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Article

Novel Multi Epitope-Based Vaccine against Monkeypox Virus: Vaccinomic approach

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Abstract:

Background: While mankind is still dealing with the COVID-19 pandemic, on May 7, 2022, a case of monkeypox virus (MPXV) has been reported to the WHO. Monkeypox is a viral zoonotic disease with characteristics comparable to those seen in smallpox cases in the past. It has been a public health threat, particularly in Africa, but recently have been circulating the world, consequently, may become a global public health threat in a very short period. Thus, the current work was planned and then constructed a multi-epitope vaccine that can evoke an immunological response against MPXV utilizing cell surface-binding protein as a target in order to develop a novel vaccine that is both safe and almost free of side effects. **Results:** The proposed vaccine composed of 304 amino acids and was shown to be antigenic in Vaxijen server (0. 5311) and nonallergenic in AllerTop server. The 3D structure of the designed vaccine is predicted, refined and validated by various *in silico* tools to assess the stability of the vaccine. Moreover, solubility of the vaccine construct was found greater than the average solubility provided by protein-Sol server indicating the solubility of the vaccine construct. Moreover, the most promising epitopes bound to MHC I and MHC II alleles were found having good binding affinities with low energies ranging between −7.0 - −8.1kcal/mol. **Conclusion:** We conclude from our research that the cell surface-binding protein is one of the primary proteins involved in MPXV pathogenesis. The most promising epitopes were selected using a rigorous procedure and used for vaccine design. As a result, our study will aid in the development of appropriate therapeutics and prompt the development of future vaccines against MPXV, which could serve as an important milestone in the production of an antiviral vaccine against MPXV.

Keywords: Multi Epitope; monkeypox virus (MPXV); Vaccine; Immunoinformatics; *In silico*; Molecular docking

1. Introduction:

While mankind is still dealing with the COVID-19 pandemic, on May 7, 2022, a case of monkeypox virus (MPXV) was reported to the WHO. The patient has a history of traveling from the United Kingdom to Nigeria and back [1, 2].

Monkeypox is a viral zoonotic disease with characteristics comparable to those seen in smallpox cases in the past, though it is physiologically less acute. It is induced by the monkeypox virus, which belongs to the *Orthopoxvirus* genus in the family *Poxviridae* [3-5]. Considering half of the world's population without immunity to the *Orthopoxvirus*, [6], Poxviruses have a strong proclivity to emerge outside of their regular ecological range by transmissioning to a naive community [1].

Monkeypox is clinically nearly identical to conventional smallpox; thus, since the global termination of smallpox in 1977, great attention has been devoted to monkeypox as a smallpox-like disease and potential

bioterrorism agent [7]. This virus received significant attention when it first appeared in the Western Hemisphere in the spring of 2003, causing a cluster of cases in the US Midwest [7, 8]. Yet, as of June 4, 2022, there are approximately a hundred verified monkeypox cases in the United States, the United Kingdom, and many other European countries. Monkeypox presents a variety of clinical manifestations, including flu-like symptoms, fever, malaise, back pain, headache, and a distinctive rash [7]. There are two clades circulating the world, the Central African clade and the West African clade. The Central African clade had a case fatality rate of 10.6 percent versus 3.6 percent for the West African clade. The United States is the first country to disclose that at least two strains of monkeypox are now circulating in the country; the majority of cases in the US are the same strain that has been spreading in the United Kingdom and Europe, but experts have detected a mutation [9]. This could mean that monkeypox has been spreading in the US prior to the CDC's investigation beginning in May.

MPXV can be diagnosed by real-time polymerase chain reaction (PCR) of samples collected via dry swabs of unroofed lesions or ulcers [10-12]. From January to June 1, 2022, seven endemic countries reported 1408 suspected and 44 confirmed cases, causing 66 deaths. Cameroon, the Central African Republic, the Democratic Republic of the Congo, Gabon, and Ghana are among the nations where monkeypox is endemic (identified in animals only). The scenario is changing, and WHO predicts that additional cases of monkeypox will be detected as the outbreak advances and surveillance increases in both endemic and non-endemic countries [13].

Scientists in Portugal have published the first draft genome of the monkeypox virus to an online database on May 19, 2022, as well as other genomes have also followed. These basic genetic research shows that the monkeypox virus strain discovered thus far is connected to a viral strain prevalent primarily in West Africa, when compared to the type that spreads in Central Africa, this strain causes milder symptoms and has a lower death rate of roughly 1% in poor rural populations (which can have a death rate of up to 10%) [14].

At the molecular level, the monkeypox virus genome consists of a linear double-stranded DNA [15]. The inverted terminal repeats are composed of hairpin loops, tandem repeats, and some open reading frames that are covalently connected at their ends. Despite being a DNA virus, MPXV spends its whole life cycle in the cytoplasm of infected cells. The MPXV genome encodes all of the proteins essential for viral DNA replication, transcription, virion assembly, and egress [16, 17].

There are no approved vaccines or drugs to treat the human monkeypox virus until 2019. Dryvax, a smallpox vaccine has been used for both smallpox and monkeypox treatment [18]. Nevertheless, the many negative effects influenced both the vaccinated and those in contact with the vaccinated [19, 20].

The primary premise behind all vaccinations is the vaccine's ability to generate an immune response faster than the virus itself. Although classical vaccines based on biochemical experiments elicited strong neutralizing and protective antibodies in vaccinated animals, they are expensive, allergic, and time-consuming, and they involve in vitro cultivation of dangerous viruses, raising serious safety concerns [21, 22]. Peptide-based vaccine production is exceptionally safe and cost-effective, particularly in comparison to traditional vaccinations. The need for safe and efficacious vaccines is highly vital. Therefore, this study was dedicated to design a peptide-based vaccine to predict epitopes from MPXV protein using immunoinformatics combined with molecular docking studies.

2. Material and Methods:

The Immunoinformatic guided rational design of MPXV Vaccine are shown in **Figure (1)**.

2.1. Sequence Retrieval:

The sequence of cell surface-binding polyprotein of all Monkeypox Virus variants including those of the new variant strain of USA and Italy were retrieved from the National Center of Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/) on 17 June 2022

2.2. Antigenicity prediction:

To confirm the immunogenic character of all epitopes fragments Vaxijen 2.0 server (http://www.ddgpharmfac.net/vaxijen/VaxiJen/VaxiJen.html) [23]. It is based on the alignment independence method which predict antigenicity using physiochemical properties and ACC methods for antigenicity assessments peptide fragment with a threshold greater than 0.4 were marked as potentially antigenic [23].

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2.3. Phylogenetic analysis:

In order to identify similar sequences, sequence comparison and alignment of E8L were performed using BLASTp (http://blast.ncbi.nim.nih.gov//Blast.cgi?PAGE=Proteins) and Molecular Evolutionary Genetics Analysis (MEGA-X) (version 10.1.18)

2.4. B-cell epitope prediction

In order to create antibodies against disease, B-cells must be activated by particular antigens/epitopes. The BCPRED server (http://ailab.ist.psu.edu/bcpred/) was used to search the indicated antigenic protein of the Monkeypox virus for opportunistic linear B-cell epitopes [24]. The BCPRED service generates 20mer epitopes with a standard specificity of 75% for B-cell receptors using a FASTA sequence as input. For further designing of a multiepitope vaccine, the epitope with the highest score was chosen [24].

2.5. T-cell epitope prediction tools:

2.5.1. MHC class I binding predictions:

To predict the interaction with different MHC I alleles, the Major Histocompatibility Complex class I (MHC I) binding prediction tool on the IEDB (http://tools.iedb.org/mhcI) [24] was used. It services distinct approaches to measure the binding affinity of selected sequence to a definite MHC class I molecule. The half maximal inhibitory concentration (IC50) values of peptide binding to MHC class I molecule was calculated by artificial neural network (ANN) approach [25-27]. All alleles having a binding affinity of IC50 equal to or less than 100 nM were selected for further analysis.

2.5.2. MHC class II binding predictions:

To predict MHCII peptides, the protein sequence was again submitted to IEDB sever using NN-align2.3 as a prediction method and a peptide length of 15. Peptides were chosen if he IC50 were less than 500 [28].

2.6. Potential Cytotoxic T-Lymphocyte (CTL) Epitopes Prediction:

CTL epitope predictions that are consistent are essential for building a coherent vaccine. This was accomplished using NetCTL1.2 36 [29], an internet-based server designed to detect human CTL epitopes in a protein of interest. TAP transport efficiency, proteasomal cleavage, and MHCI molecules binding affinity data were added to get the overall score. The variable was set to 0.5, which has 0.89 and 0.94 sensitivity and specificity, respectively [29].

2.7. Population coverage:

Predicting the distribution of HLA-alleles in the world population is essential for effective multi-epitope vaccine design; thus, the IEDB server (http://tools.iedb.org/tools/population/iedb_input) was used to do a population coverage investigation of the selected MHC classes I and II epitopes [30].

