JOURNAL OF PROTEINS AND PROTEOMICS
Vol. 1, No. 2, July-December 2010, pp. 53–63

Structural Simulation of MHC-peptide Interactions using T-cell Epitope in Iron-acquisition Protein of *N. meningitides* for Vaccine Design

Namrata Mishra*, Priyanka Chaubey*, Ankita Mishra* and Kavita Shah#**

Abstract: *The present work uses a structural simulation approach to identify the potential target vaccine candidates or T cell epitopes (antigenic region that can activate T cell response) in two iron acquisition proteins from Neisseria. An iron regulated outer membrane protein frpB: extracellular, [NMB1988], and a Major ferric Iron-binding protein fbpA: periplasmic, [NMB0634] critical for the survival of the pathogen in the host were used. Ten novel promiscuous epitopes from the two iron acquisition proteins were identified using bioinformatics interface. Of these epitopes, ^{630}VQKAVGSIL^{638} present on frpB with high binding affinity for allele HLA*DR1 was identified with an anchor position at P2, an aliphatic residue at P4 and glycine at P6 making it thereby a potential quality choice for linking peptide-loaded MHC dynamics to T-cell activation and vaccine constructs. The feasibility and structural binding of predicted peptide to the respective HLA allele was investigated by molecular modeling and template-based structural simulation. The conformational properties of the linear peptide were investigated by molecular dynamics using GROMOS96 package and Swiss PDB viewer.*

Keywords: *Iron-Acquisition Protein, MHC-peptide, N. meningitides, Simulation, T-cell epitope.*

I INTRODUCTION

The genomic sequence provides a catalogue of virtually all protein antigens that the pathogen can express at any time. Immunomics is a comparatively new discipline that uses high throughput techniques to explore immune mechanisms which has made it possible to envisage the design of vaccines based on an ensemble of epitopes (Epitope Driven Vaccine design, EDVD), derived from the genome of a pathogen, using tools that have been developed in the field of immuno-informatics (De Groot et al., 2008). These epitopes, or the proteins from which they are derived are then considered as putative candidate vaccine.

In the case of larger bacterial and viral pathogens, the traditional vaccine targets include surface proteins and secreted proteins, toxins and virulence factors. However, many other proteins can also be worthy of consideration; in particular, proteins expressed in high amounts, proteins over expressed during growth or replication, or under stress conditions. In the context of vaccines against infectious diseases, it may be prudent to exclude proteins that are highly conserved across species; such proteins may cross react with unrelated and virulent organisms or with self proteins to which there may be pre-existing tolerance; however conservation across species should not be confused with conservation within species variants. Sequence conservation within species variants is a highly desirable trait for vaccine components and one that the Immunome derived vaccine (IDV) approach is particularly well suited to harness (Vivona et al., 2007; De Groot et al., 2008)

Iron is an essential metal for nearly all living systems; however, in biological fluids it exists only as a complex with iron-binding proteins, making it essentially unavailable. To establish an infection, invasive microorganisms must depend on their ability to use the iron found in the host which is complexed to high affinity iron binding proteins such as transferrin, or lactoferrin or is part of heme in the red cells. *Neisseria meningitidis* causes meningitis and sepsis in adults (Tettelin et al., 2000; Shah et al., 2010). T cell immunity is implicated in the clearance of a disease (Romero and Poolman, 1994; Shah et al., 2010). If one can restrict the iron uptake of bacteria at outer membrane and periplasmic levels, it may be possible to decrease the pathogenicity of the bacteria (Shah et al., 2010). For a vaccine to be effective it must evoke a strong response from both T and B cells and therefore epitope mapping (Romero and Poolman, 1994; De Groot

* Bioinformatics Division, MMV, Banaras Hindu University, Varanasi- 221005, India
**# Corresponding author: Biochemistry and Bioinformatics Lab, Department of Zoology, Mahila Mahavidyalaya, Banaras Hindu University, Varanasi-221 005, India; Tel.: +91-542-6701082; fax: +91 542 2317074; Email: kavitashah@bhu.ac.in

and Berzofsky, 2004; Ekins and Schryvers, 2006) and MHC binding predictions are a central issue in its design. The vaccine aims to cover the HLA haplotype of the target population, be effective against the wider spectrum of meningitis strains and expected not to have any self reactive epitopes. In this communication we have used bioinformatics tools to analyze the proteome of *N. meningitidis* iron acquisition proteins *viz* an iron regulated outer membrane protein frpB [NMB1988, NCBI] and a periplasmic protein, Major ferric Iron-binding protein fbpA [NMB0634, NCBI] so as to identify the putative T cell epitopes specific to human HLA class I and II molecules for the formulation of the chimeric vaccine. The protein frpB [NMB1988] is also among the proteins encoded by the very important phase variable genes in *Neisseria meningitides* strain MC589 (Tettelin et al., 2000; Haq et al., 2010). Using molecular dynamic simulations the prediction has been evaluated for atomic interaction with the MHC peptide and the binding energy of the MHC-peptide complex thus providing a quality assessment for reliable immunological conclusions (Omasits et al., 2008; Haq et al., 2010).

II METHODOLOGY

Computational Resources and Antigen Processing Pathway Prediction

The project was carried out on a Linux system within the computational cluster of the Banaras Hindu University at Varanasi.

FbpA and frpB protein sequences were retrieved from the Swiss Prot database (Tzeng and Stephens, 2000) with accession number NMB0634 and NMB1988 followed by a BlastP search (Altschul,1999) to look for protein homologs in *Neisseria meningitidis* variants. The program compared the iron acquisition protein sequences to the sequence database and statistically significant matches were obtained. The Sub-cellular localization of both the proteins (NMB1988 and NMB0634) was reconfirmed with Sub Loc v 1.0 (Gardy and Brunkman, 2006).

The functional annotation of the frpB and frpA proteins for immunogenicity prediction was done using SignalP 3.0 server (Bendtsen et al., 2004) and PrediSi (Hiller et al., 2004) tools. We report a new peptide identified from frpB protein *Neisseria* that bind with high affinity to class 1 HLA*DR1 and has not been reported yet. The peptide is predicted to form stable complex through molecular modeling and does not contain any human peptide of contiguous five amino acids or longer.

Prediction of T-cell Epitope

Prediction of MHC Class 1 and class 2 binding peptides for the targeted frpB and fbpA proteins were performed using computational tools. The prediction of promiscuous MHC Classs1 binding peptides was carried out using several algorithms like ProPred1 (Singh and Raghava, 2003) SYFPEITHI (Rammensee et al., 1999) and nHLAPred [17] computational tools. Both the protein sequences (NMB1988 and NMB0634) were analyzed at a threshold setting of 10%. Proteasome and immuno-proteasome filtering were also carried out by keeping the proteasome filters ON.

