# Immunoproteomics approach for development of MHC binders and fragment based peptide vaccines from *Tetrahymena pyriformis*

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**Abstract**- *Tetrahymena pyriformis* are free-living ciliate protozoa that Guppies are easily susceptible to Tetrahymena. Peptide fragments of antigen protein can be used to select nonamers for use in rational vaccine design and to increase the understanding of roles of the immune system in parasitic diseases. Analysis shows MHC class II binding peptides of antigen protein from *Tetrahymena pyriformis* are important determinant for protection of host form parasitic infection. In this assay, we used PSSM and SVM algorithms for antigen design and predicted the binding affinity of antigen protein having 389 amino acids, which shows 381 nonamers. Binding ability prediction of antigen peptides to major histocompatibility complex (MHC) class I & II molecules is important in vaccine development from *Tetrahymena pyriformis*.
***Keywords****- antigen protein, epitope, PSSM, SVM, MHC, peptide vaccine*

*Abbreviations: Goldman, Engelberg and Steitz, (GES); major histocompatibility complex, (MHC); Position Specific Scoring Matrices, (PSSMs); Support Vector Machine, (SVM)*

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## I. Introduction

*Tetrahymena pyriformis* are free-living ciliate protozoa that can also switch from commensalistic to pathogenic modes of survival. They are common in fresh-water. Guppies do succumb to the disease quite easily and it can cause big problems where guppies are produced commercially. [1, 2]. *Tetrahymena pyriformis* bacterial peptides are most suitable for subunit vaccine development because with single epitope, the immune response can be generated in large population. This approach is based on the phenomenon of cross-protection, whereby a host infected with a mild strain of pathogen is protected against a more severe strain of the same pathogen. The phenotype of the resistant transgenic hosts includes fewer centers of initial pathogenic infection, a delay in symptom development, and low pathogenic accumulation. antigen protein from *Tetrahymena pyriformis* is necessary for new paradigm of synthetic vaccine development and target validation [3-5].

## II. Methodology

In this research work antigenic epitopes of antigen protein from *Tetrahymena pyriformis* is determined using the Gomase in 2007, Hopp and Woods, Welling, Parker and Protrusion Index (Thornton) antigenicity [6-8]. The major histocompatibility complex (MHC) peptide binding of antigen protein is predicted using neural networks trained on C terminals of known epitopes. In analysis predicted MHC/peptide binding of antigen protein is a log-transformed value related to the IC50 values in nM units. RANKPEP predicts peptide binders to MHCI and MHCII molecules from protein sequences or sequence alignments using Position Specific Scoring Matrices (PSSMs). Support Vector Machine (SVM) based method for prediction of promiscuous MHC class II binding peptides. SVM has been trained on the binary input of single amino acid sequence [9-14]. In addition, we predict those MHC ligands from whose C-terminal end is likely to be the result of proteosomal cleavage [15].

## III. Results and Interpretations

We found binding of peptides to a number of different alleles using Position Specific Scoring Matrix. A antigen protein sequence is 389 residues long, having antigenic MHC binding peptides. MHC molecules are cell surface glycoproteins, which take active part in host immune reactions and involvement of MHC class-I and MHC II in response to almost all antigens. PSSM based

server predict the peptide binders to MHCI molecules of antigen protein sequence are as 11mer_H2_Db, 10mer_H2_Db, 9mer_H2_Db, 8mer_H2_Db and also peptide binders to MHCII molecules of antigen protein sequence as I_Ab.p, I_Ad.p, analysis found antigenic epitopes region in putative antigen protein (Table 1). We also found the SVM based MHCII-IAb peptide regions; MHCII-IAd peptide regions; MHCII-IAg7 peptide regions and MHCII- RT1.B peptide regions, which represented predicted binders from parasitic antigen protein (Table 2). The predicted binding affinity is normalized by the 1% fractil. We describe an improved method for predicting linear epitopes (Table 2). The region of maximal hydrophilicity is likely to be an antigenic site, having hydrophobic characteristics, because terminal regions of antigen protein is solvent accessible and unstructured, antibodies against those regions are also likely to recognize the native protein (Fig. 1, 2, 5). It was shown that a antigen protein is hydrophobic in nature and contains segments of low complexity and high-predicted flexibility (Fig. 3, 4). Predicted antigenic fragments can bind to MHC molecule is the first bottlenecks in vaccine design.