2.8. Secondary structural prediction:

By using reference sequences as input, PSIPRED sever (http://bioinf.cs.ucl.ac.uk/psipred/) was used to generate the secondary structures of the developed vaccine [31]. The PSIPRED is a simple and accurate 2D homology modelling platform that contains two feed-forward neural networks to assess PSI-BLAST output [32, 33].

2.9. Homology modeling and protein validation:

The MPXV protein's tertiary structure was created using the SWISS-Model sever. (https://swissmodel.expasy.org/). The most promising peptides for vaccine creation were visualized using Discovery Studio 2020 (https://www.3ds.com/products-services/biovia/products/molecular-modeling-simulation/biovia-discovery-studio/visualization/). The proposed three-dimensional structure was validated using QMEAN. (https://swissmodel.expasy.org/qmean/),

2.10. Physiochemical analysis and Allergenicity prediction:

ProtParam server available at (https://web.expasy.org/protparam/) was used to predict the physicochemical features of the protein under study and to understand the fundamental nature of the vaccine [34]. The allergenicity of the predicted peptides were further assessed using AllerTop v 2.0 server [35].

2.11. 3D model refinement and validations

The SWISS-Model server's best protein model was redesigned and refined using the GalaxyRefine web server (http://galaxy.seoklab.org/cgi-bin/submit.cgi?type=REFINE). The molecular dynamic simulation replaces amino acids with high-probability rotamers, resulting in total structural relaxation [36]. The server

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recommends five refined models that differ in GDT-HA, RMSD, MolProbity, Clash Score, Poor rotamers, and Rama favored. Following that, the improved model required validations on two servers (ProSA-web and PROCHECK servers). The ProSA-web server calculates the total quality score based on a Z-score for all known protein structures; while the protein quality was assessed using the PROCHECK server and the Ramachandran plot (https://www.ebi.ac.uk/thornton-srv/software/PROCHECK/) [37].

2.12. Molecular docking:

For proper computational calculations, the promising epitopes and the target protein were prepared. LigPrep tool interfaced with Maestro module of Schrödinger suite was used for the ligand preparation. The 3 D structures including all possible tautomers and ionization states at pH 7.0 ± 2.0 of all the epitopes were generated and minimized using optimized potential liquid simulations (OPLS4) force field. Schrödinger's multi-step Protein Preparation Wizard PrepWizard was used for the protein preparations [38]. As an initial step, high-resolution protein crystal structure of MHC I allele HLA-A*02:01 (PDB ID: 4UQ3) at 2 Å resolution was retrieved from RCSB Protein Data Bank. Charges and bond orders were assigned, hydrogens were added to the heavy atoms, all water molecules and heteroatoms were then removed. OPLS4 force field was used for optimization and energy minimization for both the epitopes and protein final structures. Glide XP (extra precision) module of Schrödinger Suite was used to dock the promising epitopes into the active site of the crystal structures [38]. Best-scoring docked pose of the molecule obtained was superimposed against X-ray crystal orientation and conformation of the bound ligand and RMSD was calculated. Visualization was then conducted using Schrödinger Suite and DS visualizer client.

2.13. Solubility properties prediction:

Protein-sol (https://protein-sol.manchester.ac.uk/) is a web-based tool for performing numerical simulations and predicting protein solubility [39]. The Protein-Sol server estimated that the multi-epitope protein would be soluble when expressed in *E. coli*.

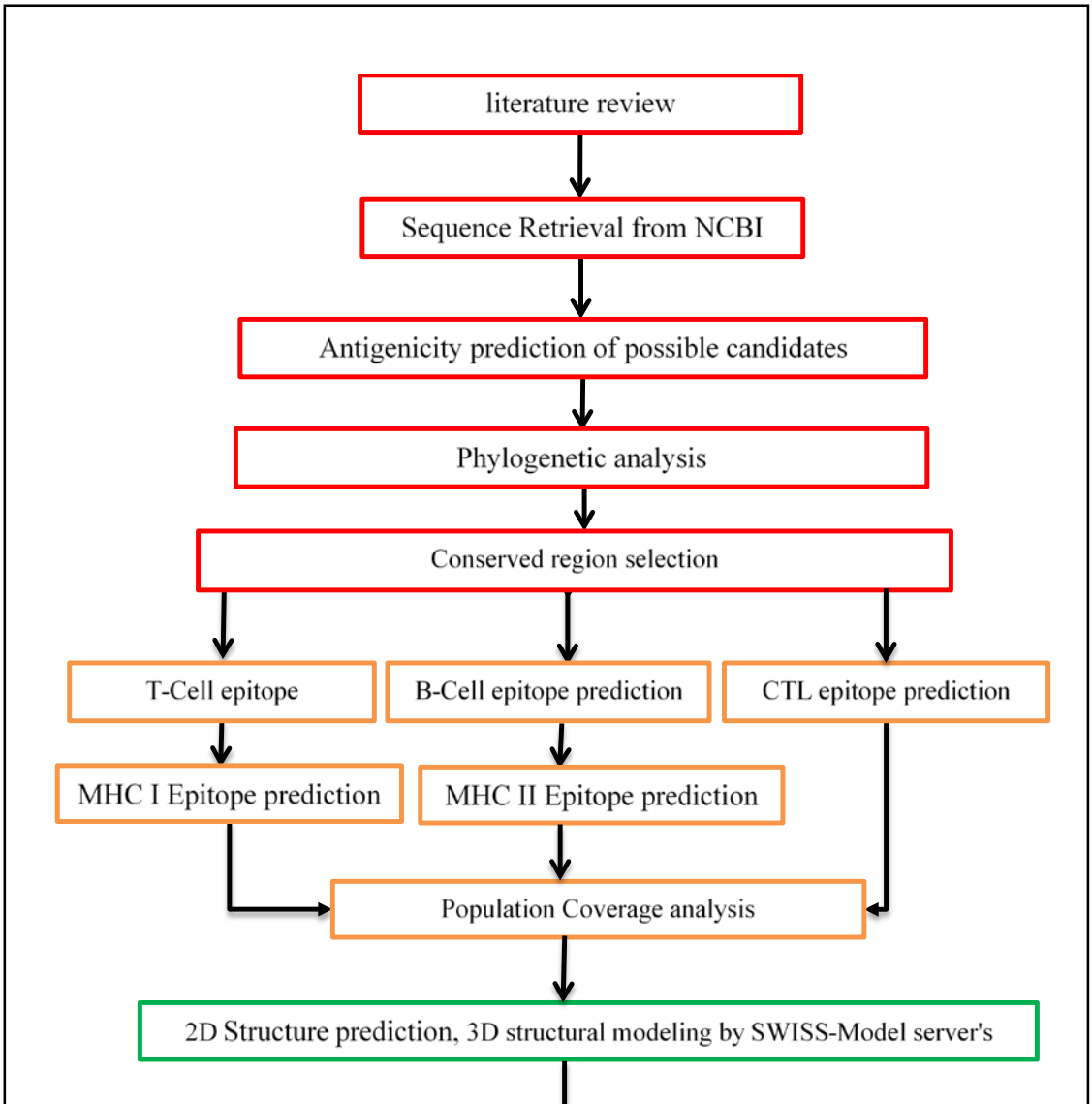

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Figure 1: Demonstrated the immunoinformatics approaches used for vaccine design against MPXV.

3. Results

3.1 Antigenicity prediction and conservation analysis:

The alignment of 7 E8L sequences yielded 6 conserved peptides with a length greater than 10 amino acids. The antigenicity was then tested using the VaxiJen server with score threshold for viruses is 0.5, which means that proteins with a score greater than 0.5 are deemed antigenic, whereas proteins with a score less than 0.5 are considered non-antigenic. The VaxiJen score for Monkeypox Virus's tested protein was more than 0.5 (0.5311). Moreover, analysis of those conserved regions showed only four antigenic regions that met VaxiJen's default criterion of 0.5. (Table 1).

Table1: Conserved regions of Cell surface binding protein:

Conserved peptide	Score
MPQQLSPINIETKKAISD	0.9972
RLKTLDIHYNESKPTTIQNTGKLVRINFKGGYISGGFLPNEYV LSTIHIYWGKEDDYGSNHLIDVYKYSGEINLVHWNKKKYSS YEE	0.7807
KKHDDGIIIIAIFLQVSDHKNVYFQKIVNQLDSIRSANMSAPF DSVFYLDNLLPSTLDYFTYLGTTINHSADA	0.4468
WIIFPTPINIHSDQLSKFRTLLSSSNHEGKP	0.0067
YITENYRNPYKLNDDTQVYYSGEIIRAATTSPVRENYFMKWL SDLR	0.1645
CFSYYQKYIEGNKTFAIIAIVFVFILT	0.6078

Phylogenetic analysis:

Sequence comparison and alignment results revealed that E8L protein was conserved, with 98.36% to 100% identity among all Monkeypox virus genomes isolates in till June 8, 2022.

