Multi-Protein HIV-1 Vaccine Design: In Silico Prediction, Structural Assessment, and Immunoinformatic Validation

By

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A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons.)

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

The thesis titled "Multi-Protein HIV-1 Vaccine Design: In Silico Prediction, Structural Assessment, and Immunoinformatic Validation" submitted by Tahsina Islam (21346040) of Summer, 2021 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons.) on September 2025.

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Ethics Statement

This thesis was conducted in compliance with all the relevant ethical standards and did not involve human or animal trials.

Abstract

HIV-1 remains a global health concern since there is no effective vaccine to date. *In silico* multi-protein vaccine design offers a promising strategy to induce widespread and durable immunity. Using linker and adjuvant sequences, including the PADRE universal helper T-cell epitope, conserved epitopes from several HIV-1 proteins were combined into a single construct. The design incorporated HTL ($CD4^+$ helper T lymphocyte) epitopes to enhance cytokine secretion and B-cell help, CTL ($CD8^+$ cytotoxic T lymphocyte) epitopes to promote targeted killing of infected cells, and B-cell epitopes to induce neutralizing antibody responses. Physicochemical properties, antigenicity, toxicity, and allergenicity were evaluated through immunoinformatics pipelines. The coverage was analysed using IEDB Population Coverage Calculation. The 3D structure was modeled and validated using MolProbity, Ramachandran analysis. Molecular docking with TLR-3 was performed to assess receptor interaction, and immune simulations were conducted to predict immune responses. The construct showed stability, antigenicity, and non-allergenicity, with >90% residues in favored Ramachandran regions. Docking confirmed stability of TLR-3 binding, while immune simulation predicted strong IgM and IgG responses and robust T-cell activation.

Keywords: HIV-1; In Silico Vaccine; Multi-Protein; Molecular Docking; Immune Simulation, Immunoinformatics.

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List of Acronyms

CTL	Cytotoxic T Lymphocyte ($CD8^+$ T cell)
ERRAT	Structure Verification Algorithm for Non-Bonded Atomic Interactions
HTL	Helper T Lymphocyte ($CD4^+$ T cell)
PADRE	Pan HLA-DR Epitope (universal helper T-cell epitope sequence)
QMEAN	Qualitative Model Energy Analysis
TLR-3	Toll-Like Receptor 3

Chapter 01

Introduction

Human immunodeficiency virus type 1 (HIV-1) is a type of retrovirus belonging to the family *Retroviridae* and the genus *Lentivirus*. The virus primarily targets $CD4^+$ T lymphocytes, leading to progressive immune system deterioration. If left untreated, this immunosuppression leads to malignancies, opportunistic infections, and eventually AIDS. Despite revolutionary antiretroviral treatment (ART), HIV-1 continues to pose a serious threat to world health, as ART does not eliminate latent viral reservoirs, and requires lifelong adherence. An effective preventive vaccination is quite needed, as evidenced by the anticipated 40.8 million individuals living with HIV in 2024, 1.3 million new infections, and 630,000 AIDS-related deaths (*Global HIV & AIDS Statistics — Fact Sheet | UNAIDS*, n.d.; *Global HIV Programme*, n.d.). HIV-1's remarkable sequence variation, rapid development, and intricate immune-evasion tactics make it challenging to develop such a vaccine (Seabright et al., 2019).

1.1 Pathogenesis and Immune Evasion Mechanisms

HIV-1 primarily infects $CD4^+$ T lymphocytes (very important in helping coordinate the body's immune response), macrophages, and dendritic cells, establishing lifelong infection by incorporating its genome into host DNA. This forms long-lived latent reservoirs, in spite of antiretroviral therapy (ART) (Cohn et al., 2020; *Global HIV & AIDS Statistics — Fact Sheet | UNAIDS*, n.d.). Due to error-prone reverse transcription and recombination, viruses evolve quickly, producing a wide range of sequence diversity that facilitate immune evasion. A thick "glycan shield" (around 90 N-linked glycans) envelops the envelope (Env) trimer at the protein level, concealing sensitive epitopes and influencing the production and selectivity of broadly

neutralizing antibodies (bnAbs) (Cao et al., 2017; Crispin et al., 2018). Additionally, HIV-1 undermines cellular immunity; for instance, the accessory protein Nef reduces the recognition and clearance of cytotoxic T-lymphocytes (CTLs) by downregulating HLA-I on infected cells (Kuang & Brockman, 2018; Lubben et al., 2007). Collectively, these characteristics- latency, glycan shielding, antigenic diversity, and MHC-I down-regulation; all work together to facilitate immune evasion and prolonged reproduction**.** The RV144 regimen only provided modest, waning protection, and next-generation regimens (HVTN 702 "Uhambo," HVTN 705 "Imbokodo," and HVTN 706 "Mosaico") failed to show efficacy, which led to a shift toward new antigen designs and adjuvant concepts.

1.2 Challenges in HIV-1 Vaccine Development

A preventative vaccine is urgently needed since, despite significant improvements in ART coverage, the worldwide burden of HIV remains high, with about 40.8 million people living with the virus, 1.3 million new infections, and 630,000 AIDS-related deaths in 2024 (*Global HIV & AIDS Statistics — Fact Sheet | UNAIDS*, n.d.). Extreme genetic diversity across clades, conformational plasticity, and the glycan barrier that hides neutralization targets have all contributed to the difficulties faced by traditional vaccination platforms against HIV-1(Cao et al., 2017; Crispin et al., 2018). The RV144 regimen only provided modest, waning protection, and next-generation regimens (HVTN 702 "Uhambo," HVTN 705 "Imbokodo," and HVTN 706 "Mosaico") failed to show efficacy, which led to a pivot toward new antigen designs and adjuvant concepts (*An Empirical Approach to HIV Vaccine Development | NIAID: National Institute of Allergy and Infectious Diseases*, n.d.; *Experimental HIV Vaccine Regimen Safe but Ineffective, NIH Study Finds | NIAID: National Institute of Allergy and Infectious Diseases*, n.d.; Factsheet, 2023). These challenges are highlighted by large efficacy trials. However, these data collectively

support tactics that (i) focus responses on conserved regions across multiple proteins and (ii) coordinate balanced humoral and cellular immunity.

