ECRONICON OPEN ACCESS

PHARMACOLOGY AND TOXICOLOGY
Research Article

Prediction of MHC Binding peptides and Antigenic peptides of Hsp70 from GWD

Sonu Mishra* and Virendra S Gomase

Department of Biotechnology, Mewar University, India

***Corresponding Author:** Sonu Mishra, Department of Biotechnology, Mewar University, Chittorgarh, India.

Received: February 09, 2016; **Published:** February 25, 2016

Abstract

The parasitic nematode disease (Dracunculus medinensis) which is directly related to the environmental declension and contamination are major concern of the public health, not only by the morbidity and mortality that these waterborne pathogens cause, but mostly by the higher cost represents their prevention and treatment. Despite the continued efforts to maintain water safety, waterborne outbreaks are still reported globally and its systematic treatment is the major issue. In this assay, we predicted the binding peptides of the MHC class I and MHC class II by PSSM and SVM algorithms. Antigenicity, Solvent accessibility, polar and nonpolar residue of protein is also analyzed. The regions that are likely exposed on the surface of proteins which are potentially antigenic that allows potential drug targets to identify active sites against infection as well as to design effective drug to treat the diseases or infections. In this assay, we predicted the binding affinity of Hsp70 having 647 amino acids, which shows 639 nonamers. In this study, we found the SVM based MHCII-I_Ab peptide regions, 371-AYGAAVQAA,603-VCNPIITKM,429-FTTYSDNQP,460- FELSGIPPA(optimal score is35.632); MHCII-I_Ad peptide regions, 237-RMVNHFVAE,33-QGNRTTPSY,533-QKDRIAAKN,374-AAVQAAILS(optimal score is 53.145);MHC-II I_Ag7 peptide regions 519-MVQEAEKYK,188-KKGHGERNV,181-AIAYGLDKK,229-LGGEDFDNR, 365-NPDEAVAYG (optimal score is 40.873) which represented the predicted binders from Hsp70 protein. The method integrates prediction of peptide MHC class I binding; proteosomal C terminal cleavage and TAP transport efficiency of the protein of GWD. Thus a small fragment of antigen can stimulate immune responses against whole antigen. This theme can be implemented in designing subunit and synthetic peptide vaccines.

Keywords: *Dracunculiasis; Epitopes; Antigenic peptides; MHC-Binders; Tappred; PSSM; SVM; Nonamers; heat shock protein 70*

Abbreviations

MHC I: Major Histocompatibility Complex-Class I

MHC I: Major Histocompatibility Complex-Class II

PSSM: Position Specific Scoring Matrices

SVM: Support Vector Machine

GWD: Guinea worm disease

UniProt: The Universal Protein Resource

NCBI: National Center for Biotechnology Information

TAP: Transporter Associated with Antigen Processing

HPLC: High Performance Liquid Chromatography

TapPred: TAPPred is an on-line service for predicting binding affinity of peptides toward the TAP transporter. The prediction of TAP binding peptides is important in order to identify the MHC class-1 restricted T cell epitopes. The Prediction is based on cascade SVM, using sequence and properties of the amino acids. The correlation coefficient of 0.88 was obtained by using jack-knife validation test.

Rankpep: This server predicts peptide binders to MHCI and MHCII molecules from protein sequence/s or sequence alignments using Position Specific Scoring Matrices (PSSMs). In addition, it predicts those MHCI ligands whose C-terminal end is likely to be the result of proteasomal cleavage.

Introduction

The mild and non-lethal heat shock protects cells of various origins induced by a subsequent severe heat shock as well as lethal stimuli [1-5] against cell death , which has been noticed by the several individual group s of the investigators. Later on ,its cleared that the enhanced cell survival was intimately linked to the induction and accumulation of heat-inducible proteins and especially to that of a 70kD protein that was designated as heat shock protein 70(Hsp70) [6-9] Hsp70 found to be the most conserved protein in evolution [10-12] and the Hsp70 protein family is highly homologous protein with overlapping and distinct function [13]. Study reveals that the membrane-bound or extracellularly located HSPs act as danger signals and elicit immune responses mediated by the adaptive or innate immune system.

Heat shock proteins (HSP) belong to the protein family where cell produces the response with respect to the exposure to stressful conditions such as cold [14], UV light [15] and during wound healing or tissue remodeling [16].There are group of the HSP protein which function as the chaperone like by stabilizing new proteins in order to ensure correct folding or by helping to refold proteins which are undergone any damaged due to cell stress [17]. The HSP's virtual abundance has been noticed from bacteria to humans i.e almost all living organisms. HSP-70 protein is named according to its molecular weight. Hsp 70s (70-kDa) proteins provide assistance in wide range of folding processes , which includes the folding of protein, assembly of newly synthesized proteins, refolding of misfolded protein and aggregated proteins, membrane translocation of organellar and secretory proteins, and control of the activity of regulatory proteins[18-24]. This protein has also perform housekeeping functions in the cell in which they are built-in components of folding and signal transduction pathways, and in quality control functions this protein proofread the structure of proteins and repair misfolded conformers. This functional activity of this protein is based on the attribute of Hsp70 to interact with hydrophobic peptide segments of proteins in an ATP-controlled fashion. The two most distinct functional regions of HSP 70 are (1) peptide binding domain (PBD) and (2) the amino-terminal ATPase domain (ABD). Peptide binding domain holds a groove with an affinity for neutral, hydrophobic amino acid residues. Whereas C-terminal /amino terminal domain – rich in alpha helical structure acts as a 'lid' for the substrate binding domain. When the HSP70 is ATP bounded ,it open up the lid for peptide binding and release relatively rapidly, whereas, when this protein is ADP bounded, it usually shut down the lid, and peptides are closely bound to the substrate binding domain. In the malignant melanoma the over expressed Hsp 70 protein has been found [25] and under expression in renal cell cancer [26]. The extracellular HSPs act as powerful way of sending a "Risk signal" to the immune system, to generate response against infection or disease. The predicted antigen protein from GWD might play an important role in the new paradigms of synthetic vaccine development and target validation. By considering the importance of the HSP protein, we have used this protein to study the antigenicity of protein, its solvent accessibility, polar and nonpolar residue to analyse the regions that are likely exposed on the surface of proteins which could be the potentially antigenic that allows potential drug targets to identify active sites against infection as well as to design effective drug to treat Dracunculiasis. D.medinesis (a little dragon from Medina) is the causative agent and it is the only species from the 12 species of *Dracunculus* [27-30] which infects humans, commonly known as "Guinea worm disease (GWD)". The other Dracunculus species generally resides in the internal tissues and body cavities of non-human mammals and reptiles (snake and turtles) [31]. This little dragon undergo a very unusual life cycle of six developmental stages with incubation period last for 1 to one an half years approximately [32]. This is one of the most neglected tropicalparasites which bears clinical importance and needs to be eradicated after small pox [33]. After reaching to the maturation stage, these worms copulate and an adult female produces millions of eggs in its uterus whereas male dies. Later on stage, this female worms release the larvae which trigger a painful blister of diameter of 1 to 6cm, unremarkably and predominantly localized on the skin of lower limbs (80-90%) in most of the reported cases). The infected person develops slight fever, localskinredness, swelling and severe pruritus around the blister. Other symptoms includes: diarrhea, nausea, vomiting and dizziness. The blister burst within three days and female worms one or more slowly comes out from the wounds which causes an excoriating burning sensation and pain [34]. Immersing or pouring water over the blister provides pain relief. But this is only the moment that adult female is exposed to the external environment [35]. The uniqueness of this worm can be observed when the patients emerge the limbs in open water sources, this worm smartly recognizes the temperature difference and start releasing milky white liquid in the water which holds millions of immature larvae, when larvae released in water are ingested by copepods where they mount twice and become infective larvae within two weeks [36].

