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An *In Silico* Vaccine Designing Approach against Fish Pathogens *Flavobacterium columnare*

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Abstract: The development of peptide vaccines is a highly avant-garde approach to computer-aided vaccine design. Using various *in silico* approaches, an effort has been undertaken to identify potential peptides that could be used in developing a peptide vaccination. Research into vaccine design has advanced due to the creation of multiple sophisticated experiments that examine T-cell reactions to various vaccine candidates. This study identified potential peptides that bind specifically to Major Histocompatibility Complex (MHC) class I alleles in the outer membrane protein of fish pathogens. The results show that the majority of peptides bind effectively to the alpha1 and alpha 2 grooves of the MHC class I complex. There were substantial hydrogen bonding connections between MHC and even the peptide domains.

Keywords: Vaccine design, T-cell, MHC class I, Peptides, Fish pathogens

Citation: Sarkar Diptendu: An *in silico* vaccine designing approach against fish pathogens *Flavobacterium columnare.* Intern. J. Zool. Invest. 9(2): 359-368, 2023.

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Introduction

The majority of fish are prone to different bacterial diseases. More environmental infections come into direct contact with fish. As a result, these infections can quickly bind to and damage fish tissues (Ellis, 2021). Huge economic loss in fisheries is mostly driven by pathogen and disease induced illnesses in fish tissue. The world's greatest sector of livestock farming is aquaculture. As a result, there is a big need to find solutions to fight fish infections. The most common intracellular bacterial infection that causes columnaris in fish is identified as *Flavobacterium columnare.* Effective and efficient vaccines and strong preventative and preventive medications against bacterial fish illnesses are being sought for by fish farming scientists (Sudheesh *et al*., 2012). *F. columnare* is a widely distributed, motile Gram-negative rod. All around the world, it causes freshwater fish to contract the columnaris illness. Columnaris has a significant negative impact on a number of fish species. Salmonid species, carp, peaches, eels, tilapia, channel catfish, and goldfish are the most commonly impacted by columnaris (Abbott, 2007). Fish with columnaris have

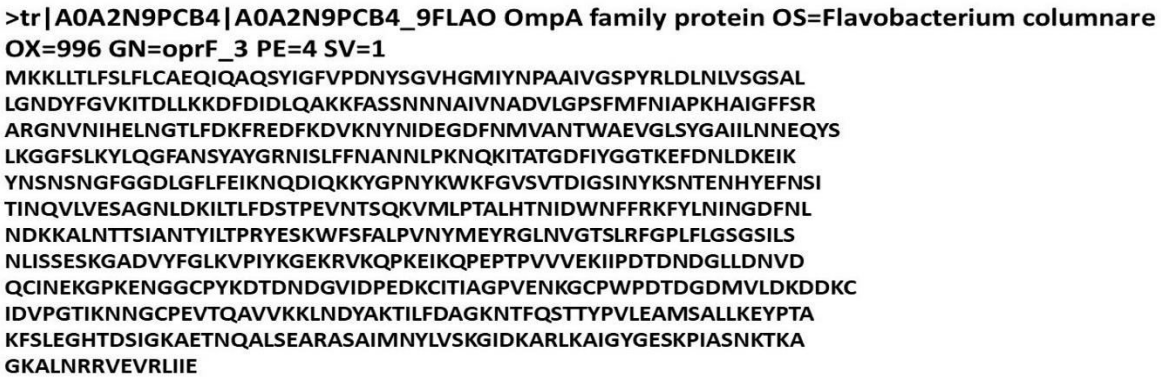


Fig. 1: UniProt protein sequence of *Flavobacterium columnare.*

brownish-yellow scars on their skin, gills, and fins. Each bacterial cell has its own gill filament. These factors raise the necessity for the creation of novel vaccinations to protect against columnaris (Plumb, 1999). Resistance to antibiotics among microorganisms is a result of the use of antibiotics to treat illnesses. There are already vaccinations available to treat columnaris. For live attenuated vaccinations, there is a risk of reversion (Mohanty *et al*., 2007).

This study employed immunoinformatic methods and software including databases to identify possible T-cell epitopes that might serve as efficient vaccine candidates. Which peptide from the pathogenic bacterial pathogen's proteome will attach to the major histocompatibility complex (MHC) molecules is to be determined by the T-cell epitope (Stock *et al*., 2001). A key factor in influencing T-cell immunogenicity is the degree of MHC molecule binding to epitopes (Farmer et al., 2007). Using the proper immunoinformatics methods, this study have modelled peptide-MHC complexes and examined their interactions in this investigation.

