Immunol Genet J, Vol. 8, No. 4, 2025, pp.310-328 **DOI: https://doi.org/10.18502/igj.v8i4.20099**

Original Article

Unraveling the M1R Protein of Monkeypox Virus: An Integrated Structural Bioinformatics, Immunological Profiling, and Molecular Dynamics Simulation Approach

Cena Aram[1,2†], Kiarash Saleki[3,4,5,6†], Amirreza Mazloomi[4,6], Maryam Barancheshmeh[7], Nima Rezaei[3,4,8*]

[1] *Department of Cell & Molecular Biology, Faculty of Biological Sciences, Kharazmi University, Tehran, Iran*

[2] *Universal Scientific Education and Research Network (USERN), Babol, Iran*

[3] *Research Center for Immunodeficiencies, Children's Medical Center Hospital, Tehran University of Medical Sciences, Tehran, Iran*

[4] *Student Research Committee, Babol University of Medical Sciences, Babol, Iran*

[5] *Network of Immunity in Infection, Malignancy and Autoimmunity (NIIMA), Universal Scientific Education and Research Network (USERN), Stockholm, Sweden*

[6] *USERN MUBabol Office, Universal Scientific Education and Research Network (USERN), Babol, Iran*

[7] *Universal Scientific Education and Research Network (USERN), Nowshahr, Iran*

[8] *Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*

†Co-first authorship and contributed equally.

Received: 03 July 2025; Accepted: 08 October 2025

Abstract

Background: Monkeypox virus (MPXV) is a zoonotic pathogen that affects both humans and animals, posing a significant public health concern due to its emergence and circulation. The structural dynamics and features of several MPXV proteins, including M1R, are not completely studied.

Methods: This experiment focuses on the prediction and analysis of the secondary and tertiary constructs for the M1R protein. Briefly, its amino acid sequence was collected from the UniProt database. A wide range of in silico approaches were employed, including ProtParam, SOPMA, PSIPRED, CD Search, GalaxyTMB, Robetta, I-TASSER, and GROMACS, in order to explore the physicochemical properties, structural features, and functional insights of the M1R protein. The tertiary structure models were evaluated to detect the most reliable solution, which was then used for Immunoinformatics analyses such as MHC I/II and B-cell epitope prediction using the IEDB and Ellipro tools, respectively. Epitopes from the M1R protein were evaluated based on antigenicity, affinity of binding, along solubility. Furthermore, active sites were forecast by the CASTp v3.0 tool.

Results: Physicochemical calculations indicate that M1R had favorable thermostability and hydrophilic features. Structural analyses suggested that M1R is a lipid membrane protein component of DNA viruses, suggesting it as a robust antigenic target. Immunogenicity analyses indicated it as a potentially suitable target for immunogenic protein design. As well, molecular dynamics simulations (MDS) were carried out for 100-ns using an all-atom forcefield. Analysis of various molecular dynamics parameters of M1R throughout the MDS trajectory, including RMSD, RMSF, radius of gyration (Rg), and solvent accessible surface area (SASA), indicated good stability of the M1R and unveiled important molecular dynamics characteristics such as the flexibility of certain protein regions. Multiple epitopes were detected in our experiment, with 12 B-cell epitopes identified using the Robetta model and 6 B-cell epitopes predicted by the Galaxy model, alongside 3 MHC-I and 3 MHC-II epitopes, which scored favorably.

Conclusion: The results of the present computational analysis provide clues to unleash the potential of M1R as an immunotherapy target for the development of antiviral solutions against MPXV in the future.

Keywords: Monkeypox; Molecular Mechanics, Molecular Dynamic Simulation; M1R Protein; Structural Immunology

***Corresponding Author:** Nima Rezaei, MD, PhD

Research Center for Immunodeficiencies, Pediatrics Center of Excellence, Children's Medical Center Hospital, Tehran University of Medical Sciences, Tehran, Iran

E-mail: rezaei_nima@tums.ac.ir

How to cite this article

Aram C, Saleki K, Mazloomi A, Barancheshmeh M, Rezaei N. Unraveling the M1R Protein of Monkeypox Virus: An Integrated Structural Bioinformatics, Immunological Profiling, and Molecular Dynamics Simulation Approach. Immunol Genet J, 2025; 8(4): 310-328. DOI: https://doi.org/10.18502/igj.v8i4.20099

Introduction

Monkeypox virus (MPXV) is a structurally complex and large virus from the Orthopox virus (OPXV) genus, Chordopoxvirinae subfamily, and Poxviridae family (1,2). The genome of MPXV comprises 197kb double-stranded (ds) DNA forming an oval-shaped virion with 190 nonoverlapping open reading frames (ORFs) as well as inverted terminal repeat sequences (3). MPXV structure resembles an oval or a brick. The virus is sized about 200-250 nanometers (4). Clinical manifestations of infection by MPXV include a wide range of symptoms including fever, headache, myalgia, lymphadenopathy, and dermatological presentations (5–9). In addition, multi-system adverse sequelae have been reported, including neurological disorders, as well as other manifestations such as encephalitis, sepsis, secondary bacterial infection, bronchopneumonia, conjunctivitis, corneal scarring, as well as miscarriage in pregnant individuals (10).

The MPXV was first detected among laboratory monkeys in Copenhagen in the late1950s with the first case of human monkeypox infectious being reported in a 9-month-old boy in 1970 in Congo (11–13). This virus could be disseminated either from animals to humans or between humans. Zoonotic transmission often occurs via direct contact with infected body fluids as well as touch, bites, scratches, or during handling of dead animals (14,15). This mode of transmission was implicated in the onset of the MPX outbreaks in Central and Western Africa in 1970. The second form of transmission, from human to human, may be responsible for the increasing number of MPXV cases worldwide. The main source of human-to-human transmission is by direct contact with respiratory secretions, skin lesions, genital fluids or prolonged face-to-face interactions (16–18).

Diagnosis of MPXV consists of a combination of genetic, phenotypic, immunologic, and electron microscopic techniques PCR is the gold standard method for confirmation (19). Monkey Pox is on the rise in the world and is a global concern. Emerging reports have documented an increase in cases, notably in non-endemic nations, such as the USA, the UK, Nigeria, and Singapore (20,21). According to the worldwide outbreak map by centers for disease control (CDC), by late 2022, over about 58,000 verified cases have been reported in a wide range of countries (22). A CDC report also indicated 30,123 cases and 28 deaths in the United States from 2022 to February 1, 2023. In total, reports indicated 85,536 cases and 91 events of mortality from 110 and 71 different regions, respectively (23).

The MPXV genome comprises approximately 197,205 base pairs, with variable regions at its ends and conserved central region (24). This central genomic region contains structural proteins such as M1R protein. MPXV exists in two primary infectious forms: extracellular enveloped virus and intracellular mature virus (IMV) (25,26). On the other hand, M1R, a surface membrane protein found in IMV, is known to be highly conserved and plays a crucial role in viral particle assembly and entry (27). Studies have shown that this protein is one of the significant targets for neutralizing antibodies (26,28). Focus of studies has been on new experimental drugs along with controlled trials, which has led to the approval of multiple antiviral medications including tecovirimat and brincidofovir (29) as well as two vaccines, JYNNEOS and ACAM2000, for the treatment of critical infections by MPX (30).

Targeting the immune system using key disease or infection-related proteins has gained interest in recent years (31–33). Even though outbreaks in recent years and current treatment options show that M1R is a useful drug target, additional exploration and pursuit of novel therapeutics focusing on proteins such as M1R are highly needed. It could have implications for the genetic bioengineering of M1R as a pharmaceutical target, however, there could be restrictions that should be addressed in the vaccine development procedure to ensure vaccine efficacy and accessibility. A difficult task is to provoke specific immune response to special antigens in groups that are immunologically naïve to a fraction of antigens. For instance, even though JYNNEOS has been suggested to be a safe candidate.

On the other hand, this vaccine is a non-replicating immunotherapy candidate that been associated with varying efficacy, ranging from as low as 36% following one dose to 85.9% following complete vaccination protocol. (34). These differences point out the need for utilizing novel adjuvants and immunostimulatory sequences.

Another example ACAM2000 has demonstrated optimal efficacy, however, it causes complications like myocarditis and pericarditis (35). These points indicate the need for safer alternatives that sustain antigenic stability without safety being compromised.

A difficulty in MPXV immunotherapy is the maintenance of a stable antigen during the production processes, storage, and the delivery. A handful problems related to thermostability and the protection of antigens should be tackled, in particular in vaccine platforms, such as the mRNA-based strategy which derives its success from improved stability, manufacturing consistency and strong immunity. The development of safer alternatives with a better delivery systems and formulation strategies will lead to effective and accessible MPXV vaccines (36).

In recent years, the focus of Immunoinformatics has revolved around the rapid study of biological mechanisms as well as the acceleration of vaccine and drug development efforts (37–44). In this study, a structural analysis of the M1R protein to gain insights regarding its physicochemical and tertiary structure has been performed. In order to achieve this, we conducted several computational screenings to elucidate the biological functionality and the construct of the M1R protein, which may provide a reference for a vaccine or therapeutic target for MPXV. Molecular dynamic simulations were performed to validate the stability of the M1R protein. This study provides informative structural data on the M1R protein as well as suggests it as an invaluable immunotherapeutic target for the design of an MPXV vaccine candidate.

Method and Material

Retrieval of M1R Protein for Characterization of M1R

The M1R protein sequence (Uniprot ID: QJQ40223.1) was obtained from Uniprot (uniprot.org). Moreover, Blastp was utilized to look into its relationships with other proteins, together with their functional characteristics and related data. A search for structural information on the M1R protein was conducted using the RCSB database (rcsb.org), however, a proper structure was not found.

