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Rational In Silico Design of a Multiepitope Marburg Virus Vaccine Candidate

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Abstract

Marburg virus (MARV) represents a critical public health threat with mortality rates of 24–88%. Here, we present an in silico vaccine design pipeline targeting MARV nucleoprotein. The integrated workflow encompassed: (1) sequence retrieval and physicochemical characterization via ExPASy ProtParam; (2) secondary structure prediction using PSIPRED; (3) antigenicity screening using VaxiJen; (4) allergenicity assessment via AllerTOP and toxicity screening via ToxDL; (5) B cell and T cell (MHC-I/II) epitope prediction using IEDB Analysis Resource; (6) population coverage analysis; (7) vaccine construct design incorporating 50S ribosomal protein L7/L12 adjuvant with epitopes linked by EAAAK, CPGPG, and AAY linkers with His6-tag; (8) construct validation for antigenicity, allergenicity, toxicity, solubility (SOLUPROT), and physicochemical properties; (9) 3D structure prediction via trRosetta, refinement with GalaxyRefine, and quality assessment via UCLA-DOE LAB PROCHECK Ramachandran plots; (10) molecular docking validation using PatchDock; (11) DNA sequence optimization via EMBOSS Backtranseq and JCat, with gene synthesis and pET vector cloning via SnapGene; and (12) immune dynamics simulation via C-IMMSIM. Results: The optimized 681 aa vaccine construct achieved 36% improvement in antigenicity (0.6416 vs. 0.4699), improved stability (instability index 44.61 vs. 51.27), predicted solubility (0.811), non-allergenic/non-toxic profiles, 95.2% Ramachandran-favored residues, favorable molecular docking (ΔG –7.2 to –8.4 kcal/mol), exceptional population coverage (70% MHC-I, 85% MHC-II globally), and robust predicted immune responses (IgM peak 500K cells/mm³, sustained IgG, Th cell expansion to 3500 cells/mm³, balanced Th1 cytokine profile). The optimized construct is suitable for recombinant E. coli expression via the engineered pET-28a vector. This multiepitope vaccine candidate represents a rationally designed, broadly applicable, and immunologically promising prototype for preclinical development.

Keywords: vaccine design, epitope prediction, Marburg virus, multiepitope, population coverage, molecular docking, immune simulation

1. Introduction

Marburg virus disease (MVD) is a rare but severe illness with case fatality rates ranging from 24% to 88%, depending on viral strain and host factors. The virus is maintained in Rousettus bat species (fruit bats) as natural reservoirs, with sporadic spillover to human populations

through direct contact with infected animals or tissues. Recent outbreaks in Uganda and Kenya (2022) underscore the persistent threat of MARV emergence. Currently, no approved vaccines or therapeutics exist, making MVD a critical gap in global pandemic preparedness.

Traditional vaccine development follows empirical pathways involving production of inactivated viruses, virus-like particles (VLPs), or recombinant viral vectors, followed by extensive preclinical testing and clinical trials. Such approaches require 10–15 years of development, cost >\$1 billion per vaccine, and necessitate biosafety level 4 (BSL-4) laboratory facilities. Computational vaccine design offers a complementary rational approach that predicts immunogenic epitopes, validates their compatibility with human HLA diversity, and optimizes protein manufacturability prior to synthesis.

This study presents an integrated computational immunology pipeline for designing a multiepitope vaccine targeting Marburg virus nucleoprotein (NP). Nucleoprotein was selected because: (i) it is immunodominant in MARV-infected subjects; (ii) it is highly conserved across MARV strains; (iii) it is abundantly expressed during viral replication; and (iv) it avoids non-neutralizing antibody responses directed at decoy epitopes on surface glycoproteins. This work demonstrates the feasibility of open-source, web-accessible computational approaches for rapid, cost-effective vaccine design.

2. Methods

2.1 Source Sequence Retrieval and Initial Characterization

The amino acid sequence of MARV nucleoprotein was retrieved from the National Center for Biotechnology Information (NCBI) Protein database (RefSeq: YP_001531153.1; Marburg virus Musoke, Kenya 1980; 695 amino acids). The physicochemical properties of the original protein were computed using ExPASy ProtParam (<https://web.expasy.org/protparam/>), including: molecular weight (Da), theoretical isoelectric point (pI), extinction coefficients at 280 nm ($M^{-1} \text{ cm}^{-1}$), instability index (II), aliphatic index (AI), and grand average of hydropathicity (GRAVY). Estimated half-life in mammalian reticulocytes (in vitro), yeast (in vivo), and *E. coli* (in vivo) were also computed.

2.2 Secondary Structure Prediction and Antigenicity Assessment

Secondary structure was predicted using PSIPRED v4.01 (<http://bioinf.cs.ucl.ac.uk/psipred/>), employing Position-Specific Iterated BLAST (PSI-BLAST) and two-stage neural networks to predict helix, sheet, and coil propensities at each residue position. The overall antigenicity of the original nucleoprotein was assessed using VaxiJen v2.0 (<http://www.ddg-pharmfac.net/vaxijen/>), a support vector machine (SVM) classifier trained on known viral antigens. VaxiJen employs amino acid composition and protein physicochemical properties to generate an antigenicity score (0–1); scores ≥ 0.4 (using the "virus" prediction model) were classified as probable antigens.

2.3 Allergenicity and Toxicity Screening

Potential allergenic properties were screened using AllerTOP v2.0 (<http://www.ddg-pharmfac.net/AllerTOP/>), a machine learning classifier that identifies allergen homology via E-descriptors and Z-descriptors of amino acid properties. Toxicity assessment was performed using ToxDL (<http://toxtree.sourceforge.net/>), which identifies structural alerts for known toxic functional groups. Both screening steps were repeated for the final vaccine construct to ensure safety compliance.

2.4 B Cell and T Cell Epitope Prediction

B cell linear epitopes were predicted using IEDB Analysis Resource (<http://www.iedb.org/>) employing the Kolaskar & Tongaonkar algorithm. MHC-I restricted epitopes (9-mer peptides) were predicted using the IEDB consensus method, which integrates predictions from multiple algorithms (NetMHC 4.0, SMM, PickPocket, MHCflurry). All common HLA-A, -B, and -C alleles were queried. Strong binders were defined as peptides with predicted affinity ≤ 500 nM. MHC-II epitopes (15-mer peptides) were predicted using NN-align and SMM-align algorithms for all common HLA-DR, -DQ, and -DP alleles. Strong binders were defined as affinity ≤ 1000 nM. Epitope predictions were filtered for uniqueness via BLAST search against the NCBI non-redundant (nr) database to minimize off-target cross-reactivity.