Materials and Methods

Retrieval of protein sequences:

The choice of outer membrane protein for this investigation was made possible by the high immunogenicity and considerable antigenicity of these proteins. The UniProt database, a well structured protein sequence database with precise structural and functional information about proteins, is where outer membrane protein was found. From UniProt, the outer membrane proteins' amino acid sequences in FASTA format was obtained (Fig. 1).

Forecasting of antigenic locations:

Using the Kolaskar and Tongaonkar antigenicity tool (http://tools.immuneepitope.org/tools/ bcell/iedb input), antigenic sites were predicted (Park *et al*., 2012). The prediction of peptides employs a semiempirical method that is based on the physical, chemical, and physico-chemical parameters of the amino acids as well as the frequency of occurrence of residues in experimentally known epitopes. The threshold value is 1.000, and it can predict antigens with an effectiveness of approximately 75%.

CTL epitope prediction:

The NetCTL.1.2 server (http://www.cbs.dtu.dk/ services/ NetCTL) was used to predict CTL epitopes (Woo *et al*., 2011; Tekedar *et al*., 2012). CTLs attack invading pathogens, and they are often activated by antigen-presenting MHC molecules on their exterior. Prediction of TAP transportation effectiveness, MHC class I binding, plus proteasomal C-terminal cleavage are all included in the NetCTL 1.2 server (Hawke *et al*., 1992; Declercq *et al*., 2013). The organism's FASTA sequence was used as input. Human

leukocyte antigen (HLA) alleles and peptide length were chosen and uploaded. As a result, T-cell epitopes were produced. To forecast MHC class I binding and proteasomal C-terminal cleavage, artificial neural networks are applied. In order to forecast TAP transport effectiveness, weight matrix is being used. Further VaxiJen 2.0 software was used to determine antigenic property of all listed epitope (Gianfrani *et al*., 2000; Zhu *et al*., 2012).

Protein-peptide complex modelling and docking:

Protein-peptide complexes were modelled using the MODPROPEP programme (Gillespie *et al*., 2000). The protein-peptide complex was modelled using the input peptide sequence and the crystal structure of the MHC alleles (HLA-B2705) from Protein Data Bank (PDB). High-resolution peptide docking was performed using the Rosetta FlexPepDock tool (http://flexpepdock.furmanlab.cs.huji.ac.il) (Lazarski *et al*., 2005; Sarkar *et al*., 2023). For simultaneous docking and *de novo* folding of peptides, FlexPepDock makes use of the Rosetta fragment library and a representation of the receptor and peptide structures. High-resolution models of the results are presented in the publication. FlexPepDock precisely refines the peptide structure, starting from up to 5.5 of its native conformation's root mean square deviation (RMSD). Additionally, it enables the full flexibility of the peptide and side chain flexibility of the receptor. By employing fragment-based sampling, the overall conformation of the peptide in a centroid mode is discovered. The Biovia Discovary studio was used to retrieve the residues involved in the hydrogen bond interaction between the peptide and the MHC protein (Sarkar *et al*., 2022).

Results and Discussion

The development of peptide vaccines depends critically on the prediction of antigenic sites in outer membrane proteins. Using the Kolaskar and Tongaonkar antigenicity tool, this study predicted the antigenic sites of the outer membrane protein of *F. columnare.*

The present study discovered 25 antigenic sites from *F. columnare*'s A0A2N9PCB4 (Table 1). Peptide having Cys(C) Isoleucine (I) Aspartic acid (D) Valine (V) Proline (P) Glycin (G) sequence showed maximum highest antigenic property 1.8790. This site is located in the CTL epitope in between 540 to 545. Rest all other probable CTL epitope which also can be exhibited as antigens are illustrated in Table 1.

Possible candidates for the development of peptide vaccines against various illnesses include CTL epitopes. Using the prediction tool in NetServer 1.2, the present study predicted CTL epitopes (Sarkar, 2021). Based on the peptides' MHC binding affinity, proteasomal C-terminal cleavage, and transport affinity, these predictions were made. MHC supertype A1 was used for assessment. From the outer membrane protein of *F. columnare*, 24 peptide sequences were predicted to be CTL epitopes. Out of 24, only 11were found to have antigenic property (Table 2).

