Computational Approach to Identify the Major Histocompatibility Complex Binding Antigenic Peptides from ‘Ascariasis’
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Abstract
The Ascaris lumbricoides cause ‘ascariasis’ in human and its elimination is the major public concern. In this current investigation, we predicted the MHC (Major Histocompatibility Complex) class I & II binding peptides with computational approach like PSSM (Position Specific Scoring Matrices) and SVM (Support Vector Machine) algorithms. We predict the peptide binders of Cytochrome b (mitochondrion) protein from Ascaris lumbricoides sequence to MHC-I molecules are as 11mer_H2_Db, 10mer_H2_Db, 9mer_H2_Db, 8mer_H2_Db. Also study integrates prediction of peptide MHC class I binding; proteasomal C terminal cleavage and TAP transport efficiency by using sequence and properties of the amino acids. We also found the binding of peptides to different alleles by using Position Specific Scoring Matrix. Cytochrome B from Ascaris lumbricoides (365 residues long) with 357 nonamers having antigenic MHC binding peptides. PSSM based server will predict the peptide binders from sequence to MHCI alleles are as I_Aa, I_Ad, I_Ag7, which are found antigenic epitopes region in Cytochrome B from Ascaris lumbricoides. This investigation can be useful in rational vaccine design and simultaneously increase the understanding the role of the immune system against antigenic.

Keywords: Ascaris; Ascaris lumbricoides; Epitopes; Antigenic peptides; MHC-Binders; TapPred; PSSM; SVM; Nonamers; Cytochrome b (mitochondrion)

Abbreviations: STH: Soil Transmitted Helminthes; SAC: School Age Children; MDA: Mass Drug Administration; MHC I: Major Histocompatibility Complex Class I; MHC II: Major Histocompatibility Complex Class II; PSSM: Position Specific Scoring Matrices; SVM: Support Vector Machine; UniProt: The Universal Protein Resource; NCBI: National Center for Biotechnology Information; TAP: Transporter Associated with Antigen Processing

Introduction
“Ascariasis” infection caused in humans and other mammals via intestinal round worms (A. lumbricoides) of genus ‘Ascaris’ with incubation period of 10-24 months in the jejunum and middle ileum of the human intestine. The adult worm are in size varies up to 15-35 cm long and appeared in color white or yellow. Female worm produces 240,000 eggs per day. The fertilized eggs turn infectious within 5-10 days when it expelled into favorable soil [1] and stay viable for up to 17 months. The route of the disease propagation is majorly from the worm contaminated food hand and soil, through ingestion of contaminated food and the opportunistic parasite get success in hatching of eggs in the small intestine. This worm infectious occurrence are also observed as a zoonotic infection in pigs and use of hog manure [2] but, in endemic areas, the transmission of this infection is basically from person to person [3]. The diseases symptoms experienced by the individuals are pneumonitis include wheezing, dyspnea, nonproductive cough, hemoptysis, and fever and eosinophilia. The larvae mature in jejunum in approximately 65 days which ultimately feeds on host (human) digestion (Figure 1). The infected children’s with nutritional conditional like malnourished diet may found to be susceptible to protein, caloric, or suffer from Vitamin A deficiency, which ultimately interfere in their normal growth and causes growth [4] and the relative immunodeficiency also been observed in overexposed situation [5]. In the minimal load this worm expression remain asymptomatic whereas in the overload situation it creates severe complications. The predominant occurrence of this disease found in the warm and moist climatic geographical regions and also in those tropical and subtropical regions where the poor sanitation and hygiene are practice. In the worldwide the estimated preponderance of this disease outbreak is recorded is 25% (0.8-1.22 billion people) [6]. Children’s are supposed to be the majorly prone to this disease of tropical and arising countries [7-8]. The differential diagnoses includes are acute pancreatitis, biliary colic and community-acquired pneumonia. The recent advanced strategy for STH infection control is preventive chemotherapy with combination of albendazole or mebendazole [9]. In another study, conducted by researcher in Chencha district, where the mass drug administration (MDA) therapy is implied to most risky population and SAC as the central strategy to control soil-transmitted helminthes (STH) infection [10]. However, Identification of antigenic peptide that binds to MHC [Major Histocompatibility Complex] molecule improves the understanding of specificity of immune responses and is important for discovery of vaccines. MHC molecules are cell surface proteins that found in all vertebrate animals and are play an important role in the immune system.