NetCTL 1.2 server (Larsen et al., 2007) was used to predict CTL epitopes in protein sequences. The method integrates prediction of peptide MHC Class1 binding, proteasomal C cleavage by neural network and TAP transport efficiency by weight matrix. The server allows for predictions of CTL epitopes restricted to 12 MHC Class1 super types. Both the protein sequences were analyzed by scoring on C terminal cleavage, weight on TAP transport efficiency and threshold for epitope identification by default.

The protein frpB and fbpA were analyzed using POPI server which predicts the immunogenicity of the epitopes according to its physicochemical properties (Larsen et al., 2007; Tung and Ho, 2007).

All the high affinity HLA binding peptide binders commonly obtained by different computational tools above were analyzed for the presence of human self peptides using HLAPred (Adams and Koziol, 1995). The population coverage of the selected peptides was calculated using the population coverage tool of IEDB (Saffari et al., 2008) that calculates the fraction of individuals predicted to respond to a given set of epitopes with known MHC restrictions based on HLA genotyping frequencies assuming non linkage disequilibrium between HLA loci. The selected epitopes were subjected to structural modelling and simulation studies.

Structural Simulation of MHC-peptide

Motif based methods describe general position based patterns of recurrent amino acids favourable for MHC-peptide binding. The selected peptides obtained by the above bioinformatics tools were listed with their anchor residues. For structural simulations, the molecular models of the peptides were constructed using a web based interface MHCsim (Todman et al., 2008) available online. Sample peptides of HIGH affinity binders for a few alleles whose crystal structures are known (HLA-B*5102, HLA-B*2705, HLA-DRBI*0101) were obtained from the Protein Data Bank (Bergman et al., 2000) and modelled employing structural templates *(viz.* 1K5N for HLA-B and 1AQD for HRA-DR1) (Bergman et al., 2000) to assess the feasibility and structural basis of binding peptides to

their respective HLA alleles. Their Energy minimization (EM) scores that describes the deviation of the bond length, bond angles and torsion angles away from the equilibrium values plus terms for non-bonded pairs of atoms describing Van der Waals and electrostatic interactions were recorded (Omasits et al., 2008). To save on computational time only the essential parts of the protein are considered in the simulation i.e. the antigen binding α-1 and α-2 domain of the MHC molecule (human leukocyte antigens (HLA) molecules). The MHC molecule is reported to include 5 pockets (A-F) of different sizes forming the binding grooves carrying specific amino acid residues *viz.* Pocket A (M5, Y7, Y59,E63, Y159, E163, W167 and Y171), pocket B (H9, T24, E45, L66, C67 and Y99), pocket C (H9, K70, T73, D74 and R97), pocket D (Y99, H114, L156, Y159 and L160) and pocket F (D77, T80, L81, Y84, D116,Y123, T143, K146 and W147) of which the Pockets F and A are the largest (Omasits et al., 2008). The binding of the high and moderate affinity epitopes with the amino acid residues contributing to the MHC pockets were visualized with Chimera. Changes in the amino acid residues in the epitopes at anchor positions were also noted to understand their quality for immunological purposes.

III RESULTS

Antigen Processing Pathway Prediction

The fasta format of the major ferric iron binding protein (fbpA, accession number NMB0634) and the iron regulated outer membrane protein (frpB, accession number NMB1988) revealed 331 and 714 amino acids long sequence, respectively. Sub Loc server predicted NMB1988 (frpB) as an extracellular protein with 91% accuracy and NMB0634 (fbpA) as periplasmic protein with 92% accuracy. BlastP search showed presence of 100 homologous proteins that were present in different strains of the pathogen implying thereby that both the proteins are specific to *Neisseria meningitidis.* None of the similar proteins were observed in humans.

SignalP tool predicted signal peptides and the presence of cleavage site in the two proteins between position 22 and 23, however the S–Score (probability of position belonging to Signal Peptides) and C-Score (probability of position being the first in mature protein) for the frpB was 0.988 and 1.00 and for fbpA 0.52 and 0.92, respectively. PrediSi tool reconfirmed the results of SignalP.

Prediction of MHC Class1 Binding T-cell Epitopes

The three different analytical procedures (ProPred1, SYFPEITHI, nHLAPred) resulted in 272 peptides for the two fbpA and frpB proteins in all. The total 272 peptides when tested using POPI 2.0 server resulted in 28 high binders, 44 moderate, 34 little and 100 non-binding peptides for frpB protein (Fig. 1A).The fpbA protein however resulted in 6 high affinity, 8 little, 12 moderate ad 40 non-binding peptides (Fig.1B).

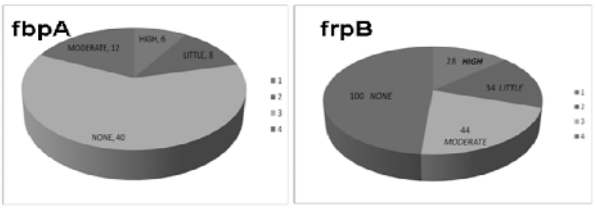

Figure 1: Pie Chart Showing Immunogenicity of Predicted Peptides of fbpA (A) and frpB (B) Proteins, Respectively as Obtained Through POPI Server Analysis.

When the binding scores of the predicted epitopes with the common HLA alleles were studied it was observed that alleles like HLA-A*0202, HLA-B*2705, HLA-B*5102 bind to the most of the peptides showing the tallest bar in the graph (Fig. 2). The rare peptide binders like HLAA* 3101, HLA-B*4403, HLA-B*51, HLA-B*3804 binds to very less peptides and some of them do not bind at all (Fig. 2). The high scoring peptides showing binding to a higher number of HLA molecules by the above tools were *s* elected for further studies.