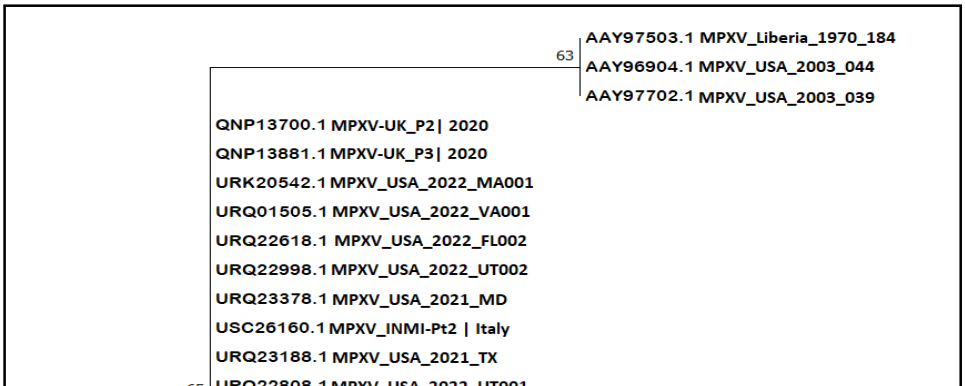

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Figure 2: Phylogenetic analysis of the top similar sequences of MPXV.

3.2. Identification and prediction of B cell epitopes:

The BCPRED of B cell proposed 5 epitopes, and all epitopes were found 100% conserved using IEDB conservation tools, and thus can generate immune response (Table 2)

Table 2: List of linear B-cell epitopes with their location and score by using the BCPRED server:

Position	Epitope	Score
94	VHWNKKKYSSYEEAKKHDDG	0.966
64	STIHIYWGKEDDYGSNHLID	0.935
238	IRAATTSPVRENYFMKWLSD	0.912
204	SSSNHEGKPHYITENYRNPY	0.891
43	VRINFKGGYISGGFLPNEYV	0.851

3.3. Prediction of T cell epitopes

Tables 3 summarizes the most promising peptides bound to MHC I along with their immunogenicity and allergenicity predication.

Table 3: Most promising epitopes and their immunogenicity profile

Residue position	Peptide	Alleles	IC50	Allergen.	Predicted binding affinity	C-terminal cleavage efficiency	TAP transport efficiency	Combi score
274-282	KTFAIIAIV	HLA-A*02:01, HLA-A*02:06, HLA-A*30:01, HLA-A*32:01,	57.58	Non-allergen	0.0849	0.5324	0.6530	0.4730

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		HLA-A*68:02, HLA-C*12:03						
142-150	RSANMSAPF	HLA-A*32:01, HLA-B*15:01, HLA-B*58:01, HLA-C*03:03	5.13	Non-allergen	0.1234	0.3332	2.8450	0.7160
276-284	FAIIAIVFV	HLA-A*02:06, HLA-A*68:02, HLA-C*03:03, HLA-C*12:03	14.07	Non-allergen	0.0772	0.1201	0.3510	0.3633
146-154	MSAPFDSVF	HLA-B*15:01, HLA-B*35:01, HLA-B*57:01, HLA-C*12:03	10.1	Non-allergen	0.2462	0.9564	2.7800	1.3277

Regarding MHC II related peptides. 3256 peptides were retrieved from IEDB sever along with the associated alleles. Then each peptide were linked to the alleles associated with and the data was sorted according to the number of associated alleles. Six peptides were found to have the highest number of associated alleles while in the same time having IC-50 less than 500 (Table 4).

Table 4: MHC II associated peptides and alleles.

Start	End	Core Sequence	Combined alleles	Allergen.	Alleles NO.	Combined score
234	248	IRAATTSPV	HLA-DQA1*01:02/DQB1*05:01, HLA-DQA1*01:02/DQB1*06:02, HLA-DQA1*02:01/DQB1*03:01, HLA-DQA1*05:01/DQB1*03:03, HLA-DQA1*05:01/DQB1*03:01, HLA-DRB1*04:01,HLA-DRB1*04:05,HLA-DRB1*01:01, HLA-DRB1*13:02,HLA-DRB1*10:01,HLA-DRB1*09:01,HLA-DRB1*08:02, HLA-DRB1*07:01,HLA-DRB3*03:01, HLA-DRB4*01:03,HLA-DRB1*15:01,HLA-DRB3*02:02,HLA-DRB1*16:02,HLA-DRB5*01:01	Allergen	19	0.3878
248	262	FMKWLSDLR	HLA-DPA1*03:01/DPB1*04:02,HLA-DPA1*02:01/DPB1*01:01, HLA-DQA1*01:02/DQB1*05:01,HLA-DQA1*01:02/DQB1*05:02, HLA-	Non- allergen	16	0.3910

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			DRB1*04:05,HLA-DRB1*04:04,HLA-DRB1*04:01,HLA-DRB1*13:01,HLA-DRB1*10:01,HLA-DRB1*09:01,HLA-DRB1*16:02,HLA-DRB4*01:03,HLA-DRB4*01:01,HLA-DRB1*15:01,HLA-DRB1*16:02,HLA-DRB5*01:01			
195	209	FRTLLSSSN	HLA-DPA1*01:03/DPB1*03:01,HLA-DQA1*01:02/DQB1*05:01, HLA-DQA1*05:01/DQB1*03:03,HLA-DRB1*01:01,HLA-DRB1*04:05,HLA-DRB1*04:01,HLA-DRB1*10:01,HLA-DRB1*08:02,HLA-DRB1*11:01,HLA-DRB1*09:01,HLA-DRB1*07:01,HLA-DRB1*12:01,HLA-DRB1*16:02,HLA-DRB5*01:01,HLA-DRB1*15:01,HLA-DRB3*02:02	Non- allergen	16	0.2164
59	73	YVLSTIHIY	HLA-DPA1*01:03/DPB1*06:01,HLA-DPA1*01:03/DPB1*02:01, HLA-DPA1*01:03/DPB1*04:01,HLA-DRB1*01:01,HLA-DRB1*04:05,HLA-DRB1*04:01,HLA-DRB1*13:01,HLA-DRB1*07:01,HLA-DRB1*09:01,HLA-DRB1*10:01,HLA-DRB1*08:01,HLA-DRB1*13:02,HLA-DRB3*02:02,HLA-DRB4*01:03,HLA-DRB3*03:01,HLA-DRB5*01:01	Non- allergen	16	1.339
287	301	FLMSQRYSR	HLA-DPA1*01:03/DPB1*06:01,HLA-DQA1*02:01/DQB1*04:02, HLA-DQA1*06:01/DQB1*04:02,HLA-DQA1*05:01/DQB1*04:02, HLA-DRB1*03:01,HLA-DRB1*01:01,HLA-DRB1*11:01,HLA-DRB1*08:01,HLA-DRB1*10:01,HLA-DRB1*08:02,HLA-DRB4*01:03,HLA-DRB5*01:01,HLA-DRB4*01:01,HLA-DRB1*16:02,HLA-DRB3*02:02	Non- allergen	15	0.4460
165	179	FTYLGTTIN	HLA-DQA1*01:02/DQB1*05:01,HLA-DQA1*02:01/DQB1*04:02, HLA-DQA1*02:01/DQB1*03:01,HLA-DQA1*02:01/DQB1*03:03, HLA-DQA1*05:01/DQB1*04:02,HLA-DQA1*06:01/DQB1*04:02, HLA-DQA1*05:01/DQB1*03:02,HLA-DRB1*01:01,HLA-DRB1*04:05,HLA-DRB1*04:01,HLA-DRB1*10:01,HLA-DRB1*07:01,HLA-DRB1*08:01,HLA-DRB1*09:01,HLA-DRB5*01:01	Allergen	15	0.2527

3.4. Population coverage:

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The world coverage for the predicted epitopes are summarized in figures 3 and 4.

Figure 3: Shows the global coverage for the top 10 MHC-I peptides.