MHC class I antigen
In the process of the antigen-presentation in the proteasome, antigenic protein is cleaved into oligopeptides [11-13]. The cleaved antigenic peptide fragment were brought through TAP protein into the endoplasmic reticulum (ER) and then these peptides combines with MHC class I molecule and forms a complex [14-18]. The resultant complexes were translocated towards the surface of the antigen presenting cell, which are thereafter recognized and identified by T-cell
and enable immune system to elicit an immune response [19-23]. Henceforth, TAP binding peptides prediction is very important for identification of the MHC class-I restricted T cell epitopes.

Proteasomal degradation

The process of antigen presentation the proteasomal degradation is important step to regulate and maintain the equilibrium among intracellular proteins [24]. Through the action of the proteasine inside the antigenic protein are cleaved into oligopeptides [25] and then after these oligopeptides binds to the TAP, which later on transports peptides into the ER.

Transport of peptide by TAP

TAP is heterodimeric transmembrane protein, and belongs from ABC transporter family. This transports antigenic peptide to ER [26]. Study suggest that most of the MHC binding peptides are unable to diffuse across membrane, but TAP able to carry them inside the ER where it binds to MHC class I molecules. These MHC-peptide complexes will be translocate on the surface of antigen presenting cells [21] and are recognized by T-cell receptors to elicit an immune response.

MHC class II antigen

The prediction of peptides binding to a MHC class II molecule is difficult due to different side chains and longer length in the extracellular antigen presentation [27-29]. In the MHC class II antigen presentation process, antigenic protein is ingested by antigen-presenting cells via endocytosis or phagocytosis process, and the after cleaved by cathepsins (a class of protease) into oligopeptides in the endosomes, than are fuse with lysosomes containing MHC class II molecules [30] and present them at the cell surface for recognition by T cells [31-39]. Where T helper cells trigger an immune response by inflammation and swelling due to phagocytes or may lead to an antibody-mediated immune response via B-cell activation. Since MHCs have a key role in enabling immune system to elicit an immune response [19-23], henceforth, TAP binding peptides prediction is very important for identification of the MHC class-I restricted T cell epitopes.

Antigenicity identification of protein

The identification of the antigenicity of the Cytochrome b (mitochondron) protein are predicted through utilizing the different types of the computational tools, methods and relevant scales like Hopp and Woods, Welling, Parker, Bepipred, Kolaskar and Tongaonkar antigenicity methods [48-59] and identified the antigenic peptide which are capable to eliciting an antibody response.

MHC binding peptide prediction

The earlier sequence patterns method for prediction of MHC molecules have proven to be too elementary and also the complexity of the binding motif cannot be precisely interpreted by the few residues present in the pattern [60]. MHC binding peptide is predicted using neural networks trained on C terminals of known epitopes. By using RANKPEP, we predict peptide binders to MHC1 molecules from protein sequences or sequence alignments using Position Specific Scoring Matrices (PSSMs) whose C terminal end is likely to be the result of proteosomal cleavage [61-64].

Antigenic peptides prediction by cascade SVM based TAP pred method

By using TAPPred we predict TAP binders on the basis of sequence and the properties of amino acids [65]. We found the MHC binding regions, the binding affinity of protein from Ascaris lumbricoides having 365 amino acids long residues with 357 nonamers.

Results

Retrival of the protein sequence from database

The Cytochrome b protein from Ascaris lumbricoides [gi|319656158|gb|ADV58572.1|], contain a long residue of 365 amino acids having 365 amino acids long residues with 357 nonamers.