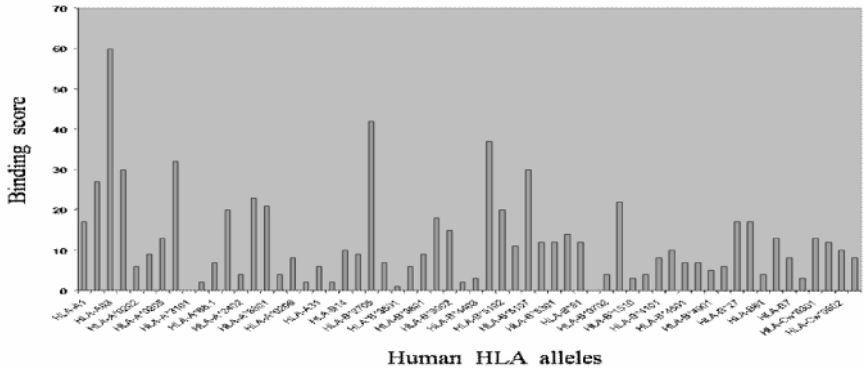

Figure 2: The Binding of the Predicted to the Common HLA Alleles like HLA-A*0205, HLA-A*68.1, HLA-B*4501, HLA-B61, HLA-B7. Alleles like HLA-A*0202, HLA-B*2705, HLA-B*5102 Bind to the Most of the Peptides (tallest bar). The Alleles, like HLA-A*3101, HLA-B*4403, HLA-B*51, HLA-B*3804 Binds to Very Few Peptides and some of them do not Bind at all.

Five nonamers ^{121}LVKVVAVQK^{130}, ^{148}KTVDAQDLL^{157}, ^{151}DAQDLLKGL^{160}, ^{60}ATDMRELLK^{69} and ^{488}HGKRGIISI^{497} were predicted for frpB by ProPred1 of which the first four showed high immunogenicity as predicted by POPI whereas nonamer ^{488}HGKRKIISI^{497} showed moderate immunogenicity for MHC 1 by POPI (Table 1).

Table 1: Prediction of MHC Class 1 and Class 2 Peptide Binders for frpB [NMB1988 (Extracellular)] Iron Acquisition Protein of *N. meningitidis.*

NMB 1988 [EXTRACELLULAR]															
ProPred1					*SYFPEITHI*					*nHLAPred*					*Net CTL*
Pos	*Peptide*	*Score*	*HLA*	*POPI*	*Pos*	*Peptide*	*Score*	*HLA 1*	*POPI*	*Pos*	*Peptide*	*Score*	*HLA*	*POPI*	*HLA super type*
121	LVKV VSVQK	360	HLA-B	High	121	LVKVVSVQK	29	HLA *03	High	121	LVKVVSVQK	4.78	HLA 68.1	High	A_3
148	KTVD AQDLL	2000	HLA-B*2705	High	148	KTVDAQDLL	27	HLA-A26	High	148	KTVDAQDLL	2.07	HLA CW*0301	High	A_3
151	DAQD LLKGL	200	HLA-B*5101	High	151	DAQDLLKGL	23	HLA-A5701	High	151	DAQDLLKGL	8.39	HLA B*60	High	A_{24}
60	ATDM RELLK	2000	HLA-A	High	60	ATDMRELLK	20	HLA-A*1101	High						A_1
488	HGKR GIISI	200	HLA-B*5102	Mod						488	HGKRGIISI	7.99	HLA-B*5703	Mod	B_{84}
629	VQKAV GSILVAG	200	HLA-DRB1*0101	High											B_{48}
630	VQKA VGSIL	360	HLA-DRB1*0101	High											B_{48}
635	GSIL VAGQK	200	HLA-DRB1*0101	High											A_3

Both SYFPEITHI and nHLAPred tools resulted in four nonamers each for frpB protein where peptides ^{488}HGKRGIISI^{497} could not be obtained with SYFPEITHI and the peptide ^{60}ATDMRELLK^{69} was absent with nHLAPred. On the other hand ProPred1 predicted all these absent peptides. Protein fbpA showed two nonamers with ProPred1 and nHLAPred tools of which 9-mer ^{136}EKDLEKSVL^{145} and ^{44}RATGIKVKL^{53} showed high and moderate immunogenicity with POPI server. With SYFPEITHI, one additional peptide ^{268}KFVAFLASK^{277} was predicted that showed high immunogenicity by POPI server (Table 2).

Prediction of MHC Class 2 Binding Epitopes

The protein sequences for the protein frpB and fbpA were taken in HTML and graphical formats and subjected to the tool ProPred. The 10 peptides *viz.* ^{121}LVKVVSVQK^{130}, ^{148}KTVDAQDLL^{157}, ^{151}DAQDLLKGL^{160}, ^{60}ATDMRELLK^{69}, ^{488}HGKRGIIS^{497}, ^{630}VQKAVGSIL^{639} from frpB and ^{136}EKDLEKSVL^{145}, ^{268}KFVAFLASK^{277}, ^{44}RATGIKVKL^{53}

Table 2: Prediction of MHC Class 1 and Class 2 Peptide Binders for fbpA [NMB0634 (Periplasmic)] the Iron Acquisition Protein of *N. meningitidis*.

NMB 0634 [PERIPLASMIC]															
ProPred1					*SYFPEITHI*					*nHLAPred*					*Net CTL*
Pos	*Peptide*	*Score*	*HLA*	*POPI*	*Pos*	*Peptide*	*Score*	*HLA 1*	*POPI*	*Pos*	*Peptide*	*Score*	*HLA*	*POPI*	*HLA super type*
136	EKDL EKSVL	1000	HLA-B*2705	High	136	EKDLEKSVL	21	HLA-B*2705	High	136	EKDLEKSVL	2.99	HLA-B*3902	High	B_{39}
44	RATG IKVKL	1000	HLA-B*5101	Mod	44	RATGIKVKL	20	HLA-A*03	Mod	44	RATGIKVKL	2.27	HLA-B*66	Mod	B_7
					268	KFVAFLASK	21	HLA-B*2705	High						A_3
192	KPYAKN SVALQAV	1100	HLA-DRB1*0101	High											A_{24}
193	PYAK NSVAL	1100	HLA-DRB1*0101	High											A_{24}

and [193]PYAKNSVAL[202] from fbpA (Table 2) were tested and confirmed with NetCTL and POPI to be overlapping, moderate or highly immunogenic and to have high binding with HLA supertypes. The POPI tool predicted the two iron acquisition proteins to have high immunogenicity for MHC class 2 binding. One 13-mer and a 12-mer peptide was observed for the fbpA and frpB proteins namely [192]KPYAKNSVALQAV[205] and [629]VQKAVGSILVAG[641], respectively. Confirmation with NetCTL suggested the overlapping nonamers [193]PYAKNSVAL[120] (Table 1 & 2) to be highly immunogenic and to have a high binding with HLA super types (A_{24}). Similarly the two overlapping nonamers of frpB protein [630]VQKAVGSIL[639] and [635]GSILVAGQK[644], respectively (Table 1) were highly immunogenic for HLA supertypes (B and A_3).

Determination of Self Peptides and Population Coverage Analysis

Analysis of the 10 selected peptides of both Class 1 and 2 against human proteome by HLAPred tool suggested the predicted potential to be completely dissimilar with human host peptides. Population coverage calculations for all the 10 peptides of our interest showed almost all the above 10 peptides (epitopes) had more than 95 percent population coverage (Fig. 3).