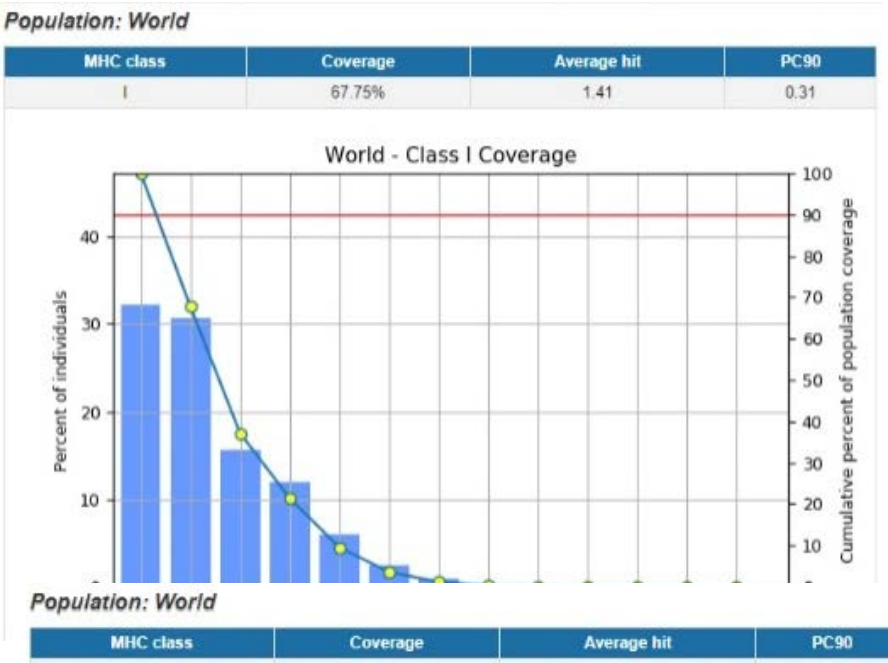
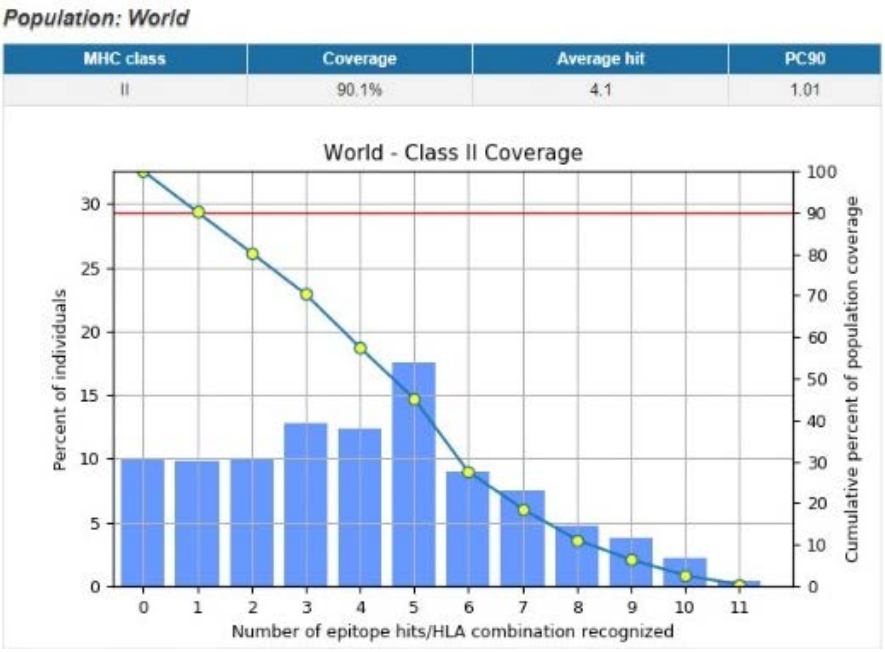


Figure 4: Shows the global coverage for the top 10 MHC-II peptides.



3.5. Secondary structure prediction:

PSIPRED was used to predict the secondary structures of the Monkeypox virus vaccine. The alpha helix residues are in pink, the beta strand residues are in yellow and the coil residues are in grey. According to the predicted secondary structure, the final vaccine contains 27.63% alpha helix, 21.71% beta strand, and 42.43% coil (Figure 5).

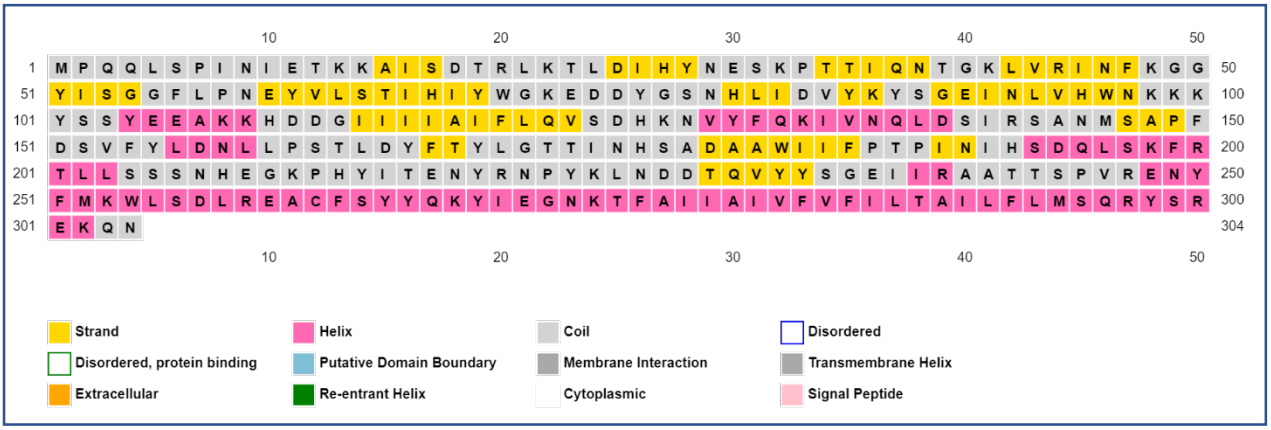


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Figure 5: Secondary structure analysis.

3.6. Homology modeling and protein validation:

The obtained 3D structure and the validation analysis are shown in Figures 6 & 7.

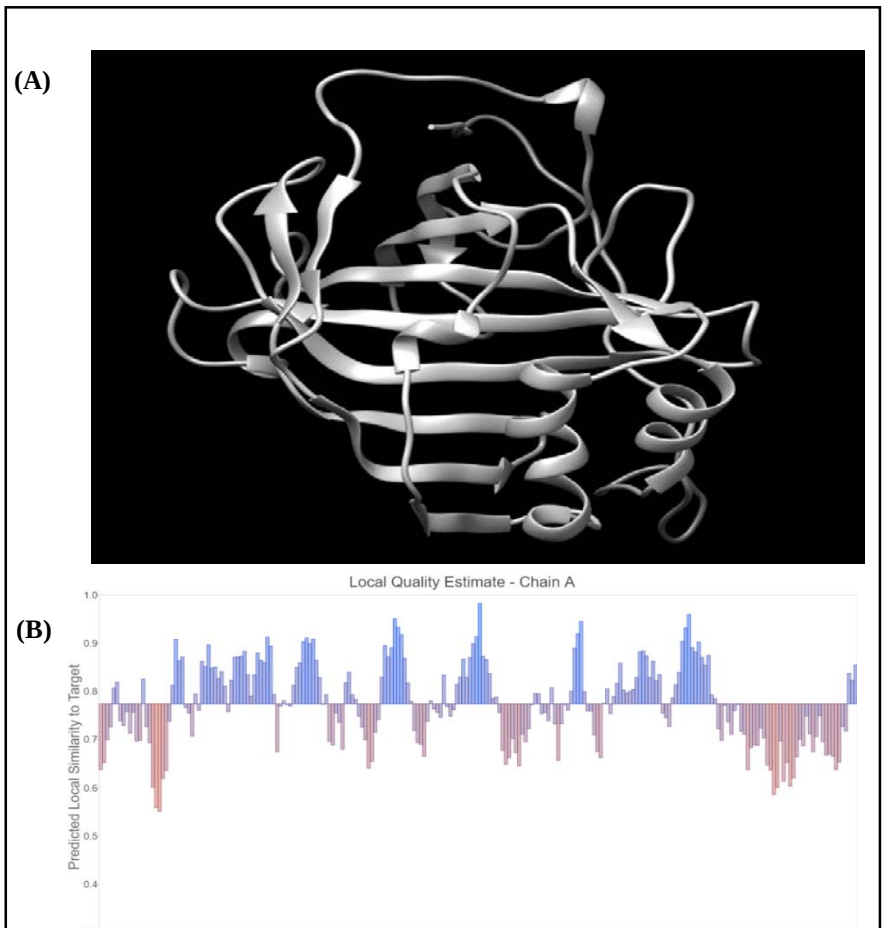

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Figure 6: shows 3D modeling of vaccine construct and validation (A) Naïve structure. (B) QMEAN server evaluates the structural superiority of cell surface-binding protein**.**

3.7. Physicochemical properties

The obtained results are summarized in Table 5.

