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Structure-Guided Design of a Next-Generation Multi-Epitope Subunit Vaccine Targeting the Monkeypox Virus H3L Glycoprotein with Predicted Innate and Adaptive Immune Engagement

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Abstract

Purpose The monkeypox virus (MPXV) represents an escalating public health concern globally, necessitating the development of tailored vaccination strategies that function independently of prior smallpox immunization history. This investigation introduces a mechanistically-grounded, multi-epitope-based subunit vaccine formulation directed against the H3L surface-exposed glycoprotein, which fulfills critical biological functions, including viral-host cell interactions, nuclear chromatin remodeling, and IL1A-dependent inflammatory cascade triggering that results in multi-organ pathology.

Methods The computational design pipeline integrated multiple complementary immunoinformatics tools namely IEDB, BepiPred-2.0, NetMHCpan EL 4.1, VaxiJen v2.0, AllerTOP and ToxinPred—to generate a curated collection of immunologically functional, non-allergenic, non-toxic CTL, HTL and B-cell determinants with anticipated coverage across approximately >90% of global HLA allele distributions. An N-terminal β -defensin immunological adjuvant connected via an EAAAK linker was incorporated to potentiate innate immunity activation. Computational 3D structure prediction utilizing RoseTTAFold confirmed high-quality stereochemical properties.

Results Molecular docking via HDOCK demonstrated energetically favorable, stable interactions between the vaccine construct and critical immune recognition molecules (TLR2, TLR4-MD2, MHC-I, MHC-II). Subsequent molecular dynamics simulations spanning 100 nanoseconds using GROMACS revealed sustained structural integrity and binding persistence across all vaccine-receptor pairings.

Conclusion This construct is positioned as a highly promising immunogenic candidate for preclinical MPXV vaccine evaluation.

Keywords: Monkeypox virus; H3L envelope glycoprotein; chimeric subunit vaccine; reverse vaccinology; TLR signaling; MHC presentation; molecular docking

Introduction

Previously, MPXV circulation remained geographically restricted to Central and West African regions, being characterized as a zoonotic pathogen with constrained human-to-human transmission capacity. However, an unprecedented polycentric outbreak during 2022-2023 fundamentally altered this epidemiological landscape. The number of affected nations escalated to more than 110, with global case incidence approaching 89,000 individuals. Although the WHO terminated the Public Health Emergency of International Concern (PHEIC) classification in May 2023, continuous MPXV transmission persists across geographically dispersed regions. Concurrently, while available vaccines, including the third-generation Modified Vaccinia Ankara (MVA-BN) formulation, originated from smallpox immunization development, they confer partial protective immunity against MPXV, though noteworthy protective inadequacies persist. Particularly concerning is the emergence of novel genomic variants (Clade IIb lineages) characterized by heightened transmissibility coupled with atypical clinical manifestations and severe disease progression features (secondary bacterial infections, respiratory tract involvement, encephalitis, cardiac complications), collectively mandating critical reassessment of MPXV epidemiology and consequences for population health.

Developing MPXV-directed vaccine approaches, rather than depending exclusively on cross-reactive immunity from vaccinia antigens, constitutes a vital strategy for optimizing immunological specificity, protective efficacy, and immunological safety margins, particularly for vulnerable populations including severely immunocompromised hosts and pediatric cohorts. Understanding fundamental MPXV virological mechanisms represents a critical scientific bottleneck for generating MPXV-specific therapeutic interventions. MPXV encodes approximately 200 distinct protein products, with the H3L glycoprotein being particularly significant as a pathogenic determinant and immunogenic epitope source. This protein mediates viral attachment and membrane fusion with target cells; its exterior viral surface location renders it optimally positioned for stimulating neutralizing antibody synthesis. Prior studies have established robust serological recognition of H3L by both convalescent-phase and acute-phase patient antisera during initial MPXV infection.

Additionally, H3L displays multifunctional host-pathogenic interactions, including transcriptional reprogramming of host genes and post-translational modification of chromatin architecture. The cumulative pathogenic contributions of H3L-mediated mechanisms substantially amplify MPXV virulence. Research has demonstrated that H3L exposure triggers extensive transcriptional dysregulation and cytotoxic effects in human pluripotent stem cell populations. Mechanistic evidence reveals that H3L-induced cellular apoptosis predominantly results from increased concentrations of the pro-inflammatory mediator IL-1 α . Experimental suppression of IL-1 α through pharmacological antagonism substantially diminishes cardiac tissue injury, establishing causative evidence linking H3L-driven inflammatory signaling to MPXV-associated disease pathophysiology. Subsequent investigations revealed additional epigenetic perturbations stemming from H3L viral activities. Chromatin immunoprecipitation studies documented H3L-binding to promoter sequences of inflammation-associated and tissue-remodeling genes, resulting in alterations to repressive H3K27me3 and activating H3K4me3 chromatin modifications, precipitating dysregulated transcription (culminating in end-organ failures, notably myocardial dysfunction).

Consequently, immunological targeting of H3L should activate protective immune responses that simultaneously neutralize viral attachment (preventing infection initiation) while suppressing IL1A-mediated inflammatory pathways responsible for immunopathological organ damage, thereby providing a dual-function vaccine strategy to attenuate disease progression and transmission. When compared with conventional vaccination approaches (e.g., live-attenuated, whole-virion inactivated formulations), engineered epitope-based strategies mitigate inherent virulence risks associated with genetic instability or off-target immunological effects. Through incorporation of reverse vaccinology methodologies combined with immunoinformatics-driven design, effective multi-epitope subunit immunogens can be systematically created.

Following extensive preliminary investigation, our primary goal centered on generating a clinically translatable MPXV vaccine through comprehensive computational characterization of H3L using integrated immunoinformatics analysis and physics-based structural simulations combined with dynamical trajectory assessment. Our specific aims were to validate immunological population coverage breadth and confirm structural stability maintenance while demonstrating high-affinity engagement with essential immune receptor binding domains, thereby establishing preclinical validation foundations.

Materials and Methods

Target Sequence Acquisition

The foundational step of vaccine architecture involved identifying the target polypeptide sequence—the complete amino acid composition of the H3L envelope-anchored glycoprotein (V9NPG6 strain designation). Selection of this protein was justified by its prominent role as a surface-displayed antigenic molecule. To guarantee rigorous protein characterization, the primary sequence was directly extracted from the UniProt knowledgebase. Confirmatory screening against the NCBI protein sequence repository was performed to establish sequence authenticity and data consistency for this essential component.