Prediction of antigenic peptides

In antigenic prediction, we focused on the area of greatest local Hydrophilicity through antigenic determinants. In Hopp-Woods scale

![Figure 1: Lifecycle of the Ascaris lumbricoides.](image-url)
Hydrophilicity Prediction method, result data found high in Position: 197 with Score: 0.956 (max) 194-TRSTSK-200 in a protein, assuming the probability of antigenic determinants would be exposed on the surface of the protein and hence, there might be chances to be located in hydrophilic regions (Figure 2). Welling et 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: 189 Score: 0.841 (max) 186-LLHLVF-192 (Figure 3). We also study Hydrophilicity plot of HPLC/Parker Hydrophilicity Prediction Result Data found in Position: 197 (Residue: S) i.e., 194-ETRSTSK-200 with highest score: 5.871 (Figure 4), BepiPred predicts the location of linear B-cell epitopes Result found in Position: 197 (Residue: S) with highest score: 1.023 (193-LVFLHETR-200) (Figure 5), Kolaskar and Tongaonkar antigenicity methods (Figures 6 and 7), Table 1). Predicted peptides result found 4-DFVNSMVVSLPSSKVLT-20, 28-MLGMVLGFQILTFLAFY-46, 52-ALAFLSVQYMY-63, 69-WIFRVSH-75, 80-SMFFIFLYLHLFK-92, 100-

![Figure 2: Hydrophobicity plot (result data found high in position: 197 with score: 0.956 (max) 194-TRSTSK-200) [49].](image)

![Figure 3: Hydrophobicity plot (result found highest in position: 189 Score: 0.841 (max) 186-LLHLVF FL-192) [50].](image)

Table 1: The 11 antigenic determinants of cytochrome b (mitochondrion) protein.

<table>
<thead>
<tr>
<th>n</th>
<th>Strat</th>
<th>Sequence</th>
<th>End Position</th>
</tr>
</thead>
<tbody>
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<td>1</td>
<td>4</td>
<td>DFVNSMVVSLPSSKVLT</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>28</td>
<td>MLGMVLGFQILTFLAFY</td>
<td>46</td>
</tr>
<tr>
<td>3</td>
<td>52</td>
<td>ALAFLSVQYMY</td>
<td>63</td>
</tr>
<tr>
<td>4</td>
<td>69</td>
<td>WIFRVSH</td>
<td>75</td>
</tr>
<tr>
<td>5</td>
<td>80</td>
<td>SMFFIFLYLHLFK</td>
<td>92</td>
</tr>
<tr>
<td>6</td>
<td>100</td>
<td>RLKAWSGIVLVM</td>
<td>116</td>
</tr>
<tr>
<td>7</td>
<td>120</td>
<td>FMGYVLWVA</td>
<td>128</td>
</tr>
<tr>
<td>8</td>
<td>132</td>
<td>FWAVSVITSSLSSVWPWGAIATCVWSTQFTVSSATKXFFVLHFLPVGLVFLHLVLVFH</td>
<td>193</td>
</tr>
<tr>
<td>9</td>
<td>199</td>
<td>SKSYCHGHYDKVCFSPFYWKFLNFVWIFVIFLSFLGFPL</td>
<td>241</td>
</tr>
<tr>
<td>10</td>
<td>253</td>
<td>NNSPVHIVPEYWFLFAYAILRAIPNCGLGVSLSLAFLVLFVLVNPYVSM</td>
<td>306</td>
</tr>
<tr>
<td>11</td>
<td>308</td>
<td>LMKLFVVFIFVFLYSLWGLGQLVEDDPVFLSMVFSFLYYFFVFLFLVYYVFG</td>
<td>361</td>
</tr>
</tbody>
</table>

There are 11 antigenic determinants in your sequences.
RLKKAWSGIVLILVM-116,120-FMGYLVWA-128,132-FWASV
VITSLLSVIPVWGFATCTIWSGFTVSSATLKFHFLVFWGLL
FLVLLHVFLH-193,199-SKSYCHGHYDVFSPEYWVKEFNLV
VVWFVFIFFSLGFPELL-241,253-MMPYHVPEWYFLAYLRA-
IPNKVLSFLASILVVFVLFVNNYSVMS-306,308-LNKFL
VFVFFFLVVLWLGQCLVEDPFVLSMVFSLYFFVIFLLFLYY
YFVG-361 and there might be chances that the predicted antigenic
fragments can bind to MHC molecule and it can be the first chokepoints
in synthetic vaccine design.

**MHC binding peptide prediction**

We found the binding of peptides to a number of different alleles
using Position Specific Scoring Matrix. MHC molecules are cell surface
proteins, which actively participate in host immune responses to almost

**Figure 4:** Hydrophobicity plot of HPLC (result data found in position: 197 (Residue: S) i.e., 194-ETRSTSK-200 with highest score: 5.871) [51].