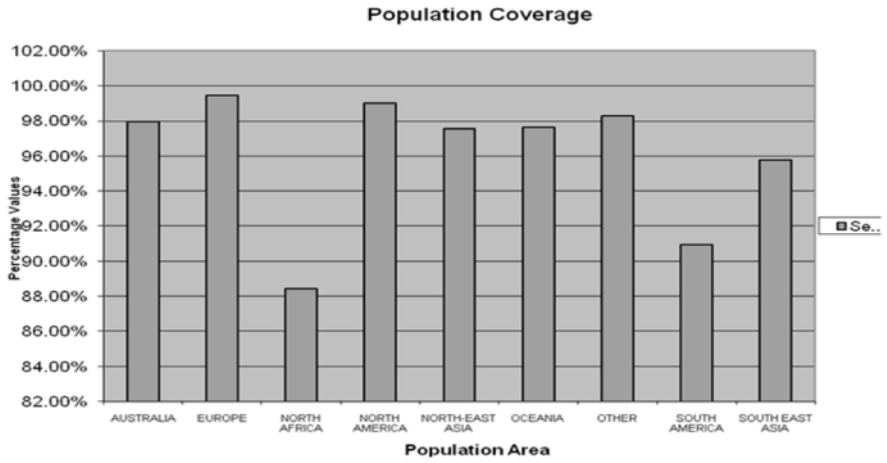

Figure 3: The Population Coverage Graph Obtained from HLA Pred Tool Showing the Selected Promiscuous Peptides that Bind to Different Human HLA Alleles with Varying Specificities as Potential Targets for Peptide Based Vaccines.

Anchor Motifs and Hydrophobicity

Table 3 shows the anchor motifs at P2 and P9 position of the nonamers as well as the hydrophobicity of selected peptides (epitopes) with the experimentally validated and corresponding MHC Class 1 allelic form (Hiller et al., [148]KTVDAQDLL[157] did not exhibit anchors at P2 for HLA-B*2705. Similarly in protein fbpA the nonamer [268]KFVAFLASK[277] did not show any anchor motifs at P2. Results suggest that although these two peptides do not have the common anchor motifs normally predicted for HLA-B*2705, however they have high hydrophobicity and are immunogenic as predicted by other tools.

In the case of the MHC Class 2 alleles (HLA–DR1) in complex, the spacing of pockets in the HLA allele is such that they interact with the peptide at P1, P4, P6, P7 and P9 and not at P2. The 12-mer peptide [629]VQKAVGSILVAG[643] present in the protein frpB (Table 3), binds with high affinity to HLA-DRB1*0101 (HLA DR1) satisfies all the requirements of close binding with the MHC allelic form and has 75.00% hydrophobicity. In the protein fbpA the peptide [192]KPYAKNSVALQAV[204] has aliphatic amino acid residue alanine at both P4 and P9 position with a hydrophobicity of 65.0% (Table 3). There is the presence of Pro residues at P2 in the peptide which might contribute towards protection of the epitope by exopeptidases.

Structure Simulation and Molecular Modeling

By using molecular dynamics we intended to simulate the immunologically relevant processes at an atomic level in detail. Modeling and simulation was carried out for all 10 peptides selected above and their interactions with MHC molecules was investigated by molecular dynamics simulation using GROMOS96 (Scott et al., 1994) and visualized by Chimera (Petersen et al., 2004). The peptide-MHC complex appeared in different conformational states. The resulting stable MHC-Peptide complexes with different epitopes when compared for peptide–specificity between moderately [488]HGKRGIISI[497] and high binding [151]DAQDLLKGL[160], Class 1 peptides with closely related MHC alleles HLA-B*5102 and HLA-B*5101 (they differ in P1 specificity but share similar motif) showed additional anchor residues (other than P2 and P9) that bind with the pocket A and F of MHC groove as visualized in Chimera (Fig. 4).

Table 3: MHC Class1 and Class 2 Peptide Motif Groups Showing Anchor Positions and Hydrophobicity in frpB [NMB1988] and fbpA [NMB0634] Protein of *N. meningitidis*.

NMB 1988 (EXTRACELLULAR)				
Peptides	*Amino terminal anchor position*	*Carboxy terminal anchor (P-9)*	*E.g. of MHC allelic form*	*% Hydrophobicity*
LVKVVSVQK	Val/Thr (P2)	Positive	HLA-A 68.1	44.4%
KTVDAQDLL	-	Positive or Hydrophobic	HLA-B* 2705	44.4%
DAQ DLLKGL	Pro/ Ala/Gly (P2)	Hydrophobic	HLA-B* 5101	44.4%
ATDMRELLK	Val/Thr (P2)	Positive	HLA-A 68.1	44.4%
HGKRGIISI (moderate)	Pro/Ala/Gly (P2)	Hydrophobic	HLA-B* 5102	55.55%
VQKAVGSILVAG	Val (P1)/Ala (P4)/ Gly (P6)/Ser (P7)	Hydrophobic	HLA-DRB1*0101	75.00%
NMB 0634 (PERIPLASMIC)				
EKDLEKSVL	-	Positive or Hydrophobic	HLA-B* 2705	22.2%
KFVAFLASK	-	Positive or Hydrophobic	HLA-B* 2705	66.6%
RATGIKVKL	Pro/ Ala/Gly (P2)	Hydrophobic	HLA-B* 5101	55.5%
KPYAKNSVALQAV	Ala (P4)	Hydrophobic	HLA-DRB1*0101	65.00%