1.3 Role of Multi-Protein and Multi-Epitope Vaccines

Multi-epitope constructions by immunoinformatics, which combine conserved and filtered CTL, HTL, and B-cell epitopes from various HIV-1 proteins, reduces off-target reactivity and increases coverage across a variety of strains (Ahmed et al., 2025; Tang et al., 2025). Rational linkers maximize processing and presentation (e.g., EAAAK, GPGPG, KK), while helper-T "universal" epitopes like PADRE improve $CD4^+$ T-cell assistance and cytokine production, enhancing antibody and CTL responses (Kim et al., 2008; Pompano et al., 2014). To facilitate the prediction of immunogenicity prior to wet-lab work, modern pipelines incorporate leading-edge predictors (e.g., NetMHCpan/NetMHCIIpan for HLA presentation, BepiPred for B-cell epitopes), in-silico safety filters (antigenicity, allergenicity, toxicity), and innate-sensing considerations (e.g., docking to TLR3 ligands) (Jespersen et al., 2017a; Ko et al., 2023; Reynisson et al., 2020a). First-pass predictions of antibody kinetics, T-cell dynamics, and cytokine profiles are provided by simulators like C-ImmSim, which can direct iterations of the construct (Rapin et al., 2010a).

As part of a cohesive in-silico workflow, we use that approach in this thesis to build a multi-protein HIV-1 vaccine using PADRE, suitable adjuvant and optimized linkers, assess innate-receptor engagement and immunological simulations, and evaluate anticipated safety and immunogenicity.

Chapter 02

Methodology

2.1 Selection of Suitable Proteins and its Sequences

For the development of an HIV-1 vaccine, three target protein sequences were chosen based on their capacity to elicit a strong immune response. These proteins were obtained in FASTA format from UniProt and the NCBI Protein Database (*UniProt*, n.d.). The Vaxijen 2.0 tool was then used to assess the antigenicity of the chosen protein sequences. With an acceptable threshold for antigenicity set at 0.44- 0.48, the tool categorized the proteins as either antigens or non-antigens (Doytchinova & Flower, 2007). "Virus" was the designated target organism for screening, guaranteeing that the program concentrated on proteins having immunological potential pertinent to viral pathogens such as HIV-1. Vaxijen uses an alignment-independent methodology to find suitable candidates early in the vaccine development process by predicting the immunogenicity of protein sequences, eliminating irrelevant proteins and concentrating on those that are most likely to trigger an immune response. The identified proteins were further analyzed in greater detail for their suitability as vaccine targets (Vélez-Segarra et al., 2019).

2.2 Identifying Cytotoxic T Lymphocyte (CTL) Epitopes

Cytotoxic T lymphocyte (CTL) epitopes (short peptide fragments) are presented on MHC class I molecules and are identified by $CD8^+$ T lymphocytes. This further destroys infected host cells. The NetCTL 1.2 tool, which incorporates several factors such as MHC class I binding affinity, proteasomal cleavage efficiency, and TAP transport efficiency, was used to predict CTL epitopes. In order to balance sensitivity and specificity in epitope prediction, the threshold was set at 0.47 and 0.48 and the sequences were taken in FASTA format. Identifying these epitopes guarantees

that peptide sequences capable of eliciting a cell-mediated immune response are included, which is essential for limiting viral reproduction.

2.3 Analyzing CTL Epitopes

To further validate the predicted epitopes, NetMHCpan 4.1 was employed. This tool provides robust pan-allelic predictions across a wide range of HLA class I molecules. Although it can evaluate peptides of any length and considers all HLA alleles, this program primarily concentrates on 9-mer peptides. A threshold of 0.5 was maintained in the event of Strong Binding (SB). Epitopes that met the specified thresholds, showed strong binding affinities, were shortlisted for further evaluation. The completed CTL epitopes are required to be antigenous without any signs of toxicity and allergenicity, evaluated by ToxinPred and AllerTOP v2.1 tools.

2.4 Identifying Helper T Lymphocyte (HTL) Epitopes

HTL epitopes were retrieved from protein 3 (Gag polyprotein). NetMHCIIpan 4.0 tool was used, which scans peptides (usually 15-mers) against an extensive panel of HLA-DR/DP/DQ alleles (Reynisson et al., 2020b). Candidates were screened for antigenicity (Vaxijen 2.0), non-allergenicity (AllerTop v2.1), and non-toxicity (T3DB) after being shortlisted based on their projected binding strength (affinity) and promiscuity (binding to numerous alleles). $CD4^+$ T-cell assistance is needed for the coordination of CTL priming and B-cell maturation (Bui et al., 2006). A rational HTL selection increases the chance that the final multi-epitope vaccination will generate balanced humoral and cell-mediated immunity by improving broad (multi-allelic coverage), quality (strong $CD4^+$ help that sustains CTL and antibody responses), and durability (memory formation).

2.5 Analyzing HTL Epitopes

The functional immunological potential of the predicted HTL epitopes was also examined along with the allergenicity, antigenicity, and toxicity. Cytokine-inducing potential was assessed using instruments like IFNepitope, IL-2Pred, and IL-4Pred server. Antiviral Th1 responses are linked to IFN-γ induction, T-cell proliferation is supported by IL-2, and humoral immunity is enhanced by IL-4 through B-cell activation (Nagpal et al., 2017). This process guarantees that the chosen epitopes efficiently support immunological signaling pathways in addition to binding MHC-II molecules. The inclusion of cytokine-inducing epitopes enhances the general quality and scope of the immune response that the vaccination produces.

2.6 Selection of B cell Epitopes

The IEDB Analysis Resource's B cell epitope prediction tool was used to find B-cell epitopes. BepiPred Linear Epitope Prediction 2.0 is one of the most advanced tools for linear epitope prediction. Utilizing a score-vs-position profile and a working threshold to enrich for accessible, antibody-recognizable areas, linear B-cell epitopes were identified (Jespersen et al., 2017b). Before being included, candidates passed antigenicity/safety screening and cross-checked within the protein context.

2.7 Evaluation of B cell Epitopes

A number of bioinformatics techniques were used to further screen these epitopes for their toxicity, allergenicity, and antigenicity profiles before final selection. ToxinPred was used to assess toxicity, AllerTOP v.2.0 was used to predict allergenic potential, and VaxiJen v2.0 was used to confirm antigenicity. For the multi-epitope vaccine design, only antigenic, non-allergic, and non-toxic epitopes were chosen.