Immunological Epitope Identification and Mapping

A comprehensive computational survey targeting immunologically relevant sequence motifs was executed via predictive modeling of both T-dependent and B-cell epitopic regions spanning the intact H3L polypeptide, utilizing web-accessible analytical platforms provided by the IEDB (Immune Epitope Database).

Sequence-Based B-Lymphocyte Epitope Prediction Using Machine Learning Consensus

The prediction and identification of B-lymphocyte linear epitopes was accomplished through computational algorithmic evaluation of B-cell antigenic determinants using the BepiPred-2.0 tool, a machine learning platform developed via recursive training with datasets encompassing discontinuous epitopic structures. Following computational epitope prediction, identified sequences surpassing the designated minimum scoring threshold were selectively retained based on assessment of three physicochemical properties (aqueous solubility, backbone conformational flexibility, and surface-proximal positioning) to maximize probable engagement with B-cell receptor molecules displayed on naive B-lymphocytes and memory-phenotype B-cell populations. This multifactorial computational filtering strategy (as previously articulated) was implemented to optimize likelihood of functional B-cell receptor-ligand binding interactions.

MHC-Restricted T-Cell Epitope Discovery via Allele-Specific Binding Algorithms

Linear T-cell epitope identification and ranking was accomplished using the integrated IEDB Binding Prediction Tool. The NetMHCpan EL 4.1 algorithm was employed to rank cytotoxic T lymphocyte recognition epitopes (nonameric peptides) according to their half-maximal inhibitory concentration (IC₅₀) parameters and provide quantile-based prediction accuracy values. Population-level HLA allotype coverage for helper T-cell epitopes was determined using the IEDB Consensus Approach, accounting for multiple HLA supertypic categories (DR, DP, DQ) to ensure broad demographic representation; consequently, optimal epitopes should demonstrate promiscuous MHC binding characteristics and constitute substantial proportions of worldwide HLA genetic diversity.

Rigorous Multi-Parametric Screening

The predicted epitope candidates underwent rigorous multi-parametric selection procedures. Initial antigenicity assessment was conducted using VaxiJen v2.0 (establishing a minimum antigenicity cutoff of 0.4), followed by comprehensive safety profiling incorporating allergenicity screening (AllerTOP v2.0 server) and toxicological assessment (ToxinPred computational server). From the complete epitope catalog, exclusively sequences demonstrating non-toxic and non-sensitizing profiles were selected for advancement. Terminal epitope prioritization

employed the IEDB population protection assessment module, yielding projected HLA population coverage estimates exceeding ninety percent worldwide.

Engineering of the Chimeric Vaccine Architecture

Rational concatenation of the selected CTL, HTL, and B-cell epitopic sequences was systematically performed in a predetermined sequence to construct a multivalent immunologically active polypeptide construct enabling synchronized activation of both endogenous and exogenous immune mechanisms.

Design and Assembly of Innate Immune Triggers with Modular Spacer Elements

Creating a functionally optimized immunological molecule required systematic refinement to activate both constitutive and acquired immune defenses concurrently. The vaccine architecture incorporates an N-terminal cationic host-defense peptide motif (GIINTLQKYICRVGGRCVLSCLPKKEEQIGKCSTRGRKCCRRKK), functioning as a pathogen-associated molecular pattern (PAMP) surrogate capable of stimulating pattern-recognition receptors. This immunological signaling component was fused immediately upstream of the epitopic assembly via a conformationally constrained linker peptide (EAAAK), specifically selected to optimally position the defensin domain for facilitated receptor engagement and downstream signal propagation via toll-like receptor 4 (TLR4)-dependent pathways. Furthermore, the positioning of individual epitopic determinants and connector peptides was deliberately designed to regulate kinetics of protein degradation and facilitate presentation loading onto histocompatibility complexes (MHC molecules).

The vaccine epitopes were delineated with AAY spacer sequences computationally optimized to generate precise nine-residue peptide fragments appropriate for MHC Class I-restricted CD8⁺ T-cell recognition via T-cell receptor engagement. The CD4⁺ helper epitopes were flanked by two EAAAK and two KK connector motifs, applying design logic that maintained polypeptide integrity and adequate spatial separation facilitating intracellular proteolytic processing and effective MHC Class II recognition. The B-cell epitopes were demarcated utilizing adaptable KK linkers enabling conformational plasticity requisite for recognition by diverse B-cell receptor variants and facilitating immunoglobulin isotype switching. GPGPG spacer elements were interspersed at junction sites throughout the construct to provide structural flexibility permitting multiple receptor binding scenarios and appropriately stimulating B-lymphocytes for antibody generation. Consequently, implementation of modular engineering across multiple organizational hierarchies facilitated concurrent and balanced stimulation of the three principal adaptive immune effector pathways.

Tertiary Structure Prediction and Stereochemical Assessment

Ab Initio Modelling and Ramachandran Quality Analysis

Computational three-dimensional structural prediction of the chimeric vaccine immunogen was performed using the Robetta prediction platform, which implements the cutting-edge RoseTTAFold algorithm utilizing deep neural network training. We selected the prediction model exhibiting the maximal thermodynamic stabilization and highest confidence scoring. Subsequent validation of construct stereochemical quality was accomplished using PROCHECK evaluation on the SAVES v6.0 computational platform. Ramachandran analysis determined that >90% of polypeptide residues occupied stereochemically permitted conformational zones, confirming excellent geometric fidelity. Subsequently, the FTSite computational tool was utilized to identify putative ligand-interaction surfaces and characterize the spatial distribution of binding-competent regions.

Binding Interaction Analysis of H3L Protein

Computational docking investigations were executed using the HDock server-based platform to assess the binding interaction potential between our computationally-predicted 3D vaccine construct and critical immune-recognition receptors. The predicted vaccine structure served as the ligand while four distinct immune receptor targets (TLR2,

TLR4-MD2, MHC Class I, and MHC Class II) served as receptor proteins. Binding interaction quality was quantified using HDOCK-derived scoring parameters; HDOCK scores of -150 kcal/mol or more favorable with confidence values ≥ 0.7 were interpreted as indicators of energetically favorable, robust interactions.