Drug resistance among microorganisms is a result of the application of antibiotics to treat illnesses. There are already vaccinations available to treat columnaris. For live attenuated vaccinations, there is a risk of reversal. The pathogens' peptide sequences are bound by the MHC molecule before being shown on the cell surface (Sarkar *et al*., 2022). The CTLs kill the infected cell after identifying the peptide-protein combination. The peptide is introduced into the MHC I molecule by a protein complex known as T cell activating protein (TAP), after which it is delivered to the cell surface (Sarkar, 2022). The class I MHC groove's invariant pocket, which is present at one end, is where the peptide's terminal amino group attaches. The class I MHC molecule's consistent pocket is located at the opposite end of the groove, where the terminal carboxyl group of the peptide attaches. Hydrogen bond interactions and networks hold the peptide's N- and C-termini in place (Koronakis et al., 1997).

The major objective of this research was to locate possible T-cell epitopes for peptide vaccine development. To do prediction analysis and

Table 1: Notes: Number of antigenic sites identified in the outer membrane protein of *F. columnare* is 25 with residue score of 1.170 (threshold =1.000). Only the probable CTL epitope antigens are highlighted in bold

Predicted peptides					
No.	**Start**	**End**	**Peptide**	**Length**	**VaxiJen 2.00 prediction (Threshold = 0.4)**
1	4	36	**LLTLFSLFLCAEQIQAQSYIGFVPDNYSGVHGM**	33	Overall Prediction for the Protective Antigen = **0.5369** (Probable ANTIGEN).
2	39	47	NPAAIVGSP	9	Overall Prediction for the Protective Antigen = **0.1741** (Probable **NON-ANTIGEN).**
3	49	59	**RLDLNLVSGSA**	11	Overall Prediction for the Protective Antigen =**1.7312** (Probable ANTIGEN).
4	67	73	**GVKITDL**	7	Overall Prediction for the Protective Antigen = **1.7312** (Probable ANTIGEN).
5	80	87	**IDLQAKKF**	8	Overall Prediction for the Protective Antigen = **1.5175** (Probable ANTIGEN).
6	97	104	**NADVLGPS**	8	Overall Prediction for the Protective Antigen = **1.0670** (Probable ANTIGEN)
7	110	118	APKHAIGFF	9	Overall Prediction for the Protective Antigen = **-0.2300** (Probable NON-ANTIGEN).
8	164	172	**EVGLSYGAI**	9	Overall Prediction for the Protective Antigen = **0.5103** (Probable ANTIGEN).
9	186	192	SLKYLQG	7	Overall Prediction for the Protective Antigen = **0.2745** (Probable NON-ANTIGEN)
10	274	282	**KFGVSVTDI**	9	Overall Prediction for the Protective Antigen = **0.6165** (Probable ANTIGEN)
11	302	310	INQVLVESA	9	Overall Prediction for the Protective Antigen = **0.1906** (Probable NON-ANTIGEN)
12	315	320	KILTLF	6	Overall Prediction for the Protective Antigen = **-0.4470** (Probable NON-ANTIGEN).
13	331	337	KVMLPTA	7	Overall Prediction for the Protective Antigen = **-0.2289** (Probable NON-ANTIGEN).
14	373	380	NTYILTPR	8	Overall Prediction for the Protective Antigen = **-0.2289** (Probable NON-ANTIGEN).
15	387	394	**SFALPVNY**	8	Overall Prediction for the Protective Antigen = **1.0057**

					(Probable ANTIGEN).
16	408	417	FGPLFLGSGS	10	Overall Prediction for the Protective Antigen = **0.3531** (Probable NON-ANTIGEN).
17	430	441	**ADVYFGLKVPIY**	12	Overall Prediction for the Protective Antigen = **1.1402** (Probable ANTIGEN).
18	458	466	PTPVVVEKI	9	Overall Prediction for the Protective Antigen = **0.1133** (Probable NON-ANTIGEN).
19	476	482	LDNVDQC	7	Overall Prediction for the Protective Antigen = **-1.0975** (Probable NON-ANTIGEN).
20	507	517	PEDKCITIAGP	11	Overall Prediction for the Protective Antigen = **0.1465** (Probable NON-ANTIGEN).
21	540	545	**CIDVPG**	6	Overall Prediction for the Protective Antigen = **1.8790** (Probable ANTIGEN).
22	552	563	**CPEVTQAVVKKL**	12	Overall Prediction for the Protective Antigen = **0.5660** (Probable ANTIGEN).
23	568	573	KTILFD	6	Overall Prediction for the Protective Antigen = **-1.2059** (Probable NON-ANTIGEN).
24	582	597	TTYPVLEAMSALLKEY	16	Overall Prediction for the Protective Antigen = **-0.1615** (Probable NON-ANTIGEN).
25	629	636	MNYLVSKG	8	Overall Prediction for the Protective Antigen = **0.0114** (Probable NON-ANTIGEN).

