

An immunoinformatics based approach to identify the and design synthetic peptide vaccine for Loiasis

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ABSTRACT

The causative agent 'Loa loa' responsible for the "Loiasis" (African eye worm). The deerflies is the intermediate host for the parasitic worm transmission to the human through the repetitive bites. The protein cytochrome c oxidase subunit III (COX3) of the Loiasis (Loa loa) is a 259 amino acid residue with 251 nonamers. In this assay we predicted the binding affinity of COX3 protein, we found the high affinity TAP Transporter peptide regions as 73-YDYRMFNQG (Score: 8.644), 102-DSSLCSLTW (Score: 8.62), 66-VSGQYSFYD (Score: 8.62), 36-SMGCFFSIF (Score: 8.6), 126-IGINGMASL (Score: 8.594), 155-FNCEVFLLI (Score: 8.58), 104-SLCSLTWL (Score: 8.562), 161-LLICIFIGS (Score: 8.555), 123-PDYIGINGM (Score: 8.543), 21-GFGILGIDV (Score: 8.503). We also predicted the SVM based MHC-IAb peptide regions, 12-EYSYPLMF (optimal score: 0.836), 52-YVSFLWLKD (optimal score: 0.596), 56-LWLKDVMLE (optimal score: 0.588), 220-IKLFNFNWY (optimal score: 0.567); MHC-IAd peptide regions, 168-GSFFLCFQF (optimal score: 0.721), 38-GCFFSIFIC (optimal score: 0.717), 127-GINGMASLF (optimal score: 0.658), 23-GILGIDVSL (optimal score: 0.605); MHC-IAg7 peptide regions, 26-GIDVSLALF (optimal score: 1.309), 77-MFNQGFRLF (optimal score: 1.305), 49-YIYVSFLW (optimal score: 1.289), 126-IGINGMASL (optimal score: 1.286); MHC-RT1.B peptide regions, 223-FNFNWYHTQ (optimal score: 0.645), 136-LMMNSQLLK (optimal score: 0.606), 77-MFNQGFRLF (optimal score: 0.548), 84-LFLFSELAL (optimal score: 0.513) which represented predicted binders from cytochrome c oxidase subunit III (COX3) of the Loiasis (Loa loa). We can assume that even these predicted small antigenic peptide fragment can induce the immune response against the whole antigen. On the basis of this finding the subunit and synthetic peptide vaccines can be developed.

Keywords—Loiasis; Loa loa; Epitopes; Antigenic peptides; MHC-Binders; TapPred; PSSM; SVM; Nonamers; cytochrome c oxidase subunit III (mitochondrion)

I. INTRODUCTION

"Loiasis" commonly known as African eye worm and causative agent is "Loa loa", [1-3] a parasitic worm which is transmitted through the intermediate host of the genus Chrysops through the repetitive bites of the deerflies, well known mango flies or mangrove flies [4]. Usually this parasite breeds in the high-rainfall forest region of the West and central Africa. The symptoms of this infection is Calabar swellings which is a itchy swellings (local angioedema). This infection remains asymptomatic for several months in many individuals. The non-painful swelling appears in any parts of the body more commonly near joints. The detection of this infection is found in the blood test which shows high eosinophils count, which is mostly known to associate with the parasitic infections.

Recognition of *Loa loa* infections has become more important in Africa because the presence of *Loa loa* infection has limited programs to control or eliminate onchocerciasis and lymphatic filariasis. This infection is non-contagious. The intermediate host i.e., *Chrysops* becomes infectious when they bit and suck the blood of infected person. mangrove flies usually attracted by the people movement and wood fires smoke .The occurrence of these flies were also seen in those area where the rubber plantation are in practice . The worm can be surgically removed in order to provide immediate relief but this not the full proof elimination techniques because worm can be localized in other parts of body too.The medical practitioner usually provide the medication to kill the worm and eliminated from the infected individuals such as diethylcarbamazine, or DEC .The worm killing through medication also not the safe way of treatment because there is small risk of the serious side effect which is associated with the worm killing. The health practitioner before selecting the treatment method to treat the patient they do test so that they can provide safe treatment to the infected person. The precaution measures are bring into this practice to avoid the infection such as wearing long pants and long-sleeved shirts and avoid the smoke of wood fires. Due to increasing travel and the migration of people from the endemic countries of West Africa to Europe and the USA, there are possible emergence of loiasis [5]. *Loa loa* macrofilariasis in the eyelid is also reported in India [6]

II. MATERIALS AND METHODS

II(a). Protein sequence analysis

The antigenic protein sequence of cytochrome c oxidase subunit III (mitochondrion) of parasite *Loa loa* was analyzed to study the antigenicity , solvent accessible regions and MHC class peptide binding, which allows potential drug targets to identify active sites against parasitic infection.

II(B). Prediction of antigenicity

Prediction of antigenicity program predicts those segments from antigenic protein that are likely to be antigenic by eliciting an antibody response. Antigenic epitopes are determined using the Gomase [7], Hopp and Woods, Welling, Parker, B-EpiPred Server and Kolaskar and Tongaonkar antigenicity methods [8-13].

II(c) . 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 [14].

II(d). 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 [15-35]

II(e) Prediction of MHC binding peptide

The MHC peptide binding is predicted using neural network strained on C terminals of known epitopes. In analysis predicted MHC/ peptide binding is a log-transformed value related to the IC₅₀ values in nM units. MHC2Pred predicts peptide binders to MHCI and MHCII molecules from protein sequences or sequence alignments using Position Specific Scoring Matrices (PSSMs). Support Vector Machine (SVM) based method for prediction of promiscuous MHC class II binding peptides. The average accuracy of SVM based method for 42 alleles is ~80%. For development of MHC binder, an elegant machine learning technique SVM has been used. SVM has been trained on the binary input of single amino acid sequence. In addition, we predicts those MHCI ligands whose C-terminal end is likely to be the result of proteosomal cleavage [36-46].