Physicochemical Properties

The ExPASy (the Expert Protein Analysis System) tool comprises SWISS-PROT and TrEMBL, PROSITE, ENZYME, SWISS-2DPAGE, and the SWISS-MODEL repository. Evaluation tools are accessible for special tasks related to proteomics, resemblance searches, pattern and profile exploration, post-translational modification forecasting, topology estimation, as well as primary, secondary and tertiary construct analysis and alignment of biological sequences (45). The evaluation of physicochemical properties was performed by the ExPASy Protparam tool web server (https://web.expasy.org/protparam).

Protein Function Prediction

Domain prediction was conducted via NCBI CD search and DeepGOweb server to comprehend the mechanism of protein and their function (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi,https://deepgo.cbrc.kaust.edu.sa/deepgo/).

Prediction of Secondary Structure

The PSIRED Workbench is provides a wide range of machine learning (ML) reliant evaluations for determining protein structure and function. In 2024, the tool has been revised to benefit further from Deep Learning approaches since the development of AlphaFold (46). The secondary structure of the protein was predicted by PSIPRED and SOPMA server (http://bioinf.cs.ucl.ac.uk/psipred/) to analyzed the 2D structure of M1R protein and also discovering of the disorder region of M1R protein predicted. The SOPMA showed the percent of the helix, sheet, turn, and coil.

Prediction of Tertiary Structure of M1R and Analysis of Protein

Initially, we conducted a comparative analysis of homologous proteins within the Vaccinia virus to identify those with significant similarities. The L1 protein (QTC35412.1) was extracted from NCBI that compare to M1R protein that used the MEGA11 program (47). In the RCSB there is no experimentally verified 3D structure for M1R protein. Accordingly, we used 3 sperate program, GalaxyTMB, Robetta, and I-TASSER web server to model the M1R protein structure. The Gal-

axyTMB (https://galaxy.seoklab.org/) is a kind of web server that predicted the structure from sequence by template based modeling and it refines the loops by ab initio method (48) that used for 3D structure. The Robetta server (https://robetta.bakerlab.org/) is a protein structure prediction that evaluated via CAMEO that based on RoseTTaFold, Comparative modeling (CM), and ab initio method. In addition, I-TASSER server was used to predict the tertiary structure, this server used the different template to design the best model for 3D structure. Furthermore, the analysis of transmembrane, cytoplasmic, and outer membrane was implemented by Philius Server (https://www.yeastrc.org/philius/) and TMHMM server (https://services.healthtech.dtu.dk/services/TMHMM-2.0/). Additionally, we employed AlphaFold2 to predict the 3D structure of the M1R protein, ensuring the selection of the best model. To further enhance the accuracy and reliability of the predicted structure, we refined the model using GalaxyRefine2 (49). For analyzing the residue interaction network (RING), we utilized the RING server (https://ring.biocomputingup.it/), applying it to the selected best model to comprehensively examine the interactions. (50).

Molecular Dynamic Simulation of M1R Protein

Molecular dynamics simulation (MDS) involves the prediction of system evolutions based on Newtonian motion laws. The approach has been refined over the years to enable the comprehensive analysis and investigation of the intricate physical conveyance and translational behavior of atoms, as well as the complex molecular interactions (51). MDS was performed by GROMACS 2022.4v. The structure was prepared for MDS and parametrized via the Amber99SB-ILDN force field and TIP3P water model in a cubic box enclosing the system at a 1 nm distance from the edges of the protein. VMD program (version 1.9) was utilized to visualize the simulation and confirm that the size of the cubic box was large enough to accommodate the protein without exceeding boundaries. In addition, the simulation system was neutralized with ions, and also the energy minimization (Fmax > 1000 KJ.mol-1. nm-1) was carried out after that the two phases of equilibration such as NVT and NPT were done at 298 oK and 1 bar in 100 ps. The final step of the MD simulation was run for 100-ns to analyze the structure of the M1R protein and its dynamics. The trajectory data was used to analyze and we used Root mean square deviation (RMSD), Root mean square fluctuation (RMSF), Radius of gyration (Rg), and Solvent accessible surface area (SASA).

Prediction of Epitopes

To characterize the M1R protein used different method to detect the immunological properties that the antigenicity, allergenicity, toxicity was predicted by VaxiJen, AlgPred, and ToxinPred. Then the antibody epitopes were predicted by Ellipro server (52) and analyzed the position of conformational epitopes. At last the MHC I/II epitopes was predicted by IEDB server that we selected the high score epitopes that had analyzed with Vaxijen (53), Allertop (54), Solpro (55) to introduce the best epitopes for designing vaccine (56).

Prediction of Active Site

Computed Atlas of Surface Topography of Proteins (CASTp) is a tool that enables the locating, delineating and evaluation of geometric and topological features for protein constructs. CASTp v3.0 server (57) was used to predict the active site of M1R protein. The top candidate model of M1R was test as the input for this web server and the output showed the active site. The workflow of this investigation has been summarized in **Figure 1.**

Result

Physicochemical Properties of the M1R Protein

The FASTA sequence of M1R protein was retrieved by Uniprot. The protein is assessed by physicochemical approaches to detect the nature of the protein. The M1R protein consists of 250 amino acids and has an overall molecular weight of 27303.24 Da. The Theoretical pI was computed to be 6.72 and the protein molecular formula was determined to be C1198H1920N320O376S15. The frequency of the amino acids is shown in **Figure 2** and the most frequency of the protein was Ala (11.2%), Thr (10.8%), Ilu (8.8%). Additionally, there were 20 positively charged residues (Arg+Lys) and 20 negatively charged residues

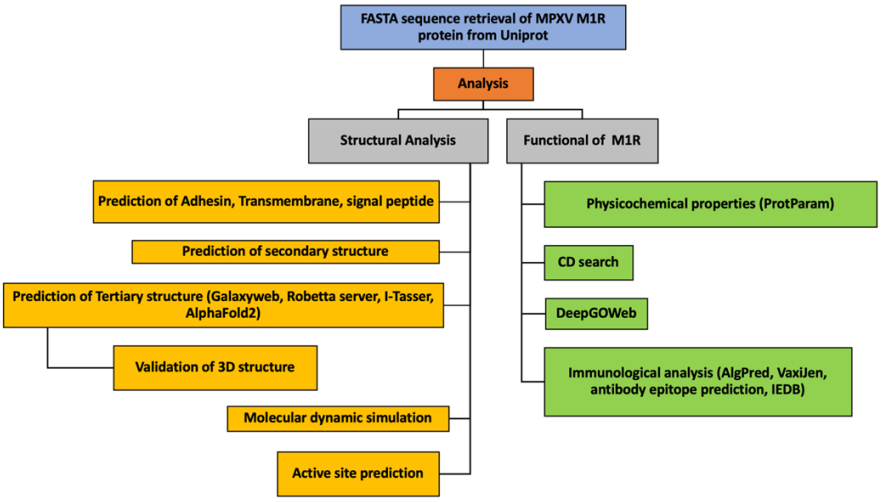

Figure 1. The workflow of the proposed investigation.

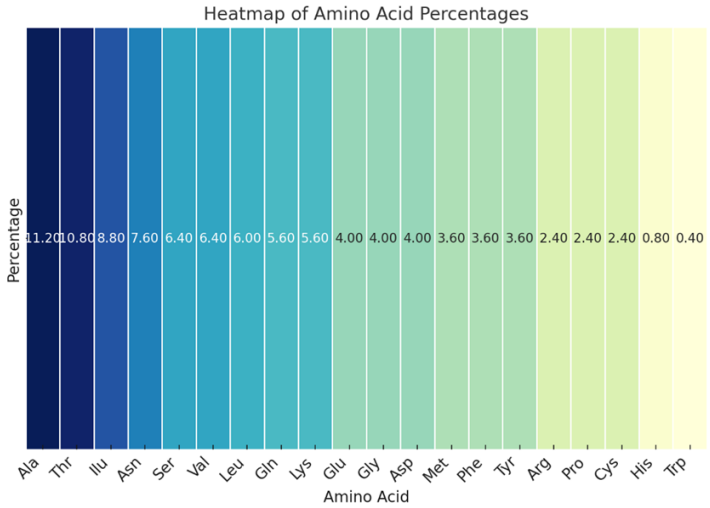

Figure 2. The detail of amino acid that showed the frequency of the variety amino acid.

Table 1. Physicochemical analysis of M1R protein.

Properties	Result
Number of amino acids	250
Molecular weight	27303.24
Theoretical pI	6.72
Total number of negatively charged residues (Asp + Glu)	20
Total number of positively charged residues (Arg + Lys)	20
Formula	$C_{1198}H_{1920}N_{320}O_{376}S_{15}$
Total number of atoms	3829
Extinction Coefficient (all pairs of Cys residues from Cysteines)	19,285
Extinction Coefficient (all Cys residues are reduced)	18,910
Half-time (mammalian, in vitro)	30h
Instability index	33.47
Aliphatic index	87.48
GRAVY	-0.004

(Asp+Glu). Therefore, the overall charge of the protein is neutral. The presence of Cys, Trp, and Tyr residues is in-dicated by a high Extinction coefficient of 19,285. The Aliphatic index profile

Table 2. Predicted functions of M1R protein.

Name	Accession	Description	Interval	Bit score
L1R_F9L	Pfam02442	Lipid membrane protein of large eukaryotic DNA viruses	1-185	2.1649
PHA02955	PHA02955	Hypothetically protein; Provisional	14-206	0.56
PHA02947	PHA02947	S-S bond generation pathway protein; Provisional	1-173	0.49
Ontology	**GO ID**	**Name**		**Bit score**
Cellular component	GO:0110165	cell anatomical entity (A constituent of a cellular organism that could be an immaterial entity or a material entity with granularity beyond the level of a protein complex yet below that of an anatomical system.)		0.349
Biological process	GO:0009987	Cell pathway (Any mechanism which may be carried out at the cellular level, yet not necessarily limited to a single cell)		0.311

showed high stability over the high temperature. The instability index of the M1R protein is 33.47 that is less than 40 and it is a stable protein in nature. **Table 1** visualizes detailed information on physicochemical analysis that help to identify of the drug and vaccine target.