Table 2: Antigenic properties of selected peptodes. Note: NetCTL-1.2 predictions using MHC supertype A1. Threshold 0.750000

NetCTL-1.2 predictions using MHC supertype A1. Threshold 0.750000								
Residue No	**Peptide Sequence**	**Predicted MHC binding affinity**	**Rescale binding affinity**	**C-terminal cleavage affinity**	**Transport affinity**	**Prediction score**	**MHC ligands Prediction score should be >.0.75000**	**VaxiJen 2.00 prediction (Threshold = 0.4)**
279	VTDIGSINY	0.7769	3.2986	0.9669	2.843	3.5858	<-E	Overall Prediction for the Protective Antigen = **-0.0256** (Probable **NON-ANTIGEN**)
218	ITATGDFIY	0.6433	2.7312	0.9615	3.044	3.0276	<-E	Overall Prediction for the Protective Antigen = **-0.2422** (Probable **NON-ANTIGEN**).
367	NTTSIANTY	0.6413	2.723	0.9741	2.952	3.0167	<-E	Overall Prediction for the Protective Antigen = **0.3352**

								(Probable **NON-ANTIGEN**).
30	**YSGVHGMIY**	0.6022	2.5569	0.7027	2.923	2.8084	<-E	Overall Prediction for the Protective Antigen = **0.4571** (Probable **ANTIGEN**).
57	GSALLGNDY	0.5191	2.2042	0.8141	2.857	2.4691	<-E	Overall Prediction for the Protective Antigen = **0.2279** (Probable **NON-ANTIGEN**).
189	YLQGFANSY	0.3329	1.4134	0.9739	2.993	1.7091	<-E	Overall Prediction for the Protective Antigen = **-0.2175** (Probable **NON-ANTIGEN**).
386	**FSFALPVNY**	0.3206	1.3612	0.8333	2.926	1.6325	<-E	Overall Prediction for the Protective Antigen = **1.9108** (Probable **ANTIGEN)** .
373	**NTYILTPRY**	0.2876	1.2213	0.973	3.281	1.5313	<-E	Overall Prediction for the Protective Antigen = **0.6539** (Probable **ANTIGEN)** .
339	**HTNIDWNFF**	0.2993	1.2708	0.1019	2.376	1.4049	<-E	Overall Prediction for the Protective Antigen = **1.7521** (Probable **ANTIGEN)** .
389	**ALPVNYMEY**	0.2068	0.8781	0.9769	2.968	1.173	<-E	Overall Prediction for the Protective Antigen = **1.7521** (Probable **ANTIGEN)** .
312	NLDKILTLF	0.2086	0.8855	0.9713	2.385	1.1504	<-E	Overall Prediction for the Protective Antigen = **-0.8025** (Probable **NON-ANTIGEN**).
625	ASAIMNYLV	0.248	1.0531	0.4035	0.648	1.146	<-E	Overall Prediction for the Protective Antigen = **0.3344** (Probable **NON-ANTIGEN**).
22	YIGFVPDNY	0.1817	0.7714	0.8761	2.924	1.049	<-E	Overall Prediction for the Protective Antigen = **0.0918** (Probable **NON-ANTIGEN**).
368	**TTSIANTYI**	0.2161	0.9175	0.6493	0.629	1.0464	<-E	Overall Prediction for the Protective Antigen = **0.4509** (Probable **ANTIGEN)** .
394	**YMEYRGLNV**	0.2094	0.889	0.8452	0.413	1.0364	<-E	Overall Prediction for the Protective Antigen = **0.6380** (Probable **ANTIGEN)** .
589	AMSALLKEY	0.1757	0.7461	0.9041	3.019	1.0326	<-E	Overall Prediction for the Protective Antigen = **-0.1313** (Probable **NON-ANTIGEN**).
40	PAAIVGSPY	0.1699	0.7215	0.9653	2.655	0.999	<-E	Overall Prediction for the Protective Antigen = **0.3491** (Probable **NON-ANTIGEN**).
287	**YKSNTENHY**	0.1605	0.6813	0.969	3.091	0.9812	<-E	Overall Prediction for the Protective Antigen = **0.7321** (Probable **ANTIGEN)** .
88	**ASSNNNAIV**	0.1711	0.7265	0.9348	0.39	0.8862	<-E	Overall Prediction for the Protective Antigen = **0.6252**