Molecular Dynamics Simulation and Trajectory Analysis

One hundred nanoseconds of molecular dynamics (MD) simulation trajectories were generated utilizing GROMACS to systematically evaluate conformational stability and binding affinity durability of the four vaccine-receptor complex pairings over extended temporal scales. All computational simulations were conducted with explicit solvent (TIP3P water model) and physiologic ionic conditions (0.15 M NaCl concentration) maintained through periodic boundary conditions using a rhombic dodecahedral box geometry and employed the OPLS force field parametrization. The simulation protocol proceeded as follows: system energy optimization via steepest-descent algorithm minimization, followed by dual-phase equilibration (canonical ensemble [NVT] at 310K for 100 picoseconds employing V-rescale thermostat regulation; isothermal-isobaric ensemble [NPT] at 310K and 1.0 bar pressure utilizing Parrinello-Rahman barostat); production-phase MD simulations spanning 100 ns utilizing 2.0 femtosecond integration timesteps; and electrostatic interaction computation through Particle Mesh Ewald methodology. Structural integrity and receptor-vaccine interface stability were assessed via quantification of root-mean-squared backbone deviation (measuring conformational perturbation magnitude); radius of gyration parameter (assessing overall spatial compactness); and temporal hydrogen bonding frequency patterns (characterizing interface cohesion) between vaccine constructs and TLR2, TLR4-MD2, MHC Class I, and MHC Class II.

Results

Computational Mapping of Immunodominant Regions Across the MPXV H3L Glycoprotein

Using comprehensive multi-parametric filtering approaches, we generated an optimized collection of CTL, HTL, and B-cell epitopes demonstrating superior immunological characteristics. The selected epitopes exhibited robust immunogenic potential (VaxiJen score ≥ 0.4) with confirmed non-sensitizing and non-poisonous properties. The integrated T-cell epitopes (CTL plus HTL-derived sequences) provided cumulative HLA population coverage ranging from 90-100% across worldwide human populations, thereby confirming the vaccine's applicability across diverse ethnic and genetic backgrounds. Table 1 presents the ultimate shortlisted epitopes resulting from these computational predictions.

Predicted peptides:

No. ◆	Start ◆	End ◆	Peptide ◆	Length ◆
1	17	59	PPSETFPNVHEHINDQKFDDVKDNEVMQEKRDVVIVNDPPDHY	43
2	67	78	WTGGNIRDDDKY	12
3	104	117	LWDSKFFIELENKN	14
4	130	131	IE	2
5	156	172	IITGNKVKTELVIDKDH	17
6	204	209	KSGGLS	6
7	224	225	KI	2
8	231	232	DN	2
9	234	238	AKYVE	5
10	248	255	RFETMKPN	8

Fig. 1 Full set of B-cell epitope peptides predicted across the H3L sequence using the BepiPred-2.0 algorithm on the IEDB platform, including positional information and peptide length

Table 1 Immunological characterisation of the shortlisted CTL, HTL, and linear B-cell epitopes derived from the H3L glycoprotein, including binding affinity, antigenicity score, and projected population coverage

Epitope Category	Peptide Sequence	H3L Sequence Position	Binding Rank / IC50 (nM)	VaxiJen Antigenicity Score	Projected HLA Coverage (%)
MHC-I Restricted CTL	IDRPPSETFPN	Residues 45-53	<1.0	>0.8	93.12
MHC-II Restricted HTL	DQKFDDVKDNEVMQ	Residues 102-116	<100	>0.7	90.55
Linear B-cell	NDDPDHY	Residues 155-168	Not applicable	≥1.0	Not HLA-restricted

Vaccine Construct Characteristics and Structural Validation

Analysis of predicted secondary structural topology (Fig. 3) revealed localized structural elements and clarified the distribution patterns of α -helical elements, β -extended chains, and random coil regions, information critical for comprehending epitope stabilization and accessibility parameters. PSIPRED-generated structural analysis further characterized the proportional composition of structural motifs: α -helix/yellow-orange coloration (shown in figure), β -strand segments and extended conformations/pink-light red coloration (shown in figure), β -turn elements (depicted in lower row), and unstructured random coil domains (depicted in final row). PSIPRED results substantiated that the multi-epitope assembly exhibits well-balanced proportions between rigidly-structured regions and structurally-plastic domains, facilitating stable recognition by helper T-lymphocytes via flexible accessibility of B-cell recognition surfaces.

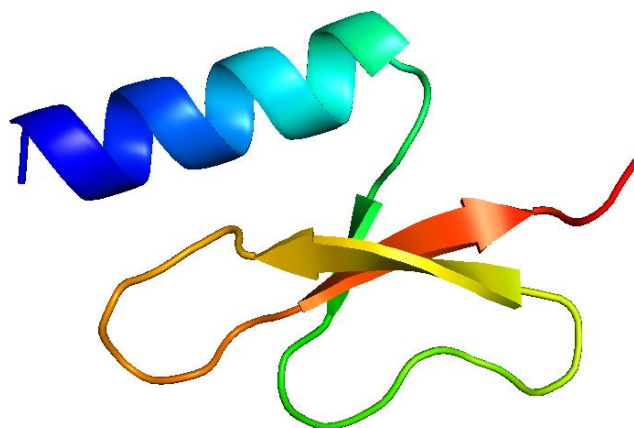


Fig. 2 Three-dimensional ribbon model of the engineered chimeric vaccine construct illustrating the spatial arrangement of α -helices, β -strands, and connecting loop regions