2.8 Construction of Vaccine with Incorporation of Suitable Linkers

A single multi-epitope vaccine design was finally designed by combining the finished CTL, HTL, and B-cell epitopes with the appropriate linkers and adjuvant. Rigidity and structural separation were provided by EAAAK linkers, solubility was improved by KK linkers, and optimum processing of HTL epitopes was made possible by GPGPG linkers (Arai et al., 2001; Chen et al., 2013). Furthermore, as a universal helper T-cell epitope, the Pan HLA-DR epitope (PADRE) was added at the N-terminal (Alexander et al., 1994). PADRE significantly increases $CD4^+$ T-cell activation and can bind to a broad range of HLA-DR alleles. While PADRE improves overall immunogenicity by offering constant helper activity across a range of populations, linkers ensure proper epitope processing and hBD-2 (human β-defensin-2) play role in innate immunity and stability.

2.9 Evaluation of Antigenicity, Toxicity and Allergenicity of Constructed Vaccine

To make sure that the combination of epitopes and linkers maintained the desired immunological qualities, the constructed vaccine construct was reevaluated. VaxiJen v2.0 was used to confirm antigenicity, AllerTOPv2.1, AllergenFPv1.1, AllerPred2 were used to screen for allergenicity, and T3DB was used to check for toxicity. This procedure demonstrated that the final construct retained immunogenicity while staying non-allergenic and non-toxic. Prior to moving further with structural modeling and docking studies, this validation is necessary.

2.10 Biochemical Analysis of Constructed Vaccine

The ProtParam tool was used for evaluating the vaccine's physicochemical characteristics. A number of important parameters are predicted by the tool, such as the estimated half-life, aliphatic index, instability index, GRAVY (Grand Average of Hydropathicity), molecular weight, and

theoretical isoelectric point (pI) (Gasteiger et al., 2005). The instability index gives an indication of how stable a protein is in vitro conditions. A protein is considered to be stable if its value is less than 40, and unstable if it is greater than 40. Protein turnover in yeast, E. coli, and human systems is predicted by estimating the half-life based on the identity of the N-terminal amino acid of the protein sequence. This indicates how long it takes for half of the protein to break down inside the cell.

The GRAVY score is determined by dividing the total number of residues by the cumulative hydropathy value of all amino acids. In contrast to a high GRAVY number, which shows hydrophobicity, a negative GRAVY value implies a hydrophilic nature, encouraging solubility (Kyte & Doolittle, 1982). The protein's thermostability is connected with the aliphatic index, which shows the relative volume filled by aliphatic side chains (alanine, valine, isoleucine, and leucine). The extinction coefficient, which is helpful for determining the concentration of proteins in solution and is related to the protein's ability to absorb light at a particular wavelength, was also predicted.

In combination, these characteristics promote the vaccine construct's appropriateness for experimental expression and further validation by offering crucial insights into its stability, solubility, hydrophilicity, and usability.

2.11 Generating a 3D Model of the Constructed Vaccine

The vaccine construct's 3D structure was predicted utilizing the Phyre2 tool, an advanced three-dimensional protein structure prediction method. It provides output in PDB format. (Kelley et al., 2015).

2.12 Evaluation of Coverage of Constructed Vaccine

IEDB population coverage analysis was performed by selecting CTL and HTL epitopes from the constructed vaccine mapped to multiple prevalent HLA class I and class II alleles, spanning major supertypes and diverse ethnic groups. The combined class I/II set yielded broad theoretical coverage across all WHO regions. Such breadth reduces the risk of population-specific blind spots and supports global deployability of the construct. These findings align with prior reports that multi-epitope, HLA-promiscuous designs enhance projected coverage and translational potential. This revealed significant worldwide immunological coverage, supporting the construct's biological significance (Pandey et al., 2018).

2.13 Generation and Analysis of Ramachandran Plots

Ramachandran plot was generated using the Swiss Model Expasy tool to validate the 3D model of the constructed vaccine, also to confirm the precision of anticipated three-dimensional structure. In Ramachandran plot analysis, it measures the phi (φ) and psi (ψ) torsion angles of amino acid residues, and was used to evaluate the stereochemical quality of the vaccine construct (Lovell et al., 2003a). While outliers point to areas that could need refining, a large percentage of residues falling in preferred and permitted regions implies proper backbone geometry. It is an important quality check prior to experimental validation that is provided by this analysis.

2.14 3D Model Quality Assessment by ERRAT and QMEAN Analysis

In order to further test the model quality, ERRAT and QMEAN analysis was done. QMEAN provides global and local quality ratings in relation to experimentally solved structures, and ERRAT evaluates non-bonded atomic interactions (Benkert et al., 2011a; Colovos & Yeates, 1993a).

ERRAT scores above 80% and QMEANDisCo Global scores near 0-1 range is considered to be a reliable model. In addition to ensuring robustness, employing different validation methods were applied to lessen reliance on a single evaluation system.

2.15 Molecular Docking Analysis of the Vaccine

To evaluate the binding affinity between the constructed vaccine and immunological receptors, molecular docking was carried out using the ClusPro tool. ClusPro predicts how the vaccine may bind to receptors using an energy-based technique, generating information on the vaccine's stability and binding potential for immune activation (Kozakov et al., 2017). Immune receptor interactions were simulated using the Phyre2 PDB file and uploaded as receptor molecule. Whereas, the PDB file for Toll-like receptor-3 was sourced from RSCB and uploaded as receptor molecule (Bird, 2008).

2.16 Immune Response simulation of the vaccine

The C-ImmSim tool, which is a popular in-silico immunology simulation platform, was utilized to perform immune response simulations in order to estimate the immunogenic potential, simulating humoral (B-cell) and cellular (T-cell) reactions of the constructed vaccine. It also simulates the release of important cytokines that are essential for coordinating the immune response, such as interleukins and interferons (Rapin et al., 2010b).

It helps to predict how the vaccine would interact with the immune system especially, how B-cells would produce antibodies, how CD4 helper T-cells (HTL) would aid in immune activation, and how CD8 cytotoxic T-cells (CTL) would target infected cells. This method saved time and money by evaluating possible immunological reactions to the vaccination without the need for laboratory testing.