Table 5: The obtained physicochemical properties:

Characteristics	Finding	Remark
Number of amino acids	304	Suitable
Molecular weight	35.28kDa	Average
Theoretical pI	7.77	Slightly basic
Chemical formula	$C_{1614}H_{2454}N_{412}O_{468}S_5$	-
Extinction coefficient (at 280nm in H_2O)	56270	-
Estimated half-life (mammalian reticulocytes, in vitro)	30h	-
Estimated half-life (yeast-cells, in vivo)	>20h	-
Estimated half-life (E. coli, in vivo)	>10h	-
Instability index of vaccine	39.84	Stable
Aliphatic index of vaccine	87.89	Thermostable
Grand average of hydropathicity (GRAVY)	-0.367	Hydrophilic

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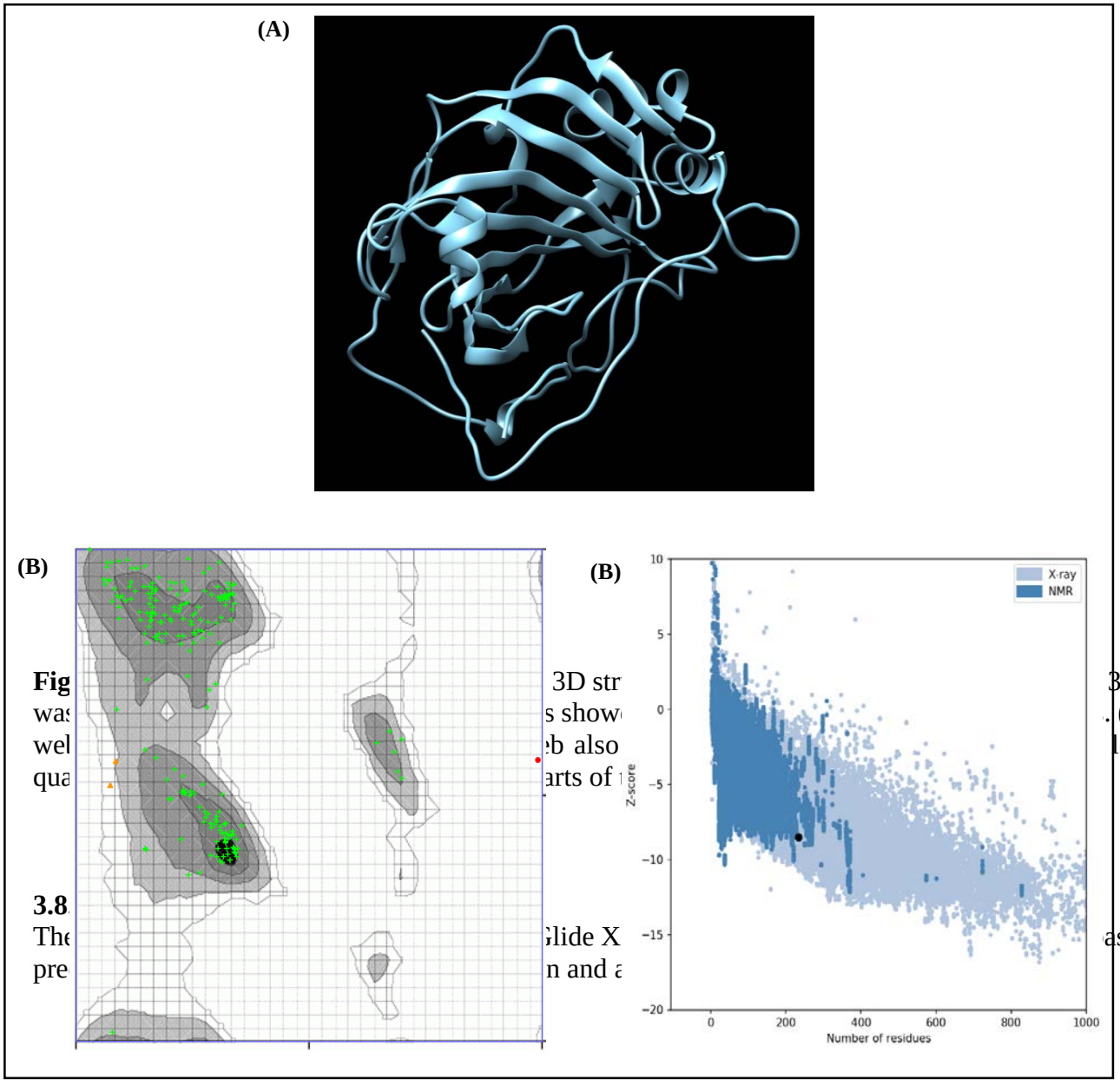

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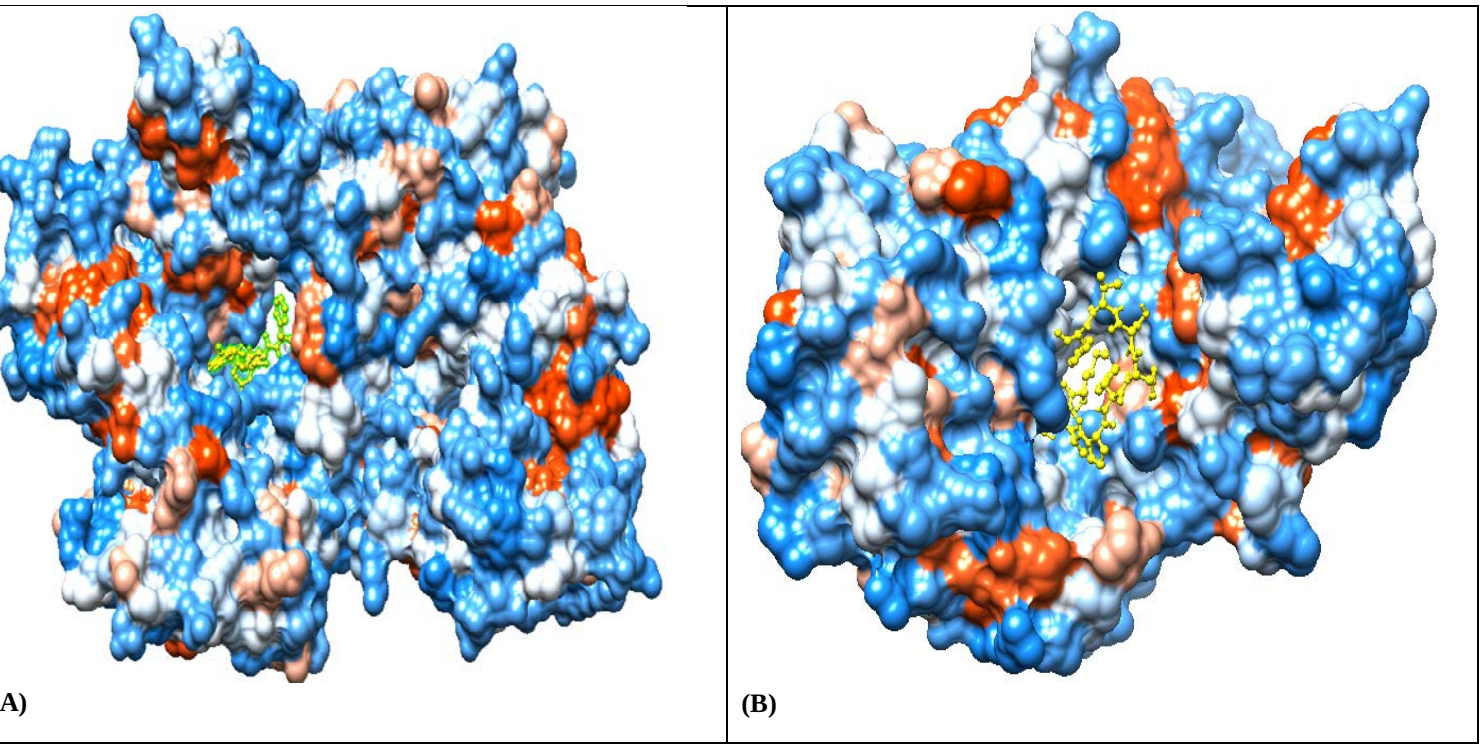

Figure 8: Representative surface view for Docking analysis of the predicted epitope: (A) RSANMSAPF with HLA-A*02:01. and (B) MSAPFDSVF with HLA-B*15:01.

Figure 9: 2D structure for the most promising peptides bound to MHC I alleles.