**Figure 5:** Bepipred linear epitope prediction plot (result found in position: 197 (Residue: S) with highest score: 1.023 and sequence is 193-LVFLHETR-200).

**Figure 6:** Antigenicity plot [53].
all antigens. We have been able to predict MHC-I peptide binders of cytochrome b (mitochondrion) from *Ascaris lumbricoides*. We found predicted MHC-I peptide binders of protein for Matrix: 8mer_H2_Db.p.mtx, Consensus: QNWNCCTI, Optimal Score: 52.494, Binding Threshold: 33.04; Matrix: 9mer_H2_Db.p.mtx, Consensus: FCHNCDYMY, Optimal Score: 50.365, Binding Threshold: 17.96; Matrix: 10mer_H2_Db.p.mtx, Consensus: SGYYNFFWCL, Optimal Score: 58.858, Binding Threshold: 41.32; Matrix: 11mer_H2_Db.p.mtx, Consensus: CGVYNFYYCCY, Optimal Score: 79.495, Binding Threshold: 56.96 (Table 2) and MHC-II peptide binders for Matrix: I_Ab.p.mtx, Consensus: YYAPWCNNA, Optimal Score: 35.632, Binding Threshold: 9.52; Matrix: I_Ad.p.mtx, Consensus: QMVHAAHAE, Optimal Score: 53.145, Binding Threshold: 7.10; Matrix: I_Ag7.p.mtx, Consensus: WYAHAFKYV, Optimal Score: 40.873, Binding Threshold: 7.54 for MHC II allele (Table 3) was tested and opted the result.

Antigenic peptides prediction by cascade SVM based TAP pred method

We also use a cascade SVM based TAPPred method which found 105 High affinity TAP Transporter (Transporter peptide regions) (Tables 3 and 4) peptide regions which represents predicted TAP binders residues which occur at N and C termini from *Ascaris lumbricoides* antigen cytochrome c oxidase subunit I (mitochondrion). The important transporter that transports antigenic peptides from cytosol to ER is TAP. It binds and translocate the selective antigenic peptides from cytosol to ER for MHC molecules specific binding. By using the approach of jackknife validation test the correlation coefficient of 0.88 was achieved.

Conclusion

The antigenic proteins cytochrome b from *Ascaris lumbricoides* involved in different multiple antigenic components to direct and