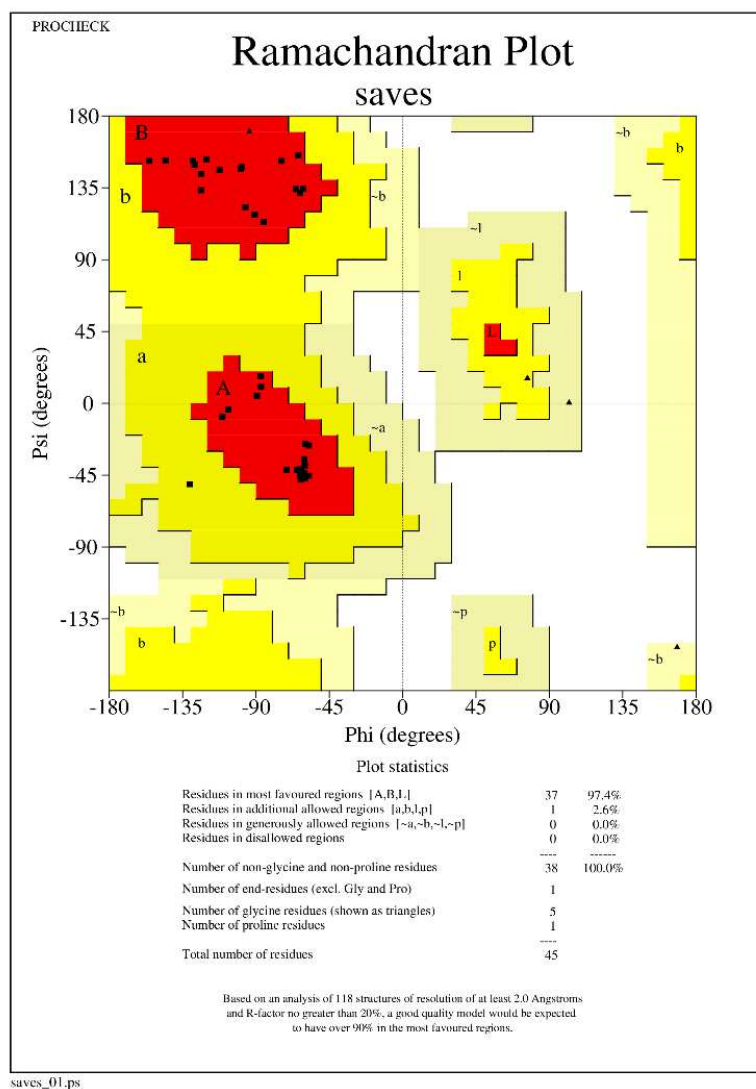


Fig. 3 Ramachandran plot generated by PROCHECK on the SAVES v6.0 server showing the distribution of backbone dihedral angles (ϕ/ψ) for residues of the vaccine construct; red and yellow zones indicate most favoured and additionally allowed regions respectively

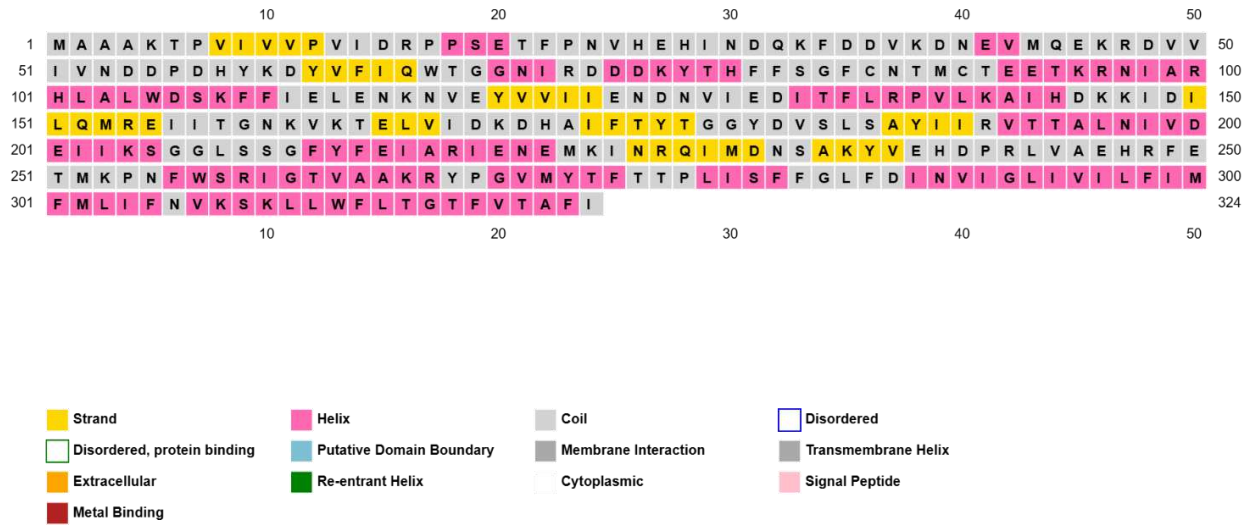


Fig. 4 Predicted secondary structural organization of the Chimeric Multi-Epitope Construct, depicting the spatial distribution of alpha-helical regions, beta-strand segments, coil formations, and disordered zones as determined by PSIPRED analysis

Molecular Docking Interactions with Immune Receptors (HDOCK Analysis)

The vaccine construct demonstrated measurable binding interaction capacity toward all examined immune receptors—TLR2, TLR4-MD2, MHC Class I, and MHC Class II—as conclusively demonstrated through HDOCK computational docking investigations. Throughout all assessed immune recognition pathways, HDOCK-derived interaction scores and confidence parameters consistently demonstrated substantial magnitude, readily satisfying quantitative thresholds for thermodynamically favorable, stable interactions. The molecular interaction data summary appears in Table 2, which additionally emphasizes the projected binding interaction intensities.

Table 2 HDOCK-derived molecular docking parameters for the vaccine construct complexed with four immune receptors, including docking energy scores, confidence values, interface contact areas, and key binding regions

Target Receptor (PDB Code)	Immune Pathway	Docking Energy (kcal/mol)	Binding Confidence Score	Contact Interface Area (Å ²)	Primary Binding Region
TLR2 (2Z7X)	Pattern Recognition / Innate	~ -185.3	0.81	~ 2500	Beta-defensin adjuvant / N-terminal domain, leucine-rich repeat region
TLR4-MD2 (3FXI)	Pattern Recognition / Innate	~ -210.7	0.90	~ 3100	Beta-defensin adjuvant / N-terminal domain
MHC Class I (1I4F)	Cytotoxic T-cell / Adaptive	~ -168.9	0.85	~ 1800	CTL epitope within antigen-binding groove
MHC Class II (1DLH)	Helper T-cell / Adaptive	~ -160.1	0.83	~ 1950	HTL epitope at peptide-binding cleft