This approach offered important insights on the effectiveness of the vaccine by assessing the immune stimulation potential, including cytokine production and the vaccine's overall capacity to trigger humoral and cellular immunity. The development of immunological memory, which is necessary for prolonged defense against the target infection, was also anticipated by the simulation. How Toll-like receptor (TLR) targets for immune activation and supports the immune response was also quantitatively predicted using the C-ImmSim program.

2.17 Key Aspects, Materials and Methods

To create a multi-epitope vaccine, the approach included in-silico epitope prediction, designing, and validation. Molecular docking was used to test the vaccine's capacity to attach to immune receptors, and immune response simulations were utilized for assessing the immunogenic potential. By combining these processes, a comprehensive pipeline for in silico vaccine design has been developed, guaranteeing better immune activation.

Chapter 03

Results

3.1 Selection of Proteins

Three different proteins were selected and the sequences were downloaded from the Uniprot database in FASTA format. Then antigenicity of each protein was checked by Vaxijen 2.0. The proteins details and sequences that have been derived from the database are mentioned below:

Protein 01

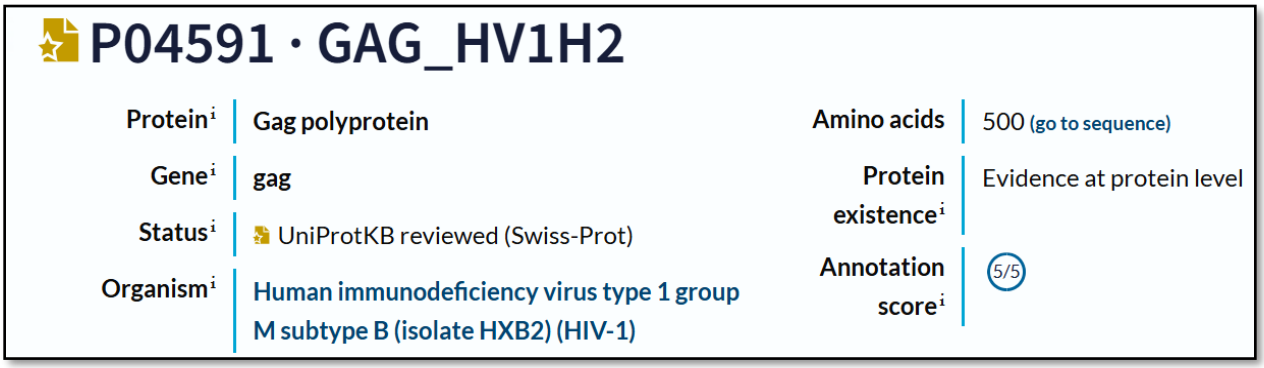
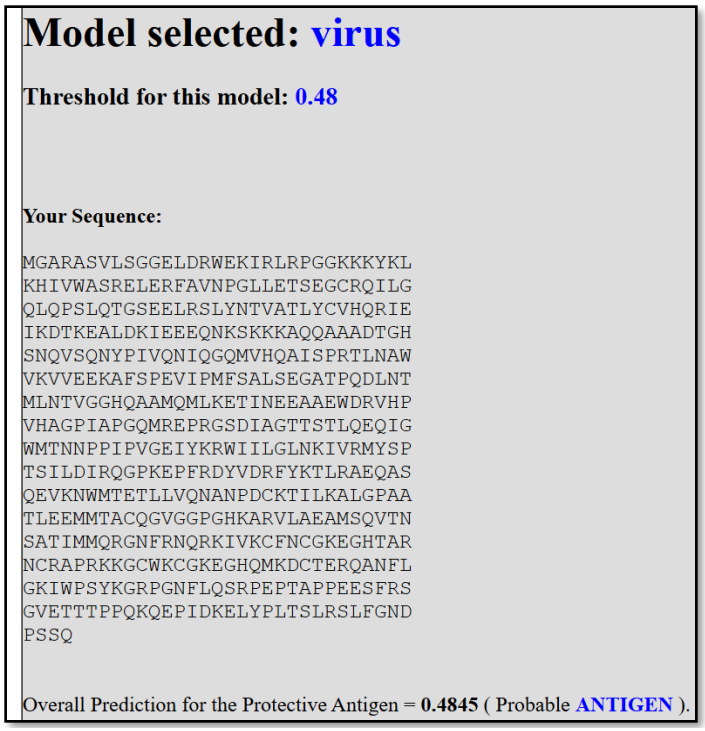


Figure 1: Gag polyprotein from Uniprot database (UniProt, n.d.)

Although VaxiJen v2.0's default viral threshold is 0.5, a significantly lower cutoff (0.48) was used in this study. Because of HIV-1's extensive genetic diversity, potentially immunogenic proteins may be excluded if rigorous standards are maintained. This protein is labeled non-antigenic at 0.5 but when reanalyzed using 0.48 (enables the identification of more conserved epitopes) it is identified as antigenic. Previous research on vaccine design based on immunoinformatics have revealed similar threshold modifications (Kar et al., 2020; Rafi et al., 2022).



Model selected: virus

Threshold for this model: 0.48

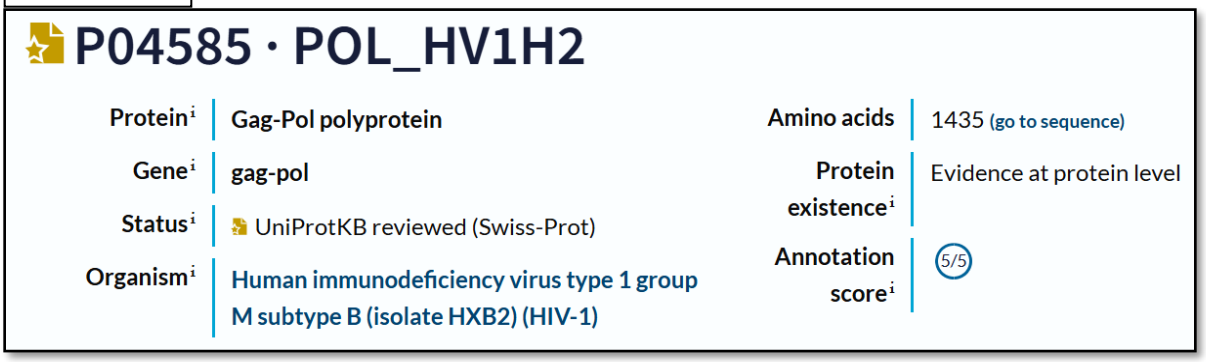
Your Sequence:

MGARASVLSGGELDRWEKIRLRPGGKKKYKL
KHIVWASRELERFAVNPGLLETSEGCRQILG
QLQPSLQTGSEELRSLYNTVATLYCVHQRIE
IKDTKEALDKIEEEQNKSKKKAQQAAADTGH
SNQVSQNYPIVQNIQGQMVHQAISPRTLNAW
VKVVEEKAFSPEVIPMFSALSEGATPQDLNT
MLNTVGGHQAAMQMLKETINEEAAEWDRVHP
VHAGPIAPGQMREPRGSDIAGTTSTLQEQIG
WMTNNPPIPVGEIYKRWIILGLNKIVRMYSP
TSILDIRQGPKEPFRDYVDRFYKTLRAEQAS
QEVKNWMTETLLVQNANPDCKTILKALGPAA
TLEEMMTACQGVGGPGHKARVLAEAMSQVTN
SATIMMQRGNFRNQRKIVKCFNCGKEGHTAR
NCRAPRKKGCWKCGKEGHQMKDCTERQANFL
GKIWPSYKGRPGNFLQSRPEPTAPPEESFRS
GVETTTPPQKQEPIDKELYPLTSLRSLFGND
PSSQ

Overall Prediction for the Protective Antigen = **0.4845** (Probable **ANTIGEN**).

Figure 2: Antigenicity score of protein 01 in VaxiJen v2.0 server (Doytchinova & Flower, 2007)

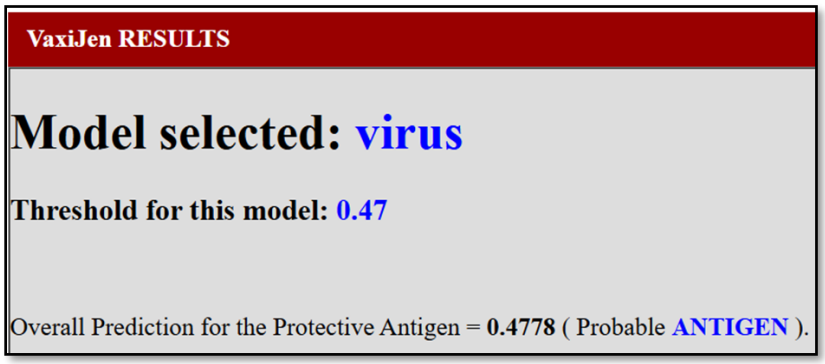
Protein 02



P04585 · POL_HV1H2

Protein	Gag-Pol polyprotein	Amino acids	1435 (go to sequence)
Gene	gag-pol	Protein existence	Evidence at protein level
Status	UniProtKB reviewed (Swiss-Prot)	Annotation score	5/5
Organism	Human immunodeficiency virus type 1 group M subtype B (isolate HXB2) (HIV-1)		

Figure 3: Gag-Pol polyprotein from Uniprot database (UniProt, n.d.)



VaxiJen RESULTS

Model selected: virus

Threshold for this model: 0.47

Overall Prediction for the Protective Antigen = **0.4778** (Probable **ANTIGEN**).

Figure 4: Antigenicity score of protein 02 in VaxiJen v2.0 server (Doytchinova & Flower, 2007)

Protein 03

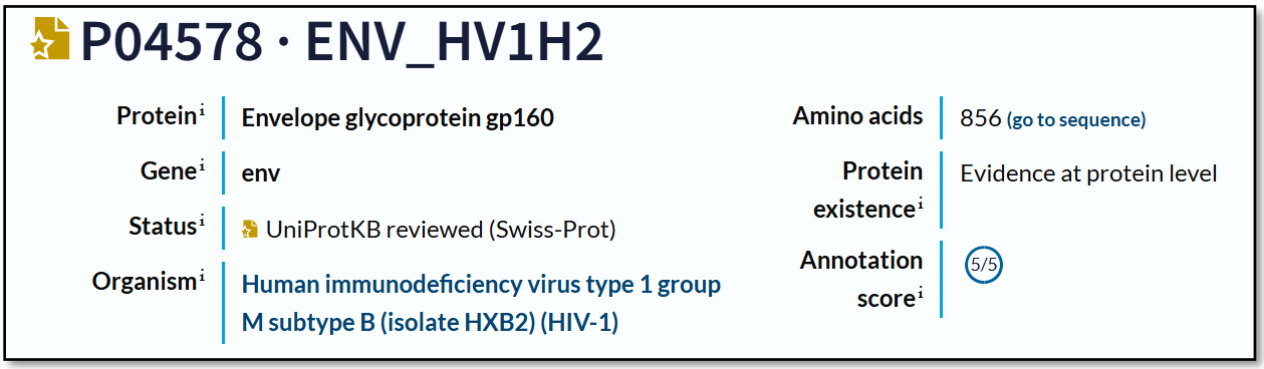


Figure 5: Envelope glycoprotein gp160 from Uniprot database (UniProt, n.d.)

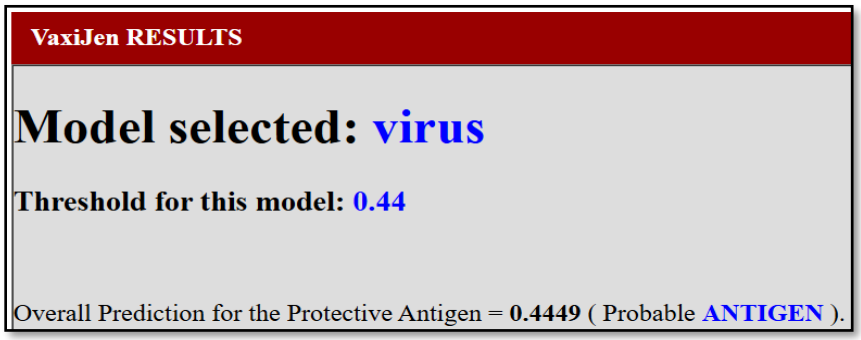


Figure 6: Antigenicity score of protein 03 in VaxiJen v2.0 server (Doytchinova & Flower, 2007)

3.2 CTL Epitopes Detection and Screening

The NetCTL server, a popular immunoinformatics tool that combines several criteria to improve prediction accuracy, was used to predict cytotoxic T cell epitopes (CTL). The parameters that were applied- A1 supertype for MHC class I allele selection, a threshold value of 0.75, a TAP transport efficiency weight of 0.05, and the computation of a combined score that incorporates proteasomal cleavage and MHC class I binding affinity were. The algorithm identified fourteen epitopes from protein 1 and twenty-four epitopes from protein 2 sequences. Out of these, four potential CTL epitopes from protein 1 and four epitopes from protein 2 with a high binding affinity score (designated by "<-E" in the output table) were taken into consideration for additional examination. To confirm their safety and appropriateness for inclusion in the multi-epitope vaccine design, these

chosen epitopes underwent further screening for toxicity, allergenicity, and antigenicity.