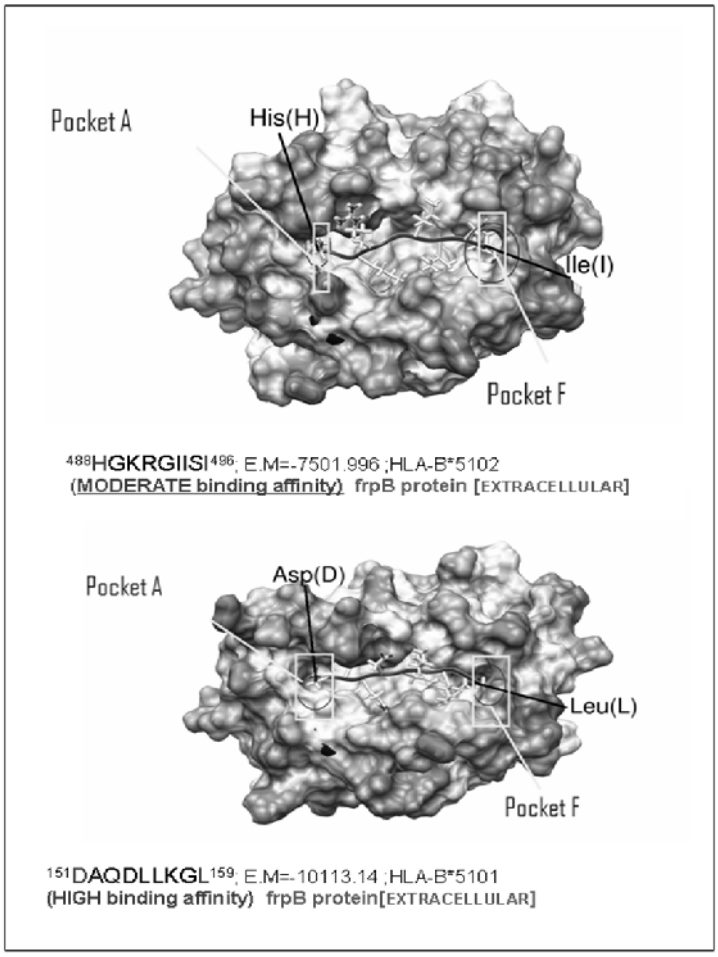

Figure 4: Simulation Modeling Showing Peptide–specificity between Moderately and High Binding Class 1 Peptides Bound to Closely Related MHC Alleles HLA-B*5102 and HLA-B*5101, Respectively, Showing Additional Anchor Residues (other than P2 and P9) that can Bind with the Pocket A and F of MHC Groove. Though the above Alleles Differ in P1 Specificity they Share Similar Motif. Template Used for MHC-Class 1 HLA-B (PDB entry 1K5N).

The binding of different Class I peptides ([268]KFVAFLASK[277]: periplasmic and extracellular) with same MHC allele HLA-B*2705 showed different confirmations where binding took place in Pocket B and F of the MHC groove. The Energy Minimization score (EM score) for the peptide-MHC complex upon binding were obtained by Swiss PDB viewer (Table 4).

Table 4: The Conformational Properties of the Selected Peptides with Efficient Binding Energy as Investigated by Molecular Dynamics Simulation Using GROMOS96 and Visualized by Swiss PDB Viewer.

NMB 1988 frpB

Peptide	*Bonds*	*Angle*	*Torsion*	*Non-bonded*	*Total KJ/mol*
LVKVVSVQK	547.409	1448.018	985.023	-4780.99	-8771.399
KTVDAQDLL	140.169	815.317	876.416	-5803.09	-4572.105
DAQDLLKGL	123.04	2899.737	844.364	-148.95	-10113.14
ATDMRELLK	866.163	1485.020	929.985	-3039.36	-7283.882
HGKRGIISI (moderate)	770.072	1918.676	954.020	-3469.70	-7501.996
VQKAVGSIL	190.479	664.080	856.152	-5610.14	-10299.63
NMB0634 fbpA					
EKDLEKSVL	161.440	802.620	872.049	-5914.77	-11608.17
KFVAFLASK	319.725	859.407	878.575	-5670.56	-11062.16
RATGIKVKL (Moderate)	707.812	1797.035	966.863	-3625.14	-7546.759
PYAKNSVAL	572.208	1665.040	873.770	-5200.44	-8217.021

It was observed that the EM scores for the binding of [MHC-[268]KFVAFLASK[277]] complex was much lower than that of [MHC-[148]KTVDAQDLL[157]] suggesting a better and strong binding of the former (Fig. 5).

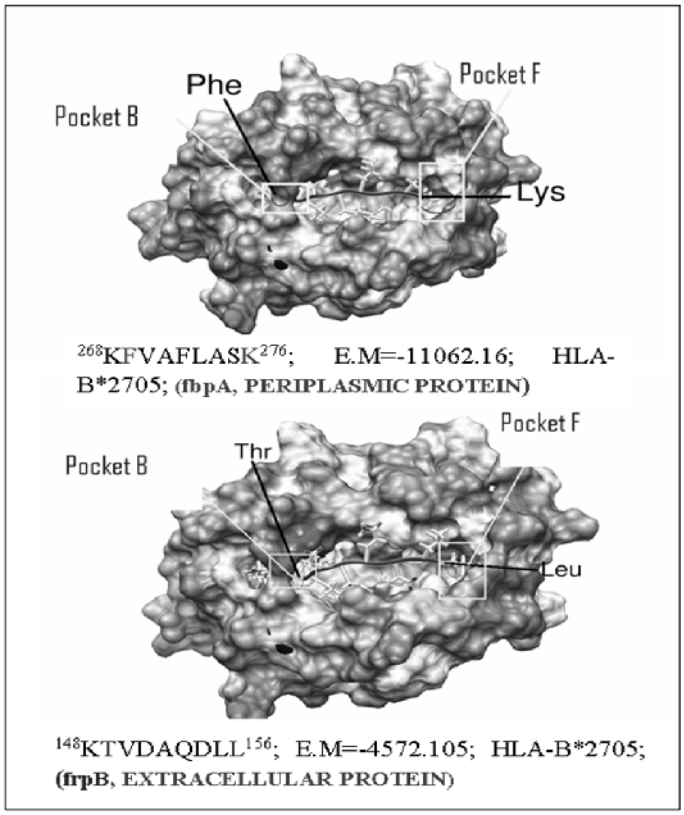

Figure 5: Different Conformations of Periplasmic and Extra Cellular Class1 Peptides when Bound to Same MHC Allele (HLA-B*2705) as Obtained by MHCsim and Visualized in Chimera. Template used for MHC-Class 1 HLA-B (PDB entry 1K5N).

MHC Class 2 epitopes ([193]PYAKNSVAL[202] and [629]VQKAVGSIL[639]) obtained from protein fbpA and frpB respectively, also showed different conformations upon binding to the same HLA allele (DR1), however the EM scores suggested the latter to have a more stable binding with MHC pockets P1, P4, P6 and P9 (Fig. 6) than the former.