III. RESULT AND INTERPRETATION

A antigenic sequence cytochrome c oxidase subunit III (mitochondrion) is 259 aa residues long as MFLKFRKFHKMEYSYYPMLFGFGILGIDVSLALFMSMGCFSSIFICLLYIIYVSFLWLKDVMLEDVSGQY SFYDYRMFNQGFRLFLFSELALFFSIFWTYLDSSLCSTWLGGVWSPLGILSPDYIGINGMASLFLMMNS QLLKYSRRYLCLNKFNCVFLLCIFIGSFLLCFQFYEYNNNCFVMNDSIYGNVIFYVGTGLHGSHV FVG VCFLIINLFRIKLFNFNWYHTQSYDMSIDYWRFLWWMWGIMFCLLYIWGA

III.A. Prediction of antigenic peptides

In these methods we found the antigenic determinants by finding the area of greatest local hydrophilicity. The Hopp-Woods scale was designed to predict the locations of antigenic determinants 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).

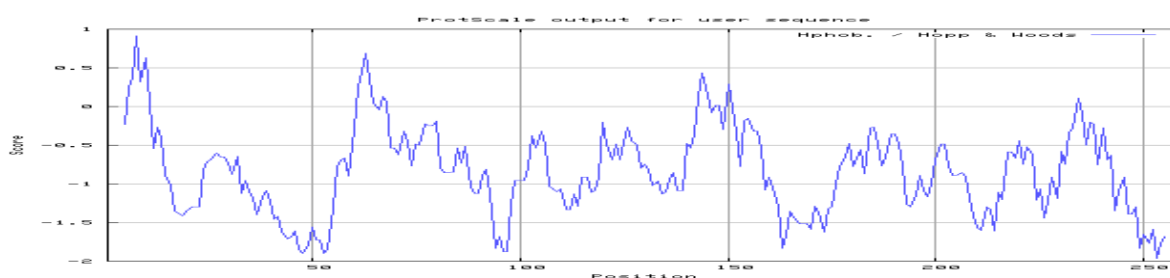


Figure 1: Hydrophobicity plot of Hopp and Woods (1981) of cytochrome c oxidase subunit III. At the Position:8 the peak is found highest with Score: 0.911 (max) and sequence is 5-FRKFHKM-11], whereas at the Position: 253, the peak is found lower with Score: -1.967 (min).

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).

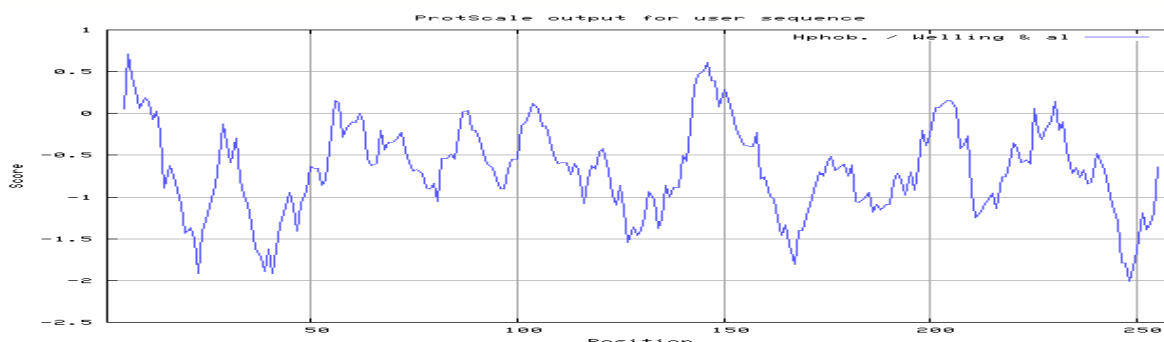
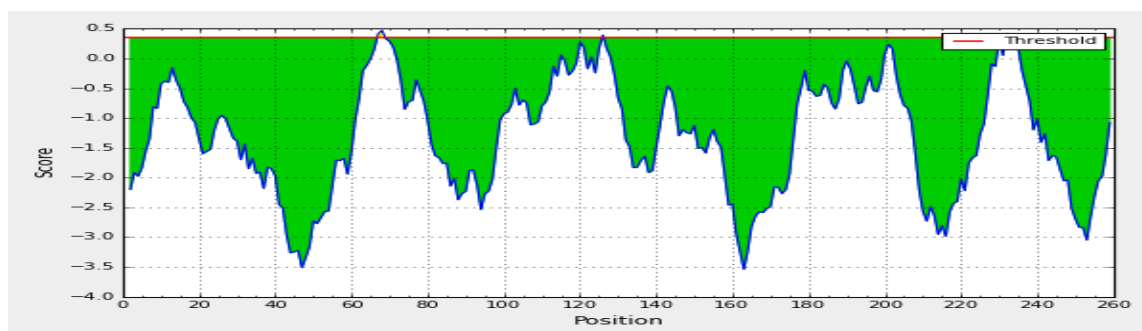


Figure 2: Hydrophobicity plot of Welling et al. (1985) of cytochrome c oxidase subunit III. The highest Score: 0.711 (max) is obtained at Position: 6 with sequence 3-LKFRKFH-9 and min score (Score: -2.007) found Position: 248

We also study B-EpiPred Server(Figure3 & Table 1), Kolaskar and Tongaonkar ntigenicity methods(Figure 4 & Table 2), Parker (Figure 5& Table 3)and the predicted antigenic fragments can bind to MHC molecule is the first bottlenecks in vaccine design (Figure 3- 5).