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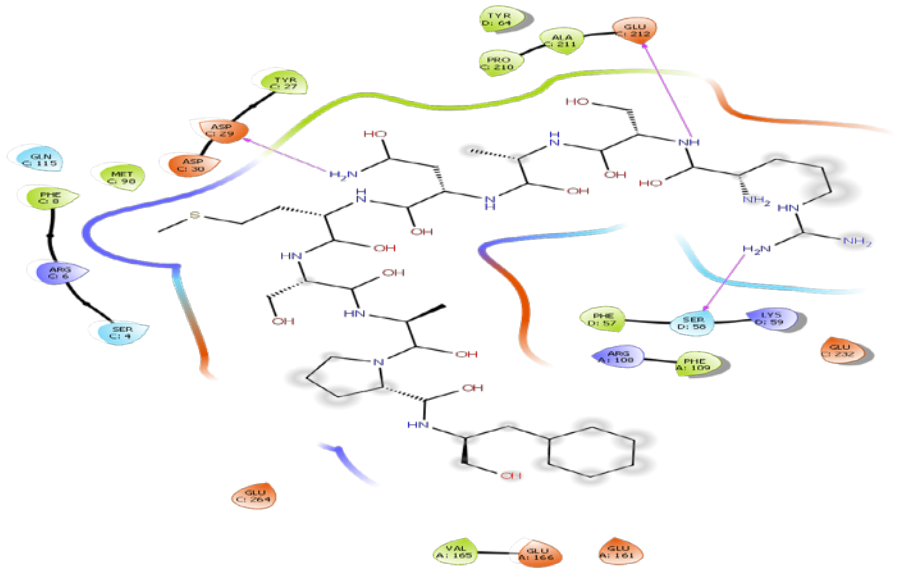
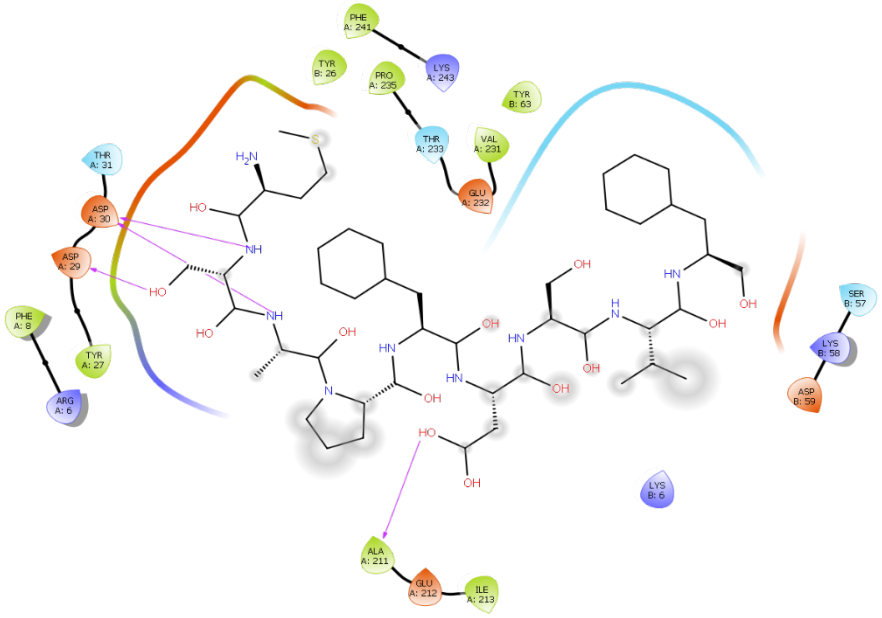
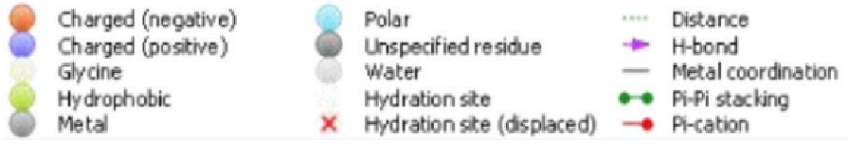


RSANMSAPF with HLA-A*02:01, E = -8.6kcal/mol



MSAPFDSVF with HLA-B*15:01, E = -7.5kcal/mol



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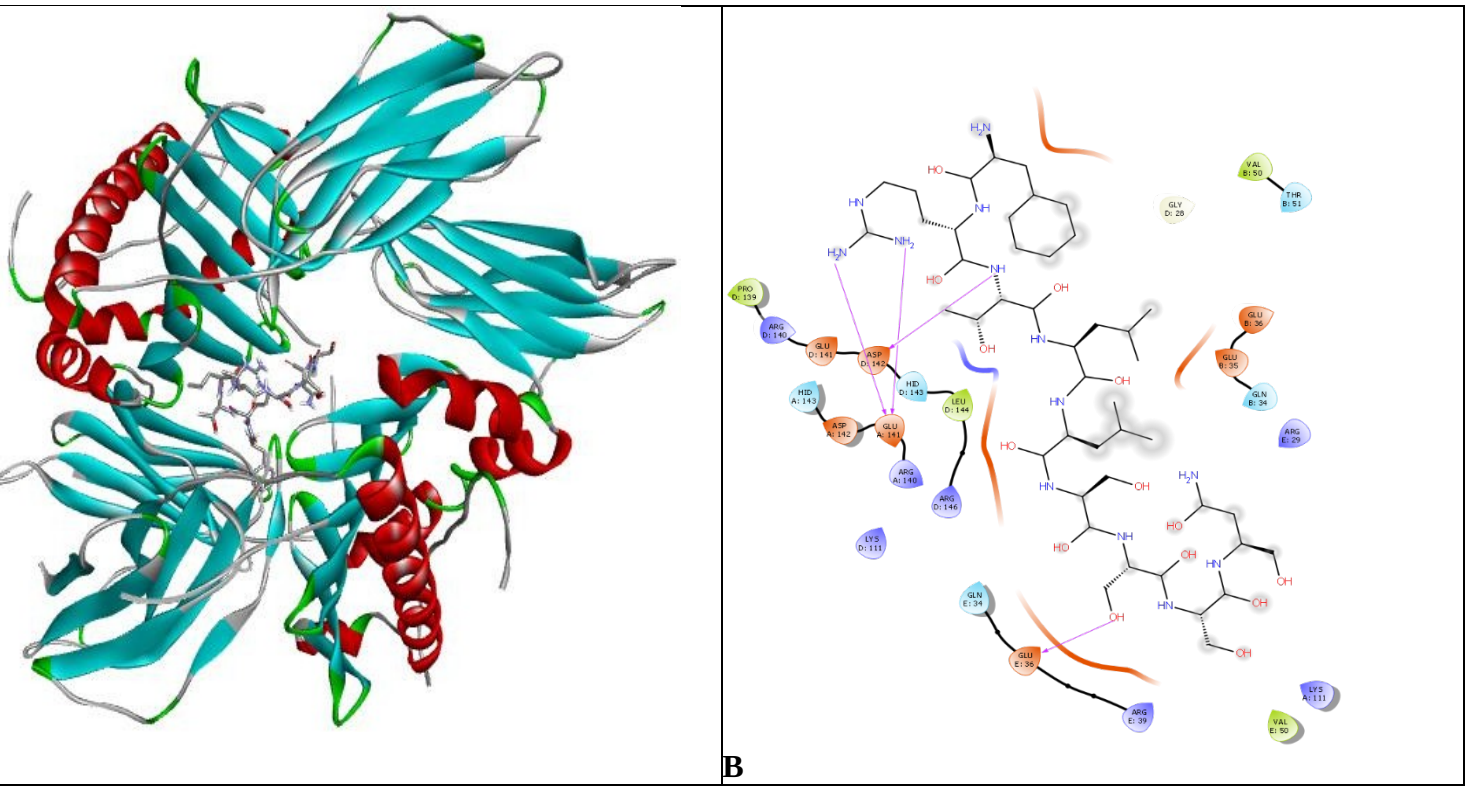

Figure 10: Representive view for Docking analysis of the predicted epitope YVLSTIHIY (A) and FLMSQRYSR (B) with HLA-DRB1.

3.9. Solubility prediction:

The obtained results are shown in Figure 11.

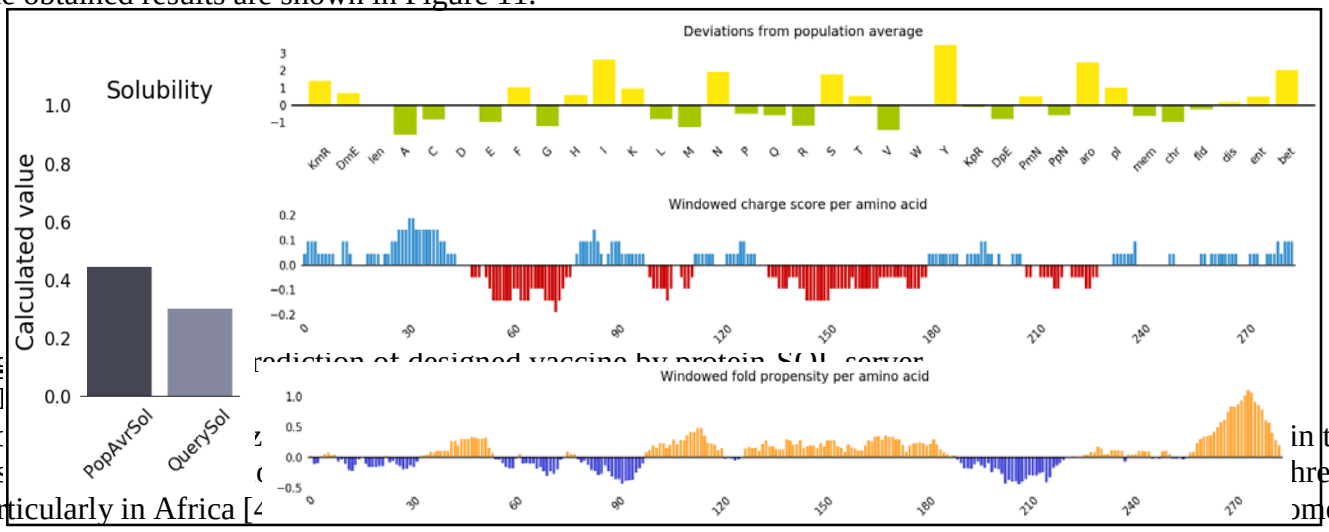

Fig
4.]
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pas hreat,
particularly in Africa [ome a
global public health threat in a very short period. Thus, the current work was planned and then manufactured a multi-epitope subunit vaccine that can evoke an immunological response against MPXV utilizing cell surface-binding protein as a target in order to develop a novel vaccine that is both safe and almost free of side effects. Cell surface-binding protein is responsible of vital biological processes for MPXV such as viral entry into host cell, virion attachment to host cell and Host-virus interaction [45].