Functional Prediction of Protein

Understanding of the domain of the protein is the key part of the to discover the function of the protein. The domain named L1R_F9L was found by CD search tool in NCBI with 2.16 bit score that showed the M1R protein is lipid membrane protein in DNA viruses and the PHA02947 domain showed that have role in S-S bond formation pathway protein. On other hand, the gene ontology (GO) analysis was performed via DeepGo web server the result was shown **(Table 2)**.

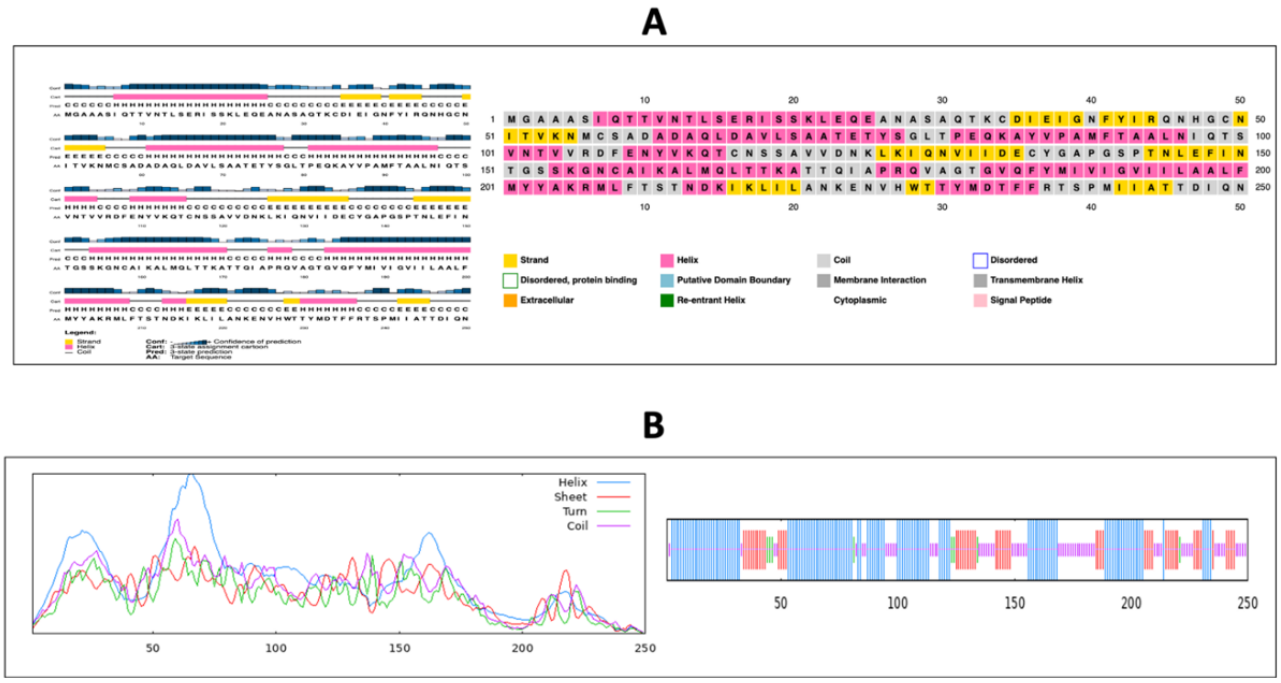

Figure 3. Analysis of M1R protein sequences and their structural features. (A) The secondary structure of the M1R protein forecasted by PSIPRED server. Sequence alignment of the target protein, highlighting key structural motifs and domains. The colored bars indicate different features: broad (yellow), helix (pink), coil (blue), disordered (gray), transmembrane (purple), and signal peptide (orange). The alignment also displays the boundaries of the predicted domains and motifs. (B) The SOPMA server prediction of 2D structure. Graphical representation of the protein's secondary structure elements over a range of residues. The plot illustrates the proportion of helix (pink), sheet (green), turn (blue), and coil (red) regions along the protein sequence, providing insight into its overall structural characteristics.

Secondary Structure Analysis

The 2D structure of the M1R protein was employed by SOPMA and PSIPRED server with default settings that showed the alpha helix, extended sheet, beta-turn, and random coil of 49.20%, 21.20%, 3.20%, and 26.40%, respectively **(Table 3)**. In addition, the PSIPRED anticipated the helix and beta sheet with high frequency **(Figure 3A)**. Also, results for SOMPA prediction are included in **Figure 3B**.

Table 3. 2D structure detailed of M1R protein

Secondary structure elements	Percent
Alpha helix	49.20%
3_{10} helix	0%
Pi helix	0%
Beta bridge	0%
Extended strand	21.20%
Beta turn	3.20%
Bend region	0%
Random coil	26.40%
Ambiguous states	0%

3D Structure Analysis and Validation

The M1R and L1 protein have 98.80% identity that analyzed via MEGA11 program. The tertiary structure of the M1R was predicted by 3 tools to compare the 3D structure and select the best model for M1R protein. The GalaxyTMB, Robetta, I-TASSER, and AlphaFold2 predicted several model model that validated via Ramachandran plot, Z-score, 3DVerify, and ERRAT that filtered to select the best model **(Table 4)**. The Robetta server, Galaxy model, and AlphaFold2 anticipated the best model via RosseTTaFold, homology modeling method, and artificial intelligence. The most favored regions, additional allowed regions, generously allowed regions, and disallowed region of the model is 89.6%, 9.5%, 0.4%, 0.4%, the galaxy model is 91.3%, 8.2%, 0%, 0.4%, and AlphaFold2 model is 92.6%, 6.1%, 1.3%, 0%, respectively. Additionally, the Z-score of models showed significant quality model that other analysis demonstrated in Table 4. The best model of other method employed considering that the GalaxyTMB and Robetta are the highest quality of M1R protein. At last, the analysis of signal peptide, trans-membrane helix, non-cytoplasmic, and cytoplasmic region of the part of the M1R protein is displayed in **Figure** 4(A-D), and 5(A-D). Also, there is no signal peptide position detected in the structure, and the position of 1-182 amino acids in non-cytoplasmic and 183-205 positions in the trans-membrane helix and the 206-

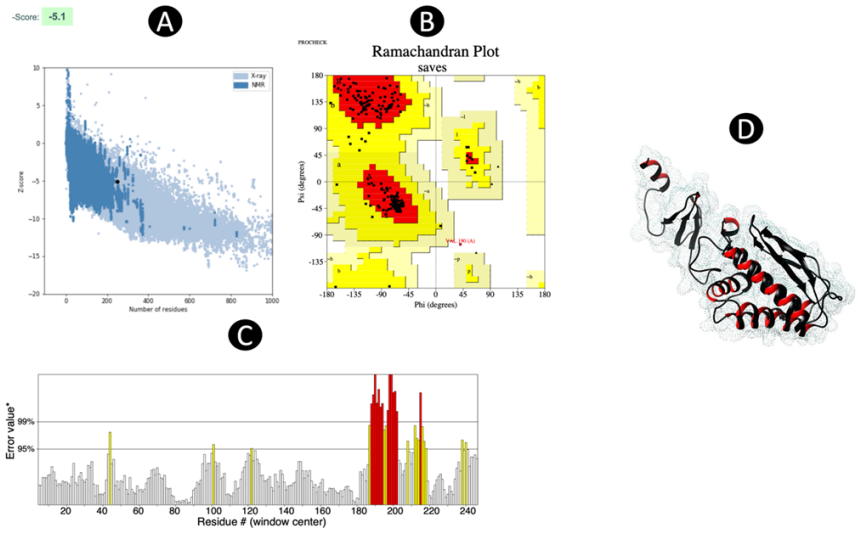

Figure 4. (A) Scatter plot displaying the relationship between the score and the number of residues in the protein sequence. The density of points is illustrated, indicating the quality of the protein model. The Z-score validation showed the -5.1 that used ProsA-web server (B) The analysis of the Ramachandran plot was carried out by PROCHECK. Ramachandran plot demonstrating the phi (φ) and psi (ψ) dihedral angles of the protein backbone. Areas in red indicate favored conformations, while yellow represents allowed regions, and black denotes outliers. (C) ERRAT plot showing the quality of the protein structure across the sequence. The bars represent the error values calculated within a sliding window, with highlighted peaks indicating regions of concern. (D) Ribbon representation of the protein structure, highlighting the secondary structure elements. The red and black ribbons indicate alpha helices and beta sheets, respectively, providing a visual summary of the protein's overall conformation.

Table 4. Comparison of validation and refinement of 3D structure with 3 tools.