It has been seen that, the single epitope can accelerate and generate the immune response in large population. This approach is usually based on the phenomenon of cross-protection. The World Health Assembly called in the 1986 in order to dracunculiasis elimination. The global Guinea Worm Eradication Program, supported by The Carter Center, World Health Organization (WHO), UNICEF, CDC, and other partners. In 1986, an estimated 3.5 million cases occurred each year in 20 countries in Africa and Asia. Dracunculiasis remains endemic in four countries in 2014 (South Sudan, Chad, Mali, and Ethiopia), but the number of overall reported incidence is decreases in 2013 by 73%and in 2014 by 71% compared with 2012. The failures in surveillance and containment is due to lack of clean drinking water, insecurity in Mali and parts of South Sudan, and an unusual epidemiologic pattern in Chad are the main remaining challenges to dracunculiasis eradication [37]. A case of Onchocerca volvulus has been reported in the Cameroon which is mimicking Dracunculus-medinensis [38]. More than two decades after the International Drinking Water Supply and Sanitation Decade (IDWSSD) implemented by the United Nations(1981-1990) [39], the disease still lingers, underscoring the daunting challenge of disease control, as has been the case of the failure of previous attempts to eradicate diseases like malaria, hookworm and yaws [40]. Till date there is no accurate and efficient curative drug of vaccine is available against Dracunculiasis [41]. The investigation suggests that the immunity is not developed by the infected individual [42,43].The specific antibodies (total, IgG1 and IgG4) of GWD has been noticed significantly higher than the levels measured in the same individuals eight months later during the time of patency [44].It was observed that the mean level of specific IgG1 and IgG4 is higher during the month of potency of the infected individual where as variation in the IgE value is relatively negotiable and constant before, during the infection and after the recovery. There is possibility that variation in antibody production is regulated by infected larvae (i.e by transmission) and / or by adult worms (i.e by patency) is still need to be clear out. It is possibility that increased production of IgG1, IgG4 during the time of patency plays a role in blocking or protecting immune responses otherwise it could have killed ingested infected larvae [45]. The antigenic peptides from GWD can be the most desirable segment for the development of peptide vaccine [46]. In this study we have study the MHC binding peptide. MHC molecules are cell surface protein that binds to the peptides derived from host or antigenic proteins and present them to cell surface for recognition by T-cells. T cell recognition is animportant mechanism of the adaptive immune system by which the host identifies and responds to foreign antigens [46,47]. The MHC molecule exists in two polymorphic form i.e MHC I & MHC II. The peptide presented by MHC class I molecules from proteins that synthesized within the cell, whereas, MHC class II molecule present peptides derived from endocytosed extracellular proteins. MHC molecules have been well characterized due to their role in immune reactions and they take active part in host immune reactions and involvement of MHC class molecule in response to almost all antigens and it give impacts on specific sites. The participation of MHC I molecule in response to approximately all antigens makes the study more interesting. They bind to some of the peptide fragments generated after proteolytic cleavage of antigen [48]. Identification of MHC-binding peptides and T-cell epitopes helps improve our understanding of specificity of immune responses [49-52]. Antigenic peptides are most suitable for peptide vaccine development because single epitope can generate large the immune response [53-55].

Methodology

Database Searching

The antigenic protein sequence of Hsp70 from *Dracunculus medinensis* was retrieved from www.ncbi.nlm.nih.gov, UniProt databases are initially the most important [56-58].

Prediction of Antigenicity

Prediction of antigenicity program predicts those segments from Antigen Hsp70 protein that are likely to be antigenic by eliciting an antibody response. In this research work antigenic epitopes of *Dracunculus medinensis* antigen Hsp70 are determined by using the Hopp and Woods, Welling, Parker, Bepipred ,Kolaskar and Tongaonkar antigenicity methods [59-64].

Prediction of Mhc Binding Peptide

The major histocompatibility complex (MHC) peptide binding of *Dracunculus medinensis* is predicted using neural networks trained on C terminals of known epitopes. Rankpep predicting tool predicts peptide binders to MHC-I ligands using PSSMs, whose C-terminal end is likely to be the result of proteosomal cleavage. The sequence similarity is observed to the peptides that bind to a given MHC

molecule. Traditionally, the sequence patterns used for the prediction of peptides binding to MHC molecules. Such sequence patterns, however, have proven to be too simple, as the complexity of the binding motif cannot be precisely represented by the few residues present in the pattern [65]. RANKPEP uses "Position Specific Scoring Matrices (PSSMs) or profiles" from set of aligned peptides known to bind to a given MHC molecule as the predictor of MHC-peptide binding and overcome the complexity of the binding motif limitation. RANKPEP web server is a variability masking feature to focus on the prediction of conserved epitopes, which could thus help to avoid immune evasion resulting from mutation. 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 [66-69].

Prediction of Antigenic Peptides by Cascade SVM based TAPPred method

In the present study, we predicted the cascade SVM based several TAP binders which was based on the sequence and the features of amino acids [70]. We found the MHCI binding regions (Table- 3), the binding affinity of *Dracunculus medinensis.*

Solvent Accessible Regions

We also analyzed the 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., *et al*. [71] and Karplus and Schulz [72]. The different scales were used to predict the hydrophobic and hydrophilic characteristics of amino acids which is rich in charged and polar residues. The methods used are Sweet., *et al*. (1983), Kyte& Doolittle (1982), Abraham & Leo(19987), Bull and Breese (1974), Guy (1985), Miyazawa, ., *et al.* (1985), Roseman (1988), Wolfenden., *et al*. (1981), Wilson., *et al*. (1981), Cowan (1990),Chothia (1976) [73-82].

Results

The *Dracunculusmedinensis* antigen Hsp70, contain a long residue of 647 amino acids with 639 nonamers.

MAKHNAVGIDLGTTYSCVGVFMHGKVEIIANDQGNRTTPSYVAFTDTERLIGDAAKNQVAMNPNNTVFDAKRLIGRRFDDPAVQADMKHWP-
FKVINAEGSKPKVQVEYKGETKTFTPEEISSMVLLKMKETAEAFLGSTVKDAVVTVPAYFNDSQRQATKDAGAIAGLNVLRIINEPTAAAIAYGLDK-
KGHGERNVLIFDLGGGTFDVSILTIEDGIFEVKSTAGDTHLGGEDFDNRMVNHFVAEFKRKHKKDLSTNPRALRRLRTACERAKRTLSSSSQASIE-
IDSLFEGIDFYTNITRARFEELCADLFRSTMDPVEKALRDAKMDKSQMHDIVLVGGSTRIPKVQKLLSDFFSGKELNKSINPDEAVAYGAAVQAAILS-
GDKSEAVQDLLLLDVAPLSLGIETAGGVMTALIKRNTTIPTKTAQTFTTYSDNQPGVLIQVFEGERAMTKDNNLLGKFELSGIPPAPRGVPQIEVTF-
DIDANGILNVSAQDKSTGKQNKITITNDKGRLSKDEIERMVQEAEKYKADDEAQKDRIAAKNALESYAFNMKQTIDDEKLKDKLSADDRKKIEDKC-
DEIIKWLDRNQTAEKDEFEHHQKELEAVCNPIITKMYQSAGGMPGNPGGFPGGGAPGGGHQGGGGPTIEEVD