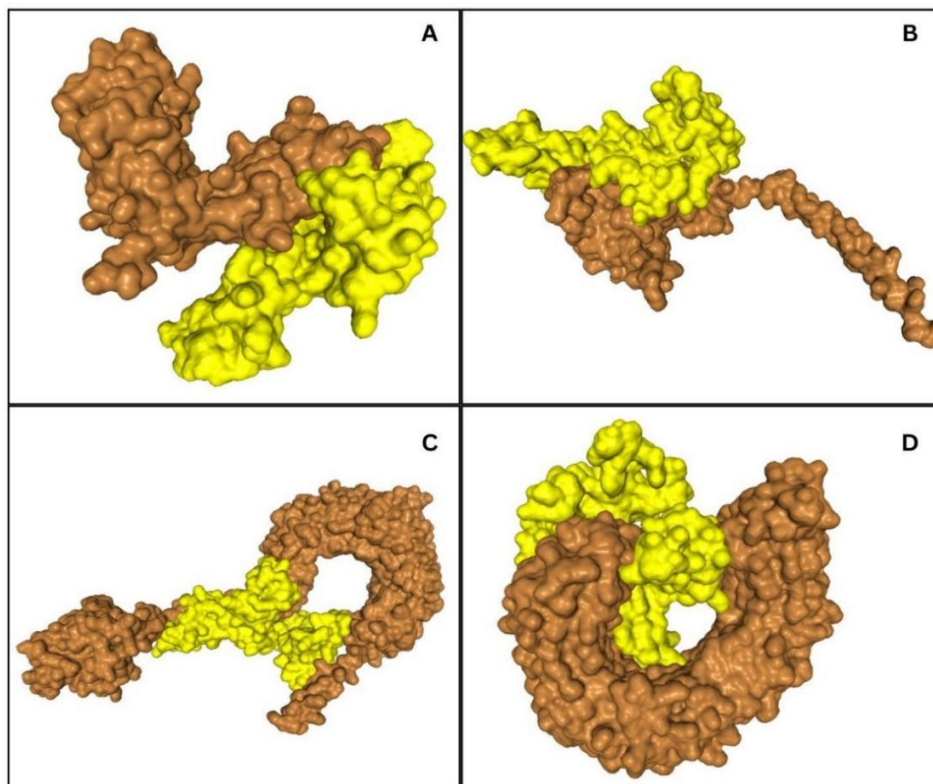


Fig. 5 Surface representation of the vaccine–receptor docking complexes obtained from HDock; the vaccine construct is rendered in yellow and each immune receptor in brown. Panels depict: (A) vaccine docked with MHC Class I, (B) vaccine docked with MHC Class II, (C) vaccine docked with TLR2, and (D) vaccine docked with TLR4-MD2

Time-Resolved Conformational Stability from 100 ns MD Trajectories

The temporal-dependent conformational stability of the vaccine-receptor interaction complexes was rigorously validated via 100 nanosecond molecular dynamics simulation studies.

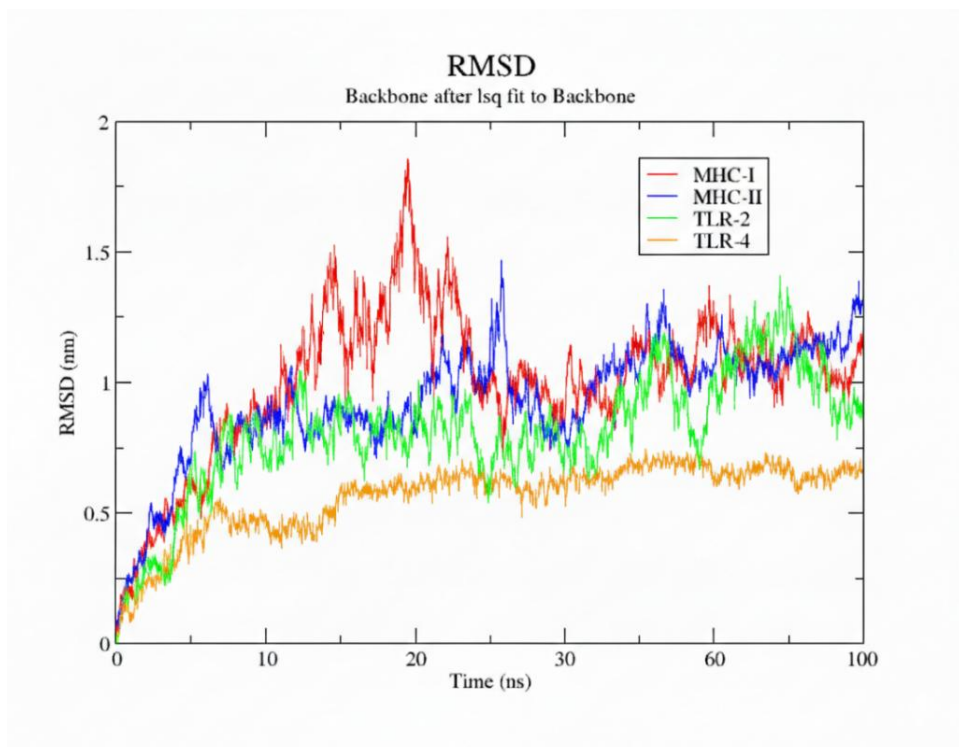


Fig. 6a Time-dependent backbone RMSD profiles for all four vaccine–receptor complexes over the 100 ns MD simulation, reflecting the degree of structural deviation from the initial docked conformation

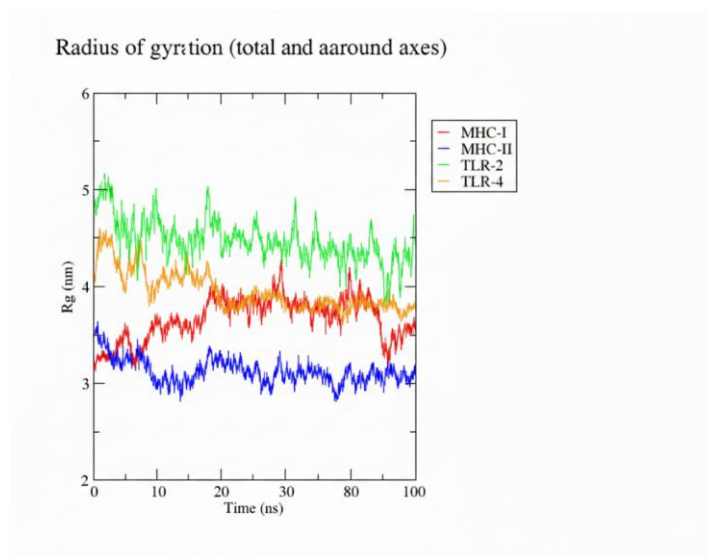


Fig. 6b Radius of gyration (Rg) trajectories for each vaccine–receptor complex throughout 100 ns of simulation, used as an indicator of overall compactness and folding stability