Peptides	Antigenicity	Allergenicity	Toxicity
QLYNTVATL	Non-Antigen	Allergen	Toxic
GSEELRSLY	Antigen	Non-Allergen	Non-Toxic
HSNQVSQNY	Non-Antigen	Allergen	Toxic
SILDISEQV	Antigen	Non-Allergen	Non-Toxic
FLSEQFLGK	Non-Antigen	Allergen	Toxic
QFRDYVDRF	Non-Antigen	Allergen	Toxic
QGLNKIVRM	Non-Antigen	Allergen	Toxic
EQYSPTSIL	Antigen	Non-Allergen	Non-Toxic
SEQGLNKIV	Non-Antigen	Allergen	Toxic
VSQNYSEQF	Non-Antigen	Allergen	Toxic
FLGKIWPSY	Non-Antigen	Allergen	Toxic
SEQYSPTSI	Antigen	Non-Allergen	Non-Toxic
GLNKIVRMY	Non-Antigen	Allergen	Toxic
IVRMYSEQY	Non-Antigen	Allergen	Toxic

Table 1: CTL epitopes from protein 01

Peptides	Antigenicity	Allergenicity	Toxicity
VAVHVASGY	Antigen	Non-Allergen	Non-Toxic
NPDIVIYQY	Antigen	Allergen	Toxic
ETKLGKAGY	Antigen	Allergen	Toxic
FKNLKTGKY	Antigen	Non-Allergen	Non-Toxic
VTNSATIMM	Non Antigen	Allergen	Toxic
VLDVGDAYF	Antigen	Allergen	Toxic
KTELQAIYL	Antigen	Allergen	Non-Toxic
YSPTSILDI	Antigen	Non-Allergen	Non-Toxic
TVLDVGDAY	Antigen	Allergen	Toxic
ETPGIRYQY	Antigen	Non-Allergen	Non-Toxic

KLNWASQIY	Non-Antigen	Allergen	Toxic
LTEEAELEL	Non-Antigen	Allergen	Toxic
LKEPVHGVY	Non-Antigen	Allergen	Toxic
KAQDEHEKY	Non-Antigen	Allergen	Toxic
WTEYWQATW	Non-Antigen	Allergen	Toxic
CTERQANFL	Antigen	Allergen	Toxic
ATDIQTKEL	Non-Antigen	Non-Allergen	Toxic
PLDEDFRKY	Non-Antigen	Non-Allergen	Toxic

Table 2: CTL epitopes from protein 02

Peptides	Combined score
GSEELRSLY	2.8964
SILDISEQV	0.8349
VAVHVASGY	0.7739
FKNLKTGKY	0.7938
ETPGIRYQY	1.0217

Table 3: Selected CTL epitopes with their respective combined scores

3.3 HTL Epitopes Detection and Screening

The HTL epitopes were selected from protein 2 (Gag polyprotein) using NetMHC-IIpan 4.0 tool. The strong binder ones are selected and then further evaluated based on their cytokine simulating profile. To see if epitopes are capable of inducing interferon-gamma (IFN-γ), IL-2 pred to predict the ability of epitopes to stimulate IL-2 for T-cell proliferation and IL-4 pred (IL-4 involved in humoral immune responses). Only epitopes with a high binding affinity and favorable cytokine-inducing characteristics were considered appropriate for inclusion in the vaccine. The total immunogenic potential of the developed multi-epitope vaccine was increased by this methodical screening, which made sure that epitopes that may effectively enhance humoral and cell-mediated immunity were incorporated. Out of all the HTL epitopes from protein 2, three potential epitopes

which matched all the criteria, were taken into consideration for additional examination.

Peptide	Antigenicity	Allergenicity	Toxicity	IFN	IL4	IL2
YVDRFYKTLRAEQAS	Non-antigen	Allergen	Toxin	Positive	Inducer	Inducer
VDRFYKTLRAEQASQ	Non-antigen	Allergen	Non-toxin	Positive	Inducer	Inducer
SPEVIPMFSALSEGA	Antigen	Non-allergen	Non-toxin	Positive	Inducer	Inducer
RFYKTLRAEQASQEV	Non-antigen	Allergen	Non-toxin	Positive	Inducer	Inducer
IWPSYKGRPGNFLQS	Antigen	Allergen	Non-toxin	Positive	Inducer	Non-inducer
TPQDLNTMLNTVGGH	Non-antigen	Allergen	Non-toxin	Negative	Inducer	Inducer
PQDLNTMLNTVGGHQ	Non-antigen	Allergen	Non-toxin	Negative	Inducer	Inducer
FYKTLRAEQASQEVK	Non-antigen	Allergen	Non-toxin	Positive	Inducer	Inducer
DYVDRFYKTLRAEQA	Non-antigen	Allergen	Non-toxin	Positive	Inducer	Inducer
RPGNFLQSRPEPTAP	Antigen	Allergen	Non-toxin	Positive	Non-inducer	Inducer
VRMYSPTSILDIRQG	Antigen	Allergen	Non-toxin	Negative	Inducer	Non-inducer
DRVHPVHAGPIAPGQ	Antigen	Allergen	Non-toxin	Positive	Non-inducer	Inducer
WDRVHPVHAGPIAPG	Antigen	Non-allergen	Non-toxin	Positive	Inducer	Inducer
LQEQIGWMTNNPPIP	Antigen	Allergen	Non-toxin	Negative	Non-inducer	Non-inducer
QEQIGWMTNNPPIPV	Antigen	Allergen	Non-toxin	Negative	Non-inducer	Non-inducer
EWDRVHPVHAGPIAP	Non-antigen	Non-allergen	Non-toxin	Positive	Inducer	Inducer
AEWDRVHPVHAGPIA	Non-antigen	Non-allergen	Non-toxin	Positive	Inducer	Inducer
LKHIVWASRELERFA	Antigen	Allergen	Non-toxin	Positive	Inducer	Inducer
ELERFAVNPGLLETS	Non-antigen	Allergen	Non-toxin	Negative	Inducer	Inducer
ATIMMQRGNFRNQRK	Antigen	Non-allergen	Non-toxin	Positive	Inducer	Inducer
NSATIMMQRGNFRNQ	Antigen	Allergen	Non-toxin	positive	Inducer	Inducer
TNSATIMMQRGNFRN	Antigen	Allergen	Non-toxin	positive	Inducer	Inducer
WEKIRLRPGGKKKYK	Antigen	Allergen	Non-toxin	Negative	Non-Inducer	Inducer
SATIMMQRGNFRNQR	Non-antigen	Non-allergen	Non-toxin	Positive	Inducer	Inducer
PKEPFRDYVDRFYKT	Non-antigen	Allergen	Non-toxin	Negative	Inducer	Inducer
LDKIEEEQNKSKKKA	Non-antigen	Allergen	Non-toxin	Positive	Inducer	Inducer
ERFAVNPGLLETSEG	Antigen	Allergen	Non-toxin	Negative	Inducer	Non-inducer
QNIQGQMVHQAISPR	Antigen	Allergen	Non-toxin	Positive	Inducer	Non-inducer