Validation	GalaxyTMB model	Robetta model	I-TASSER model	Alphafold2
Ramachandran plot				
Most favored regions	91.3	89.6%	76.6%	92.6%
Additional allowed regions	8.2%	9.5%	13.4%	6.1%
Generously allowed regions	0%	0.4%	4.8%	1.3%
Disallowed regions	0.4%	0.4%	5.2%	0%
ERRAT	88.01%	90.25%	74.46%	90.86
Verify3D	50.80%	57.60%	53.20%	37.20%
Z-score	-5.1	-6.55	-5.35	-4.64

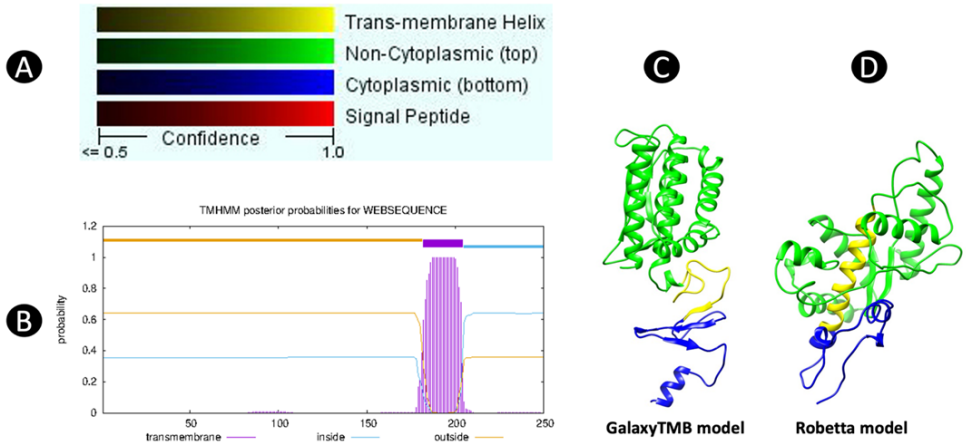

Figure 5. (A) The mapping of the M1R protein sequence that showed the position of the protein. (B) The TMHMM server prediction of the position of the sequence. The position of the trans-membrane helix, non-cytoplasmic, and cytoplasmic in the 3D structure. (C) GalaxyTMB Prediction, (D) Robetta model

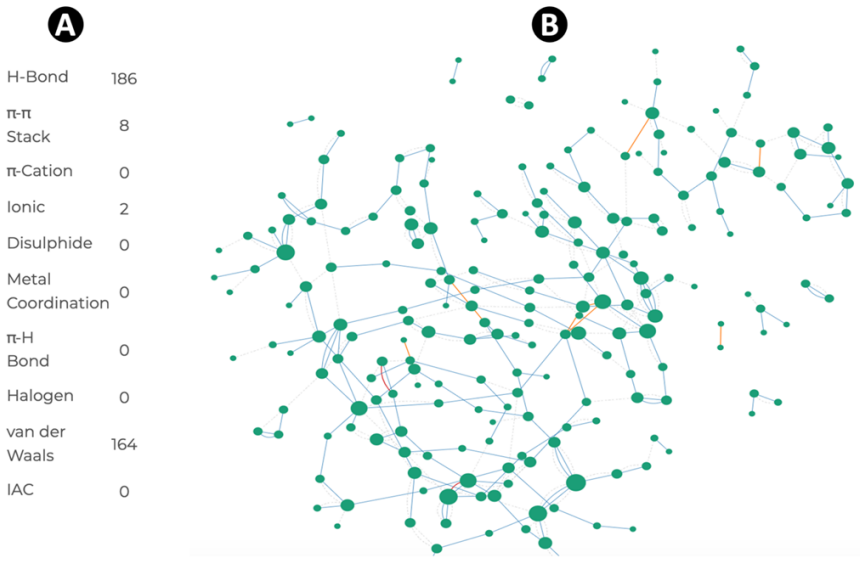

Figure 6. (A) The number of intra-chain interactions. (B) The Interaction graph showed the nodes and edges. The edges consist of different colors, such as Blue: H-bond, Orange: π–π stacking, Red: Ionic bond, and Gray: Van der Waals.

250 amino acid position in cytoplasmic. At last, the analysis of the interaction of the Galaxy M1R model showed the different intra-interactions such as 186 H-bond, 8 π–π stacking, 2 ionic, and

164 van der Walls among the residue-residue interaction. Moreover, the interaction graph shown in **Figure 6A,B** that describes the nodes (Residues) and edges (Interactions) as probabilistic network. The probability contact map showed in **Figure 7A-D**. All PDB formats of M1R protein are included in Supplementary Data.

Molecular Dynamic Simulation

In molecular dynamic simulation study, we selected the GalaxyTMB model to detect the dynamic of the structure that we employed the GROMACS tools that analyzed the different aspect.

RMSD Analysis

RMSD (Root Mean Square Deviation) is a measure of how much a protein structure deviates from its reference structure over time and commonly used to assess the stability and dynamics of proteins. In this figure, the RMSD of the protein's C-alpha backbone is plotted as a function of time

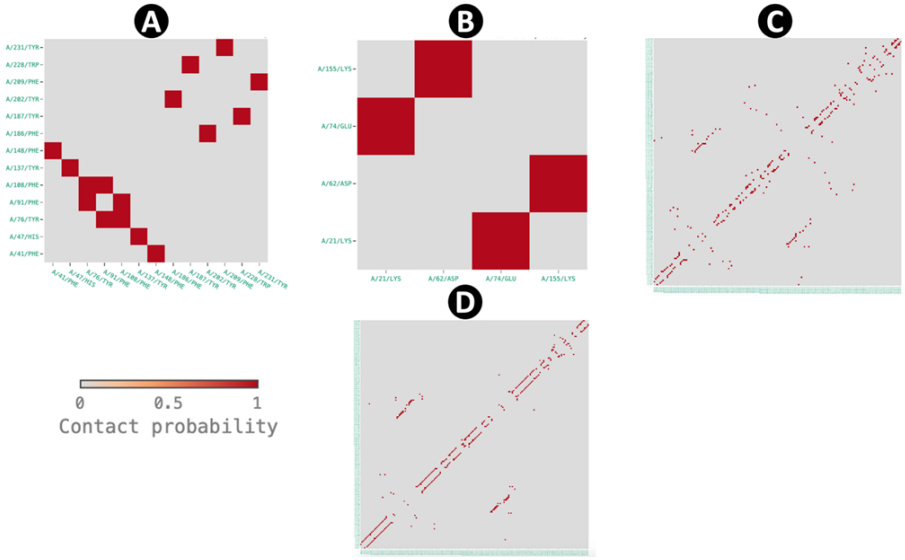

Figure 7. Probability contact map. Each residue probability of being in contact with any other residue. (A) π–π stacking (B) Ionic bond (C) Van der Waals (D) H-bond

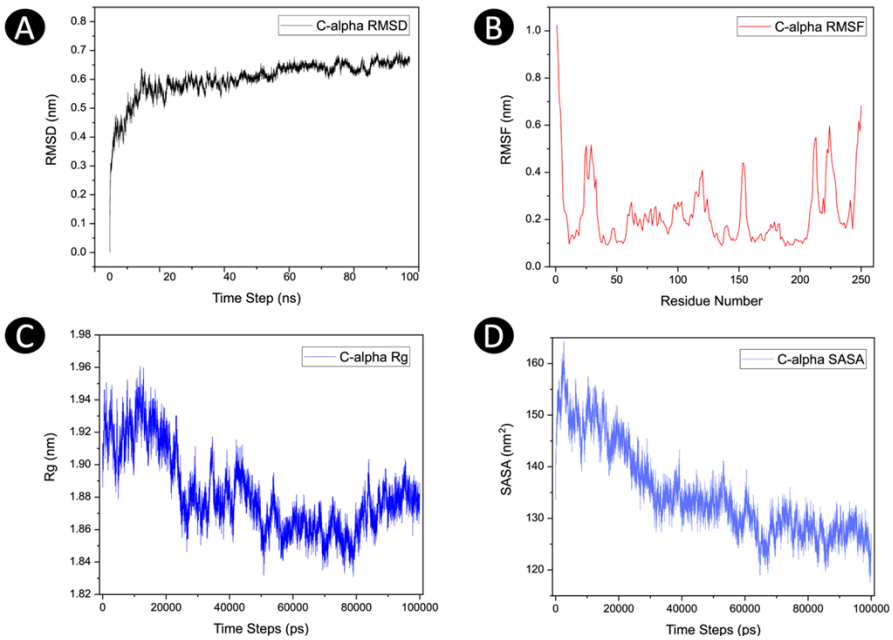

Figure 8. Molecular dynamics simulation result.)A) RMSD)B) RMSF)C) Rg)D) SASA.

(Figure 8A). The RMSD was initially very low, indicating that the protein structure was close to its native state. The RMSD follows a gradual upward trend over time, suggesting conformational changes deviating from the starting protein structure. From the 80 ns timepoint onwards, the RMSD reached a plateaued state at around 0.6 nm indicating potential stabilization of the construct. RMSD undergoes minor fluctuations following the plateau indicating only slight conformational changes. RMSD analysis shows relative stabilization of the construct with minor fluctuations.

RMSF Analysis

The root mean square fluctuation (RMSF) of the protein residues is calculate by averaging the squared distance of aminoacids C-alpha atom from its position **(Figure 8B)**. The RMSF measure is an indicator of flexibility for the area of interest within the molecule or, when analyzed globally, the whole molecule. Higher RMSF may suggest the flexibility of the studied protein area. The value of RMSF represents the fluctuation of each residue's C-alpha atom during the simulation time. Increased RMSF in the regions of 24-30, 117-120, 152-155, 210-213, and 221-225 could suggest such sequences of the protein have higher flexibility and show significant structural fluctuations across the MDS trajectory. The raised RMSF in these areas suggests that these residues are flexible and can adapt to their molecular surrounding such as solvent and ions. Flexibility could be an influential local feature of the protein regions, as it enables interaction with other compounds and represents its ability to experience conformational changes in response to different molecular environments.

Rg Analysis

The radius of gyration (Rg) is a measure of the average distance of the C-alpha atoms in a protein from the mass center of the protein. Reduced Rg may indicate higher compactness of protein. In **Figure 8C**, the Rg follows a downward trend indicating that the protein may be compacting as the MDS progresses. The Rg plateaus at 80-ns and then remains almost constant for the rest of the simulation indicating a stabilized conformation that is potentially more compact than the initial structure. The stabilization of the Rg could be an indicator that the protein has been equilibrated. In this state, the protein seems to be stably folded and may not be experiencing additional dramatic conformational changes.

SASA Analysis

The SASA of the protein as a function of time has been plotted **Figure 8D**. SASA is a parameter representing the overall surface area of the M1R that is accessible to solvent TIP3P water molecules. SASA is quantified through adding together the surface areas of all the non-hydrogen atoms in the protein that are in contact with the solvent. In the present experiment, the starting SASA of the protein was approximately 150 nm^2. Interestingly, the SASA of the protein was then attenuated as the simulation progressed, stabilizing at about 120 nm^2 at 60-ns. This downward trend in the SASA is indicative the structure is turning into a less hydrophilic conformation over the course of MD experiment. While the protein folds or unfolds, its surface area accessibility is reduced or increased, respectively. Additional investigation of specific protein subdomains and regions is highly recommended to pinpoint the source of changes to SASA, particularly for multi-domain and large proteins.