Average: -1.318

Minimum: -3.542

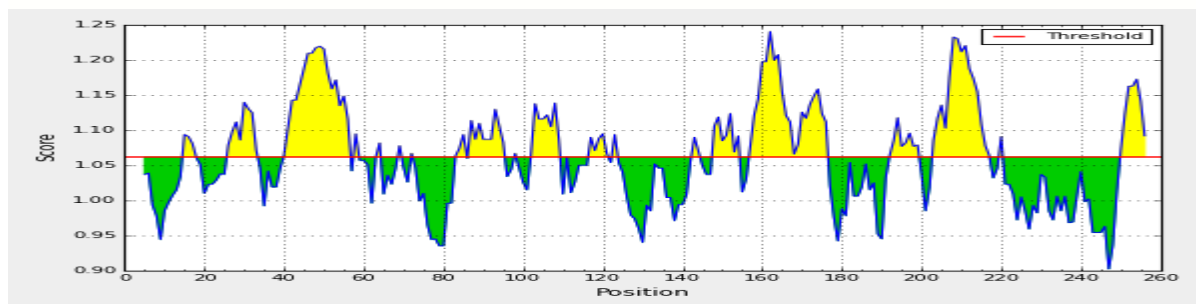
Maximum: 0.474

Figure3: B-cell epitopes are the sites of molecules that are recognized by antibodies of the immune system of the cytochrome c oxidase subunit III.

Predicted peptides:

No.	Start	End	Peptide	Length
1	67	68	SG	2
2	126	126	I	1
3	232	232	S	1
4	234	234	D	1

Table 1: B-cell predicted peptides of the cytochrome c oxidase subunit III.



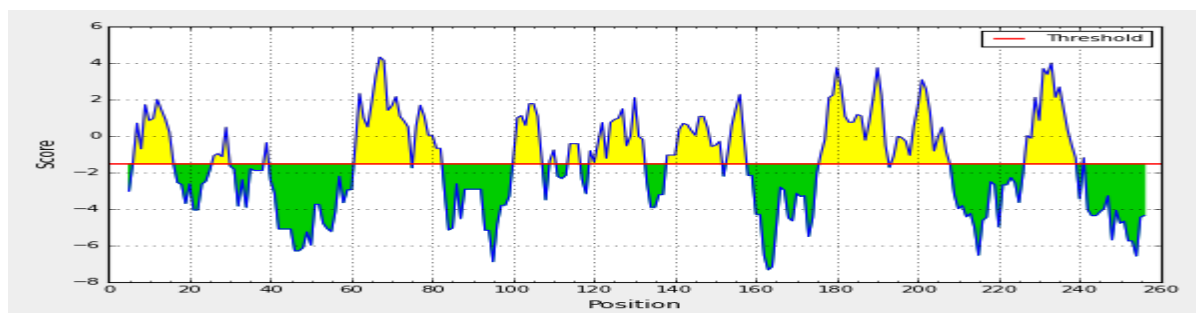
Average: 1.062 **Minimum:** 0.902 **Maximum:** 1.241

Figure 4: Kolaskar and Tongaonkar antigenicity are the sites of molecules that are recognized by antibodies of the immune system for the cytochrome c oxidase subunit III.

Predicted residue scores:

Position	Residue	Start	End	Peptide	Score
162	L	159	165	VFLLCI	1.241
208	V	205	211	HVFVGVC	1.233
209	G	206	212	VFVGVC	1.231

Table 2: Kolaskar and Tongaonkar antigenicity predicted peptides for the cytochrome c oxidase subunit III.



Average: -1.533

Minimum: -7.343

Maximum: 4.343

Figure 5: Hydrophobicity plot of HPLC / Parker et al. (1986) of cytochrome c oxidase subunit III.

Predicted residue scores:

Position	Residue	Start	End	Peptide	Score
67	S	64	70	EDVSGQY	4.343
68	G	65	71	DVSGQYS	4.157

Table 3: Hydrophobicity plot of HPLC / Parker et al. (1986) predicted peptide for cytochrome c oxidase subunit III.

III.B Secondary alignment

The Robson and Garnier method predicted the secondary structure of the cytochrome c oxidase subunit III. Each residue is assigned values for alpha helix, beta sheet, turns and coils using a window of 7 residues (Figure 6). 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.

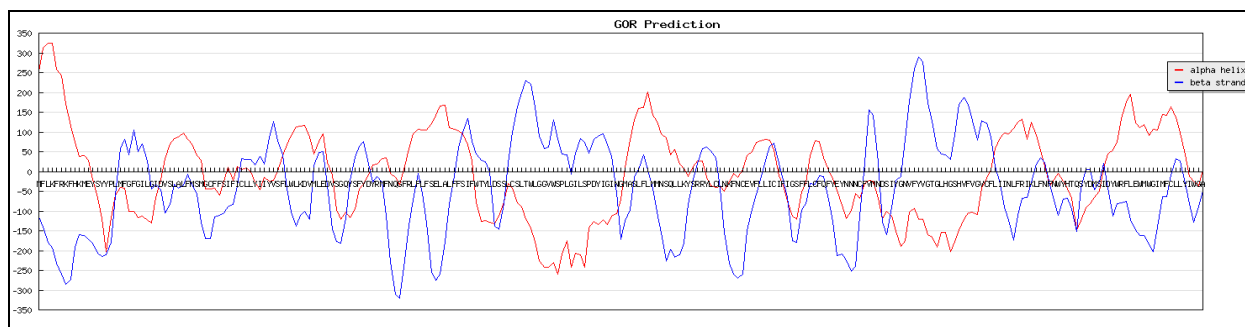


Figure 6: Secondary structure GOR plot of the cytochrome c oxidase subunit III

III.C 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 a cytochrome c oxidase subunit III is hydrophobic in nature and contains segments.