Prediction of Antigenic Peptides

In this prediction, we investigated the area of greatest local Hydrophilicity through antigenic determinants . In the Hopp-Woods scale Hydrophilicity Prediction Result Data found high in Position: 570, Score: 2.467 (max) i.e., 567-DRKKIED-573 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 & al antigenicity plot provides value as the log of the quotient between percentage in average proteins and percentage in a sample of known antigenic regions. The prediction result found highest in Position: 251 Score: 1.453 (max) i.e., 248-RKHKKDL-554 (Figure 2). We also study Hydrophobicity plot of HPLC / Parker Hydrophilicity Prediction Result Data found in Position: 531, Score: 6.600 (max) 528-ADD EAQK-534 (Figure 3), BepiPred predicts the location of linear B-cell epitopes Result found in position: 630(Residue: A) max score: 2.548 i.e., 627-MYQSAGG-633 (Figure 4), Kolaskar and Tongaonkar antigenicity methods (Figure 5) Predicted peptides result found i.e

13-TTYSCVGVFMHGKVEI-28,38-TPSYVAF-44,79-DDPAVQA-85,90-WPFKVIN-96,100-SKPKVQVE-107,120-ISSMVLL-126,132-AEAFLGSTVKDAVVTVPAY150,164-AIAGLNVLRI-173,178-TAAAIAYG-185,193-ERNVLIF-199,205-TFDVSILTI-213,216-GIFEVKS-222,239-VNHFVAE-245,260-ALRRLRTACE269,275-LSSSSQASIEIDSLFEG-291,303-FEELCADL-310,332-MHDIVLVGG-340,343-RIP-KVQKLLSD-353,366-PDEAVAYGAAVQAAILS-382,386-SEAVQDLLLLDVAPLSLG-403,436-QPGVLIQV-443,461-ELSGIPPAPRGVPQIEVTF-479, 485-GILNVSA-491,596-HQKELEAVCNP-606 and the predicted antigenic fragments can bind to MHC molecule is the first bottlenecks in vaccine design.

13-TTYSCVGVFMHGKVEI-28,38-TPSYVAF-44,79-DDPAVQA-85,90-WPFKVIN-96,100-SKPKVQVE-107,120-ISSMVLL-126,132-AEAFLGSTVKDAVVTVPAY150,164-AIAGLNVLRI-173,178-TAAAIAYG-185,193-ERNVLIF-199,205-TFDVSILTI-213,216-GIFEVKS-222,239-VNHFVAE-245,260-ALRRLRTACE269,275-LSSSSQASIEIDSLFEG-291,303-FEELCADL-310,332-MHDIVLVGG-340,343-RIP-KVQKLLSD-353,366-PDEAVAYGAAVQAAILS-382,386-SEAVQDLLLLDVAPLSLG-403,436-QPGVLIQV-443,461-ELSGIPPAPRGVPQIEVTF-479, 485-GILNVSA-491,596-HQKELEAVCNP-606 and the predicted antigenic fragments can bind to MHC molecule is the first bottlenecks in vaccine design.

Prediction of 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*. (Figure 6) predicts the highest probability in position: 249(residue: K), 247-KRKHKK-252-(max score: 8.182), 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 7) High score is found i.e. found in position: 497(residue: G), 494-STGKQN-500(max score: 1.121), position: 498(residue: K), 495-STGKQNK-501 (max score: 1.12). The hydrophobicity and hydrophilic characteristics of amino acids is determined by several other scales i.e. Sweet., *et al*. hydrophobicity prediction result data found high in position: 18 Score: 0.567 (max) i.e.,15-YSCVGVF-21 (Figure 8), Kyte & Doolittle result high in position: 396, Score: 2.211 (max)i.e.,393-LLLDVAP-399 (Figure 9), Abraham & Leo result high Position: 396, Score:1.717(max)393-LLLDVAP-399 (Figure 10),Bull & Breese use surface tension to measure in Position: 636, Score:0.814 (max) 633-GGHQGGG-639 (Figure 11), Miyazawa result high in Position: 396 Score: 6.974 (max) 393-LLLDVAP-399 (Figure 12),Guy result high in Position: 570 Score: 0.906 (max) 567-DRKK IED-573 (Figure 13), Wolfenden result high in Position: 630, Score: 1.809 (max) 627-GGGAPGG-633 (Figure 14), Roseman result high in Position: 147, Score: 0.934 (max)144-VVTVPAY-150 (Figure 15),Wilson & al Position: 396 Score: 4.378 (max) 393-LLLDVAP-399 (Figure 16), Cowan Position: 396, Score: 1.234 (max) 393-LLLDVAP-399 Figure 17), Chothia Position: 200, Score: 0.419 (max) 197-LIFD LGG-203 (Figure 18).

Prediction of MHC Binding Peptide

We found the binding of peptides to a number of different alleles using Position Specific Scoring Matrix. Hsp70 of *Dracunculus medinensis* antigen, with sequence 647 amino acid residues long, is having 639 nonamers. MHC molecules actively participate in host immune reactions and these are the cell surface proteins. We have predicted MHC-I peptide binders of Hsp70 from *Dracunculus medinensis* was tested with on a set of 4 different alleles i.e. H2-Db (mouse) 8mer, H2-Db (mouse) 9mer, H2-Db (mouse) 10mer and H2-Db (mouse) 11mer (Table-1) and MHC-II peptide binders for I_Ab.p, I_Ad.p,I_Ag7.p alleles highlighted in red represent predicted binders (Table-2). Here RANKPEP outcome by PSSM-specific binding threshold is obtained by scoring all the antigenic peptide sequences included in the alignment from which a profile is derived, and it 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 in red whereas, peptides highlighted in violet is produced by the cleavage prediction model.The cascade SVM based 'TAPPred' method has been used ,where we found more than 80 High affinity TAP Transporter peptide regions, which represents the predicted TAP binders residues which occur at N and C termini of protein from GWD (Table-3).

MHC-I Allele	RANK	POS.	N	SEQUENCE	C	MW (Da)	SCORE	% OPT.
8mer_H2_Db	1	636	GGH	QGGGGPTI	EEV	667.71	23.449	44.67%
8mer_H2_Db	2	497	KST	GKQNKITI	TND	883.04	21.607	41.16%
8mer_H2_Db	3	88	ADM	KHWPFKVI	NAE	1013.28	19.048	36.29%
8mer_H2_Db	4	434	TYS	DNQPGVLI	QVF	836.94	16.851	32.10%
8mer_H2_Db	5	34	NDQ	GNRTTPSY	VAF	876.92	14.346	27.33%
9mer_H2_Db	1	60	NQV	AMNPNNTVF	DAK	989.1	17.155	34.06%
9mer_H2_Db	2	1		MAKHNAVGI	DLG	922.1	15.959	31.69%
9mer_H2_Db	3	53	LIG	DAAKNQVAM	NPN	929.05	14.653	29.09%

9mer_H2_Db	4	307	EEL	CADLFRSTM	DPV	1025.21	11.77	23.37%
9mer_H2_Db	5	21	VGV	FMHGKVEII	AND	1055.3	11.499	22.83%
10mer_H2_Db	1	545	ALE	SYAFNMKQTI	DDE	1184.37	15.269	25.94%
10mer_H2_Db	2	496	DKS	TGKQNKITIT	NDK	1085.24	12.177	20.69%
10mer_H2_Db	3	7	HNA	VGIDLGTTYS	CVG	1007.1	9.362	15.91%
10mer_H2_Db	4	431	TFT	TYSDNQPGVL	IQV	1075.14	9.17	15.58%
10mer_H2_Db	5	33	AND	QGNRTTPSYV	AFT	1104.18	9.051	15.38%
11mer_H2_Db	1	92	HWP	FKVINAEGSKP	KVQ	1171.36	18.212	22.91%
11mer_H2_Db	2	229	DTH	LGGEDFDNRMV	NHF	1234.35	16.647	20.94%
11mer_H2_Db	3	289	DSL	FEGIDFYTNIT	RAR	1301.42	11.465	14.42%
11mer_H2_Db	4	569	DDR	KKIEDKCDEII	KWL	1315.55	8.446	10.62%
11mer_H2_Db	5	484	IDA	NGILNVSAQDK	STG	1140.25	7.837	9.86%