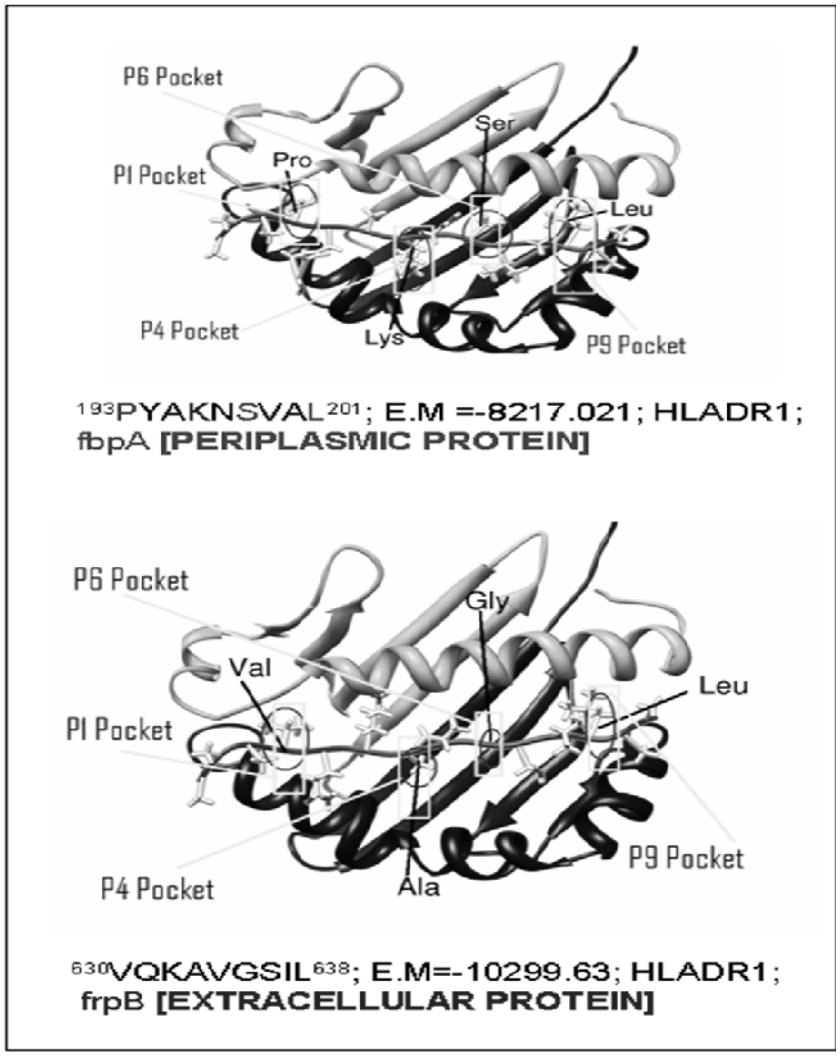

Figure 6: Conformations of MHC-peptide Complex Visualized in Chimera Showing the Binding of MHC Class II Epitopes to HLA alleles DR-1. The Anchor Positions 1, 4, 6, 9 are Shown in Red and the Pockets of MHC Alleles are Highlighted in Yellow Boxes. Template Used for MHC-Class 2 HLA-DR-1 (PDB entry 1AQD).

IV DISCUSSION

The current investigation presents *in silico* analysis of antigen presentation and mapping of epitopes on different HLA molecules so as to observe the structural interaction of MHC-peptide complex. The main focus is *N. meningitides* Serogroup B (MC58) as there is no report for an efficacious vaccine against this particular strain. The two Iron acquisition proteins frpB (NMB1988) and fbpA (NMB0634) of serogroup B were chosen to look for epitopes that could act as possible candidates for vaccine design.

BlastP resulted in several horologes for the two proteins implying thereby that the obtained horologes were specific for *Neisseria* pathogen. The subcellular localization results using integrative *in silico* approaches (PrediSi, SignalP and Sub Loc) showed the presence of signal peptides in both proteins with cleavage sites between position 22 and 23. frpB (NMB1988) was confirmed to be an extracellular protein whereas fbpA (NMB0634) had a periplasmic location. Identification of bacterial proteins and their sub-cellular localization provides valuable clues regarding its biological function. Thus, surface exposed or secreted protein and periplasmic proteins are of primary interest due to their potential as vaccine candidate and the ease with which they are accessible to drugs (Gardy and Brunkman, 2006).

The accurate prediction of peptide immunogenicity decreases several experimental efforts. In all 272 peptides were predicted from the two proteins with tools like ProPred, SYFPEITHI, nHLA Pred and tested for immunogenicity by NetCTL for cytotoxic T-lymphocytes of which only ten promiscuous peptides had high or moderate immunogenicity. The prediction of peptide immunogenicity is influenced by many factors, including intrinsic physicochemical properties and extrinsic factors such as host immunoglobulin repertoire (Saffari et al., 2008). Some of these peptides showed binding when analyzed by one algorithm but not by other. The objective of using more than one analytical tool is to select only those peptides which exhibit positive binding scores under extensive analysis by multiple methods. This would reduce the chance of failure in future experiments. Peptides which have high affinity for HLA molecules were selected because they are likely to be recognized by the TCR of specific cells.

It is advocated that a moderate binding affinity of peptide–MHC molecules is essential to induce immune responses, but the ability to induce CTL responses does not strongly correlate with their affinity for the MHC molecules (Saffari et al., 2008), hence shortlisted ten promiscuous immunogenic peptide which exhibited high affinity and are moderate or highly immunogenic (POPI server predictions) for the proteins fbpA and frpB were selected for further simulation studies.

Inclusion of peptides that bind to the rare peptide binding HLA alleles in a vaccine cocktail becomes crucial [Guo et al., 1993]. Population coverage analysis of the selected peptides showed substantial population coverage on the basis of MHC binding and/or T cell restriction data. Almost all the 10 peptides had more than 90 percent population coverage.

It has been shown that together with hydrophobicity, the charged amino acids also contribute towards the interaction with the MHC pockets (Brusic et al., 1995).The epitopes identified in this study show presence of hydrophobic and charged amino acids thus making them a good choice for inclusion in an experimental study. The specific interaction between a peptide and a MHC is mediated through anchors on the peptide side chains of amino acids at predetermined positions that protrude into complementary pockets of the Class 1 groove (Petersen et al., 2004; Parida et al., 2007).The principles of peptide interactions with both MHC Classes are rather similar. Most of the bonding forces are provided by non allele specific interactions such as the bonds between the peptides termini and the Class 1 groove, and the bonds between the peptide backbone and Class 2 (Guo et al., 1993; Stern and Wiley, 1994; Madden et al., 1993). The specificity of interactions is determined by pockets in the MHC groove that have a fixed spacing from each other, and that also have specificity for anchoring particular side chains of the peptides. Class 1 pockets are generally more specific in the amino acid residues that they bind than Class 2 pockets which compensate for their pocket degeneracy by having more of them in number (Brusic et al., 1995).In comparison to P2 and P9 anchor motifs present in MHC Class 1 peptides, peptide binding assays revealed that ligands residues at P1, P4, P6 and P9 act as anchors for the HLA-DR1, the core sequence of nonamers corresponds to the stretch of the peptide mounted in the groove, and the flanking parts of the peptide protrude out of the groove. The sequences flanking the core region appear to increase the binding affinity of peptides by non-specific interactions with the Class 1 molecule (Brusic et al., 1995; Brown et al., 1993). Although a first report on the relevance of flanking sequences to T-cell recognition indicated that they are not recognized by T cells (Malcherek et al., 1994) newer studies have indicated that they are (Brusic et al., 1995; Vignali and Strominger, 1994).Thus variations of epitopes outside the portion of the peptide mounted in the Class 2 groove can influence T cell recognition.