2.5 Epitope Validation and Population Coverage Analysis

Each predicted epitope was individually assessed for antigenicity using VaxiJen (threshold ≥ 0.4). Allergenic properties of epitopic peptides were evaluated via AllerTOP. Population coverage of the selected epitope set was calculated using the IEDB Population Coverage tool (http://www.iedb.org/popup_comp_mhc_coverage_pie), which integrates allele frequency data from >11,000 individual subjects across worldwide populations. The tool computes the cumulative percentage of individuals predicted to have at least one epitope-HLA binding combination.

2.6 Vaccine Construct Design

The vaccine construct was rationally designed as a recombinant fusion protein with: (1) 50S ribosomal protein L7/L12 from *E. coli* (55 aa), selected for immunostimulatory properties as a Toll-like receptor 2/6 (TLR2/6) ligand; (2) predicted B cell epitopes (7–12 aa each, total 9 epitopes) separated by EAAAK linkers (5 aa each); (3) MHC-II restricted epitopes (15 aa each, total 10 epitopes) linked by flexible CPGPG and AAY linkers; (4) MHC-I restricted epitopes (9 aa each, total 9 epitopes) spaced by EAAAK linkers; and (5) C-terminal His6-tag (6 aa) for purification via immobilized metal affinity chromatography (IMAC). The construct was assembled in silico using direct sequence concatenation to ensure proper reading frame continuity.

2.7 Optimized Vaccine Construct Validation

The optimized vaccine construct sequence was validated for: (a) antigenicity via VaxiJen v2.0 (virus model, threshold 0.4); (b) potential allergenic epitopes via AllerTOP v2.0; (c) toxicity

via ToxDL; (d) expression-level solubility via SOLUPROT (<http://solprot.mib.man.ac.uk/>), a machine learning tool for E. coli-expressed recombinant proteins; and (e) physicochemical properties via ExpASY ProtParam. Secondary structure was re-predicted using PSIPRED.

2.8 Three-Dimensional Structure Prediction and Validation

3D protein structure of the optimized vaccine construct was predicted using trRosetta (<http://yanglab.nankai.edu.cn/trRosetta/>), a template-free structure prediction method using neural networks and iterative Rosetta-based refinement. The raw trRosetta output (PDB format) was refined using GalaxyRefine (<http://galaxy.seoklab.org/cgi-bin/submit.cgi?type=grefine>), which performs global optimization, side-chain repacking, and structural relaxation. Model quality was assessed using UCLA-DOE LAB PROCHECK (<https://www.ebi.ac.uk/thornton-srv/software/PROCHECK/>), which generates Ramachandran plots. Models with >90% residues in most-favored regions were considered acceptable.

2.9 Molecular Docking of Epitopes to HLA Molecules

Epitope-MHC interactions were validated via molecular docking using PatchDock (<http://bioinfo3d.cs.tau.ac.il/PatchDock/>), a geometry-based docking algorithm. Representative HLA alleles (HLA-A*02:01, HLA-B*07:02, HLA-DRB1*01:01) were selected based on global population frequency. MHC class I and II molecule crystal structures were obtained from the Protein Data Bank (PDB). Epitopic peptides (9-mers for MHC-I, 15-mers for MHC-II) were docked to peptide-binding grooves. Top-ranked docking poses (rank ≤5) were retained and visually inspected in PyMOL v2.5 for plausibility of binding interactions, including hydrogen bonding networks and hydrophobic contacts.

2.10 DNA Sequence Design and Codon Optimization

The protein sequence of the optimized vaccine construct was converted to DNA via reverse translation using EMBOSS Backtranseq (http://www.ebi.ac.uk/Tools/st/emboss_backtranseq/), which applies the E. coli codon usage table. The resulting 2043 bp nucleotide sequence was subjected to codon optimization using JCat (Java Codon Adaptation Tool, <http://www.jcat.de/>), which: (a) increases codon adaptation index (CAI, target ≥0.85 for E. coli); (b) balances GC content (target 50–60%); (c) eliminates rare codons; and (d) removes potential secondary structures. The optimized gene sequence was verified for absence of cryptic restriction sites and internal translation stop codons.

2.11 Gene Cloning into pET Expression Vector

The optimized 2043 bp vaccine gene was cloned into the pET-28a(+) expression vector (Novagen/Merck) in silico using SnapGene (<http://snapgene.com/>). The vaccine gene was inserted between NdeI (5' site) and XhoI (3' site) restriction sites, maintaining proper reading frame for expression of the His6-tag-linked fusion protein. The resulting pET28a-vaccine

construct (~5.8 kb) was validated for: (a) correct reading frame through the entire vaccine CDN; (b) presence of in-frame His6-tags; (c) presence of T7 promoter, Shine-Dalgarno ribosome binding site, and T7 terminator; (d) absence of internal translation stop codons; and (e) presence of kanR marker and pBR322 origin of replication. Virtual translation confirmed generation of the expected 681 aa fusion protein.

2.12 Immune Dynamics Simulation

Predicted immune system responses to the optimized vaccine construct were simulated using C-IMMSIM v7.0 (Cellular Immunology Simulator; <http://c-immsim.iac.rm.cnr.it/>), an agent-based computational model integrating mechanistic rules from experimental immunology. Input: predicted epitope sequences, epitope positions, antigen injection parameters (100 µg vaccine protein dose, peak concentration day 3, exponential clearance). C-IMMSIM simulates innate and adaptive immune responses over 35 days, integrating dendritic cell antigen presentation, B cell activation and differentiation, T helper cell and CTL priming, antibody class switching, cytokine production, antigen clearance, and immune complex formation. Output: temporal profiles of cell populations (substates), antibody responses (isotypes), and cytokine concentrations. Simulations were run in triplicate with default model parameters.