								(Probable **ANTIGEN)** .
171	AIILNNEQY	0.1135	0.482	0.9533	3.236	0.7868	<-E	Overall Prediction for the Protective Antigen = **-0.6472** (Probable **NON-ANTIGEN**).
70	ITDLLKKDF	0.1347	0.5719	0.6083	2.461	0.7862	<-E	Overall Prediction for the Protective Antigen = **-0.1496** (Probable **NON-ANTIGEN**).
161	TWAEVGLSY	0.125	0.5307	0.5234	3.308	0.7747	<-E	Overall Prediction for the Protective Antigen = **0.1045** (Probable **NON-ANTIGEN**).
558	AVVKKLNDY	0.1092	0.4635	0.9423	3.236	0.7667	<-E	Overall Prediction for the Protective Antigen = **-0.0870** (Probable **NON-ANTIGEN**).

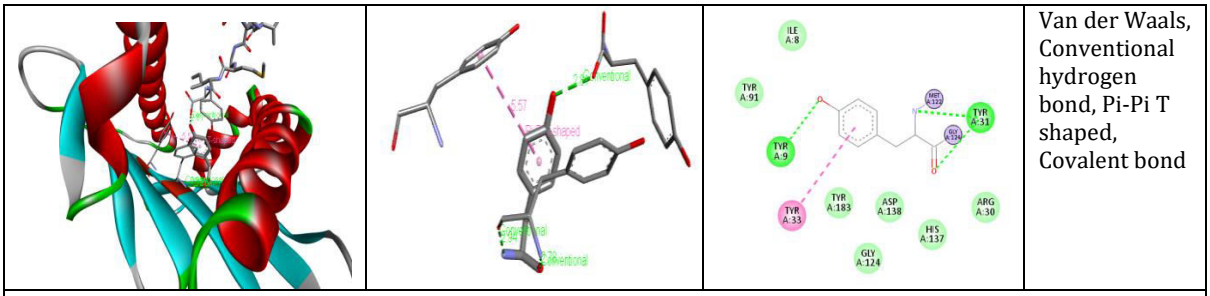
docking between the peptide and the protein, *in silico* immunoinformatics methods were employed. The limitations of conventional experimental methods are numerous. Using *in silico* immunoinformatics research, the full range of possible antigens was examined. Antigens that cannot be expressed in vitro because pathogen culture is not feasible can also be studied *in silico. In silico* vaccine candidates have been reported by several immunoresearch groups, and they have shown encouraging preclinical outcomes. The outer membrane protein of the pathogenic pathogen *F. columnare* contains potential T-cell epitopes that bind with MHC protein (Feriancikova *et al*., 2013). Antigenic domain forecasting aids in the discovery of possible outer membrane protein residues. There are nine *F. columnare* epitopes that have the potential to serve as peptide vaccine targets. YSGVHGMIY, FSFALPVNY, NTYILTPRY, HTNIDWNFF, ALPVNYMEY, TTSIANTYI, YMEYRGLNV and YKSNTENHY are the peptides in question (Table 2). Interaction between MHC bound complex with respective interaction residues and bonds involved are shown in Figures 2-9. A significant development in the field of *in silico* vaccine design is T-cell epitope forecasting. By utilising *in silico* epitope prediction methods, numerous research organisations that have been involved in the discovery of several vaccines targeting various viral diseases, including autoimmune and cancer, have achieved great outcomes (Sarkar, 2021). CTL epitope identification by this approach acts as a crucial tool in the process of creating vaccines because the *in silico* methodology simplifies the number of *in vitro* testing. A procedure used in numerous computer research alignments the MHC molecule's existing sequences and identifies residues that facilitate peptide binding (Sarkar *et al*., 2022). Knowing this data and comprehending crystallography can help you comprehend the idea underlying peptide-MHC identification (Feriancikova *et al*., 2013).

