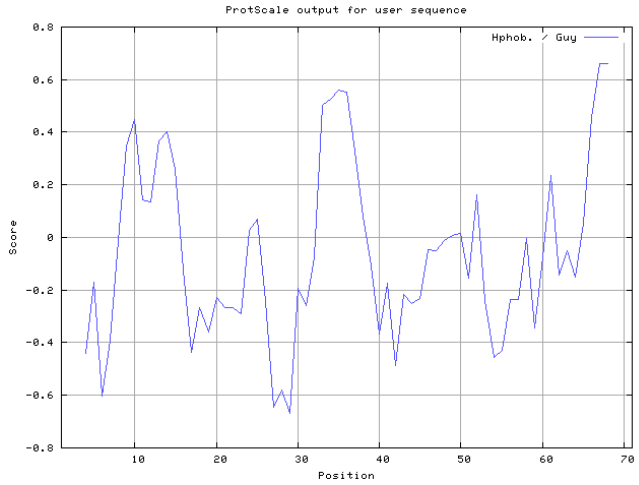

Figure 14: Hydrophobicity plot of Guy (1988) of Alpha-Cobratoxin from *N. kaouthia.*

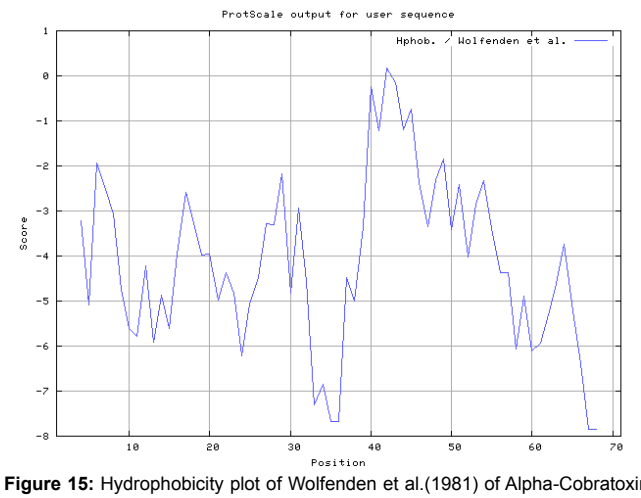

Figure 15: Hydrophobicity plot of Wolfenden et al.(1981) of Alpha-Cobratoxin from *N. kaouthia.*

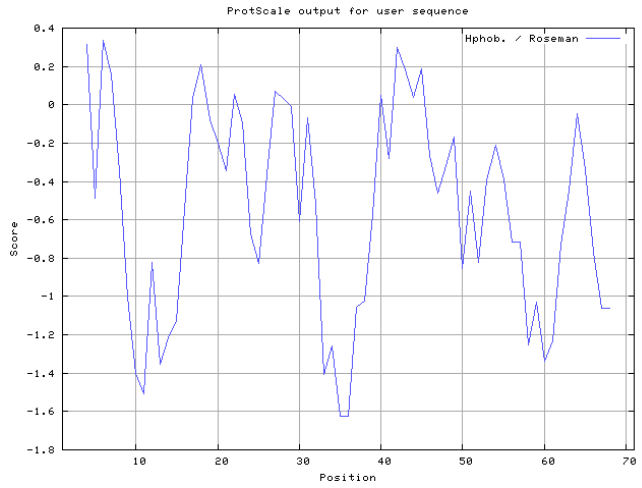

Figure 16: Hydrophobicity plot of RosemanM.A.. (1988) of Alpha-Cobratoxin from *N. kaouthia*.