## IV. Conclusion

An antigen protein from *Tetrahymena pyriformis* peptide nonamers are from a set of aligned peptides known to bind to a given MHC molecule as the predictor of MHC-peptide binding. MHCII molecules bind peptides in similar yet different modes and alignments of MHCII-ligands were obtained to be consistent with the binding mode of the peptides to their MHC class, this means the increase in affinity of MHC binding peptides may result in enhancement of immunogenicity of parasitic antigen protein. These predicted of bacterial protein antigenic peptides to MHC class molecules are important in vaccine development from Tetrahymena pyriformis.

## V. References


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*Table 1- PSSM based prediction of MHC ligands, from whose C-terminal ends are proteosomal cleavage sites*

| MHC-I | POS. | N | Sequence | C | MW (Da) | Score | % OPT. |
|---|---|---|---|---|---|---|---|
| 8mer_H2_Db | 62 | DGF | SQWDYLFY | TIP | 1080.21 | 23.964 | 45.65 % |
| 8mer_H2_Db | 308 | NCL | EGANCVTI | IEA | 787.88 | 23.827 | 45.39 % |
| 8mer_H2_Db | 368 | IVD | ISSNPFII | NKD | 872.04 | 18.998 | 36.19 % |
| 8mer_H2_Db | 73 | TIP | GIINFQMY | GIP | 967.15 | 17.925 | 34.15 % |
| 8mer_H2_Db | 54 | KYV | QHWNGDGF | SQW | 918.95 | 14.902 | 28.39 % |
| 8mer_H2_Db | 216 | DFY | IPQGGVFY | DFY | 862.0 | 14.787 | 28.17 % |
| 8mer_H2_Db | 286 | NLY | ASGSILTI | NDY | 742.87 | 12.549 | 23.91 % |
| 8mer_H2_Db | 322 | QGK | FENNSFSI | SLK | 939.0 | 10.901 | 20.77 % |
| 9mer_H2_Db | 53 | GKY | VQHWNGDGF | SQW | 1018.08 | 21.592 | 42.87 % |
| 9mer_H2_Db | 294 | LTI | NDYNNDENI | IRN | 1092.04 | 21.316 | 42.32 % |
| 9mer_H2_Db | 307 | RNC | LEGANCVTI | IEA | 901.04 | 18.755 | 37.24 % |
| 9mer_H2_Db | 32 | AAK | LMGRNLTFI | LSR | 1046.29 | 18.451 | 36.63 % |
| 9mer_H2_Db | 268 | LRS | RFLDNHFTL | YIA | 1144.3 | 15.756 | 31.28 % |
| 9mer_H2_Db | 2 | M | LHIPNYNLT | QYD | 1066.22 | 15.734 | 31.24 % |
| 9mer_H2_Db | 173 | PLM | WSFPNDDEA | LNY | 1039.07 | 14.989 | 29.76 % |
| 9mer_H2_Db | 12 | LTQ | YDMHNLNGF | GES | 1092.19 | 13.152 | 26.11 % |
| 10mer_H2_Db | 72 | YTI | PGIINFQMYG | IPF | 1121.32 | 21.156 | 35.94 % |
| 10mer_H2_Db | 353 | VII | AGISTFGNYI | SKI | 1024.14 | 19.66 | 33.40 % |
| 10mer_H2_Db | 356 | AGI | STFGNYISKI | VDI | 1111.26 | 18.805 | 31.95 % |
| 10mer_H2_Db | 210 | TNT | TGLDFYIPQG | GVF | 1092.22 | 13.971 | 23.74 % |
| 10mer_H2_Db | 294 | LTI | NDYNNDENII | RNC | 1205.2 | 13.88 | 23.58 % |
| 10mer_H2_Db | 200 | APV | VAQGNEVTNT | TGL | 1014.04 | 13.396 | 22.76 % |
| 10mer_H2_Db | 125 | SIS | QEPYAFPDYH | YVL | 1248.34 | 13.331 | 22.65 % |
| 10mer_H2_Db | 62 | DGF | SQWDYLFYTI | PGI | 1294.47 | 11.829 | 20.10 % |
| 11mer_H2_Db | 72 | YTI | PGIINFQMYGI | PFV | 1234.48 | 34.146 | 42.95 % |
| 11mer_H2_Db | 141 | SSS | IKTLNVRYQLL | KFY | 1342.64 | 20.815 | 26.18 % |
| 11mer_H2_Db | 372 | SSN | PFIINKDKQQY | DIN | 1375.59 | 16.13 | 20.29 % |
| 11mer_H2_Db | 90 | DDI | CGLNGNATPEL | CAR | 1070.18 | 12.017 | 15.12 % |
| 11mer_H2_Db | 179 | PND | DEALNYEAEFM | LGD | 1313.42 | 11.857 | 14.92 % |
| 11mer_H2_Db | 293 | ILT | INDYNNDENII | RNC | 1318.36 | 11.253 | 14.16 % |
| 11mer_H2_Db | 355 | IAG | ISTFGNYISKI | VDI | 1224.42 | 10.852 | 13.65 % |
| 11mer_H2_Db | 148 | NVR | YQLLKFYYHLF | VKE | 1516.82 | 9.687 | 12.19 % |