Our results show that besides the anchor position found at the carboxyl terminus (P-C), another anchor position at position 2 (P2) of Class 1 ligands is present. The allele HLA-DR1 absolutely requires hydrophobic residues at P1 of peptide ligands. It has a preference but not a requirements for aliphatic residues at P4 and a striking preference for small residues especially Ala or Gly at P6, whereas P7 and P9 are rather degenerate (Rammensee et al., 1999). The epitope ^{630}VQKAVGSILVAG^{642} which binds with high affinity to HLADRB1*0101 (HLA DR1) satisfies all the above conditions. The peptide ^{192}KPYAKNSVALQAV^{205} has aliphatic residue at P4 and hydrophobic at carboxyl terminus position. The high frequency of Pro residue at absolute position 2 of the Class 2 ligands is likely to reflect some processing event. Such Pro residues flanking the groove interacting with the core peptide might protect the epitope from degradation by exopeptidases some of which, like amino peptidase N, are hindered by proline (Brusic et al., 1995). In addition to the anchor residues several, if not all, of the other peptide residues can make contacts with the MHC groove but these interactions differ between different peptides. Peptide binding assays and

the study of natural ligands have both shown that within the MHC motif, certain peptide residues are either preferentially used or disfavoured which is important in fine-tuning peptide-specificity of closely related MHC molecules, and should be considered as part of the motif. For example HLAB* 5101 and HLA-B*5102 differ at only two amino acids and have very similar motifs. Again they have a different P1 preference; HLA-B*5101, has a preference for negatively charged P1 residues, whereas the non-associated molecules, HLA-B*5102, appear to disfavor negative charges at P1. A moderately ^{488}HGKRGIISI^{497} of protein frpB binds loosely (EM -7501.996 KJ/mol) with HLA-B*5102 has a positively charged residue at P1 position whereas a high immunogenic peptide ^{151}DAQDLLKGL^{160} of the same protein binds stably (EM -10113.14 KJ/mol) with HLA-B*5101 has a negatively charged residue at P1 position (Fig. 5).

When comparing the structural interactions between a periplasmic protein (^{268}KFVAFLASK^{277}), and an extra cellular protein (^{148}KTVDAQDLL^{157}), with the same MHC allele (HLA-B*2705), we observe that the P9 anchor motif is deeply embedded within the pocket of HLA-B*2705 (EM -1062.16 KJ/mol) in former whereas the P9 anchor motif is loosely attached to the pocket F of HLA allele with EM - 4572.105 KJ/mol in the latter.

V CONCLUSION

In conclusion the present study suggests that both periplasmic and extracellular iron acquisition proteins of *Neisseria* can be targeted for vaccine constructs. The energy minimization scores show a significant difference between the moderate and high affinity binders suggesting therefore that high affinity binders are better suited for wet lab studies. Class 2 epitope ^{630}VQKAVGSIL^{639} (high affinity binder from frpB) can be targeted to restrict the iron uptake of bacteria at outer membrane levels as this would lead to a decreased survival of pathogen. The molecular simulation studies show that the predicted Class 1 and 2 epitopes have different conformational bindings with same and/or different HLA alleles and are highly/ moderately immunogenic over a wide population coverage. Although both high and moderate affinity binders can be targeted for vaccine formulations in *Neisseria* they can be used only if they are able to elicit the immune response under *in vitro* tests.

VI ACKNOWLEDGEMENTS

The authors are thankful to Mr. Victor Agarwal, UK for help in English language while writing the manuscript and to the Coordinator, Bioinformatics for Computational Grid facilities at BHU. Priyanka Chaubey is grateful to BHU for providing BHU-UGC Research fellowship.

REFERENCES

[1] Adams, H.P. and Koziol, "J.A. Prediction of Binding to MHC Class-I Molecules **(**1995)." *J. Immunol. Methods*. **185**: 181-190.

[2] Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J. (1999). "Basic Local Alignment Search Tool." *J. Mol. Biol*. **215**: 403-410.

[3] Bendtsen, J.D., Nielsen, H., Heijne, G.V. and Brunak, S. (2004). "Improved Predictions of Signal Peptides: Signal P 3.0." *J. Mol. Biol.***340**: 783-795.

[4] Bergman, H.M., Westbrook, Feng, J., Z., Gilliland, G., Bhat, T. N., Weissig, H., I.Shindyalov, N. and Bourne P.E. (2000). "The Protein Data Bank." *Nucleic Acids Res*. **28**: 235-242.

[5] Brown, J.H., Jardetzky, T.S., Gorga, J.C., Stern, L.J., Urban, R.G., Strominger, J.L. and Wiley, D.C. "3-dimensional Structure of the Human Class II Histocompatibility Antigen HCA-DRI (1993)." *Nature*. **364**: 33-39.

[6] Brusic, V., Rudy, G. and Harrison, L.C. (1995). "Prediction of MHC Binding Peptides Using Artificial Neural Networks." *Complexity International*. **2 31**: 1-9. http://bic.uams.edu/mirror/ nHLApred/

[7] De Groot, A.S. and Berzofsky, J.A. (2004). "From Genome to Vaccine – new Immunoinformatics Tools for Vaccine Design." *Methods*. **34**: 425-428.

[8] De Groot, A.S., Rivera, D.S., McMurry, J.A., Buus S. and Martin, W. (2008). "Identification of Immunogenic HLA-B7 "Achilles' Heel" Epitopes within Highly Conserved Regions of HIV." *Vaccine*. **26**: 3059-3071.

[9] Ekins, A. and Schryvers, A.B. (2006). "Iron Metabolism in *Neisseria Meningitides*." In Handbook of Meningococcal Disease, (eds. M. Frosch and M.C.J. Maiden), *Wiley publications, Wiley-Vch Ve Weinheim*, pp 217-234.

[10] Gardy, J.L. and Brunkman, F.S.L. (2006). "Methods for Producing Bacterial Protein Sub Cellular Localization." *Nat. Rev. Microbiol*. **4**: 741-751. (http://www.expasy.ch/sprot/).