Table 4: HTL epitopes from protein 02

3.4 B-cell Epitopes Detection and Screening

A score vs position plot with a default threshold of 0.5 was produced by the analysis. In the output, regions with a score of 0.5 or lower (as indicated in green) were categorized as non-epitopic, whereas areas with a score of more than 0.5 (shown in yellow) highlighted potential B-cell epitopes. Epitopes with scores above the cutoff were selected as potential candidates for vaccine development based on these predictions.

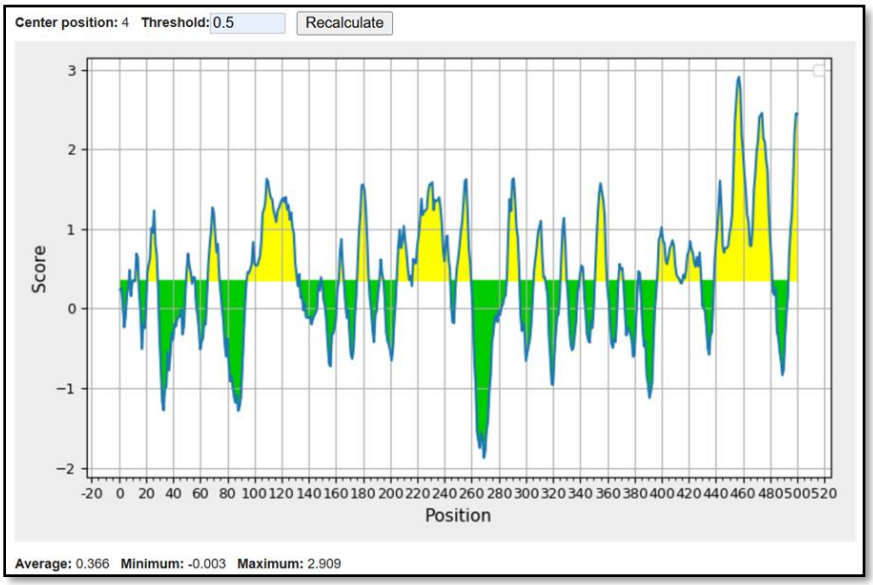


Figure 7: Score vs position graph of B cell from protein 01(Jespersen et al., 2017)

Predicted peptides:

No.	Start	End	Peptide	Length
4	21	28	LRPGGKKK	8
7	66	74	PSLQTGSEE	9
12	176	184	SEGATPQDL	9
18	306	314	AEQASQEVK	9
21	351	359	QGVGGPGHK	9
17	286	295	RQGPKEPFRD	10
14	205	215	INEEAAEWDRV	11
16	249	259	WMTNNPPIPVG	11
25	416	429	CGKEGHQMKDCTER	14
24	397	413	KEGHTARNCRAPRKKGC	17
15	217	244	PVHAGPIAPGQMREPRGSDIAGTTSTLQ	28

Figure 8: B cell epitopes from protein 01(Jespersen et al., 2017)

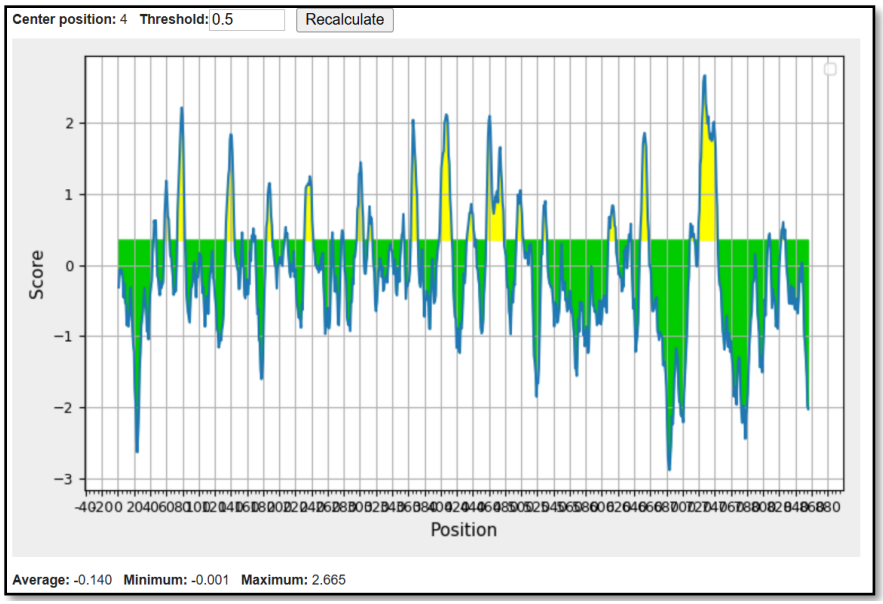


Figure 9: Score vs position graph of B cell from protein 03 (Jespersen et al., 2017)