*Table 2- SVM based prediction of promiscuous MHC class II binding peptides from antigen protein*

| ALLELE | Sequence | Residue No | Peptide Score |
|---|---|---|---|
| I-Ab | GTIFRPLMW | 165 | 1.104 |
| I-Ab | ISLKNTQEQ | 329 | 0.870 |
| I-Ab | AKLMGRNLT | 30 | 0.861 |
| I-Ab | RNLTFILSR | 35 | 0.844 |
| I-Ad | KEQGSGTIF | 160 | 0.942 |
| I-Ad | AEFMLGDYL | 186 | 0.897 |
| I-Ad | LGSLYPFSR | 107 | 0.677 |
| I-Ad | AAKLMGRNL | 29 | 0.666 |
| I-Ag7 | GESIATYEA | 21 | 1.675 |
| I-Ag7 | LNGNATPEL | 92 | 1.616 |
| I-Ag7 | SISQEPYAF | 122 | 1.612 |
| I-Ag7 | IATYEAAKL | 24 | 1.519 |
| RT1.B | NTQEQKNTQ | 333 | 0.976 |
| RT1.B | ATYEAAKLM | 25 | 0.904 |
| RT1.B | DFYNQQRYT | 224 | 0.877 |
| RT1.B | TNTTGLDFY | 207 | 0.813 |