III.D Prediction of MHC binding peptides

These MHC binding peptides are sufficient for eliciting the desired immune response. The prediction is based on cascade support vector machine, using sequence and properties of the amino acids. The correlation coefficient of 0.88 was obtained by using jack-knife validation test. In this test, we found the MHCI and MHCII binding regions (Tables 4 and 5). MHC molecules are cell surface glycoproteins, which take active part in host immune reactions and involvement of MHC class-I and MHC II in response to almost all antigens. In this assay we predicted the binding affinity of cytochrome c oxidase subunit III having 259 amino acids, which shows different nonamers (Tables 4 and 5). For development of MHC binder prediction method, an elegant machine learning technique support vector machine (SVM) has been used. SVM has been trained on the binary input of single amino acid sequence. In this assay we predicted the binding affinity of cytochrome c oxidase subunit III having 259 amino acids, which shows 251 nonamers. Small peptide regions found as High affinity TAP Transporter peptide regions as, 73-YDYRMFNQG(Score:8.644),102-DSSLCSTLW(Score:8.62),66-VSGQYSFYD(Score:8.62),36-SMGCFFSIF(Score:8.6),126-IGINGMASL(Score:8.594),155-FNCEVFLLI(Score: 8.58),104-SLCSLTWLG(Score:8.562),161-LLICIFIGS(Score:8.555),123-PDYIGINGM(Score:8.543),21-GFGILGIDV(Score:8.503).

Peptide Rank	Start Position	Sequence	Score	Predicted
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				Affinity
1	73	YDYRMFNQG	8.644	High
2	102	DSSLCSLTW	8.62	High
3	66	VSGQYSFYD	8.62	High
4	36	SMGCFFSIF	8.6	High
5	126	IGINGMASL	8.594	High
6	155	FNCEVFLLI	8.58	High
7	104	SLCSLTWL	8.562	High
8	161	LLICIFIGS	8.555	High
9	123	PDYIGINGM	8.543	High
10	21	GFGILGIDV	8.503	High

*Optimal Score for given MHC binder in Mouse

Table 4: TAP Peptide binders of cytochrome c oxidase subunit III.

Prediction method	Rank	Sequence	Residue No.	Peptide Score
ALLELE I-Ab	1	EYSYYPLMF	12	0.836
ALLELE I-Ab	2	YVSFLWLKD	52	0.596
ALLELE I-Ab	3	LWLKDVMLE	56	0.588
ALLELE I-Ab	4	IKLFNFWY	220	0.567
ALLELE I-Ad	1	GSFFLCFQF	168	0.721
ALLELE I-Ad	2	GCFSSIFIC	38	0.717
ALLELE I-Ad	3	GINGMASLF	127	0.658
ALLELE I-Ad	4	GILGIDVSL	23	0.605
ALLELE I-Ag7	1	GIDVSLALF	26	1.309
ALLELE I-Ag7	2	MFNQGFRLF	77	1.305
ALLELE I-Ag7	3	YIIYVSFLW	49	1.289
ALLELE I-Ag7	4	IGINGMASL	126	1.286
ALLELE RT1.B	1	FNFWYHTQ	223	0.645
ALLELE RT1.B	2	LMMNSQLLK	136	0.606
ALLELE RT1.B	3	MFNQGFRLF	77	0.548
ALLELE RT1.B	4	LFLFSELAL	84	0.513

*Optimal Score for given MHC II peptide binder in Mouse

Table 5: Peptide binders to MHCII molecules of cytochrome c oxidase subunit III.

We also predicted the SVM based MHC-IAb peptide regions, 12-EYSYYPLMF(optimalscore:0.836),52-YVSFLWLKD(optimalscore:0.596),56-LWLKDVMLE(optimal score:0.588),220-IKLFNFWY(optimal score:0.567); MHC-IAd peptide regions, 168-GSFFLCFQF(optimal score:0.721),38-GCFFSIFIC(optimal score:0.717),127-GINGMASLF(optimal score: 0.658),23-GILGIDVSL(optimal score:0.605); MHC-IAg7 peptide regions, 26-GIDVSLALF(optimal score:1.309),77-MFNQGFRLF(optimal score:1.305),49-YIIYVSFLW(optimal score :1.289),126-IGINGMASL(optimal score:1.286); MHC-RT1.B peptide regions,223-FNFWYHTQ(optimal score:0.645),136-LMMNSQLLK(optimal score:0.606),77-MFNQGFRLF(optimal score:0.548),84-LFLFSELAL(optimal score :0.513) which represent the predicted peptide binders from cytochrome C oxidase III from Loa loa(table:5).The predicted binding affinity is normalized by the 1% fractal. The MHC peptide binding is predicted using the neural networks trained on c terminal of known epitopes. In analysis predicted MHC –peptide binding is log-transformed value related to the IC50 values in nM units. These MHC binding peptides are sufficient for eliciting the desired immune response. Predicted MHC binding regions in an antigen sequence and there are directly associated with immune reactions, in analysis we found the MHCII binding region.