Table 1: *Promiscuous MHC ligands, having C-terminal ends are proteosomal cleavage sites of Dracunculusmedinensis. The antigenic peptide to the MHC-1 Allele i.e. 8mer_H2_Db (The binding thresholds: 33.04, optimal score: 52.494), 9mer_H2_Db (Optimal Score: 50.365, Binding Threshold: 17.96), 10mer_H2_Db (The Optimal Score: 58.858, Binding Threshold: 41.32),11mer_H2_Db(Optimal Score: 79.495, Binding Threshold: 56.96). (All rows highlighted in red represent predicted binders &A peptide highlighted in violet has a C-teminus predicted by the cleavage model used).*

MHC-II Allele	RANK	POS.	N	SEQUENCE	C	MW (Da)	SCORE	% OPT.
MHC-II I_Ab	1	371	EAV	AYGAAVQAA	ILS	802.89	10.747	30.16%
MHC-II I_Ab	2	603	LEA	VCNPIITKM	YQS	1000.27	10.619	29.80%
MHC-II I_Ab	3	429	AQT	FTTYSDNQP	GVL	1054.08	10.517	29.52%
MHC-II I_Ab	4	460	LGK	FELSGIPPA	PRG	912.07	10.292	28.88%
MHC-II I_Ab	5	600	QKE	LEAVCNPII	TKM	953.17	10.043	28.19%
MHC-II I_Ad	1	237	FDN	RMVNHFVAE	FKR	1084.26	18.933	35.63%
MHC-II I_Ad	2	33	AND	QGNRTTPSY	VAF	1005.05	17.011	32.01%
MHC-II I_Ad	3	533	DEA	QKDRIAAKN	ALE	1025.17	15.441	29.05%
MHC-II I_Ad	4	374	AYG	AAVQAAILS	GDK	824.98	15.013	28.25%
MHC-II I_Ad	5	368	NPD	EAVAYGAAV	QAA	831.93	14.436	27.16%
MHC-II I_Ag7	1	519	IER	MVQEAEKYK	ADD	1107.29	19.553	47.84%
MHC-II I_Ag7	2	188	GLD	KKGHGERNV	LIF	1006.12	17.951	43.92%
MHC-II I_Ag7	3	181	TAA	AIAYGLDKK	GHG	960.14	14.027	34.32%
MHC-II I_Ag7	4	229	DTH	LGGEDFDNR	MVN	1004.03	13.828	33.83%
MHC-II I_Ag7	5	365	KSI	NPDEAVAYG	AAV	916.95	12.379	30.29%

Table 2: *Prediction of MHCII ligands all rows highlighted in red represent predicted binders to the MHC-II Allele i.e. MHC-II I_Ab, MHC-II I_Ad, MHC-II I_Ag7. (All rows highlighted in red represent predicted binders).*

Peptide Rank	Start Position	Sequence	Score	Predicted Affinity
1	283	IEIDSLFEG	8.648	High
2	109	KGETKTFTP	8.644	High
3	429	FTTYSDNQP	8.644	High
4	519	MVQEAEKYK	8.643	High
5	555	DDEKLKDKL	8.639	High
6	526	YKADDEAQK	8.637	High
7	145	VTVPAYFND	8.635	High
8	558	KLKDKLSAD	8.635	High
9	105	QVEYKGETK	8.634	High
10	220	VKSTAGDTH	8.631	High

Table 3: *Cascade SVM based High affinity TAP Binders of Dracunculus medinensis.*

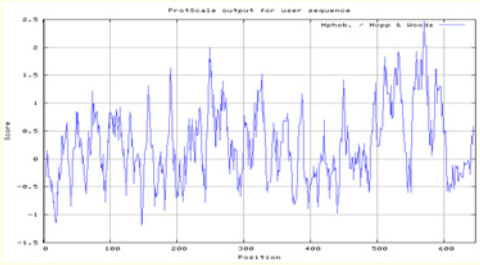

Figure 1: *Hydrophobicity plot of Hopp and Woods (1981) [59].*

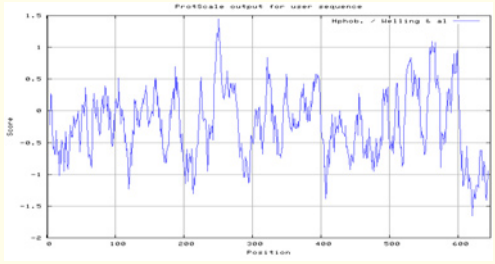

Figure 2: *Hydrophobicity plot of Welling., et al. (1985) [60].*

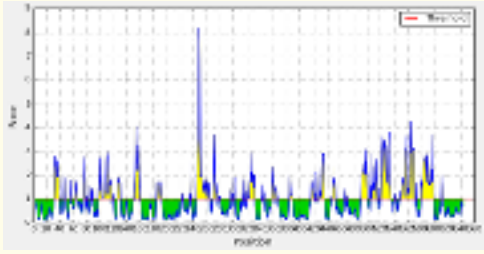

Figure 3: *Hydrophobicity plot of HPLC / Parker., et al. (1986) [61].*

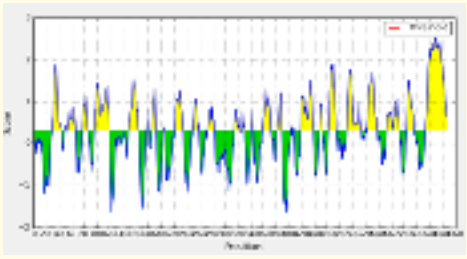

Figure 4: *Bepipred Linear Epitope Prediction plot.*

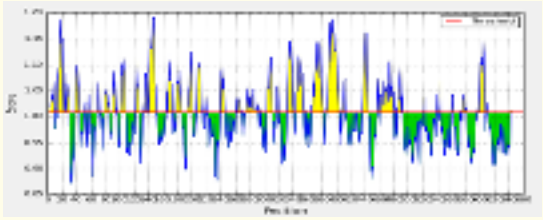

Figure 5: *Kolaskar and Tongaonkar antigenicity plot.*

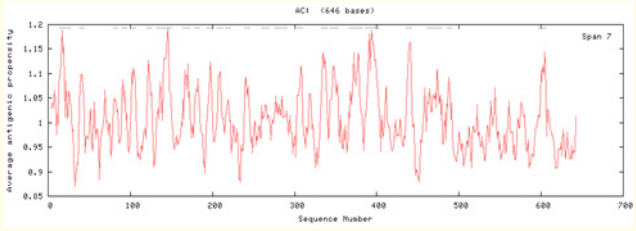

Figure 5a: *Kolaskar and Tongaonkar antigenicity plot, the average antigenic propensity for protein is 1.0067*

n	Start Position	Sequence	End Position
1	13	TTYSCVGVFMHGKVEI	28
2	38	TPSYVAF	44
3	79	DDPAVQA	85
4	90	WPFKVIN	96
5	100	SKPKVQVE	107
6	120	ISSMVLL	126
7	132	AEAFLGSTVKDAVVTVPAY	150
8	164	AIAGLNVLRI	173
9	178	TAAAIAYG	185