The scientific basis of peptide vaccines is produced by chemical process of synthesizing immunogenic B-cell and T-cell epitopes that can trigger specific antibodies. To create a target molecule immunogenic, a B-cell epitope can be coupled with a T-cell epitope. T-cell epitopes are small peptide fragments (eight to twenty amino acids), while B-cell epitopes might be proteins [46, 47].

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A 100% conserved epitope among the filtered sequence of cell surface-binding polyprotein that could be identified by B and T cells to work as vaccine candidates was suggested in this study.
Many epitopes have been reported to interact with MHCI and MHCII alleles from cell surface- binding protein polyprotein in T cells. As vaccine candidates, antigenic, non-allergic, nontoxic epitopes with high population coverage were chosen (Tables 3 & 4).
To be considered a universal vaccine, the presented epitopes must interact with the majority of ethnic polymorphic MHC I and MHC II alleles with high population coverage scores. The population coverage of predicted epitopes reacting with T lymphocytes was evaluated in this regard. The proposed epitopes interacted more strongly with MHC I and MHC II alleles and linked to various sets of alleles with high population coverage scores (Figures 3 & 4). This finding observed that the proposed epitopes as vaccine candidates could cover a large population and effectively interact with human common alleles all over the world.
A peptide must have a range of physiochemical properties in order to operate as an effective vaccine. One of these physiochemical characteristics is molecular weight. Although lymph node exposure is directly proportional, the peptide's half-life inside the body is inversely linked to its molecular weight [48]. As a result, peptides bigger than 50 kDa are recommended for maximal half-life and lymph node exposure in the formation of an active immune response. Furthermore, the hydrophobicity and hydrophilicity of the vaccine design have a substantial influence on its efficacy. The ProtParam tool, which is available on the Expasy service, was used to assess the physiochemical attributes. The molecular weight was predicted to be 35.28kDa with a predicted half-life of 30 hours in mammalian reticulocytes.
proteins with a molecular weight of less than 110 kDa are thought to be better candidates for vaccine development [49]. The vaccine structure's isoelectric point was discovered to be 7.77, indicating the playful character of the intended vaccine structure. According to the ProtParam tool, the structure's instability index is 39.84, indicating that it is a stable protein. The alpha index, which reflects the protein's stability across a large temperature range, was reported to be 87.89for this developed vaccine construct because the range of this index for stable proteins is less than 40 results. Its GRAVY value is − 0.37, which is a negative value of this index, indicating the nature of the hydrophilic structure of the vaccine, and therefore can interact strongly with water molecules.
B-lymphocytes are an important part of the humoral immune system because they produce a wide spectrum of pathogen-specific antibodies that help to neutralize antigens and eliminate viral loads. The BCPRED service was used to assess the antigenic proteome in order to add epitopes with the potential to activate B-cells into the vaccine design. Five conserved epitopes with high scores were discovered (Table 2).
The adaptive immune system is triggered by MHC class I epitopes activating Cytotoxic T-cells. The CTL epitopes are further in charge of building long-lasting immunity capable of eradicating circulating virus and infected cells [50]. As a result, MHC class I epitope prediction is critical for vaccine development. In a nutshell, multiple MHC I and II epitopes prediction web-based platforms were used to test the specified viral components of Monkeypox virus. Before being nominated for downstream investigations, T cell epitopes were rated using strict in-house criteria. Strong IEDB score, high conservancy, good binding affinity, 9mer for MHC I, 15mer for MHC II, considerably antigenic/immunogenic, and topographically accessible to membrane-bound or free antibody are only a few of the selection criteria. Lower percentile rankings and IC50 values were evaluated for high immunogenicity. According to these guidelines, only two epitopes (RSANMSAPF and MSAPFDSVF) bound to MHC I alleles (Table 3), and one peptide bound to MHC II (YVLSTIHIY) (Table 4) were selected for further analysis.
The interactions between antigenic molecules and immune receptor molecules are critical for efficient antigenic transport and immune response activation [51]. In order to study probable interactions, binding energy, and poses, docking analysis was performed between immune receptor molecules and the developed epitopes. Docking analysis was performed utilizing Schrödinger which evaluates postures, including their solid molecular surface display (Figures 8 -10)
The creation of H-bonds in the protein-ligand complex is a crucial metric for determining the stability of the conformation across the simulation period [52]. The 2D view was utilized to investigate the interactions and bonding with MHC molecules (Figure 9 & 10). RSANMSAPF and MSAPFDSVF were bonded to the groove

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of HLA-A*02:01and HLA-B*15:01 with binding energies of -8.1 kcal/mole and -7.5 kcal/mole, respectively. RSANMSAPF established three hydrogen bonds with ASP29; SER58; and GLU212 residues, while MSAPFDSVF established four hydrogen bonds with ASP29, ASP30, and ALA211 residues. While YVLSTIHIY was bound to the groove of HLA-DRB1 with binding energy of -7.1 kcal/mole.

The vaccine construct has alpha helices, extended strands, beta turns, and random coiled structures, according to the secondary structure analysis. The refined software greatly improved the three-dimensional structures of the vaccine construct, which illustrated desirable properties on Ramachandran plot prediction model. Still, one of the most difficult problems in structural biology is detecting errors in experimental and theoretical models of protein structures. [53]. As a result, the ProSA program was used to predict possible structural and modeling errors in the vaccine. ProSA measured the overall quality score for a particular input structure. The outcome was shown in a plot that included the scores of all experimentally protein chains publicly available in the Protein Data Bank. [53]. The predicted vaccine construct in this study had a Z-score of -8.5. This revealed that the overall model's reliability as a vaccine against MPXV is satisfied. The solubility of the protein being over-expressed in the *E. coli* host is important for various functional and biochemical studies [54]. When the vaccine protein was over-expressed in *E. coli*, it was revealed to be soluble. As highly soluble proteins during downstream processing exhibit ease of purification, this proves better post-production outcomes [55]. As a conclusion 0f promising results, constructing a vaccine based on the proposed peptides seems to be a high priority, with the potential to be extensively deployed as a universal epitope-based peptide vaccine against MPXV.

5. Conclusion:

The current work was planned and then manufactured a multi-epitope vaccine that can evoke an immunological response against MPXV utilizing cell surface-binding protein as a target in order to develop a novel vaccine that is both safe and almost free of side effects. We conclude from our research that the cell surface-binding protein is one of the primary proteins involved in MPXV pathogenesis. The most promising epitopes were then selected using a rigorous procedure and used for vaccine design. As a result, our study will aid in the development of appropriate therapeutics and prompt the development of future vaccines against MPXV, which could serve as an important milestone in the production of an antiviral vaccine against MPXV.

Authors' Contribution:

Study concept and design: S.W.S and M.I.M; Analysis and interpretation of data: S.W.S, M.I.M, A.H.A, H.A.F and S.G.E; Drafting of the manuscript: S.W.S and M.I.M; Statistical analysis: S.W.S and M.I.M; Critical revision of the manuscript: A.M.M.

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Conflicts of interest:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References:

1. *WHO disease outbreak news. Monkeypox - United Kingdom of Great Britain and Northern Ireland.* 2022.
2. Heymann, D.L., M. Szczeniowski, and K. Esteves, *Re-emergence of monkeypox in Africa: a review of the past six years.* Br Med Bull, 1998. **54**(3): p. 693-702.
3. Bunge, E.M., et al., *The changing epidemiology of human monkeypox-A potential threat? A systematic review.* PLoS Negl Trop Dis, 2022. **16**(2): p. e0010141.
4. Beer, E.M. and V.B. Rao, *A systematic review of the epidemiology of human monkeypox outbreaks and implications for outbreak strategy.* PLoS Negl Trop Dis, 2019. **13**(10): p. e0007791.
5. Chastel, C., *[Human monkeypox].* Pathol Biol (Paris), 2009. **57**(2): p. 175-83.