IV. DISCUSSION AND CONCLUSION

Gomase method [9], B-EpiPred Server, Hopp and Woods, Welling, Parker, Kolaskar and Tongaonkar antigenicity scales were designed to predict the locations of antigenic determinants in cytochrome c oxidase subunit III. Protein shows beta sheets regions, which are high antigenic response than helical region of this peptide and shows highly antigenicity (Figure 1-5). We also found the Sweet hydrophobicity, Kyte & Doolittle hydrophobicity, Abraham & Leo, Bull & Breese hydrophobicity, Guy, Miyazawa hydrophobicity, Roseman hydrophobicity, Cowan HPLC pH7.5 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, These scales are essentially a hydrophilic index, with a polar residues assigned negative values (Figures 6-27). In this assay we predicted the binding affinity of cytochrome c oxidase subunit III having 259 amino acids, which shows 251nonamers.Small peptide regions found as, 73-YDYRMFNQG (Score:8.644),102-DSSLCSTW(Score:8.62),66-VSGQYSFYD(Score:8.62), 36-SMGCFFSIF (Score:8.6),126-IGINGMASL(Score:8.594),155-FNCEVFLLI(Score:8.58),104-SLCSLTWLG(Score:8.562),161-LLICIFIGS(Score:8.555),123-PDYIGINGM(Score:8.543),21-GFGILGIDV(Score:8.503). Adducts of MHC and peptide complexes are the ligands for T cell receptors (TCR) (Table 1). MHC molecules are cell surface glycoproteins, which take active part in host immune reactions and involvement of MHC class-I and MHC II in response to almost all antigens (Table 5). Kolaskar and Tongaonkar antigenicity are the sites of molecules that are recognized by antibodies of the immune system for the cytochrome c oxidase subunit III, analysis shows epitopes present in the cytochrome c oxidase subunit III the desired immune response. The region of maximal hydrophilicity is likely to be an antigenic site, having hydrophobic characteristics, because C-terminal regions of cytochrome c oxidase subunit III 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 cytochrome c oxidase subunit III, we got eighteen antigenic determinant sites in the sequence. The SVM based MHCII-IAb peptide regions 12-EYSYYPLMF(optimalscore:0.836), 52-YVSFLWLKD (optimalscore:0.596), 56-LWLKDVMLE (optimal score:0.588), 220-IKLFNFWY(optimal score:0.567); MHC-IA peptide regions, 168-GSFFLCFQF (optimal score:0.721), 38-GCFFSIFIC (optimal score:0.717), 127-GINGMASLF(optimal score:0.658), 23-GILGIDVSL(optimal score:0.605); MHC-IAg7 peptide regions, 26-GIDVSLALF(optimal score:1.309), 77-MFNQGFRLF(optimalscore:1.305), 49-YIIYVSFLW(optimalscore:1.289), 126-IGINGMASL (optimal score:1.286) ; MHC-RT1.B peptide regions, 223-FNFWYHTQ(optimal score:0.645), 136-LMMNSQLLK(optimal score:0.606), 77-MFNQGFRLF(optimal score:0.548), 84-LFLFSELAL(optimal score:0.513) (Table-5). Which is a larger percentage of their atoms are directly involved in binding as compared to larger molecules.

V. FUTURE PERSPECTIVES

This method will be useful in cellular immunology, Vaccine design, immunodiagnostics, immunotherapeutics and molecular understanding of autoimmune susceptibility. cytochrome c oxidase subunit III sequence involved multiple antigenic components to direct and empower the immune system to protect the host from the dendrotoxin. MHC molecules are cell surface proteins, which take active part in host immune reactions and involvement of MHC class in response to almost all antigens and it give effects on specific sites. Predicted MHC binding regions acts like red flags for antigen specific and generate immune response against the parent antigen. So, a small fragment of antigen can induce immune response against whole antigen. The method integrates prediction of peptide MHC class binding; proteosomal C terminal cleavage and TAP transport efficiency. This theme is implemented in designing subunit and synthetic peptide vaccines.

Figures List :

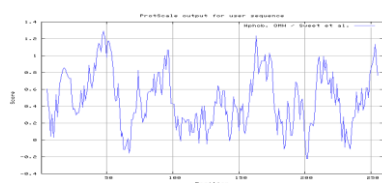


Figure7: Hydrophobicity Sweet plot of protein

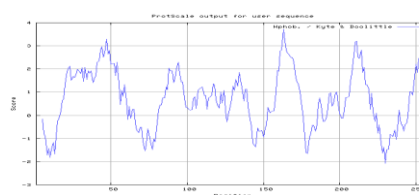


Figure 8: Hydrophobicity plot of Kyte and Doolittle (1982)

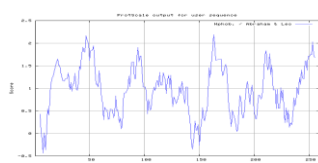


Figure9: Hydrophobicity plot of Abraham and Leo(1987)

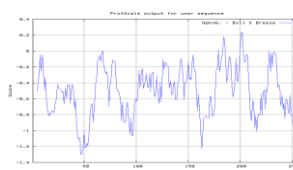


Figure10: Hydrophobicity plot of Bull and Breese(1974)

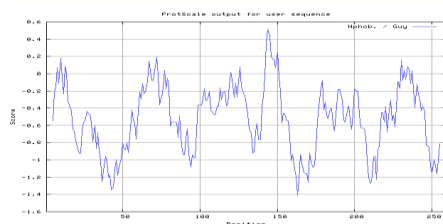


Figure 11: Hydrophobicity plot of Guy (1985)

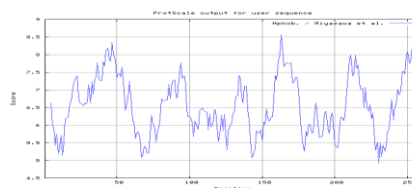


Figure 12: Hydrophobicity plot of Miyazawa, et al (1985)