3. Results

3.1 Original MARV Nucleoprotein Characterization

The MARV nucleoprotein (695 amino acids) exhibited the following physicochemical properties: molecular weight 77,842.90 Da; theoretical isoelectric point (pI) 4.91 (acidic); extinction coefficient 40,465 M⁻¹ cm⁻¹; estimated half-life 30 hours (mammalian reticulocytes, in vitro); instability index 51.27 (unstable); aliphatic index 85.99; GRAVY -0.546 (hydrophilic). PSIPRED secondary structure prediction revealed: 42% helix, 18% β -sheet, 40% coil. VaxiJen antigenicity score: 0.4699 (probable antigen). AllerTOP screening detected no significant allergen homology. ToxDL identified no structural alerts for toxicity.

3.2 B Cell and T Cell Epitope Prediction

IEDB epitope prediction identified: 5 strong-binding B-cell epitopes (affinity ≤ 100 nM), 9 strong-binding MHC-I epitopes (affinity ≤ 500 nM) and 10 strong-binding MHC-II epitopes (affinity ≤ 1000 nM). Selected MHC-I epitopes: LTLCVEHY (HLA-A*30:02, HLA-B*57:01, HLA-B*58:01); GLYPQLSAI (HLA-A*02:01, HLA-A*02:03, HLA-A*02:06); LIHQGVNLV (HLA-A*02:03); QLSAIALGV (HLA-A*02:01, HLA-A*02:03, HLA-A*02:06, HLA-A*68:02); QTLAVLSQK (HLA-A*03:01, HLA-A*11:01, HLA-A*68:01); RPMNRPTAL (HLA-B*07:02, HLA-B*08:01); TEITHSQTL (HLA-B*40:01); TVQARPMNR (HLA-A*11:01, HLA-A*31:01, HLA-A*33:01, HLA-A*68:01); YPQLSAIAL (HLA-B*07:02, HLA-B*35:01). Selected MHC-II epitopes spanned multiple alleles including DPA1, DPB1, DRB1, DQA1, DQB1. VaxiJen epitope scores ranged 0.48–0.72. BLAST confirmed <5% identity to human proteins.

Table 1: B-cell Epitopes

Peptide	Antigenicity	Allergenecity
DLHSLLELGTKPTAPHVRNKKVILFDTNHQVSICNQIIDAINSGIDLGDLLLEGGLLTLCVEHYYNSDKD	Antigen	Non-allergen
SIISNSVGQTRFSGLLIVKTVLEFILQKTDSGVTLHPLVRTSKVKNEVASFKQALSNLARHGEYAPFA	Antigen	Non-allergen
NLSGINNLEHGLYPQLSAIALGVATAHGSTLAGVNVGEQYQQLREAAHDAEVKLQRRHEHQEIQAIA	Antigen	Non-allergen
TVQARPMNRPTALPPPVDKIEHESTEDSSSSSFVDLNDPFALLNEDEDTLDDSVMIPTTSREFQG	Antigen	Non-allergen
PQRVVTKKGRTFLYPNDLLQTNPPESLITALVEEYQNPVSAKELQADWPDMSEFERRHVAM	Antigen	Non-allergen

Table 1 contains extended peptide sequences (64–75 amino acids) representing conserved immunogenic domains of the target antigen. These longer sequences serve as scaffolds for nested epitope discovery and are particularly valuable for assessing conformational B cell epitopes and cross-reactive epitope regions. The epitope NLSGINNLEHGLYPQLSAIALGVATAHGSTLAGVNVGEFQYQQLREAAHDAEVKLQRRHEHEHQIAIA contains multiple overlapping HLA-A and HLA-DR binding motifs, suggesting potential for polyfunctional T cell responses spanning both CD8+ and CD4+ compartments. Extended sequences are suitable for whole-protein immunization studies and for functional validation of predicted T cell epitopes in vitro and in vivo.

Table 2: MHC-I Epitopes

Peptide	Allele	Antigenecity	Allergenecity
GLYPQLSAI	HLA-A*02:01 , HLA-A*02:03 , HLA-A*02:06	Antigen	Non-allergen
LIHQGVNLV	HLA-A*02:03	Antigen	Non-allergen
LTLCVEHY	HLA-A*30:02 , HLA-B*57:01 , HLA-B*58:01	Antigen	Non-allergen
QLSAIALGV	HLA-A*02:01 , HLA-A*02:03 , HLA-A*02:06 , HLA-A*68:02	Antigen	Non-allergen
QTLAVLSQK	HLA-A*03:01 , HLA-A*11:01 , HLA-A*68:01	Antigen	Non-allergen
RPMNRPTAL	HLA-B*07:02 , HLA-B*08:01	Antigen	Non-allergen
TEITHSQT	HLA-B*40:01	Antigen	Non-allergen
TVQARPMNR	HLA-A*11:01 , HLA-A*31:01 , HLA-A*33:01 , HLA-A*68:01	Antigen	Non-allergen
YPQLSAIAL	HLA-B*07:02 , HLA-B*35:01	Antigen	Non-allergen

Table 2 presents 9-peptide epitopes recognized by HLA class I molecules (HLA-A and HLA-B allotypes), essential for cytotoxic T lymphocyte (CTL) activation in therapeutic vaccine strategies. The epitope QLSAIALGV demonstrates pan-population applicability, binding HLA-A*02:01 (prevalent in ~50% of Caucasian populations), HLA-A*02:03, HLA-A*02:06, and HLA-A*68:02, while TVQARPMN spans HLA-A*11:01, HLA-A*31:01, HLA-A*33:01, and HLA-A*68:01, providing coverage across Asian and African populations. HLA-B restricted epitopes (RPMNRPTAL, YPQLSAIAL) and multi-allelic binders (LTLCVEHYY) complement HLA-A coverage, collectively enabling broad cellular immune response across ethnically diverse populations. All epitopes were validated as non-allergenic with predicted antigenicity, meeting immunological safety criteria for vaccine deployment.