In silico analysis improves the accuracy and identification of functional CTL epitopes (Zhu *et al*., 2012). The CTL epitopes of conserved hepatitis C virus (HCV) proteins were predicted by the researchers, while their *in vitro* MHC class I antigen complexation was evaluated. To confirm the protective HCV CTL epitopes, they further tested the immunogenicity of the discovered epitopes in living organisms. They verified *in silico* methods and came to the conclusion that it is possible to identify and restrict the pool of potential CTL epitope candidates for the creation of therapeutic vaccines (Park *et al*., 2012). For more precise CTL epitope forecasting, they have also recommended integrating a number of algorithms for MHC class I and proteasomal degradation site assessment. These results highlight the value of the *in silico* methods for

peptide vaccine production as well as its useful in vitro implementation. This study anticipate that using the forecasted CTL epitopes from present investigation for *in vitro* research might result in the production of peptide vaccines having biomedical applications.

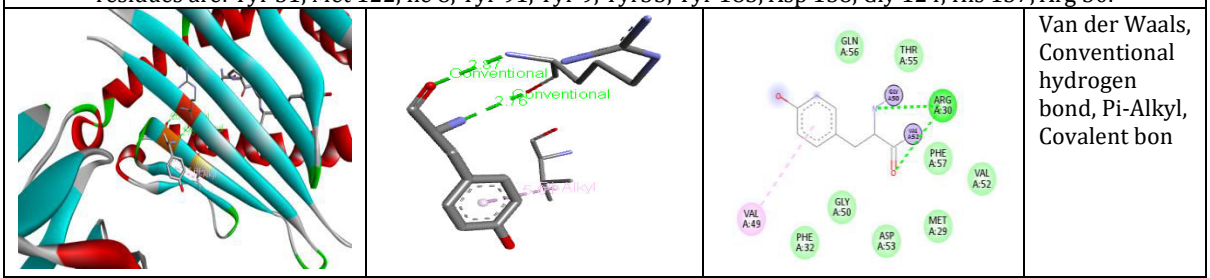
Conclusion

An extremely innovative method of computer-aided vaccine design is the creation of peptide vaccines. An effort has been made to find possible peptides that could be employed in creating



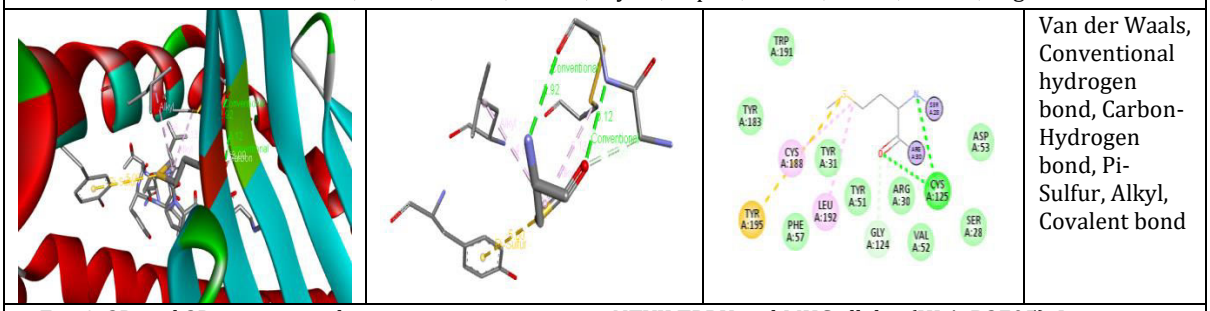
Van der Waals, Conventional hydrogen bond, Pi-Pi T shaped, Covalent bond

Fig. 2: 3D and 2D interaction between antigenic epitope YSGVHGMIY and MHC alleles (HLA-B2705). Interacting residues are: Tyr 31, Met 122, Ile 8, Tyr 91, Tyr 9, Tyr33, Tyr 183, Asp 138, Gly 124, His 137, Arg 30.