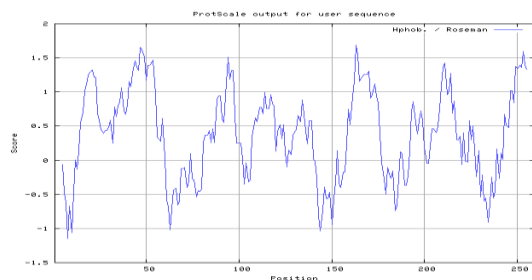


Figure 13: Hydrophobicity plot of Roseman (1988)

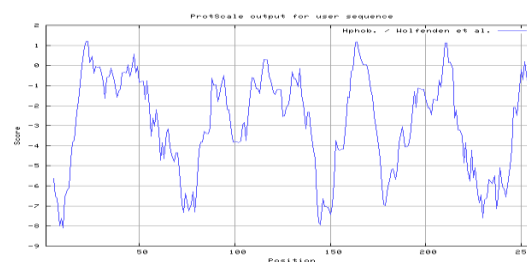


Figure 14: Hydrophobicity plot of Wolfenden et al. (1981)

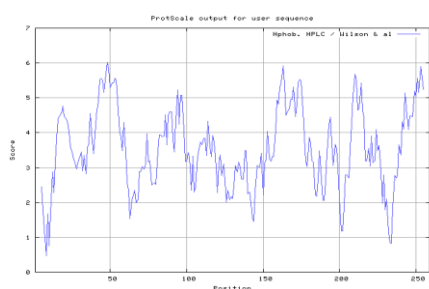


Figure 15: Hydrophobicity Wilson et al. (1981) plot

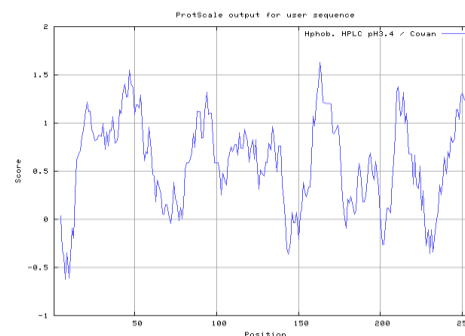


Figure 16: Hydrophobicity Cowan (1990) plot

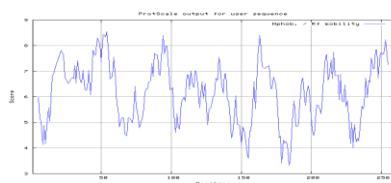


Figure 17: Hydrophobicity plot of Rf mobility

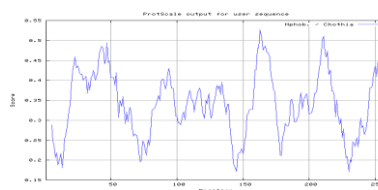


Figure 18: Hydrophobicity plot of Chothia (1976)

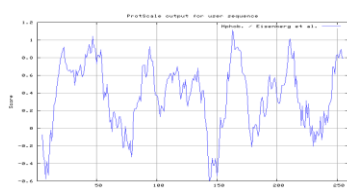


Figure 19: Hydrophobicity plot of Eisenberg et al. (1984)

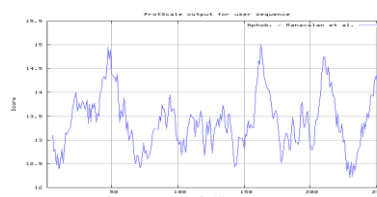


Figure 20: Hydrophobicity plot of Manavalan, et al (1978)

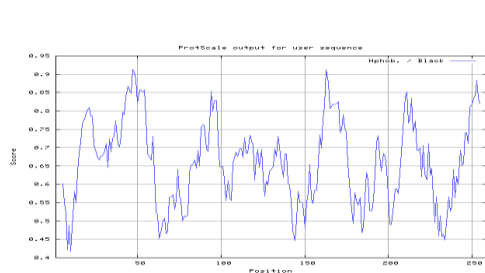


Figure 21: Hydrophobicity plot of Black (1991)

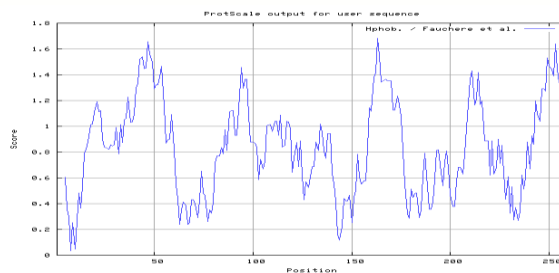


Figure 22: Hydrophobicity plot of Fauchere, et al (1983)

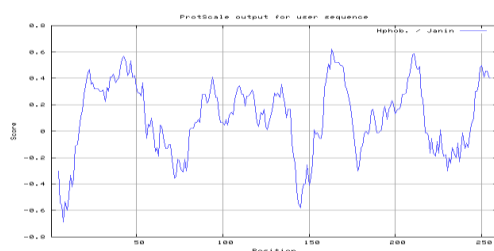


Figure 23: Hydrophobicity plot of Janin (1979)

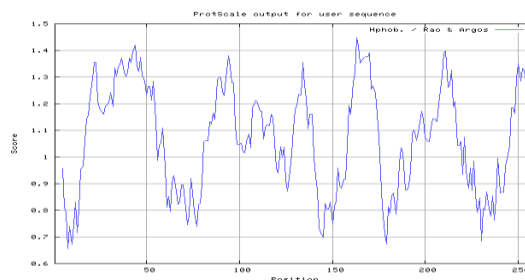


Figure 24: Hydrophobicity plot of Rao and Argos (1986)