<table>
<thead>
<tr>
<th>MHC-I Allele</th>
<th>Rank</th>
<th>POS.</th>
<th>N</th>
<th>Sequence</th>
<th>C</th>
<th>MW (Da)</th>
<th>SCORE</th>
<th>% OPT.</th>
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</thead>
<tbody>
<tr>
<td>8mer_H2_Db</td>
<td>1</td>
<td>155</td>
<td>IVT</td>
<td>CIWSGFTV</td>
<td>SSA</td>
<td>22.483</td>
<td>42.63%</td>
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<tr>
<td>8mer_H2_Db</td>
<td>2</td>
<td>146</td>
<td>SVI</td>
<td>PVWGAIV</td>
<td>TCI</td>
<td>14.929</td>
<td>28.44%</td>
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<tr>
<td>8mer_H2_Db</td>
<td>3</td>
<td>177</td>
<td>HFL</td>
<td>VPWGLFL</td>
<td>VLL</td>
<td>11.733</td>
<td>22.35%</td>
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<tr>
<td>9mer_H2_Db</td>
<td>4</td>
<td>165</td>
<td>VSS</td>
<td>ATLFFHYV</td>
<td>LHF</td>
<td>11.706</td>
<td>22.30%</td>
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<tr>
<td>8mer_H2_Db</td>
<td>5</td>
<td>224</td>
<td>FLN</td>
<td>VVVWFVI</td>
<td>FFS</td>
<td>11.667</td>
<td>22.23%</td>
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<tr>
<td>9mer_H2_Db</td>
<td>1</td>
<td>73</td>
<td>IFR</td>
<td>VSHFNGASM</td>
<td>FFI</td>
<td>21.024</td>
<td>41.74%</td>
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<tr>
<td>9mer_H2_Db</td>
<td>2</td>
<td>20</td>
<td>KVL</td>
<td>TYGWNFGSM</td>
<td>LGM</td>
<td>15.731</td>
<td>31.23%</td>
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<tr>
<td>9mer_H2_Db</td>
<td>3</td>
<td>296</td>
<td>VVF</td>
<td>VLNNYYSV</td>
<td>MSK</td>
<td>15.156</td>
<td>30.09%</td>
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<tr>
<td>9mer_H2_Db</td>
<td>4</td>
<td>233</td>
<td>FIF</td>
<td>FSFGFFLL</td>
<td>GDP</td>
<td>13.192</td>
<td>26.19%</td>
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<td>9mer_H2_Db</td>
<td>5</td>
<td>202</td>
<td>SKS</td>
<td>YCHGYDKV</td>
<td>CFS</td>
<td>12.345</td>
<td>24.51%</td>
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<tr>
<td>10mer_H2_Db</td>
<td>1</td>
<td>77</td>
<td>SHF</td>
<td>NGASMFIFIL</td>
<td>YLH</td>
<td>16.914</td>
<td>28.74%</td>
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<tr>
<td>10mer_H2_Db</td>
<td>2</td>
<td>299</td>
<td>VLV</td>
<td>NNYSVMSKL</td>
<td>NKF</td>
<td>10.744</td>
<td>18.25%</td>
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<td>10mer_H2_Db</td>
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<td>296</td>
<td>VVF</td>
<td>VLNNYYSVM</td>
<td>SKL</td>
<td>10.308</td>
<td>17.51%</td>
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<tr>
<td>10mer_H2_Db</td>
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<td>20</td>
<td>KVL</td>
<td>TYGWNFGSM</td>
<td>GMV</td>
<td>8.093</td>
<td>13.75%</td>
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<td>10mer_H2_Db</td>
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<td>73</td>
<td>IFR</td>
<td>VSHFNGASM</td>
<td>FIF</td>
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<tr>
<td>11mer_H2_Db</td>
<td>1</td>
<td>320</td>
<td>IFV</td>
<td>LVLSWLQGCL</td>
<td>VED</td>
<td>13.952</td>
<td>17.10%</td>
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<tr>
<td>11mer_H2_Db</td>
<td>2</td>
<td>115</td>
<td>LLL</td>
<td>VMMEAFLGYVL</td>
<td>VWA</td>
<td>13.422</td>
<td>16.86%</td>
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<td>LVV</td>
<td>FVLANVYVSM</td>
<td>SKL</td>
<td>12.708</td>
<td>15.88%</td>
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<tr>
<td>11mer_H2_Db</td>
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<td>20</td>
<td>KVL</td>
<td>TYGWNFGSM</td>
<td>MVL</td>
<td>12.091</td>
<td>15.21%</td>
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</tr>
<tr>
<td>11mer_H2_Db</td>
<td>5</td>
<td>339</td>
<td>VFL</td>
<td>SMVFSFLLYFV</td>
<td>IFL</td>
<td>11.309</td>
<td>14.23%</td>
<td></td>
</tr>
</tbody>
</table>

*The RANKPEP consists of a list of selected peptides binding potential (score) to the MHC molecule from the query given at a selected threshold. Peptides shown here contain a C-terminal residue that is predicted to be the result of proteasomal cleavage and also focus on the prediction of conserved epitopes that help to avoid immune evasion resulting from mutation. Proteasomal cleavage options are only applied to the prediction of MHCI-restricted peptides.

Table 2: Promiscuous MHC ligands, having C-terminal ends are proteosomal cleavage sites of *Ascaris lumbricoides*.
Useful in cellular immunology, vaccine design, immunodiagnostics, identification of T-cell epitopes. In future this methodology will be used to design synthetic peptide vaccine.


empower the immune system to protect the host from the antigenic peptide. Major histocompatibility complexes (MHC-I and MHC-II) display specificity to bind with their respective epitopes. MHC class molecules are cell surface proteins that take active part in host immune reactions to response almost all antigens.

Table 3: Prediction of MHCII ligands all rows predicted binders to the MHC-II Allele i.e., MHC-II I_Ab, MHC-II I_Ad, MHC-II I_Ag7.