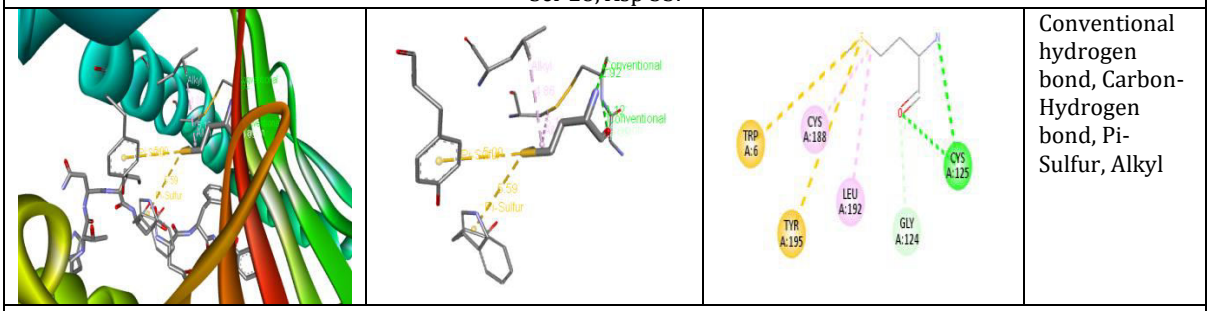
Van der Waals, Conventional hydrogen bond, Pi-Alkyl, Covalent bon

Fig. 3: 3D and 2D interaction between antigenic epitope FSFALPVNY and MHC alleles (HLA-B2705). Interacting residues are: Thr 55, Gln 56, Val 49, Phe 32, Gly 50, Asp 53, Met 29, Val 52, Phe 57, Arg 30.



Van der Waals, Conventional hydrogen bond, Carbon-Hydrogen bond, Pi-Sulfur, Alkyl, Covalent bond

Fig. 4: 3D and 2D interaction between antigenic epitope NTYILTPRY and MHC alleles (HLA-B2705). Interacting residues are: Trp 191, Tyr 183, Tyr 195, Cys 188, Phe 57, Leu 192, Tyr 31,. Tyr 51, Gly124, Val52, Arg 30, Cys 125, Ser 28, Asp 53.



Conventional hydrogen bond, Carbon-Hydrogen bond, Pi-Sulfur, Alkyl

Fig. 5: 3D and 2D interaction between antigenic epitope HTNIDWNFF and MHC alleles (HLA-B2705). Interacting residues are: Cys 125, Gly 124, Leu 192, Tyr 195, Cys 188, Trp 6.

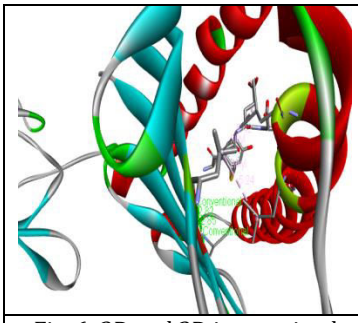
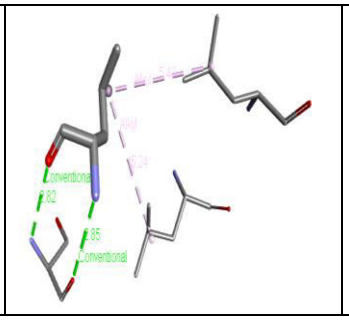
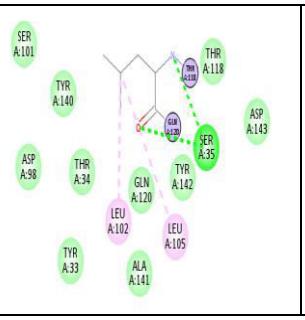
			Van der Waals, Conventional hydrogen bond, Alkyl, Covalent bond.

Fig. 6: 3D and 2D interaction between antigenic epitope ALPVNYMEY and MHC alleles (HLA-B2705). Interacting residues are: Asp 143, Thr 118, Ser 101, Tyr 140, Thr 34, Asp 98, Tyr 33, Leu 102, Ala 141, Gln 120, Leu 105, Tyr 142, Ser 35.