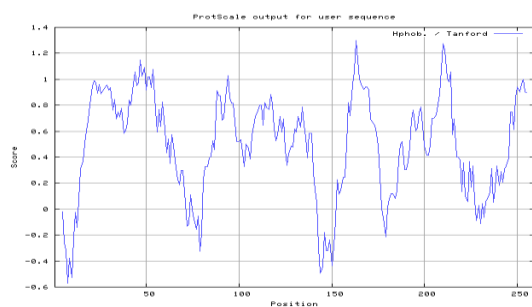


Figure 25: Hydrophobicity plot of Tanford (1962)

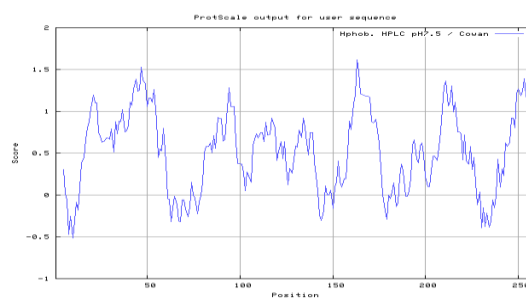


Figure 26: Hydrophobicity Cowan (1990) plot of HPLC pH7.5

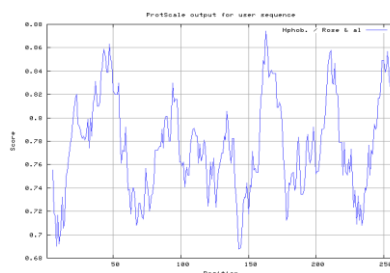


Figure 27: Hydrophobicity plot of Rose et al. (1985)

Conflicts of Interest

The authors declare no conflict of interest.

Abbreviations

1. MHC-major histocompatibility complex
2. PSSM- Position-Specific Scoring Matrix
3. SVM- Support vector machine
4. NCBI: National Center for Biotechnology Information
5. TAP : Transporter Associated with Antigen Processing

REFERENCES

- [1] Zoure' HG, Wanji S, Noma M, Amazigo UV, Diggle PJ, Tekle AH, et al. The geographic distribution of *Loa loa* in Africa: results of large-scale implementation of the rapid assessment procedure for loiasis (RAPLOA). *PLoS Negl Trop Dis* 2011;5:e1210.
- [2] World Health Organization. Guidelines for rapid assessment of *Loa loa*. TDR/ IDE/RAPLOA/02. Geneva: UNDP/World bank/WHO Special Programme for Research and Training in Tropical Diseases; 2002.
- [3] Padgett JJ, Jacobsen KH. Loiasis: African eye worm. *Trans R Soc Trop Med Hyg* 2008;102:983–9.
- [4] Boussinesq M. Loiasis. *Ann Trop Med Parasitol* 2006;100:715–31.
- [5] Antinori, Spinello et al. Imported *Loa loa* filariasis: three cases and a review of cases reported in non-endemic countries in the past 25 years. *International Journal of Infectious Diseases* , Volume 16 , Issue 9 ,2012, e649 - e662.
- [6] Bhedasgaonkar, S., Baile, R. B., Nadkarni, S., Jakkula, G., & Gogri, P. *Loa loa* macrofilariasis in the eyelid: case report of the first periocular subcutaneous manifestation in India. *Journal of Parasitic Diseases: Official Organ of the Indian Society for Parasitology*, 35(2), 2011,230–231.
- [7] Gomase VS, Kale KV, Chikhale NJ, Changbhale SS Prediction of MHC Binding Peptides and Epitopes from Alfalfa mosaic virus. *Curr Drug Discov Technol* 4: 2007 117-215.
- [8] Kolaskar AS, Tongaonkar PC. A semi-empirical method for prediction of antigenic determinants on protein antigens. *FEBS Lett* 276: 1990, 172-174.
- [9] Gomase VS. Prediction of Antigenic Epitopes of Neurotoxin Bmbktx1 from *Mesobuthus martensii*. *Curr Drug Discov Technol* 3:, 2006,225-229.
- [10] Hopp TP, Woods KR Prediction of Protein Antigenic Determinants from Amino Acid Sequences. *Proc Natl Acad Sci USA* 78: 1981, 3824-3828.
- [11] Welling GW, Weijer WJ, van der Zee R, Welling-Wester S . Prediction of sequential antigenic regions in proteins. *FEBS Lett* 188: 1985, 215-218.
- [12] Larsen JE, Lund O, Nielsen M. Improved method for predicting linear B-cell epitopes. *Immunome Res*,2006,2:2.
- [13] Parker JM, Guo D, Hodges RS 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: 1986, 5425-5432.
- [14] Garnier J, Osguthorpe DJ, Robson B. Analysis of the accuracy and implications of simple methods for predicting the secondary structure of globular proteins. *J Mol Biol* (1978) 120: 97-120.
- [15] Robson B, Garnier J Protein structure prediction. *Nature*,1993, 361: 506.