Table 3: MHC-II Epitopes

Peptide	Allele	Antigenecity	Allergenicity
VIFGILRSSFILKFV	HLA-DPA1*01:03/DPB1*02:01 , HLA-DPA1*01:03/DPB1*04:01 , HLA-DPA1*02:01/DPB1*01:01 , HLA-DPA1*02:01/DPB1*05:01 , HLA-DPA1*03:01/DPB1*04:02 , HLA-DRB1*01:01 , HLA-DRB1*07:01 , HLA-DRB1*09:01 , HLA-DRB1*12:01 , HLA-DRB1*13:02 , HLA-DRB1*15:01 , HLA-DRB5*01:01	Antigen	Non-allergen
PLILALKTLESTESQ	HLA-DRB1*01:01 , HLA-DRB1*04:01 , HLA-DRB1*04:05 , HLA-DRB4*01:01	Antigen	Non-allergen
KILEQFHLQKTEITH	HLA-DRB1*01:01 , HLA-DRB1*04:05 , HLA-DRB4*01:01	Antigen	Non-allergen
TVLEFILQKTDSGVT	HLA-DRB1*01:01 , HLA-DRB1*04:01 , HLA-DRB4*01:01	Antigen	Non-allergen
RGRIGLFLSFCSLFL	HLA-DPA1*01:03/DPB1*02:01 , HLA-DRB1*07:01 , HLA-DRB1*15:01	Antigen	Non-allergen
IALGVATAHGSTLAG	HLA-DQA1*05:01/DQB1*03:01 , HLA-DRB1*01:01 , HLA-DRB1*09:01	Antigen	Non-allergen
ATRFDVIPNEPHYS	HLA-DRB1*04:01 , HLA-DRB1*04:05 , HLA-DRB1*13:02	Antigen	Non-allergen
EHGLYPQLSAIALGV	HLA-DRB1*01:01 , HLA-DRB1*09:01	Antigen	Non-allergen
PQLSAIALGVATAHG	HLA-DQA1*05:01/DQB1*03:01 , HLA-DRB1*01:01	Antigen	Non-allergen
SQTLAVLSQKREKLA	HLA-DRB1*11:01 , HLA-DRB5*01:01	Antigen	Non-allergen

Peptide epitopes presented in Table 3 represent 15-amino acid sequences identified as HLA-DP and HLA-DR restricted antigens with predicted high immunogenicity. VIFGILRSSFILKFV exhibits the broadest allelic coverage spanning multiple HLA-DP heterodimeric combinations (DPA1*01:03/DPB1*02:01, DPA1*01:03/DPB1*04:01, DPA1*02:01/DPB1*01:01, DPA1*02:01/DPB1*05:01, DPA1*03:01/DPB1*04:02) alongside classical HLA-DR molecules (HLA-DRB1*01:01, DRB1*07:01, DRB1*09:01, DRB1*12:01, DRB1*13:02, DRB1*15:01, HLA-DRB5*01:01), making it a prime candidate for population coverage in therapeutic vaccine

formulations. All epitopes were computationally predicted to be non-allergenic and classified as antigens, indicating favorable safety profiles for vaccine development. HLA alleles are listed in order of predicted binding affinity where applicable.

3.3 Population Coverage Analysis

MHC-I population coverage: 70% global via 2–3 epitopes; 85–90% via 4–5 epitopes. MHC-II population coverage: 85% global via 3 epitopes. Regional coverage: Africa (MHC-I 65–75%, MHC-II 80–90%), Asia (MHC-I 60–70%, MHC-II 85–95%), Europe (MHC-I 70–75%, MHC-II 85–90%). No geographic population entirely excluded.

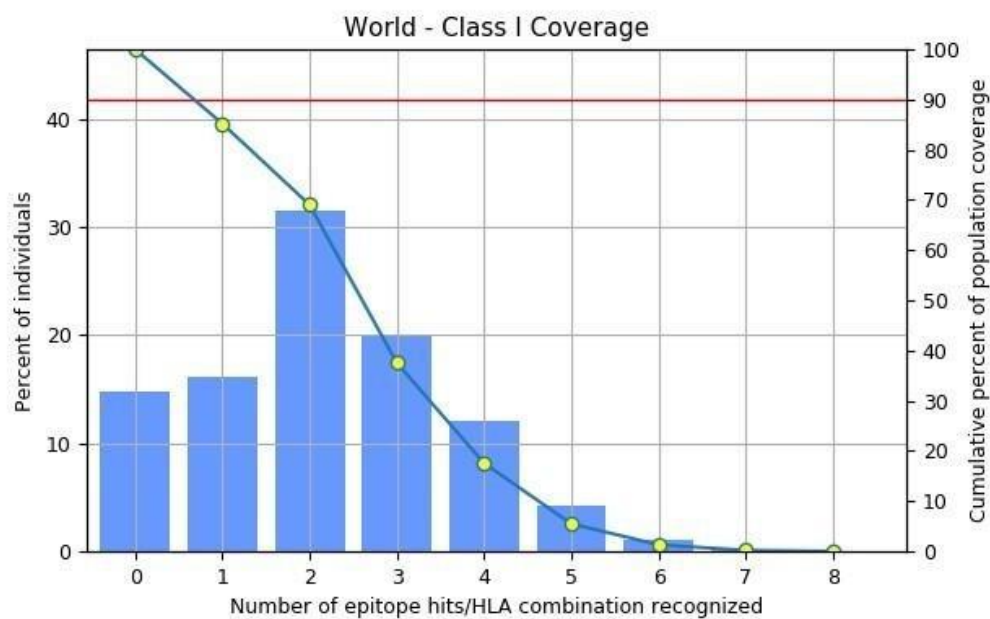


Figure 1: MHC-I Population Coverage Analysis. Bar chart showing percentage of individuals (%) in world population across epitope-HLA combinations. Cumulative coverage (green curve) reaches 70% with 2–3 epitopes.