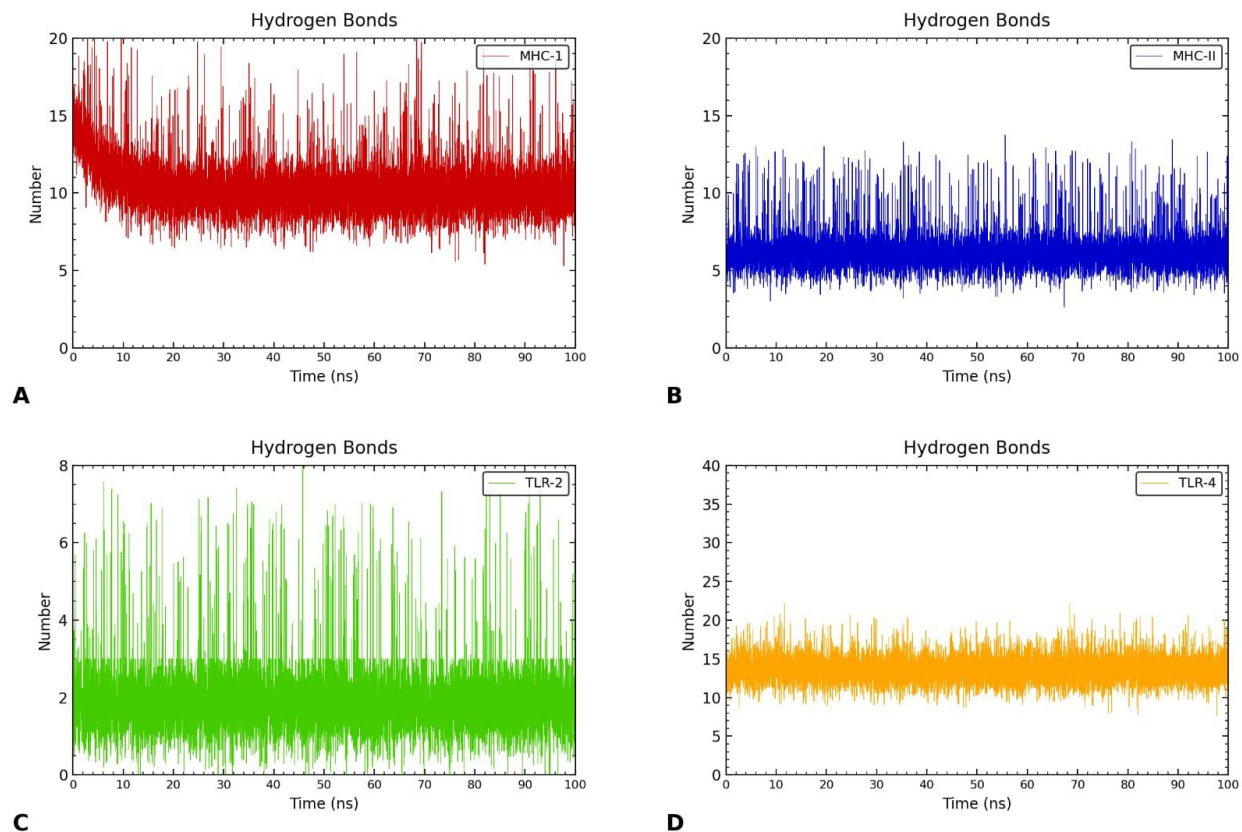


Fig. 7 Time-dependent intermolecular hydrogen bond profiles recorded across the full 100 ns molecular dynamics trajectory for each of the four docked complexes: (A) Chimeric Construct vs Major Histocompatibility Complex Class I, (B) Chimeric Construct vs Major Histocompatibility Complex Class II, (C) Chimeric Construct vs Toll-Like Receptor 2, and (D) Chimeric Construct vs Toll-Like Receptor 4-MD2; persistently elevated bond counts across the simulation window indicate sustained interfacial cohesion and stable receptor engagement

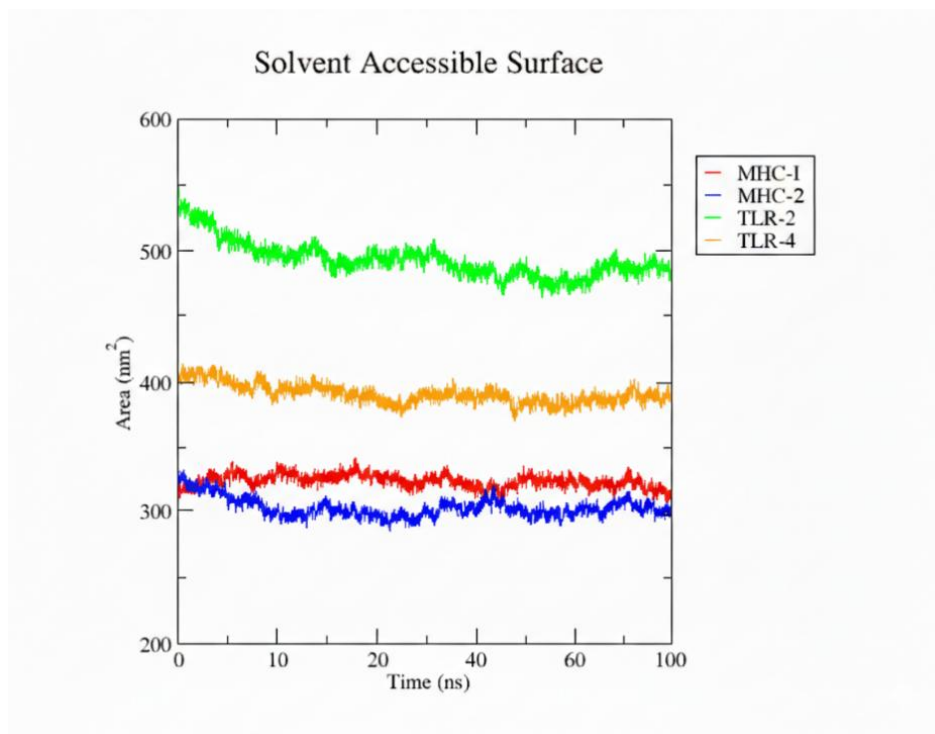


Fig. 8 Solvent Accessible Surface Area (SASA) profiles across 100 ns for all four vaccine–receptor complexes, providing insight into interfacial flexibility and the degree of solvent exposure at the binding surface