Epitope Analysis

The antigenicity of the M1R protein yielded a probability of 0.63, classifying it as a plausible antigen. Allergenicity as well as toxicity analyses confirmed its non-allergenic and non-toxic nature. **Tables 5** and **6** detail the forecasted B-lymphocyte, CTL, and HTL epitopes, respectively. In particular, the B-lymphocyte epitope evaluated detected 18 linear epitopes. Result scores were higher than 0.6. Conformational epitopes predicted by Robetta model are demonstrated in Figure 9A and predictions of the GalaxyTMB tool are demonstrated in **Figure 9B**. The CTL/HTL epitopes were forecasted to demonstrate high affinity with their respective MHC alleles, corroborating their status as potential antigens while also corroborating their non-allergenic and non-toxic features. Additionally, 3 out of 6 of epitopes showed robust water solubility **(Table 6)**.

Active Site of M1R Model

The CASTp v3.0 was conducted to unveil the

Table 5. Linear B-cell epitopes.

Robetta model				
Start	**End**	**Linear Epitopes**	**Number of residues**	**Score**
1	6	MGAAAS	6	0.805
243	250	IATTDIQN	8	0.783
16	36	ERISSKLEQEANASAQTKCDI	21	0.768
221	234	ANKENVHWTTYMDT	14	0.769
206	218	RMLFTSTNDKIKL	13	0.755
150	158	NTGSSKGNC	9	0.703
76	86	YSGLTPEQKAY	11	0.688
137	144	YGAPGSPT	8	0.673
58	62	SADAD	5	0.64
169	180	KATTQIAPRQVA	12	0.639
95	101	LNIQTSV	7	0.63
111	125	YVKQTCNSSAVVDNK	15	0.63
GalaxyTMB model				
Start	**End**	**Linear Epitopes**	**Number of residues**	**Score**
222	250	NKENVHWTTYMDTFFRTSPMIIATTDIQN	29	0.847
117	130	NSSAVVDNKLKIQN	14	0.829
206	217	RMLFTSTNDKIK	12	0.74
77	84	SGLTPEQK	8	0.7
138	143	GAPGSP	6	0.667
20	44	SKLEQEANASAQTKCDIEIGNFYIR	25	0.66

Table 6. MHC I/II epitopes.

Allele	**Position**	**MHC I**	**Percentile rank**	**VaxiJen**	**Solubility**
HLA-B*58:01 HLA-B*57:01	10-18	LANKENVHW	0.01	1.71 (Antigen)	Good water solubility
HLA-A*23:01 HLA-A*24:02	61-69	MYYAKRMLF	0.01	0.42 (Antigen)	Poor water solubility
HLA-B*15:01	37-46	RQVAGTGVQF	0.01	0.66 (Antigen)	Poor water solubility
Allele	**Position**	**MHC II**	**Percentile rank**	**VaxiJen**	**Solubility**
HLA-DRB5*01:01	3-17	TETYSGLTPEQKAYV	0.2	0.83 (Antigen)	Good water solubility
HLA-DRB1*03:01	47-61	NSSAVVDNKLKIQNV	0.33	0.99 (Antigen)	Good water solubility
HLA-DRB4*01:01	26-40	FRTSPMIIATTDIQN	0.44	1.09 (Antigen)	Poor water solubility

active site of the M1R protein. The tool executed to locate the functional place of the M1R protein. We discovered 18 residues among 250 residues in Robetta model that acts as active sites **(Red sphere Figure 10A)** for the M1R protein. Moreover, the 21 residues out of 250 resides constituted the active site in GalaxyTMB model **(Figure 10B)**.

Discussion

The world has seen the outbreak of MPX in 2022 in 117 countries with over 94,707 cases, and the increased global interest directed

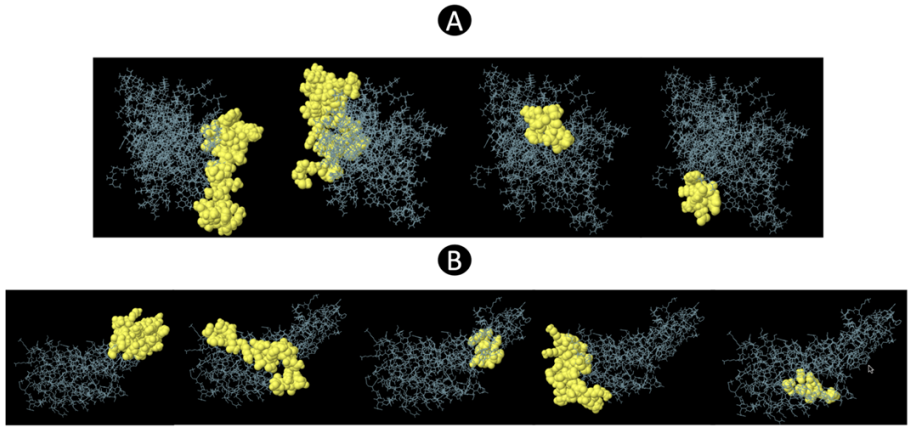

Figure 9. Prediction of the Conformational epitopes. (A) The top 4 conformational B-cell epitopes predicted by Robetta model. (B) The top 5 conformational B-cell epitopes predicted by GalaxyTMB.

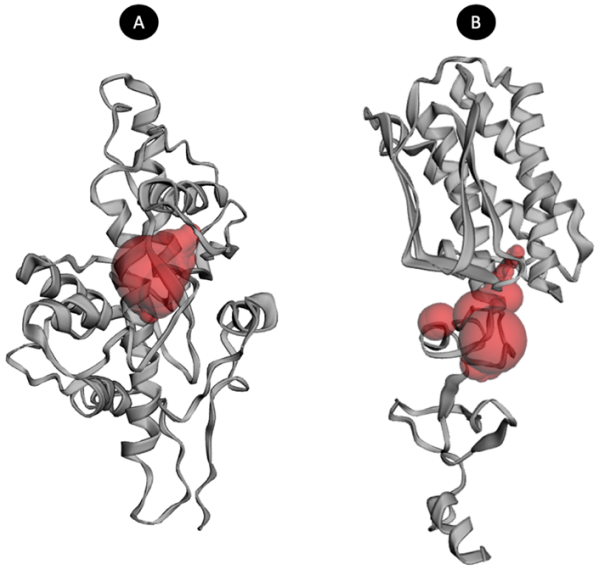

Figure 10. Active site of the M1R protein. (A) 18 amino acid residues in the active site in (B) 21 amino acid residues in the active site (Red sphere).

towards establishing potential therapeutic targets particularly M1R (58). M1R has emerged as a promising target for the treatment of MPXV infection, since it possesses several critical attributes that could enable its development not only as a vaccine target, but also as a target for antiviral strategies. In studying M1R as a therapeutic target, recent VACV work on L1R protein could inform whether it is a suitable target (59), recent evidence indicates M1R is with high confidence a target given its conserved construct, special location as well as functional importance in this protein. M1R is at the IMV exterior and enhances viral entry and assembly in a glycosaminoglycan-independent fashion (60,61). Surface accessibility is paramount for a therapeutic engagement and that mutations that provide resistance result in major fitness costs to the virus. Furthermore, due to the high conservation of this protein across orthopoxvirus and its 98% sequence homology amongst strains, it indicates higher probability of broad immunity towards various orthopoxvirus strains (62).

M1R is a target of particular interest in immunological studies and could be used to design immunogenic candidates. Immune responses and significant immunoprotection against viral challenge have been suggested due to the incorporation of M1R together with A29L, A35R, and B6R in recombinant protein immunotherapies. M1R

is highly immunogenic in comparison with other protein targets, even after the first immunization it induces significant antibody response. This highly sustained response to the immunogenic candidate after dose boosts further supports that M1R is the designed protein capable of eliciting durable immunity (63,64). Recombinant immunotherapies made with M1R activate the humoral and the cell-mediated immunity against future strains of the MPXV, that could mutate more rapidly (65–67).

The overall conservation, structural accessibility, strong immunogenicity, and functional importance of M1R makes it a suitable target for therapeutic and preventive applications targeting MPXV infections.

The MPXV M1R protein is detected on the IMV membrane and possesses similarity to the VACV L1R. As with the L1R protein, which is harbored on the outer domain of intracellular viruses and is positioned toward the cytoplasm, the MPXV M1R protein also has three intramolecular disulfide bonds (68). Of note, the myristoylated N-terminal domain of L1R is an essential interactive domain for mAbs such as 7D11. These antibodies attach VACV and abrogate its entry into BSC-1 cells in a dose-reliant manner (69). Also, the L1R protein is target for another neutralizing monoclonal antibody (mAb) 2D5 which has been unveiled and suggests its importance in the engineering of new therapeutic interventions (70).

The neutralizing mAbs 7D11 and 10F5 attach L1R. Such antibodies could simply identify and communicate with discontinuous epitopes by the presence of 2 special loops enjoined with the formation of a disulfide bond (71). These studies highlight the importance of targeting a range of key proteins in Monkeypox virus. Structural Immunoinformatics in the present work was used to rapidly examine the M1R protein (72). These tools allow the exploration of novel viral protein targets for the development of both immunotherapies and drugs (73,74).

Computational tools have renovated structural biology through rapidly uncovering structural insights for key proteins that can be used in drug and vaccine design (75). In particular, these tools could help to identify the structure of experimentally-unknown proteins and offer rapid solutions to forecast various structural dynamics and immune-inducing features of such molecules (76). In this reserach, we investigated the M1R protein of Monkeypox virus using Physicochemical properties, protein function prediction, 2D/3D structure prediction, and MDS (77,78).