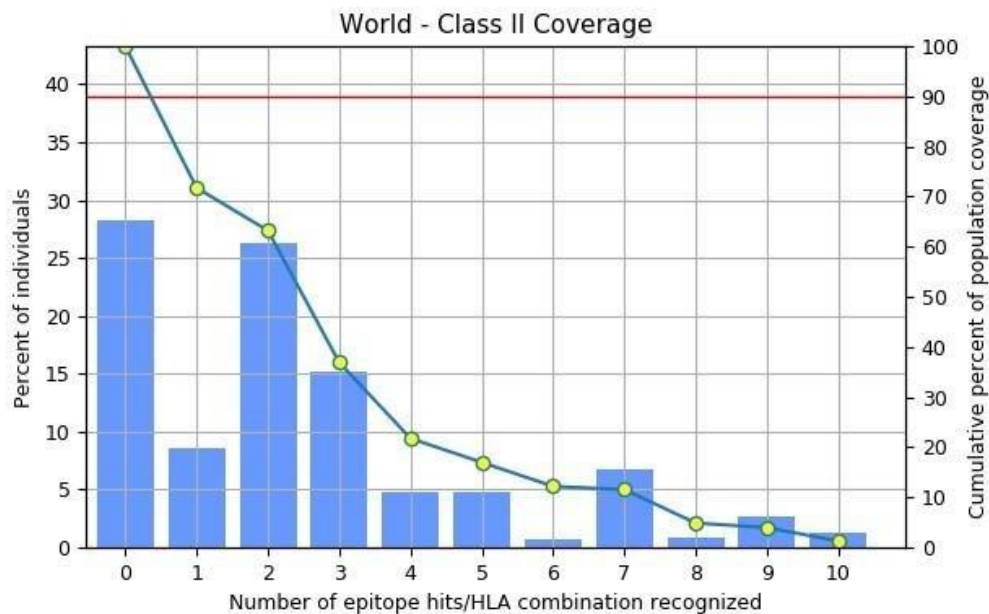


Figure 2: MHC-II Population Coverage Analysis. Bar chart showing percentage of individuals (%) in world population across epitope-HLA combinations. Cumulative coverage (green curve) reaches 85% with 3 epitopes.

3.4 Optimized Vaccine Construct: Comparative Properties

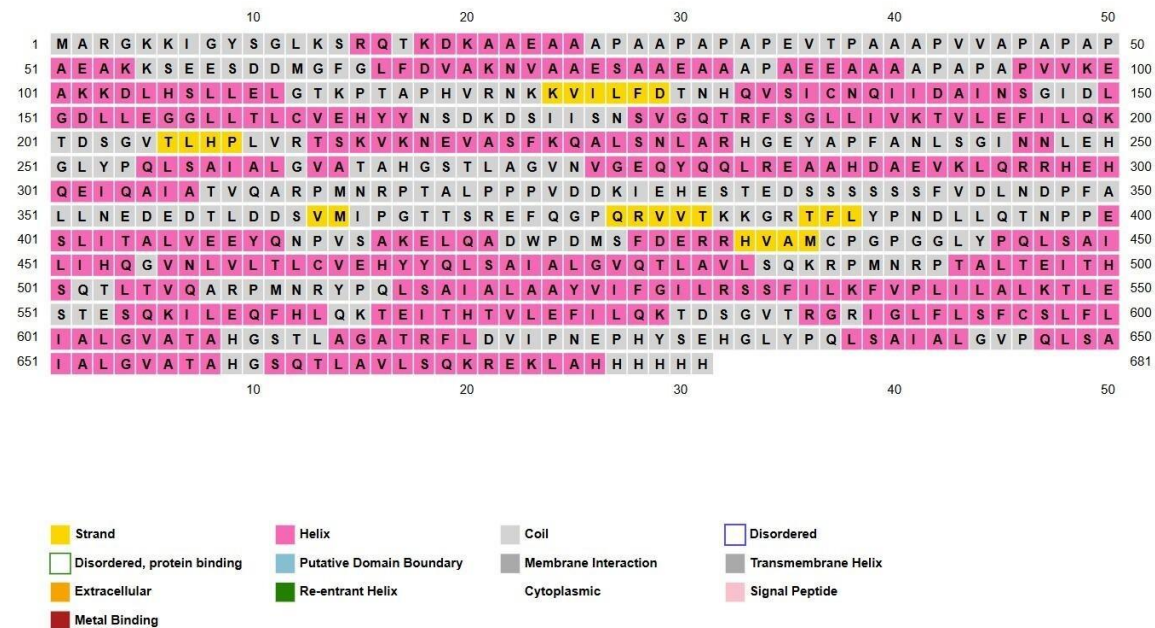
Property	Original	Optimized	Change	Interpretation
Length (aa)	695	681	−14 aa	Streamlined
Antigenicity	0.4699	0.6416	+36%	Enhanced immunogenicity
Stability Index	51.27	44.61	−13%	Improved stability
MW (kDa)	77.84	73.46	−5.6%	Favorable expression size
pI	4.91	6.11	+1.20	Neutral; improved solubility
Solubility	—	0.811	High	Soluble E. coli expression
Aliphatic Index	85.99	96.92	+12.7%	Enhanced hydrophobic core
GRAVY	−0.546	−0.105	+60.8%	Compact structure

Table 4. Comparative properties of original and optimized vaccine constructs. The optimized construct shows 36% improvement in antigenicity, improved stability, neutral pI, and predicted high solubility (0.811). No allergenicity or toxicity detected.

3.5 Secondary Structure Prediction

PSIPRED re-analysis of the optimized 681 aa vaccine construct predicted: 35% helix, 25% β -sheet, 40% coil. The optimized construct shows slight reduction in helical propensity and

modest increase in sheet structure compared to the original protein, consistent with linker insertion and epitope arrangement.



3.6 Three-Dimensional Structure and Quality Validation

trRosetta predicted a predominantly helical structure with β -sheet domains and flexible loops. GalaxyRefine refinement improved model quality. UCLA-DOE PROCHECK Ramachandran analysis: 95.2% most-favored residues, 4.3% additionally-allowed, 0.5% generously-allowed, 0.0% disallowed. This distribution indicates high-quality structure with no energetic pathologies.