6. Mazurkov, O.Y., et al., *New effective chemically synthesized anti-smallpox compound NIOCH-14.* J Gen Virol, 2016. **97**(5): p. 1229-1239.
7. Nalca, A., et al., *Reemergence of monkeypox: prevalence, diagnostics, and countermeasures.* Clin Infect Dis, 2005. **41**(12): p. 1765-71.
8. Cunha, B.E., *Monkeypox in the United States: an occupational health look at the first cases.* Aaohn j, 2004. **52**(4): p. 164-8.
9. *CDC Identifies Two Monkeypox Strains in US; Provides Updates on Investigation - Medscape - Jun 03, 2022.*
10. Li, Y., et al., *Detection of monkeypox virus with real-time PCR assays.* J Clin Virol, 2006. **36**(3): p. 194-203.
11. Li, Y., et al., *Real-time PCR assays for the specific detection of monkeypox virus West African and Congo Basin strain DNA.* J Virol Methods, 2010. **169**(1): p. 223-7.
12. Saijo, M., et al., *Diagnosis and assessment of monkeypox virus (MPXV) infection by quantitative PCR assay: differentiation of Congo Basin and West African MPXV strains.* Jpn J Infect Dis, 2008. **61**(2): p. 140-2.
13. *Multi-country monkeypox outbreak: situation update.* 2022.
14. *Monkeypox goes global: why scientists are on alert. 2022. Available: https://www.nature.com/articles/d41586-022-01421-8.* 2022.
15. Kugelman, J.R., et al., *Genomic variability of monkeypox virus among humans, Democratic Republic of the Congo.* Emerg Infect Dis, 2014. **20**(2): p. 232-9.
16. Cho, C.T. and H.A. Wenner, *Monkeypox virus.* Bacteriol Rev, 1973. **37**(1): p. 1-18.
17. Esposito, J.J. and J.C. Knight, *Orthopoxvirus DNA: a comparison of restriction profiles and maps.* Virology, 1985. **143**(1): p. 230-51.
18. Hammarlund, E., et al., *Multiple diagnostic techniques identify previously vaccinated individuals with protective immunity against monkeypox.* Nat Med, 2005. **11**(9): p. 1005-11.
19. *Supplemental recommendations on adverse events following smallpox vaccine in the pre-event vaccination program: recommendations of the Advisory Committee on Immunization Practices.* MMWR Morb Mortal Wkly Rep, 2003. **52**(13): p. 282-4.
20. Rotz, L.D., et al., *Vaccinia (smallpox) vaccine: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2001.* MMWR Recomm Rep, 2001. **50**(Rr-10): p. 1-25; quiz CE1-7.
21. Lo, Y.T., et al., *Prediction of conformational epitopes with the use of a knowledge-based energy function and geometrically related neighboring residue characteristics.* BMC Bioinformatics, 2013. **14 Suppl 4**(Suppl 4): p. S3.
22. Li, W., et al., *Peptide Vaccine: Progress and Challenges.* Vaccines (Basel), 2014. **2**(3): p. 515-36.
23. Doytchinova, I.A. and D.R. Flower, *VaxiJen: a server for prediction of protective antigens, tumour antigens and subunit vaccines.* BMC Bioinformatics, 2007. **8**: p. 4.
24. Vita, R., et al., *The Immune Epitope Database (IEDB): 2018 update.* Nucleic Acids Res, 2019. **47**(D1): p. D339-d343.
25. Bui, H.H., et al., *Development of an epitope conservancy analysis tool to facilitate the design of epitope-based diagnostics and vaccines.* BMC Bioinformatics, 2007. **8**: p. 361.
26. Kolaskar, A.S. and P.C. Tongaonkar, *A semi-empirical method for prediction of antigenic determinants on protein antigens.* FEBS Lett, 1990. **276**(1-2): p. 172-4.
27. Nielsen, M., et al., *Reliable prediction of T-cell epitopes using neural networks with novel sequence representations.* Protein Sci, 2003. **12**(5): p. 1007-17.
28. Wang, P., et al., *A systematic assessment of MHC class II peptide binding predictions and evaluation of a consensus approach.* PLoS Comput Biol, 2008. **4**(4): p. e1000048.
29. Larsen, M.V., et al., *Large-scale validation of methods for cytotoxic T-lymphocyte epitope prediction.* BMC bioinformatics, 2007. **8**: p. 424-424.
30. Bui, H.H., et al., *Predicting population coverage of T-cell epitope-based diagnostics and vaccines.* BMC Bioinformatics, 2006. **7**: p. 153.
31. McGuffin, L.J., K. Bryson, and D.T. Jones, *The PSIPRED protein structure prediction server.* Bioinformatics, 2000. **16**(4): p. 404-5.
32. Buchan, D.W.A. and D.T. Jones, *The PSIPRED Protein Analysis Workbench: 20 years on.* Nucleic Acids Res, 2019. **47**(W1): p. W402-w407.
33. Jones, D.T., *Protein secondary structure prediction based on position-specific scoring matrices.* J Mol Biol, 1999. **292**(2): p. 195-202.

34. Wilkins, M.R., et al., *Protein identification and analysis tools in the ExPASy server.* Methods Mol Biol, 1999. **112**: p. 531-52.
35. Dimitrov, I., et al., *AllerTOP v.2--a server for in silico prediction of allergens.* J Mol Model, 2014. **20**(6): p. 2278.
36. Heo, L., H. Park, and C. Seok, *GalaxyRefine: Protein structure refinement driven by side-chain repacking.* Nucleic Acids Res, 2013. **41**(Web Server issue): p. W384-8.
37. Laskowski, R., et al., *PROCHECK: A program to check the stereochemical quality of protein structures.* Journal of Applied Crystallography, 1993. **26**: p. 283-291.
38. *Schrödinger Release 2022-2: CombiGlide, Schrödinger, LLC, New York, NY, 2021.* 2021.
39. Hebditch, M., et al., *Protein-Sol: a web tool for predicting protein solubility from sequence.* Bioinformatics, 2017. **33**(19): p. 3098-3100.
40. Mauldin, M.R., et al., *Exportation of Monkeypox Virus From the African Continent.* J Infect Dis, 2022. **225**(8): p. 1367-1376.
41. Costello, V., et al., *Imported Monkeypox from International Traveler, Maryland, USA, 2021.* Emerg Infect Dis, 2022. **28**(5): p. 1002-1005.
42. Yong, S.E.F., et al., *Imported Monkeypox, Singapore.* Emerg Infect Dis, 2020. **26**(8): p. 1826-1830.
43. Kozlov, M., *Monkeypox goes global: why scientists are on alert.* Nature, 2022. **606**(7912): p. 15-16.
44. Yang, Z., *Monkeypox: A potential global threat?* J Med Virol, 2022.
45. Alkhalil, A., et al., *Gene expression profiling of monkeypox virus-infected cells reveals novel interfaces for host-virus interactions.* Virol J, 2010. **7**: p. 173.
46. Dermime, S., et al., *Vaccine and antibody-directed T cell tumour immunotherapy.* Biochim Biophys Acta, 2004. **1704**(1): p. 11-35.
47. Meloen, R.H., et al., *Synthetic peptide vaccines: unexpected fulfillment of discarded hope?* Biologicals, 2001. **29**(3-4): p. 233-6.
48. Wu, F., et al., *Fluorescence imaging of the lymph node uptake of proteins in mice after subcutaneous injection: molecular weight dependence.* Pharm Res, 2012. **29**(7): p. 1843-53.
49. Barh, D., et al., *Exoproteome and secretome derived broad spectrum novel drug and vaccine candidates in Vibrio cholerae targeted by Piper betel derived compounds.* PLoS One, 2013. **8**(1): p. e52773.
50. Kar, T., et al., *A candidate multi-epitope vaccine against SARS-CoV-2.* Sci Rep, 2020. **10**(1): p. 10895.
51. Chaplin, D.D., *Overview of the immune response.* J Allergy Clin Immunol, 2010. **125**(2 Suppl 2): p. S3-23.
52. Falk, K., et al., *Ligand exchange of major histocompatibility complex class II proteins is triggered by H-bond donor groups of small molecules.* J Biol Chem, 2002. **277**(4): p. 2709-15.
53. Wiederstein, M. and M.J. Sippl, *ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins.* Nucleic Acids Res, 2007. **35**(Web Server issue): p. W407-10.
54. Almofti, Y.A., K.A. Abd-Elrahman, and E.E.M. Eltilib, *Vaccinomic approach for novel multi epitopes vaccine against severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2).* BMC Immunol, 2021. **22**(1): p. 22.
55. Chang, C.C., et al., *Bioinformatics approaches for improved recombinant protein production in Escherichia coli: protein solubility prediction.* Brief Bioinform, 2014. **15**(6): p. 953-62.