- [16] Aboderin AA . A new fluorescent probe for protein and nucleoprotein conformation. Binding of 7-(p-methoxybenzylamino)-4-nitrobenzoxadiazole to bovine trypsinogen and bacterial ribosomes. *Biochemistry* 1971 10: 4433-4440.
- [17] Abraham DJ, Leo AJ .Extension of the fragment method to calculate amino acid zwitterions and side chain partition coefficients. *Proteins* 2: 1987, 130-152.
- [18] Sweet RM, Eisenberg D .Correlation of sequence hydrophobicities measures similarity in three-dimensional protein structure. *J Mol Biol* 171:, 1983 479-488.
- [19] Kyte J, Doolittle RF.A Simple Method for Displaying the Hydropathic Character of a Protein. *J Mol Biol* 157: 1982,105-132.
- [20] Bull HB, Breese K . Surface tension of amino acid solutions: A hydrophobicity scale of the amino acid residues. *Arch Biochem Biophys* 161:1974,665-670.
- [21] Guy HR .Amino acid side chain partition energies and distributions of residues in soluble proteins. *Biophys J* .1985 47: 61-70.
- [22] Miyazawa S, Jernigen RL .Estimation of Effective Interresidue Contact Energies from Protein Crystal Structures: Quasi-Chemical Approximation.*Macromolecules*18: 1985. 534-552.
- [23] Roseman MA Hydrophilicity of polar amino acid side-chains is markedly reduced by flanking peptide bonds. *J Mol Biol* 200: 1988, 513-522.
- [24] Wolfenden R, Andersson L, Cullis PM, Southgate CC. Affinities of amino acid side-chains for solvent water. *Biochemistry* 20: 1981 849-855.
- [25] Wilson KJ, Honegger A, Stotzel RP, Hughes GJ .The behavior of peptides on reverse-phase supports during high-pressure liquid chromatography. *Biochem J*199: 1981,31-41.
- [26] Chothia C. The nature of accessible and buried surfaces in proteins. *J Mol Biol* .1976, 105: 1-12.
- [27] Eisenberg D, Schwarz E, Komaromy M, Wall R . Analysis of membrane and surface protein sequences with the hydrophobic moment plot. *J Mol Biol*,1984, 179: 125-142.
- [28] Manavalan P, Ponnuswamy PK . Hydrophobic character of amino acid residues in globular proteins. *Nature* ,1978,275: 673-674.
- [29] Black SD, Mould DR . Development of Hydrophobicity Parameters to Analyze Proteins Which Bear Post- or Cotranslational Modifications. *Anal Biochem*,1991, 93: 72-82.
- [30] Fauchere JL, Pliska V . Hydrophobic parameters of amino-acid sidechains from the partitioning of N-acetyl-amino-acid amide. *Eur J Med Chem* , 1983,18: 369-375.
- [31] Janin J. Surface and inside volumes in globular proteins. *Nature* ,1979.277:491-492.
- [32] Rao MJK, Argos P. A conformational preference parameter to predict helices in integral membrane proteins. *Biochim Biophys Acta* ,1986,869: 197-214.
- [33] Tanford C. Hydrophobicity scale (Contribution of hydrophobic interactions to the stability of the globular conformation of proteins. *J Am Chem Soc* 1962, 84:4240-4274.
- [34] Cowan R, Whittaker RG.Hydrophobicity indices at ph 3.4 determined by HPLC. *Pept Res* 3: 75-80.
- [35] Rose GD, Geselowitz AR, Lesser GJ, Lee RH, Zehfus MH (1985) Hydrophobicity of amino acid residues in globular proteins. *Science* 1990, 229: 834-838.

- [36] Wilkins MR, Gasteiger E, Bairoch A, Sanchez JC, Williams KL, et al. Protein identification and analysis tools in the ExPASy server. *Methods Mol Biol* 1999, 112: 531-552.
- [37] Eisenberg D, Weiss RM, Terwilliger TC. The hydrophobic moment detects periodicity in protein hydrophobicity. *Proc Natl Acad Sci USA*, 1984, 81: 140-144.
- [38] Brusica V, Rudy G, Honeyman G, Hammer J, Harrison L. Prediction of MHC class II-binding peptides using an evolutionary algorithm and artificial neural network. *Bioinformatics* 1998, 14: 121-130.
- [39] Bhasin M, Raghava GP. P cleavage: an SVM based method for prediction of constitutive proteasome and immunoproteasome cleavage sites in antigenic sequences. *Nucleic Acids Res* 2005, 33: W202-W207.
- [40] Gomase VS, Changbale SS, Kale KV. In silico analysis of Mesobuthus tamulus neurotoxin from groundnut ringspot virus. *Advancements in Information Technology and Internet Security*, 2008, 370-378.
- [41] Mishra S, Gomase VS. Study Hydrophobicity and Antigenicity of Cytochrome C Oxidase Subunit II from *D. medinensis*: New Prototype of Synthetic Vaccine Development. *Immunochem Immunopathol*, 2016, 2: 113.
- [42] Mishra S, Gomase VS. "Cytochrome B"- Analysis of Hydrophobicity, Surface Accessibility, Antigenicity and Prediction of MHC I and MHC II Binders from *Dracunculiasis*. *Med chem* 2016, 6: 041-046.
- [43] Mishra S. and Virendra S Gomase. "Prediction of MHC Binding peptides and Antigenic peptides of Hsp70 from GWD". *EC Pharmacology and Toxicology* 2.1 (2016): 36-53.
- [44] Mishra S, Gomase VS. Identification of Antigenic Determinants, Solvent Accessibility and MHC Binders of Antigen NADH Dehydrogenase Subunit 5 from a Little Dragon from Medina (*Dracunculus medinensis*). *Drug Des.*, 2016, 5:126.
- [45] Mishra S, Gomase VS. Study of Hydrophobicity and Prediction of Antigenic Epitope of NADH dehydrogenase subunit 5 from *D. medinensis*. *Drug Des*, 2016, 5:127.
- [46] Mishra S, Gomase VS. "Cytochrome B"- Analysis of Hydrophobicity, Surface Accessibility, Antigenicity and Prediction of MHC I and MHC II Binders from *Dracunculiasis*. *Med chem.*, 2016, 6: 041-046.
- [47] Mishra S, Gomase VS. Computational Comparative Homology Based 3D-structure Modelling of the HSp70 Protein from GWD. *J Health Med Informat*, 2016, 7: 233.
- [48] Mishra S, Gomase VS (2016) Computational Approach to Identify the Major Histocompatibility Complex Binding Antigenic Peptides from 'Ascariasis'. *J. Data Mining Genomics Proteomics*, 2016, 7: 198.