The M1R protein sequence was retrieved from Uniprot. The physicochemical analysis of the M1R protein could inform future studies on its structural characteristics and stability. The protein consisted of 250 aa with a total molecular weight of 27303.24 Da. Neutral charge, thermostable hydrophilic nature, and equal-charged residues were found to be characteristics of M1R protein. Taken together, the aliphatic index stability across broad temperature range and low instability index supported the M1R protein stability. Functional predictions of the M1R protein were performed by domain, gene ontology and pathway analyses. The L1R_F9L region was detected by evaluating the CD expression, indicating that the DNA virus-derived M1R protein may be a lipid membrane protein. Further, the PHA02947 area was related to S-S bonds formation, giving valuable hints on potential biological functions in the viral particle assembly and entry. The secondary structure predictions for both the SOPMA and PSIPRED for this construct were predominantly helical, with α-helix making up the majority of the predictions. Prediction of tertiary structure of M1R was achieved by the Robetta, GalaxyTMB, as well as the I-TASSER tools (79). Ramachandran plot, Z-score, and other parameters for protein quality evaluated the top predicted models. The agreement between the GalaxyTMB assembly and Robetta revealed a strong and high-precision prediction of the M1R construct.

MDS was performed for 100-ns to further explore the stability and dynamics of the M1R protein. The RMSD plateaued during the MDS run confirming a stabilized structure with minor post-stabilization construct alterations. The RMSF analysis is representative of flexibility for local regions of the M1R protein. This analysis revealed flexible regions which is crucial for drug design. Evaluation of Rg exhibited a downward trend that suggests gradual compacting and was then plateaued. Moreover, SASA indicated changes to the M1R hydrophilicity throughout the sim-

ulation (80). It is advised for the future that exclusive areas of the protein are evaluated to better look into the origins of change in SASA and Rg. Furthermore, experimental validation as well as implementation of our structural and Immunoinformatics results on M1R into immune-modulating therapies design pipelines is warranted.

The epitope analysis focused on predicting B-cell, CTL, and HTL epitopes, providing valuable information for understanding the immunological aspects of the M1R protein. The identified epitopes, which were non-allergenic and non-toxic, pave the way for potential vaccine development. Experimentally identified B-cell epitopes such as 69LSAATETYSGLTPEQKAYVPAMF91 and 137YGAPGSPTNLEFINTGSSK155 (61) were confirmed by this investigation's predictions and strengthened the validity of the findings. The same specific predicted B-cell epitopes (76-86, 137-144, 77-84, 138-143) were identified via modeling and Ellipro tools (Table 5) which is the target of neutralizing monoclonal antibody (70).

Considering the efforts in this study and recent evidence regarding M1R, which pointed towards the idea of developing interventions against MPXV infection, our findings contributes to this field by highlighting M1R as a key target for therapeutic development specifically against MPXV as more studies are conducted. Its ability to act as a strong immunogen makes it a good candidate for various vaccine platforms since such vaccines could robust strong B-cell and T-cell responses that would provide an approach for preventing MPXV infection. M1R also shows a strong capability for the generation of potent neutralizing antibodies due to its role in membrane attachment and fusion (24). These characteristics will enhance the feasibility of M1R-based vaccines and also offer potent candidates for future efforts in MPXV vaccination based on high controllability and accessibility of these kinds of vaccines (81).

Furthermore, Bioinformatics analyses accelerates the development of drugs and immunotherapeutics (82–95). For the future investigation on herbal compounds as well as new pharmaceutics is recommended (96,97). The use of structural Bioinformatics in this work provides a cost-effective and rapid approach towards the better understanding of the Monkeypox virus and the potential role of the M1R protein in viral infection, which will help in developing novel vaccines in the future. Herbal and natural products, in addition to new pharmaceutical drugs, have been utilized in treating wide range of disorders (96–101). Additional studies focusing on herbal medicine-based related of M1R may be useful. Techniques utilized in this study including structural modeling, molecular dynamics simulations, epitope and active sites predictions have proven useful in uncovering the structure and important functions of the M1R protein. These computational approaches provide avenues for the development of antiviral strategies, including vaccine design and drug discovery assisting the evaluation of protein stability and interactions. This common computational basis emphasizes the potency that in silico tools have towards enhancing the understanding of protein function and assisting in the formulation of appropriate clinical intervention measure. Further experimental validation, such as protein expression and functional assays, is warranted to confirm the in-silico predictions (102,103).

Conclusion

This study implements structural Immunoinformatics for forecasting the 3D structure of M1R protein. Unveiling the structural intricacies of viral proteins is very important. We used Immunoinformatics tools to provide new data helping vaccine design, antibody development, and drug design against MPX. Through molecular dynamic study, the stability of the M1R structure was assessed and its construct characteristics were studied. Additionally, the physicochemical evaluation and epitope forecasts achieved in the present work added to knowledge into the protein's characteristics. Clues from the present study improve our comprehension of the MPXV and help progress treatment strategies and antiviral treatments.

Funding

None

Consent to Participate

Not applicable

Acknowledgements

We thank the Research Center for Immunodeficiencies (RCID), Tehran University of Medical

Sciences, and Student Research Committee and USERN MUBabol Office of Babol University of Medical Sciences, Iran.

Data and code availability statement

Data is available from corresponding author only on reasonable request.

Competing Interests

The authors declare that they have no conflict of interest with regards to the present study.

References

1. Bunge EM, Hoet B, Chen L, Lienert F, Weidenthaler H, Baer LR, et al. The changing epidemiology of human monkeypox-A potential threat? A systematic review. PLoS Negl Trop Dis. 2022 Feb;16(2):e0010141.
2. Alakunle E, Moens U, Nchinda G, Okeke MI. Monkeypox Virus in Nigeria: Infection Biology, Epidemiology, and Evolution. Viruses. 2020;12(11).
3. Kumar S, Subramaniam G, Karuppanan K. Human monkeypox outbreak in 2022. J Med Virol. 2023;95(1):e27894.
4. Cho CT, Wenner HA. Monkeypox virus. Bacteriol Rev. 1973 Mar;37(1):1–18.
5. Patauner F, Gallo R, Durante-Mangoni E. Monkeypox infection: An update for the practicing physician. Eur J Intern Med. 2022 Oct;104:1–6.
6. Meo SA, Jawaid SA. Human Monkeypox: Fifty-Two Years based analysis and Updates. Pak J Med Sci. 2022;38(6):1416–9.
7. Ihekweazu C, Yinka-Ogunleye A, Lule S, Ibrahim A. Importance of epidemiological research of monkeypox: is incidence increasing? Vol. 18, Expert review of anti-infective therapy. England; 2020. p. 389–92.
8. Yinka-Ogunleye A, Aruna O, Dalhat M, Ogoina D, McCollum A, Disu Y, et al. Outbreak of human monkeypox in Nigeria in 2017-18: a clinical and epidemiological report. Lancet Infect Dis. 2019 Aug;19(8):872–9.
9. Bunge EM, Hoet B, Chen L, Lienert F, Weidenthaler H, Baer LR, et al. The changing epidemiology of human monkeypox-A potential threat? A systematic review. PLoS Negl Trop Dis. 2022 Feb;16(2):e0010141.
10. Beer EM, Rao VB. A systematic review of the epidemiology of human monkeypox outbreaks and implications for outbreak strategy. PLoS Negl Trop Dis. 2019 Oct;13(10):e0007791.
11. Ladnyj ID, Ziegler P, Kima E. A human infection caused by monkeypox virus in Basankusu Territory, Democratic Republic of the Congo. Bull World Health Organ. 1972;46(5):593–7.
12. Marennikova SS, Seluhina EM, Mal'ceva NN, Cimiskjan KL, Macevic GR. Isolation and properties of the causal agent of a new variola-like disease (monkeypox) in man. Bull World Health Organ. 1972;46(5):599–611.
13. Magnus P von, Andersen EK, Petersen KB, Birch-Andersen A. A POX-LIKE DISEASE IN CYNOMOLGUS MONKEYS. Acta Pathologica Microbiologica Scandinavica. 1959;46(2):156–76.
14. Parker S, Buller RM. A review of experimental and natural infections of animals with monkeypox virus between 1958 and 2012. Future Virol. 2013 Feb;8(2):129–57.
15. Guarner J, Johnson BJ, Paddock CD, Shieh WJ, Goldsmith CS, Reynolds MG, et al. Monkeypox transmission and pathogenesis in prairie dogs. Emerg Infect Dis. 2004 Mar;10(3):426–31.
16. Pastula DM, Tyler KL. An Overview of Monkeypox Virus and Its Neuroinvasive Potential. Ann Neurol. 2022 Oct;92(4):527–31.
17. Brown K, Leggat PA. Human Monkeypox: Current State of Knowledge and Implications for the Future. Trop Med Infect Dis. 2016 Dec;1(1).
18. Vivancos R, Anderson C, Blomquist P, Balasegaram S, Bell A, Bishop L, et al. Community transmission of monkeypox in the United Kingdom, April to May 2022. Euro Surveill. 2022 Jun;27(22).
19. Sepehrinezhad A, Ashayeri Ahmadabad R, Sahab-Negah S. Monkeypox virus from neurological complications to neuroinvasive properties: current status and future perspectives. J Neurol. 2023 Jan;270(1):101–8.
20. Sklenovská N. Monkeypox Virus. In: Malik YS, Singh RK, Dhama K, editors. Animal-Origin Viral Zoonoses. Singapore: Springer Singapore; 2020. p. 39–68.
21. Kandi V, Pal M, Mengstie F. Epidemiology, Diagnosis, and Control of Monkeypox Disease: A comprehensive Review. American journal of infectious diseases and Microbiology. 2017 May 22;5:94–9.
22. Huang Y, Mu L, Wang W. Monkeypox: epidemiology, pathogenesis, treatment and prevention. Signal Transduct Target Ther. 2022 Nov;7(1):373.
23. Grifoni A, Zhang Y, Tarke A, Sidney J, Rubiro P, Reina-Campos M, et al. Defining antigen targets to dissect vaccinia virus and monkeypox virus-specific T cell responses in humans. Cell Host Microbe. 2022 Dec;30(12):1662-1670.e4.
24. Wang Y, Yang K, Zhou H. Immunogenic proteins and potential delivery platforms for mpox virus vaccine development: A rapid review. Int J Biol Macromol. 2023 Aug;245:125515.