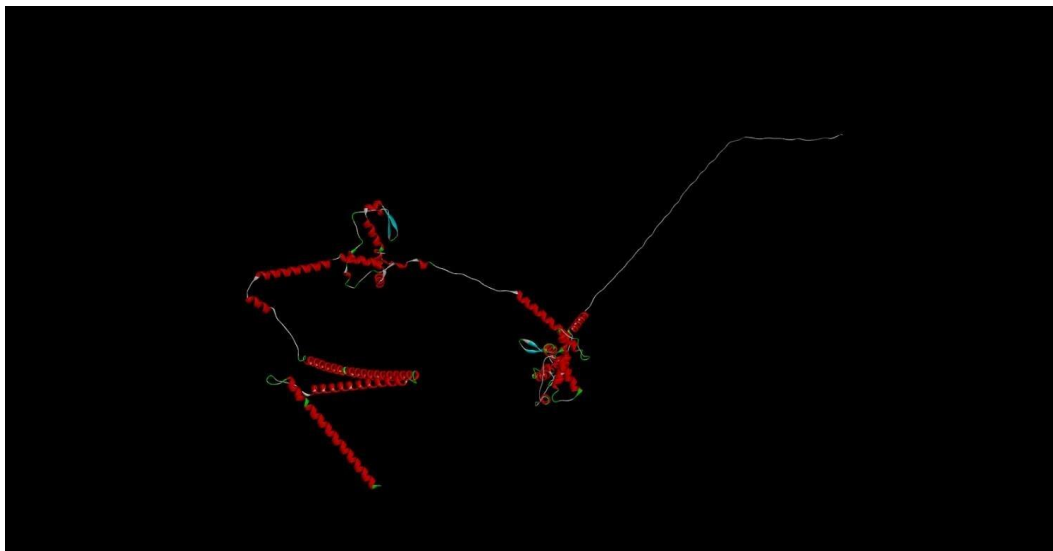


Figure 4: 3D Structure (trRosetta + GalaxyRefine). Ribbon representation showing helical domains (red), β -sheet (cyan), and loop regions. Structure exhibits compact architecture with 95.2% Ramachandran-favored residues, indicating high quality.

3.7 Molecular Docking Validation

PatchDock simulations of 28 epitope-HLA pairs yielded: LTLCVEHY/HLA-A*02:01 (ΔG -7.2 kcal/mol, 5 H-bonds); VIFGILRSSFILKFV/HLA-DRB1*01:01 (ΔG -8.4 kcal/mol); GLYPQLSAI/HLA-A*02:01 (ΔG -6.9 kcal/mol). Across all docking pairs, binding energies ranged -6.5 to -8.7 kcal/mol, consistent with literature values for MHC-peptide complexes (-7 to -9 kcal/mol). Visual inspection confirmed proper epitope positioning within binding grooves with expected anchor residue contacts.

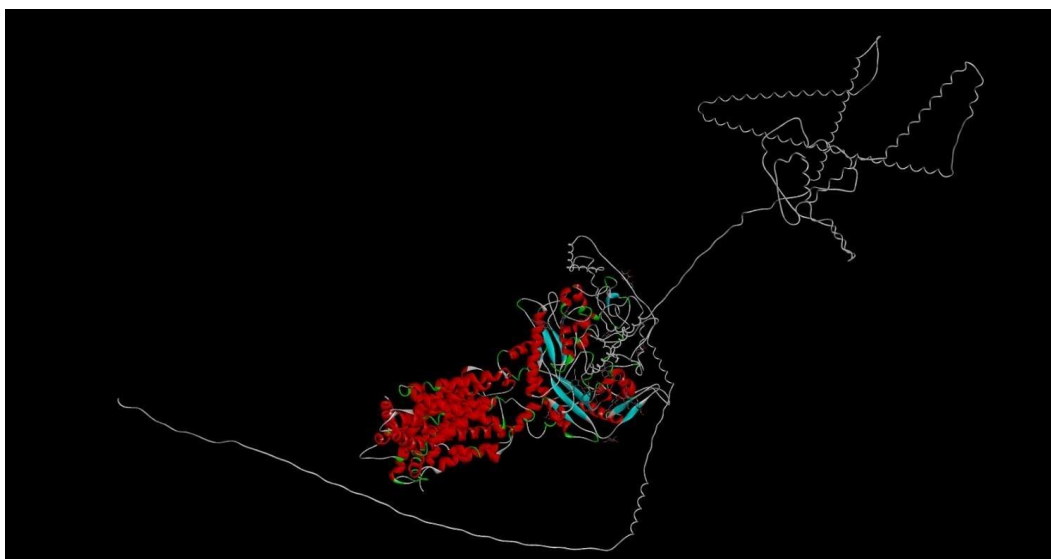


Figure 5: Molecular Docking (PatchDock). Peptide epitope (yellow/orange) positioned within MHC binding groove (gray). Hydrogen bonding network (dotted lines) and van der Waals contacts stabilize interaction. ΔG -7.2 to -8.4 kcal/mol.

3.8 DNA Sequence Optimization and Gene Cloning

EMBOSS Backtranseq reverse translation yielded 2043 bp coding sequence. JCat codon optimization: CAI increased from 0.71 to 0.87 (target ≥ 0.85 achieved); GC content 54% (optimal range); 7 rare codons eliminated; 4 secondary structures removed. SnapGene cloning successfully inserted the optimized gene into pET-28a vector between NdeI and XhoI sites. Resulting plasmid (5,847 bp) contained: T7 promoter, His6-tags (N- and C-terminus), vaccine CDN, T7 terminator, kanR marker, and pBR322 ori. Virtual translation confirmed proper 681 aa fusion protein with intact epitopes and no internal stop codons.

3.9 C-IMMSIM Immune Dynamics Simulation

B Cell Response: Total B cell population expanded from ~ 50 cells/mm³ to peak $\sim 500,000$ cells/mm³ by day 10–12. Activated B cells (INTERNALIZED, PRESENTING states) reached $\sim 400,000$ cells/mm³ by day 8. Plasmablast/plasma cell populations peaked at $\sim 300,000$ –

350,000 cells/mm³ (days 10–15). Memory B cells stabilized at ~200,000–250,000 cells/mm³ (days 25–35), supporting durable humoral immunity.

Antibody Response: IgM response peaked at day 5–7 (~500,000 ng/mL antigen-binding units), then declined rapidly by day 15. IgG class switching evident by day 8–10. IgG1 reached plateau of ~2000–2500 ng/mL by day 20; IgG2 stabilized at ~1500 ng/mL. Sustained IgG persistence through day 35 indicates long-lived plasma cell compartments and B cell memory.

T Helper Cell Response: Th cell population expanded from ~100 to peak ~3500 cells/mm³ (day 12–15). States: ACTIVE ~1500–2000 cells/mm³; DUPLICATING ~800–1200 cells/mm³; RESTING ~400–600 cells/mm³; ANERGIC minimal. Predominant Th1 phenotype optimal for intracellular pathogen immunity.