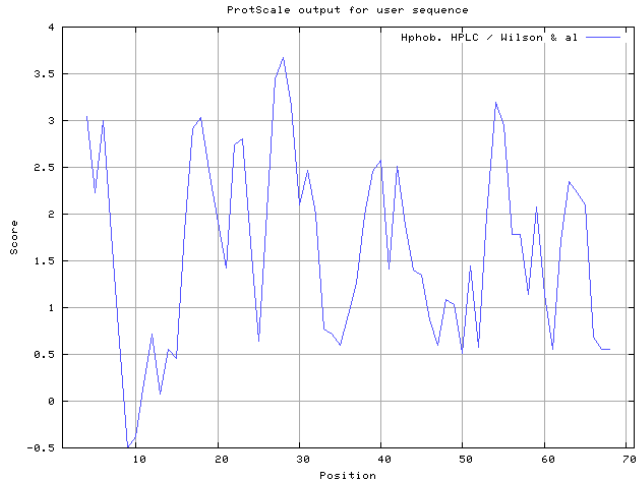

Figure 17: Hydrophobicity/HPLC plot of Wilson & al (1981) of Alpha-Cobratoxin from *N. kaouthia*.

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 highlighted in (Tables 1a-1d) and peptides produced by the cleavage prediction model are highlighted in (Table 2). We also use a cascade SVM based TAPP red method which found 25 High affinity TAP Transporter peptide regions which represents predicted TAP binders residues which occur at N and C termini from Alpha-Cobratoxin from *N. kaouthia*.

Discussion

In this study, we found the antigenic determinants by finding the area of greatest local hydrophilicity. Hopp and Woods hydrophobicity 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 9-11, 13-15, 33-37 (1.243) in a protein this scale is basically a hydrophilic index where a polar 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 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 20-21 (0.440), 36-38. 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 7-PDITSKD-13 (4.500), 8-DITSKDC-14 (4.400), 10-TSKDCPN-16 (5.414), 11-SKDCPNG-17 (5.486), 55-QCCSTDN-61 (5.357), 57-CSTDNCN-63 (5.500), 58-STDNCNP-64 (5.600) (maximum) (Figure 4). BepiPred predicts the location of linear B-cell epitopes Result found that there are 2 predicted epitopes are found 9-ITSKDCPNG-17, 45-CPTVKT-50, (Figure 5) (Table 3). There are 3 antigenic determinant sequences is found by Kolaskar and Tongaonkar antigenicity scales the results show highest pick at position 15-PNGHVCYTKT-24, 26-CDAFCSIRG-34, 36-RVDLGCAATCPTVKTGVDIQCCSTD-60, (Figure 6) (Table 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

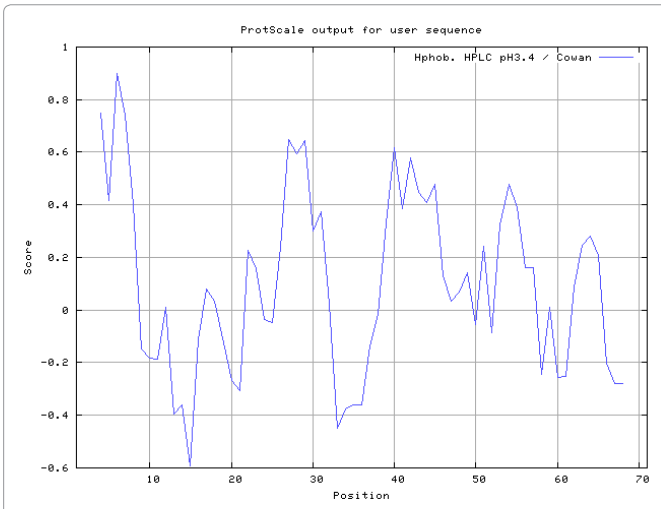

Figure 18: Hydrophobicity/HPLC pH 3.4/ plot of Cowan (1990) of Alpha-Cobratoxin from *N. kaouthia*.