[11] Guo, H.C., Madden, D.R., Silver, M.L., Jardetzky, T.S., Gorga, J.C., Strominger, J.L., Wiley, D.C., "Comparison of the P2, Specificity Pocket in Three Human Histocompatibility Antigen HLA- A* 6801, HLA- A* 0201 and HLA-B *2705 (1993)." *Proc. Natl. Acad. Sci. USA* **90***:* 8053-8057.

[12] Haq, Z.U., Khan, W., Zarina S., Sattar R. and Moin S.T. (2010). "Template-based Structure Prediction and Molecular Dynamics Simulation Study of Two Mammalian Aspartyl-tRNA Synthetases." *J. Mol. Graph. Model*. **28**: 401-412.

[13] Hiller, K., De Groot, A.S., Scheer, M., Munch, R. and John, D. (2004). "Predisi: Prediction of Signal Peptides and Their Cleavage Positions." *Nucleic Acids Res*. **32**: 375-379.

[14] Larsen, M.V., Lundegaard,C., K., Lamberth, Buus, S., Lund, O. and Nielsen. "M. Large Scale Validation of Methods for Cytotoxic T-lymphocyte Epitope Prediction (2007)." *BMC Bioinformatics*. **8**: 424-431. (http://www.cbs.dtu.dk/services/ NetCTL/).

[15] Madden, D.R., Garboczi, D.N. and Wiley, D.C. "The Antigenic Identity of Peptide-MHC Complexes -a Comparison of the Conformation of Five Viral Peptides Presented by HCA- A2 (1993)." *Cell*. **75**: 690-708.

[16] Malcherek, G., Gnau, V., Stevanovic, S., Rammensee, H.G., Jung, C. and Melms, "A. Analysis of Allelic-specific Contact Sites of Natural HLA-DR17 Ligands (1994)." *J. Immunol*. **155**: 1141-1149.

[17] Omasits, U., Knapp, B., Neumann, M., Steinbauser,O., Stockinger, H., Kobler, R. and Schreiner, W. (2008). "Analysis of Key Parameters for Molecular Dynamics of pMHC Molecules." *Mol. Sim*. **3**: 781-793.

[18] Parida, R., Shaila, M.S., Mukherjee, S., Chandra, N.R. and Nayak, R. "Computational Analysis of Proteomes of H5N1 Avian Influenza Virus to Define T-cell Epitopes with Vaccine Potential (2007)." *Vaccine*. **25**: 7530-7539.

[19] Petersen, E.F., Goddard, T.D., Huang, C.C., Couch, G.S., Greenblatt, D.M., Meng, E.C. and Ferri, "T.E. UCSF Chimera: A Visualization System (2004)." *J. Comput. Chem*. **25**: 1605-1612.

[20] Rammensee, H., Bachmann, J., Emmerich, N.P., Bachor, O.A. and Stevanoic, S. (1999). "SYFPEITHI: Database for MHC Ligands and Peptides Motifs." *Immunogenetics*. **50**: 213-219.

[21] Romero, J.D. and Poolman, (1994). "J.T. Novel Surface Proteins of *Neisseria Meningitidis*." *Clin. Microbiol. Rev*. **7**: 559-574.

[22] Saffari, B., Mohabatkar, H. and Mohsenzadeh, S. (2008). "T and B-cell Epitopes Prediction of Iranian Saffron *(Crocus Sativus*) Profiling by Bioinformatics Tools." *Protein Peptide Lett*. **15**:280-285. (http://tools.immuneepitope.org/tools/population/ie.db_input)

[23] Scott,W.R.P., Hünenberger, P.P.H, Tironi, I.G., Mark, A. E., Billeter, Fennen, S.R., J., Torda, A.E., Huber, T., Krüger, P. and Gunsteren, W.F.V. "The GROMOS Biomolecular Simulation Program Package (1999)." *J. Phys. Chem. A* **103** (19): 3596-3607.

[24] Shah,K., Chaubey, P. and Mishra, N. (2010). "Bioinformatics Approach for Screening and Modeling of Putative T cell Epitopes from P or B Protein of *N. Meningitidis* as Vaccine Constructs." *Ind. J. Biotechnol*. In press.

[25] Singh, H. and Raghava, G.P.S. (2003). "Propred: Prediction of Promiscuous MHC Class – I Binding Sites." *Bioinformatics*. **19**: 1009-1014.

[26] Stern, L.J. and Wiley, D.C. Antigenic Peptides Binding by Class-I and Class-II Histocompatibility Proteins (1994)." *Structure*. **2**: 245-251.

[27] Tettelin, H., Saunders, N.J., Heidelberg, J., Jeffries, A.C., Nelson, K.E., Eisen, J.E., K.A., Ketchun, Hood, D.W., Peden, J.F., Dodson, R.J., Nelson,W.C., Gwinn, M.L., DeBoy, R., Peterson, J.B., Hickey, E.K., Haft, D.H., Salzberg, S.L., White, O. and Fleischmann, R.D. et al. (2000). "Complete Genome sequence of *Neisseria Meningitides* Serogroup B Strain MC58." *Science*. **287**: 1809-1815.

[28] Todman, S.J., Halling-Brown, M.D., Davies, M.D., Flower, D.R., Kayikci, M., and Moss D.S. (2008). "Towards the Atomistic Simulation of T-cell Epitopes: Automated Construction of MHC-Peptide Structures for Free Energy Calculations." *J. Mol. Graphics Modeling*. **26**: 957-961. (http://grid- ext. cryst. bbk.ac.uk/MHCsim/)

[29] Tung, C.W. and Ho, Shinn-Ying (2007). "POP1: Predicting Immunogenicity of MHC Class I Binding Peptides by Mining Informative Physicochemical Properties." *Bioinformatics*. **23** (8): 942-949. (http://iclab.life.nctu.edu.tw/POPI/)

[30] Tzeng, Y.L. and Stephens, D.S. (2000). Epidemiology and Pathogenesis of *Neisseria Meningitides*." *Microbes Infect*. **6**: 687-700.

[31] Vignali, D.A.A. and Strominger, J.L. "Amino Acid Residues that Flank Core Peptide Epitopes and the Extracellular Domains of CD4 Modulate Differential Signalling through the T Cell receptor (1994)." *J. Exp. Med*. **179**: 1945-1956.

[32] Vivona, S., Gardy, J.L., Ramachandran, S., Brinkman, F.S., Raghava, G.P.S., Flower, D.R. and Fillippini, F. (2007). "Computer Aided Biotechnology: From Immunoinformatics to Reverse Vaccinology Trends." *Biotechnol*. **26**: 190-200.