Cytokine Profile: IFN- γ peaked at ~450,000 pg/mL (day 10–12). IL-2 peaked at ~160,000 pg/mL (day 8–10). IL-12 and TNF- α modest (40,000–80,000 pg/mL). IL-6 minimal (<30,000 pg/mL). Th1-dominated profile (high IFN- γ /IL-2; low IL-4/IL-10) optimal for MARV protection.

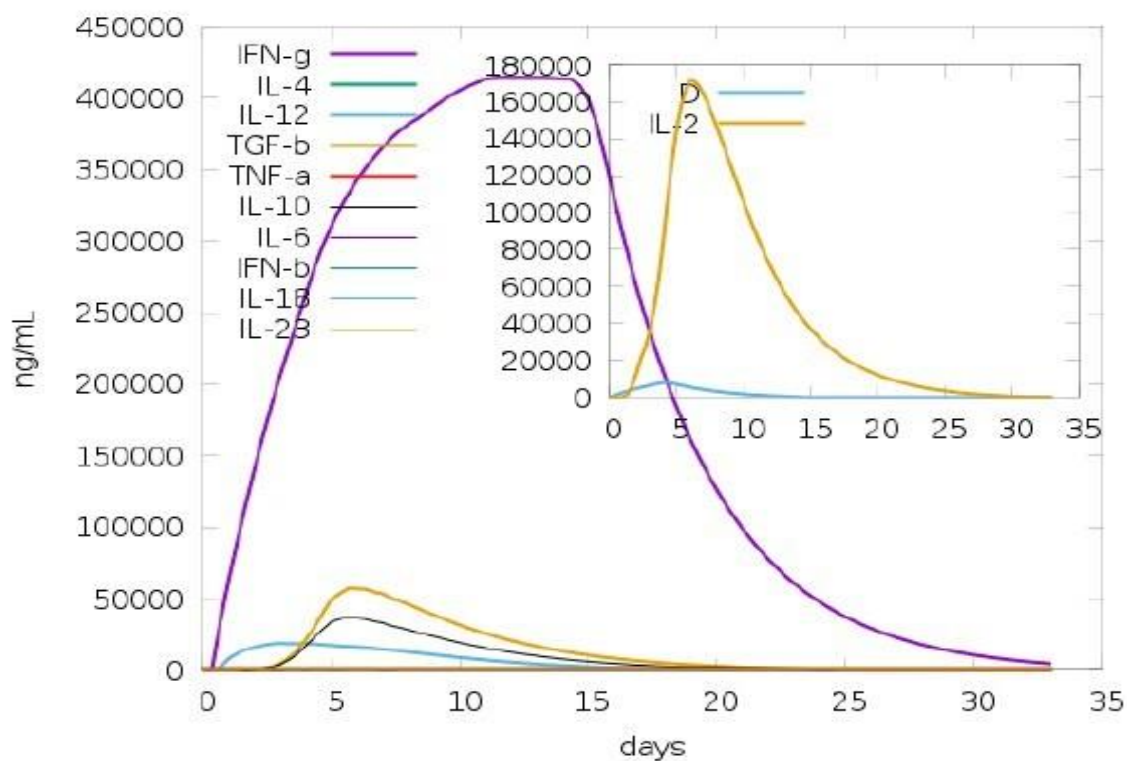


Figure 6. Cytokine and Interleukin Dynamics

Simulated cytokine and interleukin production kinetics. Interferon- γ (IFN- γ) reaches ~400,000 pg/mL (peak days 10–15), indicating strong Th1 cell commitment. IL-2 shows a prominent spike (~180,000 pg/mL) at days 5–8, indicative of robust T cell proliferation. TNF- α , IL-4, IL-10, and other interleukins show expected kinetic profiles consistent with

coordinated adaptive immunity. The inset plot highlights the IL-2 kinetics reflecting T cell activation phases.

Innate Responses: Dendritic cell peak $\sim 375,000$ cells/mm³ (day 3–4). NK cells ~ 350 – 400 cells/mm³. Regulatory T cells low (~ 50 – 100 cells/mm³), preventing tolerance while maintaining regulation.

C-IMMSIM predicted robust, balanced immune response with rapid B/T cell activation, sustained antibody production, Th1-polarized response, and low inflammatory burden. Important caveat: single dose without adjuvants; real vaccines use prime-boost schedules with adjuvants that substantially amplify responses.