10	193	ERNVLIF	199
11	205	TFDVSILTI	213
12	216	GIFEVKS	222
13	239	VNHFVAE	245
14	260	ALRRLRTACE	269
15	275	LSSSSQASIEIDSLFEG	291
16	303	FEELCADL	310
17	332	MHDIVLVGG	340
18	343	RIPKVQKLLSD	353
19	366	PDEAVAYGAAVQAAILS	382
20	386	SEAVQDLLLLDVAPLSLG	403
21	436	QPGVLIQV	443
22	461	ELSGIPPAPRGVPQIEVTF	479
23	485	GILNVSA	491
24	596	HQKELEAVCNP	606

Figure 5b: *The 24 antigenic determinants of Hsp70 protein.*

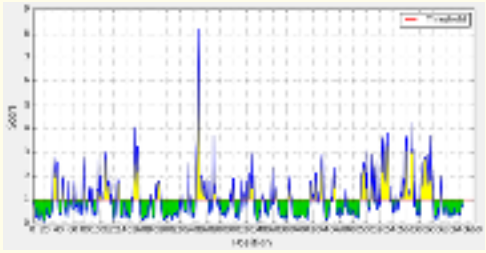

Figure 6: *Emini Surface Accessibility Prediction plot.*

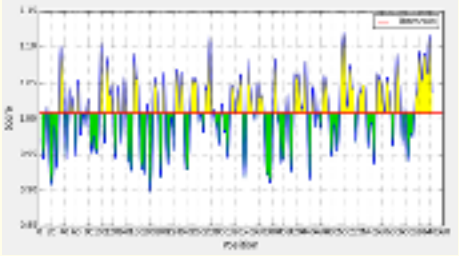

Figure 7: *Karplus& Schulz Flexibility Prediction.*

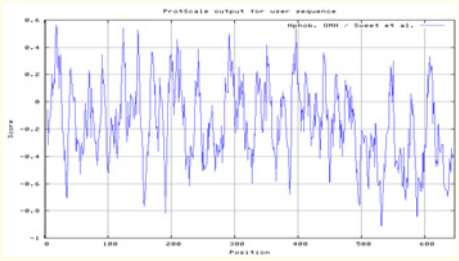

Figure 8: *Hydrophobicity plot of Sweet., et al. (1983) [73].*

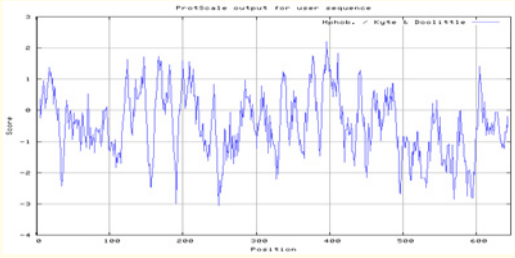

Figure 9: *Kyte& Doolittle hydrophobicity plot.*

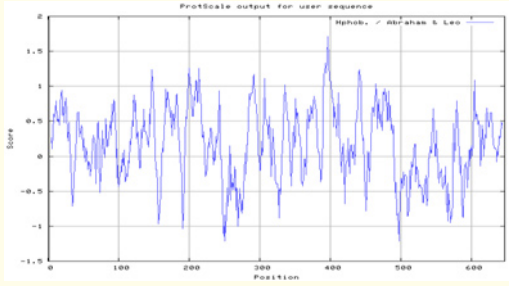

Figure 10: *Abraham & Leo hydrophobicity plot.*

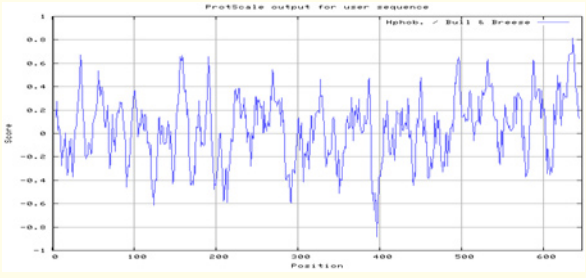

Figure 11: *Bull & Breese use surface tension to measure hydrophobicity and also uses negative values to describe the hydrophobicity of antigen Hsp70.*