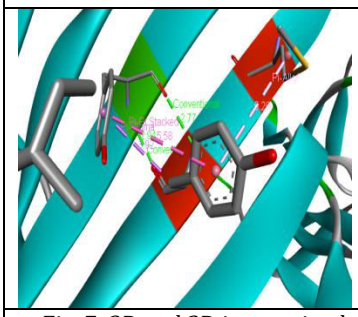
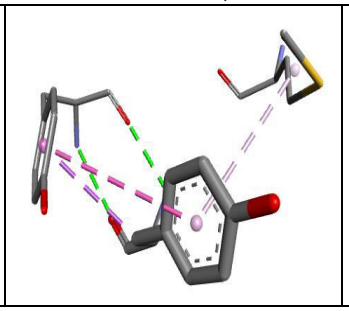
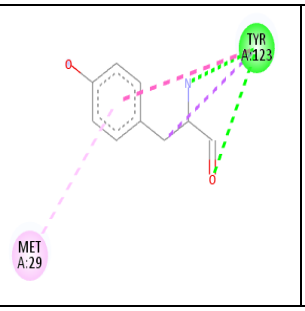
			Conventional hydrogen bond, Pi-sigma, Pi-Pi-Stacked, Pi-Alkyl

Fig. 7: 3D and 2D interaction between antigenic epitope TTSIANTYI and MHC alleles (HLA-B2705). Interacting residues are: Tyr 123, Met 29.

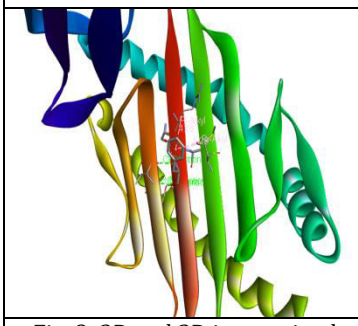
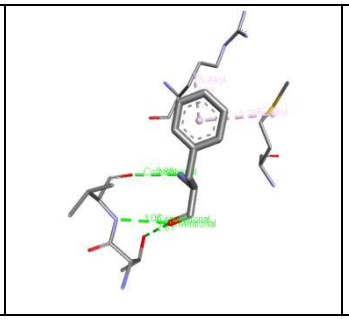
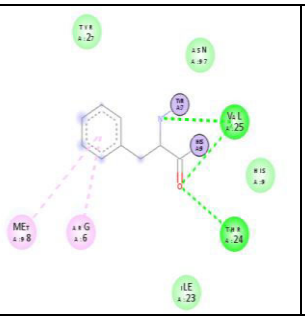
			Conventional hydrogen bond, Pi-Alkyl, Conventional hydrogen bond, Covalent bond

Fig. 8: 3D and 2D interaction between antigenic epitope YMEYRGLNV and MHC alleles (HLA-B2705). Interacting residues are: Tyr 123, Met 122.

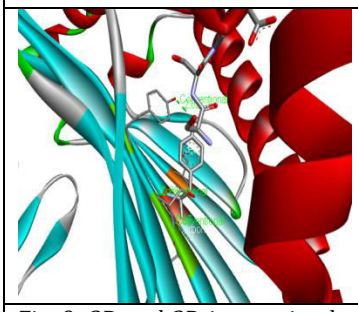
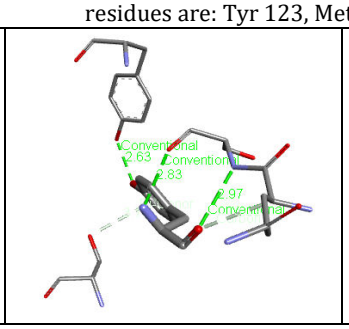
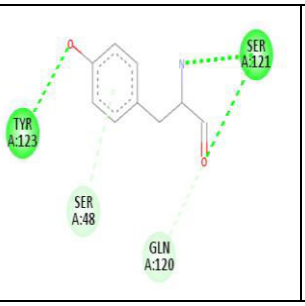
			Conventional hydrogen bond, Carbon Hydrogen bond, Pi-Donor hydrogen bond

Fig. 9: 3D and 2D interaction between antigenic epitope YKSNTENHY and MHC alleles (HLA-B2705). Interacting residues are: Ser 121, Tyr 123, Ser 48, Gln 120.

a peptide vaccination using various *in silico* methodologies. The development of numerous sophisticated experiments that test the T-cell responses against diverse vaccine candidates presently has aided in the advancement of vaccine-designing investigation. The outer membrane protein of fish pathogens contained putative peptides that bind effectively to MHC class I alleles. The findings indicate that the majority of the peptides bind efficiently to the MHC class I complex's grooves alpha1 and alpha 2. Significant hydrogen bonding linkages existed in

between MHC and even the peptide domains.

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