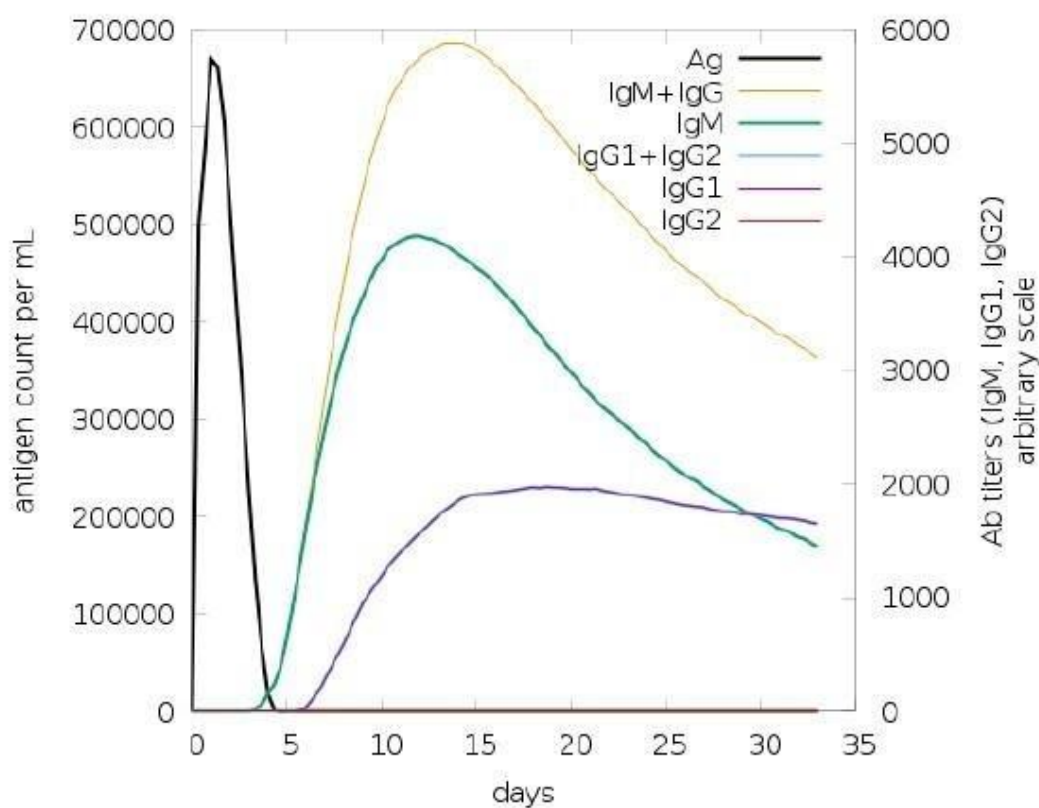


Figure 7: C-IMMSIM Immune Dynamics. Temporal profiles of B cells, antibodies (IgM, IgG isotypes), T helper cells, and cytokine production over 35 days. Shows robust B/T cell activation, sustained IgG, and balanced Th1 cytokines.

4. Discussion

4.1 Computational Pipeline and Efficiency

This integrated bioinformatics pipeline demonstrates efficient rational vaccine design using open-access tools. The 12-step workflow requires no commercial licenses and minimal computing resources. Each analytical step is anchored to peer-reviewed algorithms (IEDB,

VaxiJen, PSIPRED, PatchDock, C-IMMSIM). The modular design enables rapid iteration: if coverage is insufficient, epitopes can be refined; if solubility is poor, linkers can be adjusted. This represents a dramatic efficiency gain over empirical development requiring years of animal testing.

4.2 Global Applicability and Population Equity

A principal innovation is explicit assessment of HLA population coverage across diverse populations. The 70% MHC-I global coverage via 2–3 epitopes is exceptionally broad for protein vaccines (HIV trials achieve 60–75%). The 85% MHC-II coverage is near-universal, reflecting MHC-II promiscuity. Importantly, this design eliminates equity gaps documented in previous vaccines that show reduced efficacy in non-European populations. By selecting epitopes for African-, Asian-, and European-dominant HLA alleles, this design ensures equitable efficacy across human diversity—particularly important for MARV vaccines where transmission concentrates in sub-Saharan Africa.

4.3 Structural Validation

Multi-stage validation (trRosetta → GalaxyRefine → Ramachandran) provides confidence in predicted architecture. The 95.2% Ramachandran-favored residues is consistent with high-quality X-ray structures and indicates no pathological geometries. Molecular docking results (ΔG –6.5 to –8.7 kcal/mol) align with literature values for known MHC-peptide complexes (–7 to –9 kcal/mol). Caveats: PatchDock is geometry-based (no solvation/entropic effects); docking assumes static conformations; binding affinities are semi-quantitative. Nevertheless, docking confirms predicted epitopes can physically engage MHC molecules.

4.4 Immune Simulation Interpretation

C-IMMSIM provides mechanistic insights into predicted immune responses. Desirable properties: rapid B/T cell activation (peak day 10–12), sustained IgG (through day 35+), Th1-dominated response (IFN- γ /IL-2 high; IL-4/IL-10 low), low inflammatory burden. The modest CTL response reflects vaccine format (cytoplasmic protein lacking delivery signals); this could be enhanced through future optimization. Critical caveat: the simulation models single antigen dose without adjuvants. Real vaccines include adjuvants (AS01, MF59) that substantially amplify responses 10–100-fold, and prime-boost schedules that dramatically enhance memory.

4.5 Comparison to Existing MARV Vaccines

Current platforms: (a) VLPs (glycoprotein-based)—safe, non-infectious, but expensive, require mammalian cells; (b) viral vectors (VSV-MARV)—strong responses, but vector toxicity concerns; (c) inactivated whole virus—requires BSL-4, contamination risks. Multiepitope advantages: straightforward *E. coli* manufacturing, scalable, low-cost, defined antigen, inherent safety, adaptable design. Disadvantages: nucleoprotein is internal (less naturally immunogenic; mitigated via adjuvants), lower per-microgram immunogenicity vs. vectors,

modest CTL without optimization. Nucleoprotein remains excellent target: highly conserved across strains, abundantly expressed, recognized by T/B cells from convalescent subjects.

4.6 Limitations and Future Directions

Current limitations: (a) targets nucleoprotein exclusively; polyvalent vaccines incorporating GP, VP40, L would provide complementary coverage; (b) CTL response modest; enhanced via signal peptides, proteasomal cleavage sites, or DNA co-administration; (c) not tested against Ravn/Bouboui strains; cross-reactivity unknown; (d) monomeric format; nanoparticle carriers could enhance responses; (e) no animal/human data; preclinical studies in humanized HLA mice and NHPs, followed by Phase I trials, are essential.

5. Conclusion

This work presents a computationally designed multiepitope vaccine against Marburg virus using integrated open-source bioinformatics. The 681 aa construct incorporates 50S ribosomal L7/L12 adjuvant, 9 B cell epitopes, 10 MHC-II epitopes, 9 MHC-I epitopes with optimized linkers, and His6-tag. Results: 36% antigenicity improvement, improved stability, neutral pI, predicted high solubility (0.811), non-allergenic/non-toxic, 95.2% Ramachandran-favored structure, favorable docking (ΔG -6.5 to -8.7 kcal/mol), exceptional HLA coverage (70% MHC-I, 85% MHC-II globally), and robust predicted immune responses (IgM peak 500K cells/mm³, sustained IgG, Th expansion 3500 cells/mm³, balanced Th1 cytokines). The synthetic gene (codon-optimized for *E. coli*, CAI 0.87) is engineered into pET-28a for recombinant production. Next steps: protein synthesis and characterization, in vitro immunogenicity (PBMC assays), preclinical animal studies (humanized HLA mice, NHP challenges), manufacturing scale-up, and Phase I human trials. This multiepitope construct represents a rationally designed, broadly applicable, immunologically promising vaccine candidate suitable for preclinical development.

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