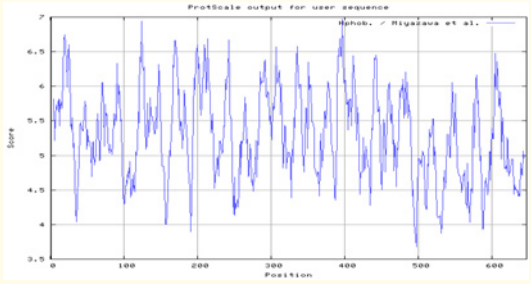

Figure 12: *Hydrophobicity plot of Miyazawa., et al. (1985) [77].*

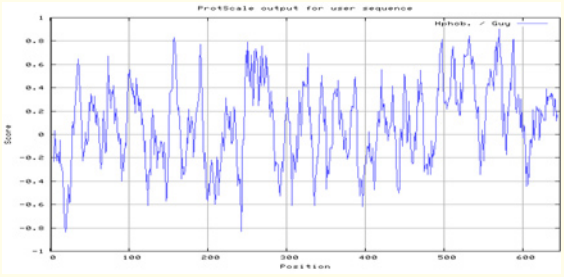

Figure 13: *Hydrophobicity plot of Guy (1988) [76].*

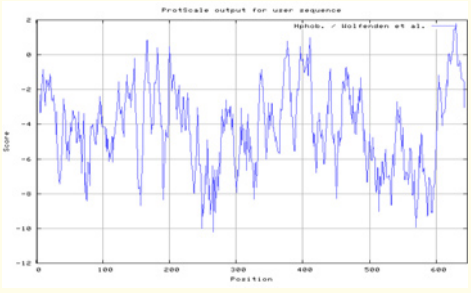

Figure 14: *Hydrophobicity plot of Wolfenden., et al.(1981) [79].*

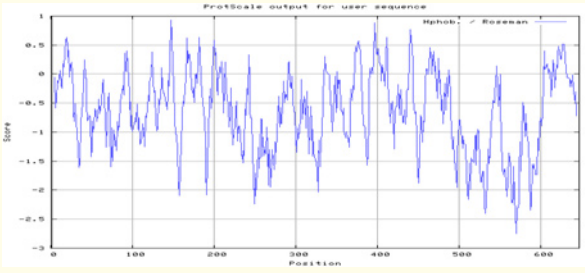

Figure 15: *Hydrophobicity plot of Roseman M.A. (1988) [78].*

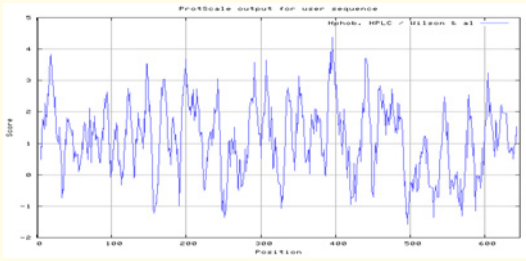

Figure 16: *Hydrophobicity/HPLC plot of Wilson & al (1981) [80].*

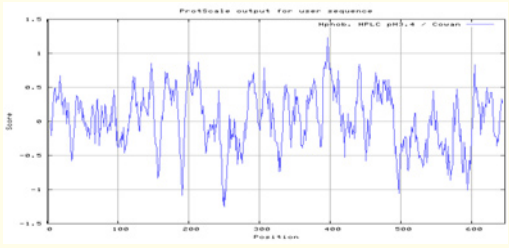

Figure 17: *Hydrophobicity/HPLC pH 3.4/ plot of Cowan (1990) [81].*

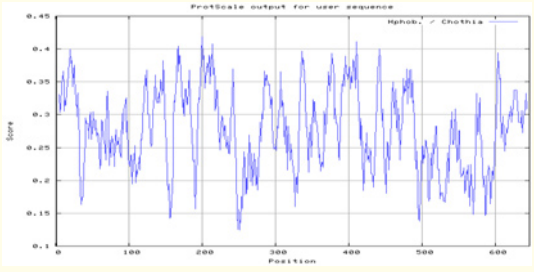

Figure 18: *Hydrophobicity plot of Chothia (1976) [82].*

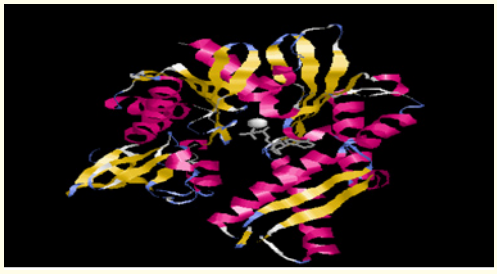

Figure 19: *Structure of Hsp70 the Dracunculus medinensis antigen (DeepView (Swiss Pdb-Viewer) program).*

Discussion

In this study, the antigenic-determinants were identified by finding the area of greatest local-hydrophilicity. Hopp and Woods hydrophobicity scale is used to identify of potentially antigenic sites in proteinsby analyzing amino acid sequences in order to find the point of greatest hydrophilic. Hydrophilicity Prediction result data found high in sequence position: 570, Score: 2.467 (max) i.e., 567-DRKKIED-573 in a protein this scale is basically a hydrophilic index where apolar residues have been assigned negative values. In the process of finding hydrophilic regions, usually the window size of 5-7 is considered to be good. The opted values greater than 0 values are consider as hydrophilic which is deliberated as antigenic. Welling & al, analysis reveals the information on the reciprocal happening of amino acids in antigenic regions to construct a scale which is utile for prediction of antigenic regions .The predicted protein result data found high in sequence in Position: 251 Score: 1.453 (max) i.e., 248-RKHKKDL-554. Welling antigenicity plot gives value as the log of the quotient between percentage in asample of known antigenic regions and percentage in average proteins. We also study Hydrophobicity plot of HPLC / Parker Hydrophilicity Prediction and the result data found highest in Position: 531, Score: 6.600 (max) 528-ADD EAQK-534. BepiPred predicts the location of linear B-cell epitopes. Through this analysis the opted result found highest in position: 630(Residue: A) max score: 2.548 i.e., 627-MYQSAGG-633. There are 24 antigenic determinant sequences is found by Kolaskar and Tongaonkar antigenicity scales(Fig. 6a &6b) the results show highest pick 13-TTYSCVGVFMHGKVEI-28,38-TPSYVAF-44,79-DDPAVQA-85,90-WPFKVIN-96,100-SKPKVQVE-107,120-ISSMVLL-126,132-AEAFLGSTVKDAVVTVPAY150,164-AIAGLNVLRI-173,178-TAAAIAYG-185, 193-ERNVLIF-199, 205-TFDVSILTI-213,216-GIFEVKS-222,239-VNHFVAE-245,260-ALRRLRTACE-269,275-LSSSSQASIEIDSLFEG-291-,303-FEELCADL-310,332-MHDIVLVGG-340,343-RIPKVQKLLSD-353,366-PDEAVAYGAAVQAAILS-382,386-SEAVQDLLLLDVAPLSLG-403,436-QPGVLIQV-443,461- ELSGIPPAPRGVPQIEVTF-479, 485-GILNVSA-491,596-HQKELEAVCNP-606. The determined antigenic site on proteins indicates that the hydrophobic residues if they occur on the surface of a protein, there is the strong possibility they are more likely to be a part of antigenic sites. The accuracy of this method 75% to predict antigenic determinants and also provides the important information of surface accessibility and flexibility. Furthermore, this region forms beta sheet which show high antigenic response than helical region of this peptide and found comparatively highly antigenicity. The Structure of the Dracunculus medinensis antigen- Hsp70 is predicted by SWISS-MODEL (automated protein structure homology-modelling server) Deep View (Swiss Pdb-Viewer) program (Figure 19). The target structure will also serve as a detailed model for determining the structure of peptide within protein structure. We predict Solvent accessibility by using Emini., et al. and the result found the with highest probability i.e. found in position :249(residue: K), 247-KRKHKK-252 (max score:8.182), 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 in position:497(residue: G),494-STGKQN-500(max score:1.121), position:498(residue: K), 495-STGKQNK-501 (max score:1.12). We predict Solvent accessibility of Hsp70 from GWD antigen for describing the hydrophobic and hydrophilic characteristics of amino acids. Solvent accessibility is generally applied to identify active site of functionally important residues in membrane proteins. The reason of varying solvent-accessible surface areas and backbone angles continuously, due to proteins nature's which 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 Position: 18 Score: 0.567 (max) i.e.,15-YSCVGVF-21, Kyte & Doolittle result high Position: 396, Score: 2.211 (max) i.e.,393-LLLDVAP-399, Abraham & Leo result high Position: 396, Score:1.717 (max) 393-LLLDVAP-399,Bull and Breese result high Position: 636, Score:0.814 (max) 633-GGHQGGG-639, Guy result high Position: 570, Score: 0.906 (max) 567-DRKK IED-573, Miyazawa result high in Position: 396 Score: 6.974 (max) 393-LLLDVAP-399, Wolfenden result high in Position: 630,Score: 1.809 (max) 627-GGGAPGG-633, Roseman result high Position: 147,Score: 0.934 (max)144-VVTVPAY-150, Wilson & al Position: 396 Score: 4.378 (max) 393-LLLDVAP-399, Cowan Position: 396, Score: 1.234 (max) 393-LLLDVAP-399, Chothia Position: 200, Score: 0.419 (max) 197-LIFD LGG-203. These scales are a hydrophilic with a polar residues (assigned negative value). Because of the N- and C- terminal regions of proteins are generally solvent accessible and unstructured, antibodies against that particular regions recognizes 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 QNWNCCTIthat 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 and I_Ab with the consensus sequence YYAPWCNNA that yields the maximum score i.e. 35.632,I_Ad with the consensus sequence QMVHAAHAE that yields the maximum score i.e. 53.145, MHC-II I_Ag7 with the consensus sequence WYAHAFKYV that yields the maximum score i.e. 40.873 for MHC II allele was tasted. We also use a cascade SVM based TAPPred method which found 160 High affinity. TAP Transporter peptide regions which represent predicted TAP binders residues which occur at N and C termini from Dracunculus medinensis antigen Hsp70. TAP is one of the important conveyor, which allow antigenic peptides to move from cytosol to ER. TAP directs binds and translocate the selective antigen peptides for binding to the particular 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 antigen Hsp70. 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 determing T-cell epitopes. Consequently, RANKPEP moreover, focus on the prediction of conserved epitopes and the sequences highlighted in purple in the output results.