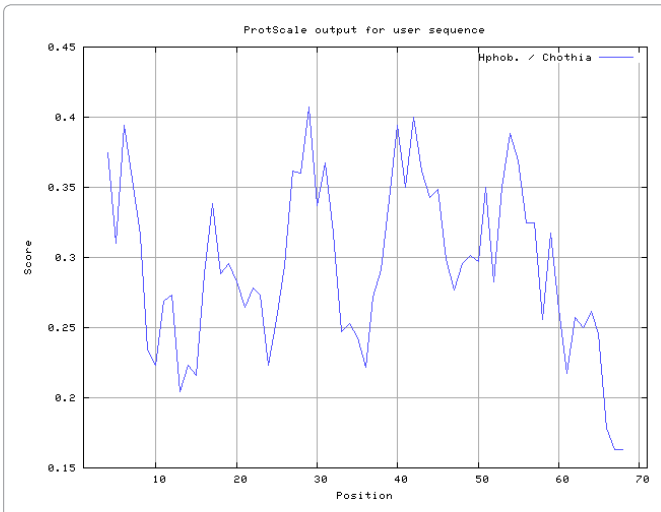

Figure 19: Hydrophobicity plot of Chothia (1976) of Alpha-Cobratoxin from *N. kaouthia*.

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. X-Ray Diffraction with Resolution 1.94 Å 3D Structure of the Alpha-Cobratoxin from *N. kaouthia* is predicted by PDB vive. We generate a purified protein for analysis of the chosen target and then structure determined the target experimentally to evaluate their similarity to known protein structures and to determine possible relationships that are identifiable from protein sequence alone. The target structure will also serve as a detailed model for determining the structure of peptide within that protein structure. We predict Solvent accessibility by using Emani et al., the result found the highest probability i.e. found 7-PDITSK-12(2.082),8-DITSKD-13 (2.248), 31-SIRGKR-36 (2.121), 33-RGKRVD-38 (2.798), 63-NPFPTR-68 (2.798), 64-PFPTRK-69 (3.480), 65-FPTRKR-70 (4.408), 66-PTRKRP-71 (7.872) (maximum), 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. 6-TPDITSK-12 (1.064), 7-PDITSKD-13 (1.077), 8-DITSKDC-14 (1.084) (maximum), 9-ITSKDCP-15 (1.078), 10-TSKDCPN-16 (1.074), 11-SKDCPNG-17 (1.062). We predict Solvent accessibility of Alpha-Cobratoxin from *N. kaouthia* 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 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 4 (0.461), 6-7, 21-23, Kyte and Doolittle result high in position 4, 6-7, 29-31, 40-44 (1.614), Abraham and Leo result high in position 6-7(1.230), 27-29, 40-42, Bull and Breese result high in position 13-16, 43-44, 58-61 (0.557), Guy result high in position 9-10, 13-15, 33-37, 66-68 (0.661), Miyazawa result high in position 4-7 (6.737), 27-31, 41-42, 54-57, Roseman result high in position 6-7 (0.334), 17-18, 42-45, Wolfenden result high in position 42 (0.170), Wilson et al. 4-6, 17-19, 22-23, 26-32 (3.671), 39-40, 54-55, Cowan 4-7(0.899), 27-29, 40-42, Chothia 4-8,27-32 (0.407), 39-45, 53-57, (Figures 9-19). 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. In this study, we found 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 TAPP red method which found 25 High affinity TAP Transporter peptide regions which represents predicted TAP binders residues which occur at N and C termini from Alpha-Cobratoxin from *N. kaouthia*. 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 steps in endogenous antigen processing. The correlation coefficient of 0.88 was obtained by using jackknife validation test. In this test, we found the MHCI and MHCII binding regions. 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 Alpha-Cobratoxin from *N. kaouthia*. 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 specifity 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 RANKPEP predict *Peptide binders of* Alpha-Cobratoxin from *N. kaouthia to MHC-I molecules* 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. All above prediction methods are based on propensity scales for the 20 amino acids to describe the tendency of each residue to be associated with properties such as hydrophilicity, surface accessibility or mobility. 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 Alpha-Cobratoxin from *N. kaouthia* 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 Alpha-Cobratoxin from *N. kaouthia*. 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.

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