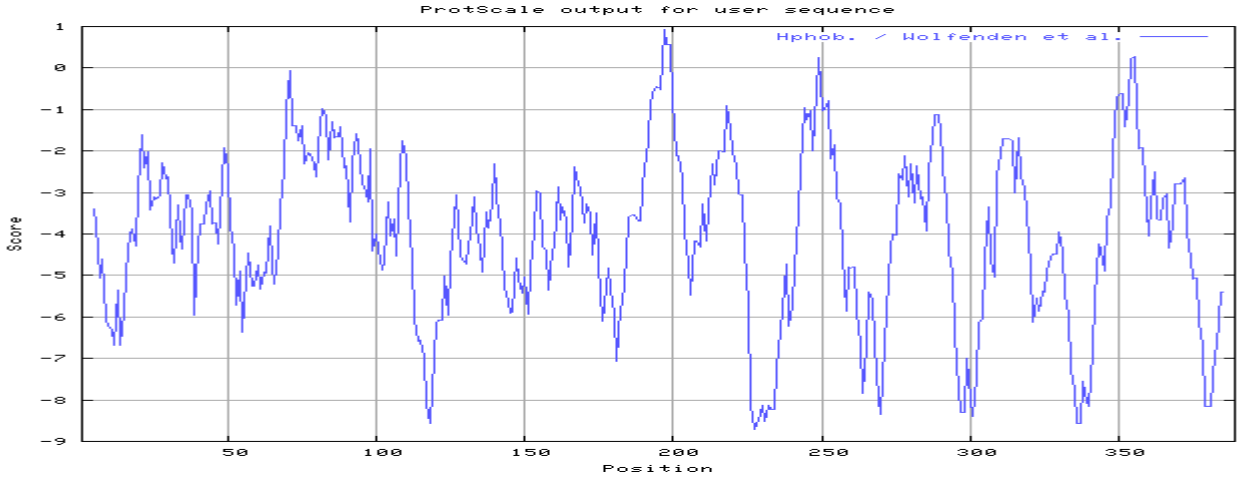


Fig. 1- Antigenicity plot of antigen protein by Wolfenden, et al., scale

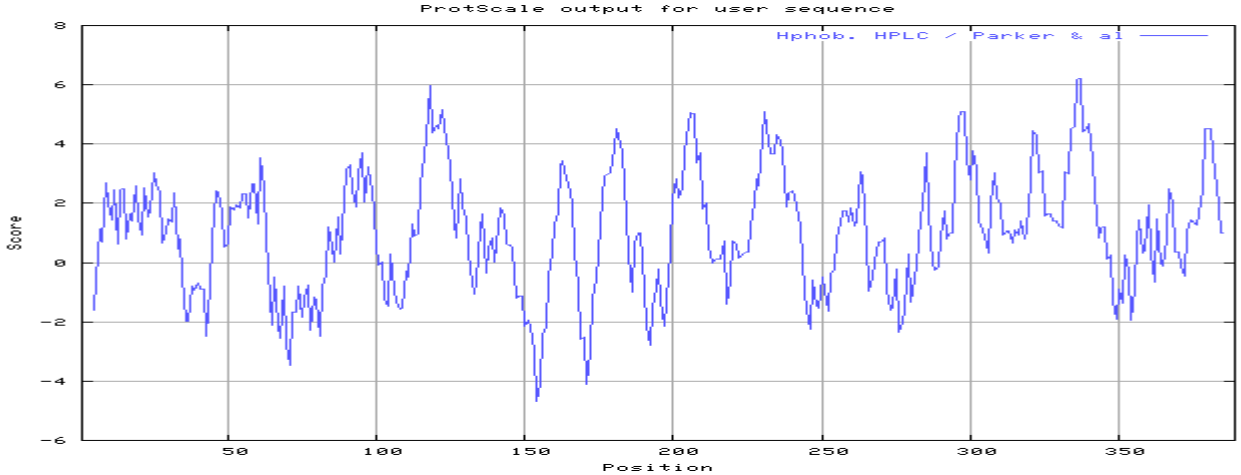


Fig. 2- Antigenicity plot of antigen protein by HPLC / Parker, et al., scale