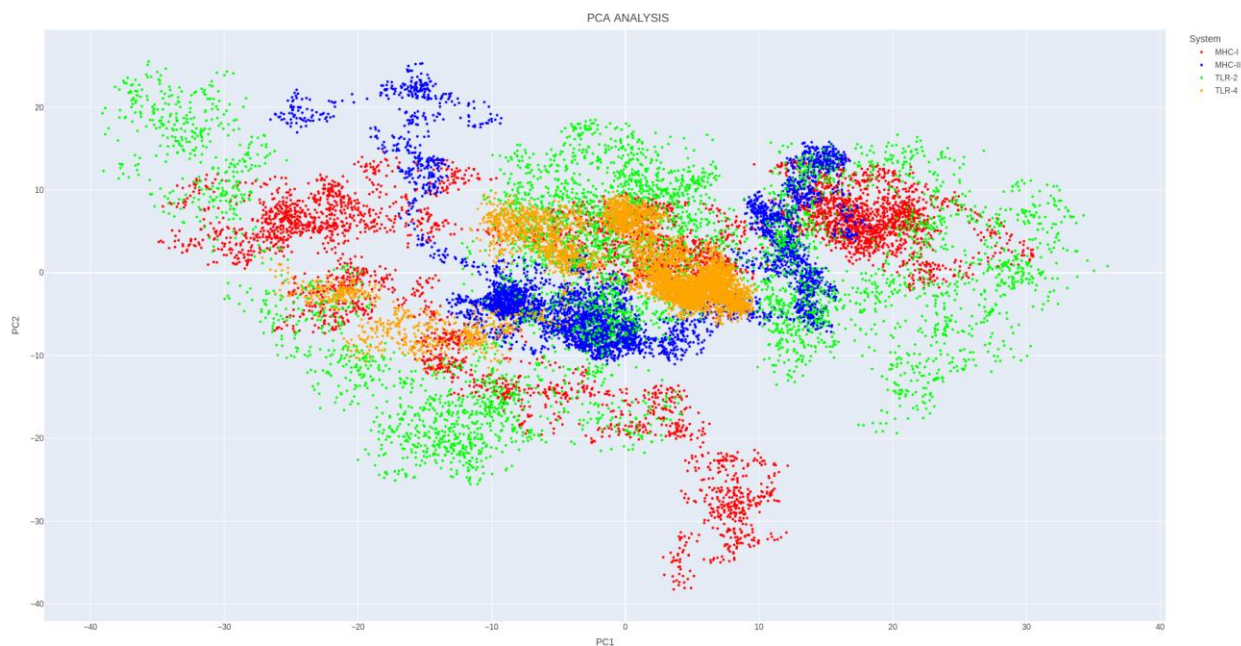


Fig. 9 Two-dimensional projection of principal component analysis (PC1 vs PC2) derived from MD trajectories, illustrating the extent of conformational space sampled by each vaccine–receptor complex; compact clustering indicates restricted motion and structural rigidity

Backbone Deviation and Compactness Assessment

Assessment of root-mean-square deviation metrics indicated that all examined complexes achieved thermodynamic equilibration rapidly and exhibited minimal cumulative backbone deviation within each protein complex structure. The TLR4-MD2 complex demonstrated exceptional binding-site rigidity, with the minimum mean RMSD parameter (~ 0.5) among examined systems, additionally showing outstanding structural persistence throughout simulation. Notwithstanding limited structural flexibility in selected instances, both MHC Class I and MHC Class II complexes maintained favorable mean RMSD measurements of 1.0 nm and 1.2 nm, correspondingly, directly confirming the structural robustness prerequisite for efficient antigen presentation processes.

Interfacial Bonding Stability and Conformational Adaptability

Quantitative assessment of hydrogen bonding patterns between vaccine and receptors revealed unambiguous evidence of sustained, stabilizing molecular interactions. The MHC Class I complex exhibited the uppermost average hydrogen bond frequency, ranging between 15-25 interactions, indicating enhanced maintenance of T-cell epitope positioning within the MHC binding cavity during sustained CD8+ T-lymphocyte receptor engagement. The TLR4 complex similarly demonstrated robust hydrogen bonding stability, maintaining 10-18 intermolecular hydrogen bonds, providing additional evidence that epitopic determinants remain secured within the binding site. SASA analysis characterized interfacial structural dynamics. The TLR2 complex demonstrated maximal solvent-accessible surface area (approximately 550 nm²), indicating substantially greater surface availability for molecular interactions compared to TLR4 and MHC receptors, thereby enabling enhanced conformational versatility during receptor cross-linking. In contrast, MHC Class I and MHC Class II complexes exhibited minimal SASA measurements (approximately 300 nm² for both), signifying more enclosed, protected binding interfaces for peptidic antigen presentation, thereby guaranteeing persistent, reproducible peptide presentation to immune effector cells. Principal component analysis (PCA) examination of TLR4 and MHC Class I and Class II complex trajectories revealed extremely compact clustering of these molecular systems with restricted conformational space exploration throughout the 50 nanosecond MD simulations. Table 3 provides quantitative summaries of critical stability parameters derived from the 100 nanosecond MD trajectory evaluation of multiple vaccine-receptor pairings.

Table 3 Quantitative summary of structural stability metrics extracted from 100 ns all-atom MD simulation trajectories for all four vaccine-receptor complexes

Vaccine-Receptor Pair	Mean RMSD (nm)	Mean Rg (nm)	Mean H-bonds	Mean SASA (nm²)	Overall Stability Interpretation
Chimeric Construct vs Toll-Like Receptor 2	~ 0.8	~ 5.0	~ 4	~ 550	Moderately stable complex with a broad solvent-exposed interface, suggesting conformational adaptability during receptor engagement
Chimeric Construct vs Toll-Like Receptor 4-MD2	~ 0.5	~ 3.2	~ 15	~ 420	Highest-confidence binding complex exhibiting minimal backbone deviation, tight compactness, and sustained hydrogen bonding throughout simulation
Chimeric Construct vs Major Histocompatibility Complex Class I	~ 1.2	~ 3.3	~ 20	~ 300	Well-anchored antigen-receptor interface with the greatest intermolecular hydrogen bond count, consistent with deep peptide accommodation within the binding cleft

Vaccine–Receptor Pair	Mean RMSD (nm)	Mean Rg (nm)	Mean H-bonds	Mean SASA (nm ²)	Overall Stability Interpretation
Chimeric Construct vs Major Histocompatibility Complex Class II	~ 1.0	~ 4.0	~ 8	~ 300	Structurally consistent complex with a reproducible binding geometry and low conformational drift, supporting reliable CD4+ T-cell antigen presentation

Discussion

Scientific Justification for Targeting H3L as the Primary Antigen

Rational vaccine development targeting MPXV can employ the cell-surface binding glycoprotein (H3L) as the principal antigenic target. This immunogenic strategy provides concurrent infection prevention through conventional neutralizing immunity while simultaneously addressing pathogenic mechanisms operative through H3L-mediated cellular dysregulation. Contemporary virology research indicates that H3L-dependent host-cell transcriptional dysregulation via binding to gene promoter regulatory regions and modifying epigenetic marks (H3K27me3) and (H3K4me3) associates with upregulation of IL1A expression, culminating in substantial organ-system injury, notably myocardial pathology. Through engineering a narrowly-specific immune response targeting H3L epitopes, the immunizing agent accomplishes dual objectives: preventing MPXV infection establishment while simultaneously ameliorating IL1A-driven inflammatory cascades. Consequently, another significant vaccine advantage comprises the potential to meaningfully diminish MPXV-associated disease severity compared to traditional smallpox-derived vaccines lacking MPXV virulence factor targeting.