<table>
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<th>Peptide Rank</th>
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<th>Predicted Affinity</th>
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<td>201</td>
<td>SYCHGHYDK</td>
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<tr>
<td>2</td>
<td>144</td>
<td>VIPVVVGFAI</td>
<td>8.64</td>
<td>High</td>
</tr>
<tr>
<td>3</td>
<td>319</td>
<td>VLVLSVVLG</td>
<td>8.64</td>
<td>High</td>
</tr>
<tr>
<td>4</td>
<td>301</td>
<td>YSVMSDKLN</td>
<td>8.624</td>
<td>High</td>
</tr>
<tr>
<td>5</td>
<td>160</td>
<td>FTVSSATLK</td>
<td>8.628</td>
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</tr>
<tr>
<td>6</td>
<td>56</td>
<td>LQOYIMYME</td>
<td>8.627</td>
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</tr>
<tr>
<td>7</td>
<td>127</td>
<td>WAGQMSFWAS</td>
<td>8.625</td>
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<td>8</td>
<td>41</td>
<td>TFLAFYYSN</td>
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</tr>
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<td>9</td>
<td>213</td>
<td>PSEYWKVDF</td>
<td>8.624</td>
<td>High</td>
</tr>
<tr>
<td>10</td>
<td>217</td>
<td>VWKDFNLNV</td>
<td>8.624</td>
<td>High</td>
</tr>
</tbody>
</table>

Table 4: Cascade SVM based high affinity TAP binders.

<table>
<thead>
<tr>
<th>MHC-II Allele</th>
<th>Rank</th>
<th>POS.</th>
<th>N</th>
<th>SEQUENCE</th>
<th>C</th>
<th>MW (Da)</th>
<th>SCORE</th>
<th>% OPT.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHC-II I_Ab</td>
<td>1</td>
<td>150</td>
<td>VVG</td>
<td>FAIVTCIWS</td>
<td>GFT</td>
<td>998.24</td>
<td>18.998</td>
<td>53.32%</td>
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<td>MHC-II I_Ab</td>
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<td>123</td>
<td>FMG</td>
<td>YVLWVAGQS</td>
<td>FWA</td>
<td>1055.29</td>
<td>14.317</td>
<td>40.18%</td>
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<tr>
<td>MHC-II I_Ab</td>
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<td>46</td>
<td>LAF</td>
<td>YSSNDGALA</td>
<td>FLS</td>
<td>955.00</td>
<td>13.721</td>
<td>38.51%</td>
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<td>MHC-II I_Ab</td>
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<td>132</td>
<td>QMS</td>
<td>FWSVVITIS</td>
<td>LLS</td>
<td>968.15</td>
<td>12.184</td>
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<td>MHC-II I_Ab</td>
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<td>VLH</td>
<td>FLVPWGLFL</td>
<td>LVL</td>
<td>1050.35</td>
<td>11.623</td>
<td>32.62%</td>
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<tr>
<td>MHC-II I_Ad</td>
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<td>LFA</td>
<td>YAILRAIPN</td>
<td>KVL</td>
<td>1012.23</td>
<td>17.555</td>
<td>33.03%</td>
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<td>MHC-II I_Ad</td>
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<td>158</td>
<td>CIW</td>
<td>SGFTVSSAT</td>
<td>LFK</td>
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<td>69</td>
<td>NFG</td>
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<td>GAS</td>
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<tr>
<td>MHC-II I_Ad</td>
<td>4</td>
<td>287</td>
<td>YFL</td>
<td>FAYAILRAI</td>
<td>PKN</td>
<td>1019.27</td>
<td>11.974</td>
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<tr>
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<td>5</td>
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<td>NKV</td>
<td>LGVSVSLFAS</td>
<td>ILV</td>
<td>874.05</td>
<td>10.238</td>
<td>19.26%</td>
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<td>FIF</td>
<td>LYLHLFKGM</td>
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<td>VMMEAFLGY</td>
<td>VLV</td>
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<td>AILRAIPNK</td>
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<td>GMVGLFOQI</td>
<td>TGT</td>
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<td>YWVKDFNLNV</td>
<td>VVW</td>
<td>1142.35</td>
<td>9.304</td>
<td>22.76%</td>
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References