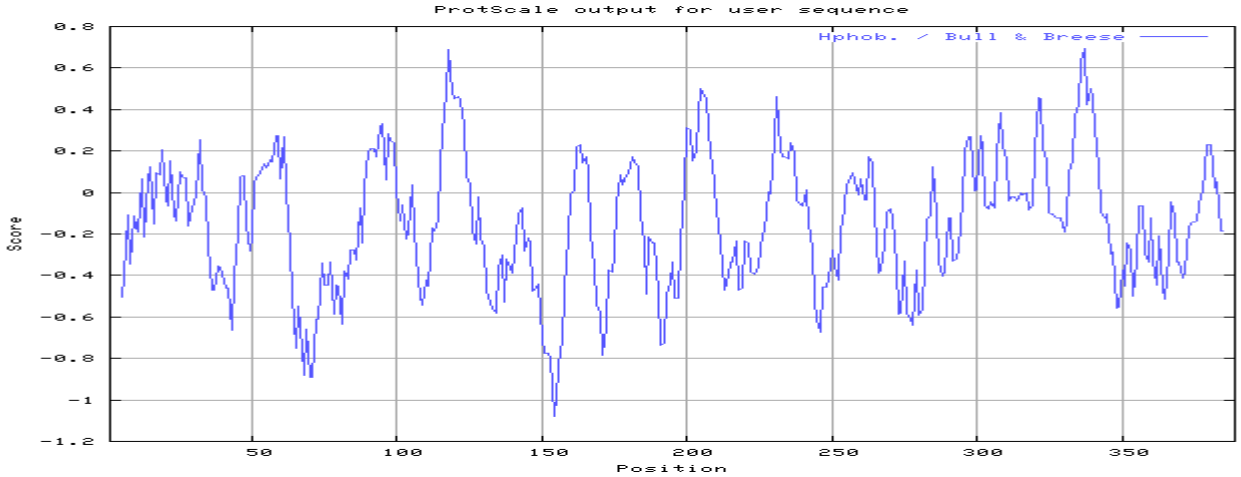


Fig. 3- Hydrophobicity plot of antigen protein by Bull &Breese, et al., scale

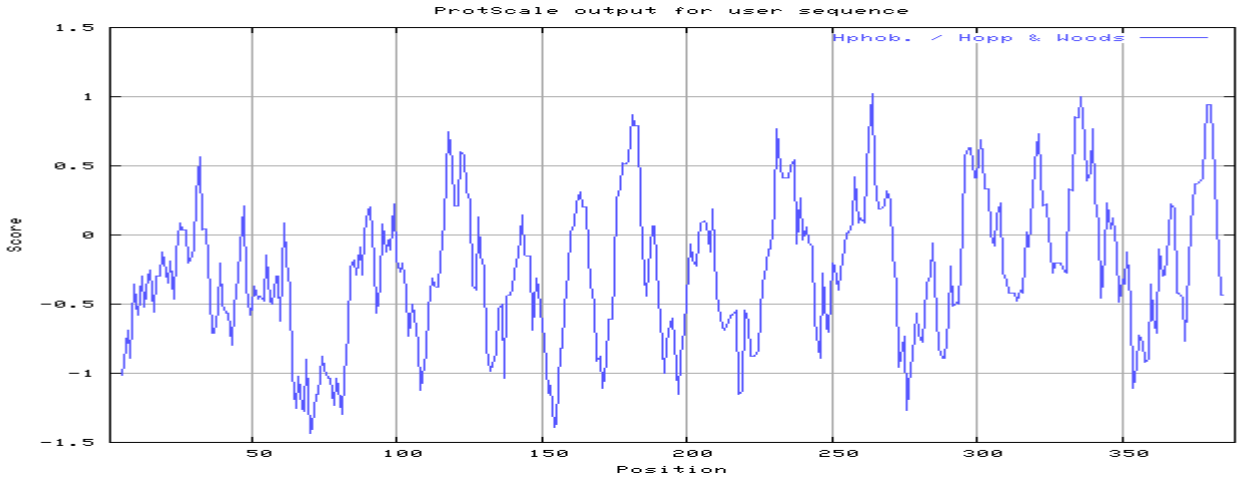


Fig. 4-Hydrophobicity plot of antigen protein by Hopp & Woods scale