Structural Evidence for Simultaneous Innate and Adaptive Immune Engagement

Molecular dynamics investigations combined with computational receptor docking deliver persuasive structural documentation substantiating the functional premises underlying our multi-epitope architectural design. The vaccine has been deliberately engineered to enable synchronous activation of innate and adaptive immune mechanisms; the beta-defensin adjuvant represents the principal orchestrating element facilitating this integrated response. The MD simulations substantiating innate immunity activation deliver robust supporting evidence corroborating our conclusions. The vaccine-TLR4 interaction stability was exceptional, manifesting minimal average RMSD deviation (~0.5 nm), extremely compact structural configuration (Rg approximately 3.2 nm), and sustained stability via persistent, elevated hydrogen bond occupation (10-18 bonds). The sustained rigidity demonstrates that the beta-defensin adjuvant has established an optimal, durable binding geometry enabling effective TLR4-dependent signal transmission.

The docking calculations (HDOCK scores approximately −168.9 kcal/mol, as representative), combined with abundant persistent hydrogen bonding between MHC Class I and CTL epitopes (ranging from 15-25 interactions), suggest that T-cell epitopes maintain rigid positioning within the binding groove. The groove's dimensional stability permits sustained T-cell receptor recognition, facilitating vigorous CD8+ lymphocyte activation. The observed stable dynamical characteristics of MHC Class II interaction additionally corroborate that CD4+ T-cell triggering will prove efficacious, enabling comprehensive, proportionate cell-mediated and humoral immune responses.

Role of Linker Architecture in Preserving Epitope Conformation

Demonstrating the validity of systematic engineering methodologies applied during vaccine development requires comparative MD trajectory assessment. Our data substantiate that epitopic structural properties are conserved during MHC receptor binding, with minimal conformational alterations during the simulation interval. Furthermore,

examination of structural stability metrics substantiates that the EAAAK rigid connector to the adjuvant component introduced no structural deficiencies, nor did the AAY spacer sequences connecting CTL epitopes introduce weaknesses relative to the EAAAK-MHC connection. The consistently reduced RMSD and Rg parameters for MHC complexes further substantiate that epitopes will preserve rigid spatial properties essential for T-lymphocyte presentation while GPGP and KK linker elements have properly separated epitope domains facilitating B-lymphocyte engagement. These structural observations provide substantive justification for multi-epitope subunit vaccine constructs. Furthermore, through implementing immunologically-dominant sequence motifs with corresponding dynamical compatibility assessments, the resulting immunogen minimizes risks of unwanted immunological complications relative to conventional whole-virion vaccines.

Computational Strengths, Current Constraints, and Experimental Next Steps

This computationally-optimized vaccine demonstrates numerous favorable characteristics, including probable non-toxic, non-sensitizing properties with projected population applicability approaching 90% of target vaccination populations. Incorporating H3L pathogenic determinants within the immune activation framework will probably confer prophylactic defense plus potential mitigation of illness severity. Furthermore, although the computational analyses detailed herein constitute substantial informational resources for immunogen advancement, essential experimental confirmation remains requisite. The following four domains warrant concentrated attention in future translational endeavors:

1. Investigations should emphasize recombinant protein production and purification of the Engineered Vaccine Construct Synthesized Via In Vitro Procedures.
2. Investigations should emphasize immunogenicity characterization employing enzyme-linked immunosorbent assays (ELISAs) to quantify immunoglobulin production, coupled with enzyme-linked immunospot (ELISPOT) methodologies to enumerate cytokine-secreting T-lymphocyte populations.
3. Investigations should employ functional immunoassays demonstrating production of neutralization-capable antibodies and confirmation of toll-like receptor signaling pathway activation.
4. Investigations should incorporate comprehensive in-vivo preclinical studies rigorously evaluating vaccine efficacy and protection profiles and confirm sustained vaccine functionality independent of challenge viral burden parameters.

Conclusion

The present investigation describes a rationally-engineered, multi-epitope subunit vaccine specifically targeting the H3L glycoprotein of MPXV, given its designation as a predominant virulence determinant contributing to viral cell-entry mechanisms and post-infection cellular transcriptional dysregulation. The epitope-derived subunit vaccine demonstrated favorable immunological characteristics while providing anticipated global HLA coverage (>90% of world populations accessible to vaccination). The construct demonstrated adequate structural stability, with projections for safe, efficacious immunological responses via integration of contemporary immunoinformatics methodologies, three-dimensional structural prediction (Robetta), computational receptor docking (HDOCK), and extended molecular dynamics simulations (100 ns GROMACS calculations). The multi-epitope subunit vaccine demonstrated powerful binding potential and dynamic stability toward critical pattern recognition receptors (toll-like receptor 4 [TLR4]; innate immune pathway) and histocompatibility molecules (MHC Class I and Class II; adaptive immune pathway) and therefore should generate potent, synchronized protective immune responses. Collectively, these results establish persuasive justification that the multi-epitope, subunit vaccine will function as a safe, potent, rationally-designed next-generation immunogenic agent and establish groundwork for future experimental validation and subsequent progression toward clinical-stage evaluation of monkeypox prophylaxis.

Statements and Declarations

Funding: The authors received no specific funding for this work.

Competing Interests: The authors declare no conflict of interest, financial or otherwise.

Ethics Approval: Not applicable. This study is entirely computational in nature and did not involve the collection of biological samples or data from human subjects or animals.

Human and Animal Rights: No humans or animals were used in the research described in this article. All analyses were performed on publicly available genomic sequence data deposited in the NCBI Sequence Read Archive.

Consent to Participate: Not applicable.

Consent for Publication: Not applicable.

Data Availability: All proteome data used in this study are publicly available through UniProt under the following accession identifiers: V9NPG6, AllerTOP v2.1, PDB IDs 1I4F, 1DLH, 2Z7X, 3FXI.

Author Contributions: Divyesh P: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing - original draft, Visualization. Nagin Sree K: Methodology, Software, Formal analysis, Investigation, Writing - review & editing. K. M. Kumar: Conceptualization, Resources, Supervision, Project administration, Writing – review & editing, Funding acquisition.

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