25. Yefet R, Friedel N, Tamir H, Polonsky K, Mor M, Cherry-Mimran L, et al. Monkeypox infection elicits strong antibody and B cell response against A35R and H3L antigens. iScience. 2023 Feb;26(2):105957.
26. Shchelkunov SN, Totmenin A V, Safronov PF, Mikheev M V, Gutorov V V, Ryazankina OI, et al. Analysis of the monkeypox virus genome. Virology. 2002 Jun;297(2):172–94.
27. Fang M, Cheng H, Dai Z, Bu Z, Sigal LJ. Immunization with a single extracellular enveloped virus protein produced in bacteria provides partial protection from a lethal orthopoxvirus infection in a natural host. Virology. 2006 Feb;345(1):231–43.
28. Papukashvili D, Rcheulishvili N, Liu C, Wang X, He Y, Wang PG. Strategy of developing nucleic acid-based universal monkeypox vaccine candidates. Front Immunol. 2022;13:1050309.
29. Ferdous J, Barek MA, Hossen MS, Bhowmik KK, Islam MS. A review on monkeypox virus outbreak: New challenge for world. Health Sci Rep. 2023 Jan;6(1):e1007.
30. Natami M, Gorgzadeh A, Gholipour A, Fatemi SN, Firouzeh N, Zokaei M, et al. An overview on mRNA-based vaccines to prevent monkeypox infection. J Nanobiotechnology. 2024 Mar;22(1):86.
31. Rezaei N. Clinical Immunology. Academic Press; 2022.
32. Saleki K, Alijanizadeh P, Azadmehr A. Is neuropilin-1 the neuroimmune initiator of multi-system hyperinflammation in COVID-19? Biomedicine & Pharmacotherapy. 2023;167:115558.
33. Saleki K, Mohamadi MH, Alijanizadeh P, Rezaei N. Neurological adverse effects of chimeric antigen receptor T-cell therapy. Expert Rev Clin Immunol. 2023;19(11):1361–83.
34. P. DN, Joseph D, N. CA, Neil S, L. MD, Eric B, et al. Vaccine Effectiveness of JYNNEOS against Mpox Disease in the United States. New England Journal of Medicine. 2023 Jun;388(26):2434–43.
35. Nalca A, Zumbrun EE. ACAM2000: the new smallpox vaccine for United States Strategic National Stockpile. Drug Des Devel Ther. 2010 May;4:71–9.
36. Garcia-Atutxa I, Mondragon-Teran P, Huerta-Saquero A, Villanueva-Flores F. Advancements in monkeypox vaccines development: a critical review of emerging technologies. Front Immunol. 2024;15:1456060.
37. Aram C, Alijanizadeh P, Saleki K, Karami L. Development of an ancestral DC and TLR4-inducing multi-epitope peptide vaccine against the spike protein of SARS-CoV and SARS-CoV-2 using the advanced immunoinformatics approaches. Biochem Biophys Rep. 2024;39:101745.
38. Saleki K, Payandeh P, Shakeri M, Pourahmad R, Banazadeh M, Alijanizadeh P. Utilizing Immunoinformatics to Target Brain Tumors; An Aid t o Current Neurosurgical Practice. Interv. Pain Med Neuromodulation. 2022;2(1).
39. Saleki K, Aram C, Alijanizadeh P, Khanmirzaei MH, Vaziri Z, Ramzankhah M, et al. Matrix metalloproteinase/Fas ligand (MMP/FasL) interaction dynamics in COVID-19: An in silico study and neuroimmune perspective. Heliyon. 2024;10(10).
40. Saleki K, Alijanizadeh P, Javanmehr N, Rezaei N. The role of Toll-like receptors in neuropsychiatric disorders: Immunopathology, treatment, and management. Med Res Rev. 2024;44(3):1267–325.
41. Saleki K, Shirzad M, Javanian M, Mohammadkhani S, Alijani MH, Miri N, et al. Serum soluble Fas ligand is a severity and mortality prognostic marker for COVID-19 patients. Front Immunol. 2022;13:947401.
42. Saleki K, Mohamadi MH, Banazadeh M, Alijanizadeh P, Javanmehr N, Pourahmad R, et al. In silico design of a TLR4-mediating multiepitope chimeric vaccine against amyotrophic lateral sclerosis via advanced immunoinformatics. J Leukoc Biol. 2022;112(5):1191–207.
43. Saleki K, Alijanizade P, Moradi S, Rahmani A, Banazadeh M, Mohamadi MH, et al. Engineering a novel immunogenic chimera protein utilizing bacterial infections associated with atherosclerosis to induce a deviation in adaptive immune responses via Immunoinformatics approaches. Infection, Genetics and Evolution. 2022;102:105290.
44. Rahmani A, Baee M, Saleki K, Moradi S, Nouri HR. Applying high throughput and comprehensive immunoinformatics approaches to design a trivalent subunit vaccine for induction of immune response against emerging human coronaviruses SARS-CoV, MERS-CoV and SARS-CoV-2. J Biomol Struct Dyn. 2022;40(13):6097–113.
45. pmc_168970.
46. Buchan DWA, Moffat L, Lau A, Kandathil SM, Jones DT. Deep learning for the PSIPRED Protein Analysis Workbench. Nucleic Acids Res. 2024;gkae328.
47. Tamura K, Stecher G, Kumar S. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. Mol Biol Evol. 2021 Jun;38(7):3022–7.
48. Ko J, Park H, Heo L, Seok C. GalaxyWEB server for protein structure prediction and refinement. Nucleic Acids Res. 2012;40(W1):W294–7.
49. Lee GR, Won J, Heo L, Seok C. GalaxyRefine2: simultaneous refinement of inaccurate local regions and overall protein structure. Nucleic Acids Res.

2019;47(W1):W451–5.
50. Del Conte A, Camagni GF, Clementel D, Minervini G, Monzon AM, Ferrari C, et al. RING 4.0: faster residue interaction networks with novel interaction types across over 35,000 different chemical structures. Nucleic Acids Res. 2024;gkae337.
51. Van Der Spoel D, Lindahl E, Hess B, Groenhof G, Mark AE, Berendsen HJC. GROMACS: fast, flexible, and free. J Comput Chem. 2005 Dec;26(16):1701–18.
52. Ponomarenko J, Bui HH, Li W, Fusseder N, Bourne PE, Sette A, et al. ElliPro: a new structure-based tool for the prediction of antibody epitopes. BMC Bioinformatics. 2008 Dec;9:514.
53. Doytchinova IA, Flower DR. VaxiJen: a server for prediction of protective antigens, tumour antigens and subunit vaccines. BMC Bioinformatics. 2007 Jan;8:4.
54. Dimitrov I, Bangov I, Flower DR, Doytchinova I. AllerTOP v.2--a server for in silico prediction of allergens. J Mol Model. 2014 Jun;20(6):2278.
55. Magnan CN, Randall A, Baldi P. SOLpro: accurate sequence-based prediction of protein solubility. Bioinformatics. 2009 Sep;25(17):2200–7.
56. Aram C, Alijanizadeh P, Saleki K, Karami L. Development of an ancestral DC and TLR4-inducing multi-epitope peptide vaccine against the spike protein of SARS-CoV and SARS-CoV-2 using the advanced immunoinformatics approaches. Biochem Biophys Rep. 2024;39:101745.
57. Tian W, Chen C, Lei X, Zhao J, Liang J. CASTp 3.0: computed atlas of surface topography of proteins. Nucleic Acids Res. 2018;46(W1):W363–7.
58. Sagdat K, Batyrkhan A, Kanayeva D. Exploring monkeypox virus proteins and rapid detection techniques. Front Cell Infect Microbiol. 2024;14:1414224.
59. Su HP, Garman SC, Allison TJ, Fogg C, Moss B, Garboczi DN. The 1.51-Angstrom structure of the poxvirus L1 protein, a target of potent neutralizing antibodies. Proc Natl Acad Sci U S A. 2005 Mar;102(12):4240–5.
60. Tang D, Liu X, Lu J, Fan H, Xu X, Sun K, et al. Recombinant proteins A29L, M1R, A35R, and B6R vaccination protects mice from mpox virus challenge. Front Immunol. 2023;14.
61. Foo CH, Lou H, Whitbeck JC, Ponce-de-León M, Atanasiu D, Eisenberg RJ, et al. Vaccinia virus L1 binds to cell surfaces and blocks virus entry independently of glycosaminoglycans. Virology. 2009 Mar;385(2):368–82.
62. Ren Z, Li M, Chen J, Gong X, Song S, Li D, et al. Identification of mpox M1R and B6R monoclonal and bispecific antibodies that efficiently neutralize authentic mpox virus. Emerg Microbes Infect. 2024 Dec;13(1):2401931.
63. Franceschi V, Parker S, Jacca S, Crump RW, Doronin K, Hembrador E, et al. BoHV-4-Based Vector Single Heterologous Antigen Delivery Protects STAT1(-/-) Mice from Monkeypoxvirus Lethal Challenge. PLoS Negl Trop Dis. 2015 Jun;9(6):e0003850.
64. Tang D, Liu X, Lu J, Fan H, Xu X, Sun K, et al. Recombinant proteins A29L, M1R, A35R, and B6R vaccination protects mice from mpox virus challenge. Front Immunol. 2023;14.
65. Hou F, Zhang Y, Liu X, Murad Y, Xu J, Yu Z, et al. Novel mRNA vaccines encoding Monkeypox virus M1R and A35R protect mice from a lethal virus challenge. bioRxiv. 2022;
66. Li E, Gong Q, Zhang J, Guo X, Xie W, Chen D, et al. An mpox quadrivalent mRNA vaccine protects mice from lethal vaccinia virus challenge. Antiviral Res. 2024;230:105974.
67. Ye T, Zhou J, Guo C, Zhang K, Wang Y, Liu Y, et al. Polyvalent mpox mRNA vaccines elicit robust immune responses and confer potent protection against vaccinia virus. Cell Rep. 2024;43(6).
68. Senkevich TG, White CL, Koonin E V, Moss B. Complete pathway for protein disulfide bond formation encoded by poxviruses. Proc Natl Acad Sci U S A. 2002 May;99(10):6667–72.
69. Wolffe EJ, Vijaya S, Moss B. A myristylated membrane protein encoded by the vaccinia virus L1R open reading frame is the target of potent neutralizing monoclonal antibodies. Virology. 1995 Aug;211(1):53–63.
70. Wolffe EJ, Vijaya S, Moss B. A myristylated membrane protein encoded by the vaccinia virus L1R open reading frame is the target of potent neutralizing monoclonal antibodies. Virology. 1995 Aug;211(1):53–63.
71. Su HP, Golden JW, Gittis AG, Hooper JW, Garboczi DN. Structural basis for the binding of the neutralizing antibody, 7D11, to the poxvirus L1 protein. Virology. 2007 Nov;368(2):331–41.
72. Meszaros B, Park E, Malinverni D, Sejdiu BI, Immadisetty K, Sandhu M, et al. Recent breakthroughs in computational structural biology harnessing the power of sequences and structures. Curr Opin Struct Biol. 2023 Jun;80:102608.
73. Vishweshwaraiah YL, Dokholyan N V. Toward rational vaccine engineering. Adv Drug Deliv Rev. 2022 Apr;183:114142.
74. Maryam Barancheshmeh, Hossein Najafzadehvarzi, Naser Shokrzadeh CA. Comparative Analysis of Fennel Essential Oil and Manganese in PCOS Rat Model via modulating miR-145 Expression