Conclusion

MHC molecules are the cell surface proteins, which actively take part in the host immune responses against infection (pathogens) and reason of its involvement in the response to almost all antigens and it gives effects on specific sites. 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, antigenic peptide that binds to MHC molecule are antigenic that means hydrophilic in nature. This means the increase in affinity of MHC binding peptides may result in enhancement of immunogenicity of Dracunculus medinensis antigen Hsp70 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 Dracunculus medinensis antigen Hsp70. The Overall conducted study and opted results are encouraging. Both the 'sites of action' and 'physiological functions' can be predicted with very high accuracies which is helping to minimize the number of validation experiments. The future perspectives of this method will be useful in cellular immunology, vaccine design, immunodiagnostics, immunotherapeutic and molecular understanding of autoimmune susceptibility.

Bibliography

1. Gerner EW., *et al*. "A transient thermotolerant survival response produced by single thermal doses in HeLa cells". *Cancer Research* 36.3 (1976): 1035-1040.
2. Sapareto SA., *et al*. "Effects of hyperthermia on survival and progression of Chinese hamster ovary cells". *Cancer Research* 38.2 (1978): 393-400.
3. Henle KJ., *et al*. "Induction of thermotolerance in Chinese hamster ovary cells by high (45 degrees) or low (40 degrees) hyperthermia". *Cancer Research* 38.3 (1978): 570-574.
4. Petersen NS and Mitchell HK. "Recovery of protein synthesis after heat shock: prior heat treatment affects the ability of cells to translate mRNA". *Proceedings of the National Academy of Science* 78.3 (1981): 1708-1711.
5. Jäättelä M., *et al*. "Heat shock protects WEHI-164 target cells from the cytolysis by tumor necrosis factors a and beta". *European Journal of Immunology* 19.8 (1989): 1413-1417.
6. Li GC and Werb Z. "Correlation between synthesis of heat shock proteins and development of thermotolerance in Chinese hamster fibroblasts". *Proceedings of the National Academy of Science* 79.10 (1982): 3122-3218.
7. Landry J., *et al*. "Synthesis and degradation of heat shock proteins during development and decay of thermotolerance". *Cancer Research* 42.6 (1982): 2457-2461.

8. Riabolow KT., *et al*. "Heat shock is lethal to fibroblasts microinjected with antibodies against hsp70". *Science* 242.4877 (1988): 433-436.
9. Johnston RN and Kucey BL. "Competitive inhibition of hsp70 gene expression causes thermosensitivity". *Science* 242 (1988): 1551-1554.
10. Lindquist S and Craig EA. "The heat shock proteins". *Annual Review of Genetics* 22 (1988): 631-677.
11. Gupta RS and Singh B. "Phylogenetic analysis of 70 kD heat shock protein sequences suggests a chimeric origin for the eukaryotic cell nucleus". *Current Biology* 4.12 (1994): 1104-1114.
12. Hunt C and Morimoto RI. "Conserved features of eucaryotic hsp70 genes releaved by comparision with the nucleotide sequence of human hsp70". *Proceedings of the National Academy of Science* 82 (1985): 6455-6459.
13. Daugaard M., *et al*. "The heat shock protein 70 family: Highly homologous proteins with overlapping and distinct functions". *FEBS Letters* 581.19 (2007): 3702-3710.
14. Matz JM., *et al*. "Characterization and regulation of cold-induced heat shock protein expression in mouse brown adipose tissue". *American Journal of Physiology* 269.1 Pt 2 (1995): 38-47.
15. Cao Y., *et al*. "TGF-β11 mediates 70-kDa heat shock protein induction due to ultraviolet irradiation in human skin fibroblasts". *Pflügers Archiv* 438.3 (1999): 239-244.
16. Laplante AF., *et al*. "Expression of heat shock proteins in mouse skin during wound healing". *Journal of Histochemistry & Cytochemistry* 46.11 (1998): 1291-1301.
17. De Maio A. "Heat shock proteins: facts, thoughts, and dreams". *Shock* 11.1 (1999): 1-12.
18. Bukau B., *et al*. "Getting newly synthesized proteins into shape". *Cell* 101.2 (2000): 119-122.
19. Hart F U. and Hayer-Hart M. "Molecular chaperones in the cytosol: from nascent chain to folded protein". *Science* 295.5561 (2002): 1852-1858.
20. Young J C., *et al*. "More than folding: localized functions of cytosolic chaperones". *Trends in Biochemical Sciences* 28 (2003): 541-547.
21. Neupert W and Brunner M. "The protein import motor of mitochondria". *Nature Reviews Molecular Cell Biology* 3.8 (2002): 555-565.
22. Ryan M T and Pfanner N. "Hsp70 proteins in protein translocation". *Advances in Protein Chemistry* 59 (2002): 223-242.
23. Pratt W B and Toft D O. "Regulation of signaling protein function and trafficking by the hsp90/hsp70-based chaperone machinery". *Experimental biology and medicine (Maywood)* 228.2 (2003): 111-133.
24. Toft D O. "Control of hormone receptor function by molecular chaperones and folding catalysts. In: Molecular Chaperones and Folding Catalysts". *Regulation, Cellular Function and Mechanism* (1999): pp. 313–327.
25. Ricaniadis N., *et al*. "Long-term prognostic significance of HSP-70, c-myc and HLA-DR expression in patients with malignant melanoma". *European Journal of Surgical Oncology* 27.1 (2001): 88-93.
26. Ramp U., *et al*. "Expression of heat shock protein 70 in renal cell carcinoma and its relation to tumor progression and prognosis". *Histopathology* 22.10 (2007): 1099-107.
27. Muller R. "Dracunculus and dracuneuliasis". *Advances in Parasitology* 9 (1971): 73-151.
28. Muller R. "Guinea worm disease: epidemiology, control and treatment". *Bull World Health Organ* 57.5 (1979): 683-689.
29. Jones hI and Mulder E. "Dracunculusmulbusn.sp. (nematode: Spirurida) from water Python Liasisfuscus (sperpentes: Boidae) in northern". *Australian Society for Parasitology* 66.3 (2007): 195-205.
30. Moravee F and Santos CP. "Dracunculus brasiliensis sp.n. (Nematoda: Dracunculidae) from the anaconda. Eunectesmurinus (Ophidia: Boidae)". *Parasitology Research* 104.3 (2009): 589-592.
31. Bimi L., *et al*. "Ginea worm infection of urinary bladder manifesting as obstructureuropathy in rural Maharastra". *Tropical Doctor* 35.4 (2005): 242.
32. 6-www.cdc.gov/parasites/guineaworm/biology.html