and Structure-Based Virtual Screening of IGF2R Protein to Address Insulin Resistance and Obesity. Obes Med. 2024;
75. Chaudary, A. S., Guo, Y., Utkin, Y. N., Baranchesh-meh, M., Dagda, R. K., & Gasanoff ES. Sphingomyelin Inhibits Hydrolytic Activity of Heterodimeric PLA2 in Model Myelin Membranes: Pharmacological Relevance. J Membr Biol. 2024;1–18.
76. Rahman MF, Hasan R, Biswas MS, Shathi JH, Hossain MF, Yeasmin A, et al. A bioinformatics approach to characterize a hypothetical protein Q6S8D9_SARS of SARS-CoV. Genomics Inform. 2023 Mar;21(1):e3.
77. Suleman M, Rashid F, Ali S, Sher H, Luo S, Xie L, et al. Immunoinformatic-based design of immune-boosting multiepitope subunit vaccines against monkeypox virus and validation through molecular dynamics and immune simulation. Front Immunol. 2022;13:1042997.
78. Saleki K, Aram C, Alijanizadeh P, Khanmirzaei MH, Vaziri Z, Ramzankhah M, et al. Matrix metalloproteinase/Fas ligand (MMP/FasL) interaction dynamics in COVID-19: An in silico study and neuroimmune perspective. Heliyon. 2024 May 30;10(10).
79. Elalouf A. In-silico Structural Modeling of Human Immunodeficiency Virus Proteins. Biomed Eng Comput Biol. 2023;14:11795972231154402.
80. Taidi L, Maurady A, Britel MR. Molecular docking study and molecular dynamic simulation of human cyclooxygenase-2 (COX-2) with selected eutypoids. J Biomol Struct Dyn. 2022 Feb;40(3):1189–204.
81. Kong T, Du P, Ma R, Wang H, Ma X, Lu J, et al. Single-chain A35R-M1R-B6R trivalent mRNA vaccines protect mice against both mpox virus and vaccinia virus. EBioMedicine. 2024 Nov;109.
82. Saleki K, Payandeh P, Shakeri M, Pourahmad R, Banazadeh M, Alijanizadeh P, et al. Utilizing immunoinformatics to target brain tumors; an aid to current neurosurgical practice. Interventional Pain Medicine and Neuromodulation. 2022;2(1).
83. Saleki K, Banazadeh M, Saghazadeh A, Rezaei N. Aging, testosterone, and neuroplasticity: friend or foe? Rev Neurosci. 2023;34(3):247–73.
84. Aram C, Alijanizadeh P, Saleki K, Karami L. Development of an ancestral DC and TLR4-inducing multi-epitope peptide vaccine against the spike protein of SARS-CoV and SARS-CoV-2 using the advanced immunoinformatics approaches. Biochem Biophys Rep. 2024;39:101745.
85. Saleki K, Mohamadi MH, Alijanizadeh P, Rezaei N. Neurological adverse effects of chimeric antigen receptor T-cell therapy. Expert Rev Clin Immunol. 2023;19(11):1361–83.
86. Saleki K, Payandeh P, Shakeri M, Pourahmad R, Banazadeh M, Alijanizadeh P, et al. Utilizing immunoinformatics to target brain tumors; an aid to current neurosurgical practice. Interventional Pain Medicine and Neuromodulation. 2022;2(1).
87. Saleki K, Aram C, Alijanizadeh P, Khanmirzaei MH, Vaziri Z, Ramzankhah M, et al. Matrix metalloproteinase/Fas ligand (MMP/FasL) interaction dynamics in COVID-19: An in silico study and neuroimmune perspective. Heliyon. 2024;10(10).
88. Mohseni Afshar Z, Babazadeh A, Janbakhsh A, Afsharian M, Saleki K, Barary M, et al. Vaccine-induced immune thrombotic thrombocytopenia after vaccination against Covid-19: a clinical dilemma for clinicians and patients. Rev Med Virol. 2022;32(2):e2273.
89. Vaziri Z, Saleki K, Aram C, Alijanizadeh P, Pourahmad R, Azadmehr A, et al. Empagliflozin in treatment of cardiotoxicity: A comprehensive review of clinical, immunobiological, neuroimmune, and therapeutic implications. Biomedicine & Pharmacotherapy. 2023;168:115686.
90. Aram C, Alijanizadeh P, Saleki K, Karami L. Development of an ancestral DC and TLR4-inducing multi-epitope peptide vaccine against the spike protein of SARS-CoV and SARS-CoV-2 using the advanced immunoinformatics approaches. Biochem Biophys Rep. 2024;39:101745.
91. Rahmani A, Baee M, Saleki K, Moradi S, Nouri HR. Applying high throughput and comprehensive immunoinformatics approaches to design a trivalent subunit vaccine for induction of immune response against emerging human coronaviruses SARS-CoV, MERS-CoV and SARS-CoV-2. J Biomol Struct Dyn. 2022;40(13):6097–113.
92. Saleki K, Mohamadi MH, Banazadeh M, Alijanizadeh P, Javanmehr N, Pourahmad R, et al. In silico design of a TLR4-mediating multiepitope chimeric vaccine against amyotrophic lateral sclerosis via advanced immunoinformatics. J Leukoc Biol. 2022;112(5):1191–207.
93. Saleki K, Shirzad M, Javanian M, Mohammadkhani S, Alijani MH, Miri N, et al. Serum soluble Fas ligand is a severity and mortality prognostic marker for COVID-19 patients. Front Immunol. 2022;13:947401.
94. Saleki K, Alijanizade P, Moradi S, Rahmani A, Banazadeh M, Mohamadi MH, et al. Engineering a novel immunogenic chimera protein utilizing bacterial infections associated with atherosclerosis to induce a deviation in adaptive immune responses via Immunoinformatics approaches. Infection, Genetics and Evolution. 2022;102:105290.

95. Saleki K, Alijanizadeh P, Javanmehr N, Rezaei N. The role of Toll-like receptors in neuropsychiatric disorders: Immunopathology, treatment, and management. Med Res Rev. 2024;44(3):1267–325.
96. Maurya VK, Kumar S, Maurya S, Ansari S, Paweska JT, Abdel-Moneim AS, et al. Structure-based drug designing for potential antiviral activity of selected natural product against Monkeypox (Mpox) virus and its host targets. Virusdisease. 2024;1–20.
97. Latifi R, Azadmehr A, Mosalla S, Saleki K, Hajiaghaee R. Scolicidal effects of the Nicotiana tabacum L. extract at various concentrations and exposure times. J Med Plants. 2022;21(82):111–8.
98. Alizadehmoghaddam S, Pourabdolhossein F, Najafzadehvarzi H, Sarbishegi M, Saleki K, Nouri HR. Crocin attenuates the lipopolysaccharide-induced neuroinflammation via expression of AIM2 and NLRP1 inflammasome in an experimental model of Parkinson's disease. Heliyon. 2024;10(3).
99. Saleki K, Payandeh P, Shakeri M, Pourahmad R, Banazadeh M, Alijanizadeh P. Utilizing Immunoinformatics to Target Brain Tumors; An Aid t o Current Neurosurgical Practice. Interv. Pain Med Neuromodulation. 2022;2(1).
100. Vaziri Z, Saleki K, Aram C, Alijanizadeh P, Pourahmad R, Azadmehr A, et al. Empagliflozin in treatment of cardiotoxicity: A comprehensive review of clinical, immunobiological, neuroimmune, and therapeutic implications. Biomedicine & Pharmacotherapy. 2023;168:115686.
101. Saleki K, Banazadeh M, Saghazadeh A, Rezaei N. Aging, testosterone, and neuroplasticity: friend or foe? Rev Neurosci. 2023;34(3):247–73.
102. Kaur M, Sharma A, Kaur H, Singh M, Devi B, Naresh Raj AR, et al. Screening of potential inhibitors against structural proteins from Monkeypox and related viruses of Poxviridae family via docking and molecular dynamics simulation. J Biomol Struct Dyn. 2023 Sep;1–16.
103. Suleman M, Rashid F, Ali S, Sher H, Luo S, Xie L, et al. Immunoinformatic-based design of immune-boosting multiepitope subunit vaccines against monkeypox virus and validation through molecular dynamics and immune simulation. Front Immunol. 2022;13:1042997.