33. Greenaway C. "Dracunculiasis (Guinea worm disease)". *Canadian Medical Association Journal* 170.9 (2004): 495-500.
34. Miillner A., *et al*. "Chemistry and pharmacology of neglected helminthic disease". *Current Medicinal Chemistry* 18.5 (2011): 767-789.
35. Ruiz-Tiben E and Hopkins DR. "Dracunculiasis (Guinea worm disease) eradication". *Advances in Parasitology* 61 (2006): 275-309.
36. IriemenamNC., *et al.* "Dracunculiasis–The saddle is virtually ended". *Parasitology Research* 102.3 (2008): 343-347.
37. Hopkins DR., *et al*. "Progress toward global eradication of dracunculiasis--January 2013-June 2014". *Centers for Disease Control and Prevention* 63.46 (2014): 1050-1054.
38. Eta Ngole Mbong., *et al*. "Not every worm wrapped around a stick is a guinea worm: a case of Onchocerca volvulus mimicking Dracunculusmedinensis". *Parasit Vectors* 8 (2015): 374.
39. Richards FO and Ruiz-TibenEand Hopkins DR. "Dracunculiasis eradication and the legacy of the smallpox campaign: what's new and innovative? What's old and principled?" *Vaccine* 29.4 (2011): 86-90.
40. Hopkins DR. "Disease eradication". *The New England Journal of Medicine* 368 (2013): 54-63.
41. Muller R. "Life cycle of Dracunculus Medinesis". In workshop on opportunities for control of dracunculiasis: contaminated papers, washinton,DC:National Academy Press] (1985).
42. Cairncross S., *et al*. "Dracunculiasis (Guinea worm disease) and the eradication initiative". *Clinical Microbiology Reviews* 15 (2002): 223-246.
43. Issaka –Tinogah A., *et al*. "Lack of effect of ivermectin on prepatent guinea – worm: a single- blind, placebo-controlled trial". *Transactions of the Royal Society of Tropical Medicine* 88 (1994): 346-348.
44. Bloch P., *et al*. "The antibody response to Dracunculusmedinensis in an endemic human population of northern Ghana". *Helminthology* 67 (1993): 37-48.
45. BlochP and Simonsen E PAUL. "Immunoepidemiology of Dracunculusmedinensis infections II.Variation in antibody respomses in relation to transmission season and patency". *The American Journal of Tropical Medicine and Hygiene* 59.6 (1998): 985-990.
46. Flower DR. "Vaccines: how they work". In Bioinformatics for Vaccinology Wiley-Blackwell, Oxford, UK. (2008): 73-112 .
47. Batalia Michael and Collins EJ. "Peptide Binding by Class 1 and Class II MHC Molecules". *Biopoly* 43.4 (1997): 281-302.
48. Marrack P., *et al*. "Review Evolutionarily conserved amino acids that control TCR-MHC interaction". *Annual Review of Immunology* 26 (2008): 171-203.
49. Chapman HA. "Endosomal proteolysis and MHC class II function". *Current Opinion in Immunology* 10.1 (1998): 93-102.
50. Watts C. "The exogenous pathway for antigen presentation on major histocompatibility complex class II and CD1 molecules". *Nature Immunology* 5.7 (2004): 685-92.
51. Neefjes J., *et al*. "Review towards a systems understanding of MHC class I and MHC class II antigen presentation". *Nature Reviews Immunology* 11.12 (2011): 823-836.
52. Kumar M., *et al*. "Identification of DNA-binding proteins using support vector machines and evolutionary profiles". *BMC Bioinformatics* 8 (2007): 463.
53. Gomase VS and Chitlange NR. "Prediction of MHC Class Antigen Peptides from Echinococcus Multilocularis: Application of Computer Intelligence". *Scientific Reports* 1.3 (2012): 191.
54. Gomase VS., *et al*. "Prediction of MHC Binding Peptides and Epitopes from Alfalfa mosaic virus". *Current Drug Discovery Technologies* 4.2 (2007): 117-215.
55. Sherkhane AS., *et al*. "Prediction of Major Histocompatibility Complex Binding Peptides and Epitopes from Najanaja Cardiotoxin (CTX)". *Drug Invention Today* 4.8 (2012): 435-438.
56. http://www.ncbi.nlm.nih.gov
57. Sayers E W., *et al*. "Database resources of the National Center for Biotechnology Information". *Nucleic Acids Research* 40.Database issue (2012): D13-25.
58. Bairoch A., *et al*. "The Universal Protein Resource (UniProt)". *Nucleic Acids Research* 33 (2005): D154-159.

59. Hoop TP and Woods KR. "Prediction of protein antigenic determinants from amino acid sequences". *Proceedings of the National Academy of Sciences of the USA* 78.6 (1978): 3824-3828.
60. Welling GW., *et al*. "Prediction of sequential antigenic regions in proteins". *FEBS Letters* 188.2 (1985): 215-218.
61. Parker KC., *et al*. "Scheme for ranking potential HLA-A2 binding peptides based on independent binding of individual peptide side-chains". *The Journal of Immunology* 152.1 (1994): 163-175.
62. Jens Erik., *et al*. "Improved method for predicting linear B-cell epitopes". *Immunome Research* 2 (2006): 2.
63. Kolaskar AS and Tongaonkar PC. "A semi-empirical method for prediction of antigenic determinants on protein antigens". *FEBS Letters* 276.1-2 (1990): 172-174.
64. Mishra Sonu and Virendra S Gomase. "Prediction of antigenic epitope from D. medinensis: new paradigm of synthetic vaccine development". *ICRRDESH* (2015).
65. Ruppert J., *et al*. "Prominent role of secondary anchor residues in peptide binding to HLA-A2.1 molecules". *Cell* 74.5 (1993): 929-937.
66. Reche PA., *et al*. "Prediction of MHC Class I Binding Peptides Using Profile Motifs". *Human Immunology* 63.9 (2002): 701-709.
67. RecheP A and Reinherz EL. "Sequence variability analysis of human class I and class II MHC molecules: functional and structural correlates of amino acid polymorphisms". *Journal of Molecular Biology* 331.3 (2003): 623-641.
68. Craiu A., *et al*. "Two distinct proteolytic processes in the generation of a major histocompatibility complex class I-presented peptide". *Proceedings of the National Academy of Sciences* USA 94.20 (1997): 10850-10855.
69. Pieters J. "MHC class II-restricted antigen processing and presentation". *Advances in Immunology* 75 (2000): 159-208.
70. Bhasin M and Raghava GPS. "Analysis and prediction of affinity of TAP binding peptides using cascade SVM". *Protein Science* 13.3 (2004): 596-607.
71. Emini EA., *et al*. "Induction of hepatitis a virus-neutralizing antibody by a virus-specific synthetic peptide". *Journal of Virology* 55.3 (1985): 836-839.
72. Karplus PA and Schulz GE. "Prediction of chain flexibility in proteins: a tool for the selection of peptide antigen". *Naturwissenschaften* 72.4 (1985): 212-213.
73. Sweet RM and Eisenberg D. "Correlation of sequence hydrophobicities measures similarity in three-dimensional protein structure". *Journal of Molecular Biology* 171.4 (1983): 479-488.
74. Kyte J and Doolittle RF. "A Simple Method for Displaying the Hydropathic Character of a Protein". *Journal of Molecular Biology* 157.1 (1982): 105-132.
75. Abraham DJ and Leo AJ. "Extension of the fragment method to calculate amino acid zwitterions and side chain partition coefficients". *Proteins* 2.2 (1987): 130-152.
76. Bull HB and Breese K. "Surface tension of amino acid solutions: A hydrophobicity scale of the amino acid residues". *Archives of Biochemistry and Biophysics* 161 (1974): 665-670.
77. Miyazawa S and Jernigen RL. "Estimation of Effective Interresidue Contact Energies from Protein Crystal Structures: Quasi-Chemical Approximation". *Macromolecules* 18.3 (1985): 534-552.
78. Roseman MA. "Hydrophilicity of polar amino acid side-chains is markedly reduced by flanking peptide bonds". *Journal of Molecular Biology* 200.3 (1988): 513-522.
79. Wolfenden RV., *et al*. "Affinities of amino-acid side-chains for solvent water". *Biochemistry* 20.4 (1985): 849-855.
80. Wilson J., *et al*. "The behaviour of peptides on reverse-phase K.supports during high-pressure liquid chromatography". *Biochemical Journal* 199 (1981): 31-41.
81. Cowan R and Whittaker RG. "Hydrophobicity indices at ph 3.4 determined by HPLC". *Peptide Research* 3 (1990): 75-80.
82. Chothia C. "The nature of accessible and buried surfaces in proteins". *Journal of Molecular Biology* 105.1 (1976): 1-12.

Volume 2 Issue 1 February 2016