Predicted peptides:

No.	Start	End	Peptide	Length
28	720	743	HLPTPRGPDRPEGIEEEGGERDRD	24
20	457	478	DGGNSNNESEIFRPGGGDMRDN	22
17	399	414	TWSTEGSNNTEGSDTI	16
10	231	242	KTFNGTGPCTNV	12
4	134	144	LKNDTNTNSSS	11
26	648	658	ESQNQQEKNEQ	11
3	74	83	CVPTDPNPQE	10
18	433	442	AMYAPPISGQ	10
23	608	617	VPWNASWSNK	10
13	296	304	CTRPNNNTR	9
21	494	502	LGVAPTKAK	9
8	184	191	IDNDTTSY	8
16	364	371	SSGGDPEI	8
2	58	64	AKAYDTE	7

Figure 10: B cell epitopes from protein 03 (Jespersen et al., 2017)

B-cell epitope	Length	Antigenicity	Allergenicity	Toxicity
LGVAPTKAK	9	Antigen	Non-allergen	Non-toxin
CVPTDPNPQE	10	Antigen	Non-allergen	Non-toxin
AMYAPPISGQ	10	Antigen	Non-allergen	Non-toxin
PSLQTGSEE	9	Antigen	Non-allergen	Non-toxin
WMTNNPPIPVG	11	Antigen	Non allergen	Non-toxin
PVHAGPIAPGQMREPRGSDIAGTTSTLQ	28	Antigen	Non allergen	Non-toxin

Table 5: Selected B cell epitopes from proteins 01 and 03

3.5 Construction of Final Vaccine Using Suitable Linkers, Pan HLA-DR Epitope (PADRE) and hBD-2 (Human Beta-Defensin-2) Adjuvant

The selected CTL, HTL, and B-cell epitopes were combined with the Pan HLA-DR epitope (PADRE) and hBD-2 (Human Beta-Defensin-2) adjuvant at the N-terminal region to construct the final multi-epitope vaccine design. In order to preserve structural stability and lessen adverse interactions between the adjuvant and downstream epitopes, PADRE (AKAKFVAAWTLKAAA) was joined using a rigid EAAAK linker, served as a universal helper epitope, expanding vaccination coverage and enhancing humoral and cellular responses (Xu et al., 2024). The adjuvant improved the overall strength and duration of the immune response. And hBD-2 is widely used as an adjuvant block at the N-terminus of multi-epitope vaccines to enhance structural stability (Mitra et al., 2022). KK was used as a polar linker to increase solubility and accessibility, AAY to enhance proteasomal cleavage and improves MHC-I presentation, GPGPG to maintain epitope individuality and prevent junctional immunogenicity and EAAAK as a rigid stabilizer for epitope assembly. This sequence arrangement maintained each epitope's immunogenicity while guaranteeing appropriate antigen processing. The addition of PADRE strengthened humoral and cellular immunity by increasing CD4 T-cell activation across a variety of HLA-DR alleles.

Constructed Vaccine:

GIGDPVTCLKSGAICHPVFCPRRYKQIGTCGLPGTKCCKKPEAAAKAKFVAAWTL
KAAAEAAAKGSEELRSLYAAYSILDISEQVAAYVAVHVASGYAAYFKNLKTGKYAA
YETPGIRYQGPGPGSPEVIPMFSALSEGAGPGPGWDRVHPVHAGPIAPGGPGPGATI
MMQRGNFRNQRKKKLGVAPTKAKKKCVPTDPNPQEKKAMYAPPISGQKKPSLQT
GSEEKKWMTNNPPIPVGKKPVHAGPIAPGQMREPRGSDIAGTTSTLQ

3.6 Antigenicity, Allergenicity, and Toxicity of Constructed Vaccine

The VaxiJen v2.0 server was used to evaluate the vaccine's antigenicity using the viral model at a threshold of 0.5. The findings demonstrated that the construct has substantial antigenic potential, implying that the immune system could detect it and trigger a protective response.

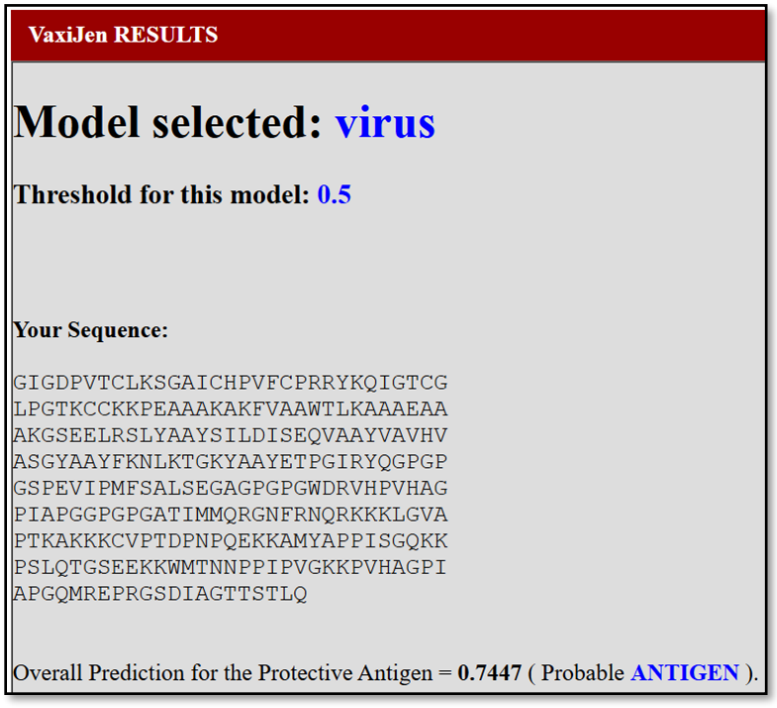


Figure 11: Antigenicity score of the constructed vaccine (Doytchinova & Flower, 2007)

Three tools were used to evaluate the vaccine's allergenicity, those are- AllerTOP v2.1, AllergenFP v1.1 and AlgPred 2.0. Each of the tools indicates that the constructed vaccine is non-allergenic and unlikely to cause hypersensitivity responses.

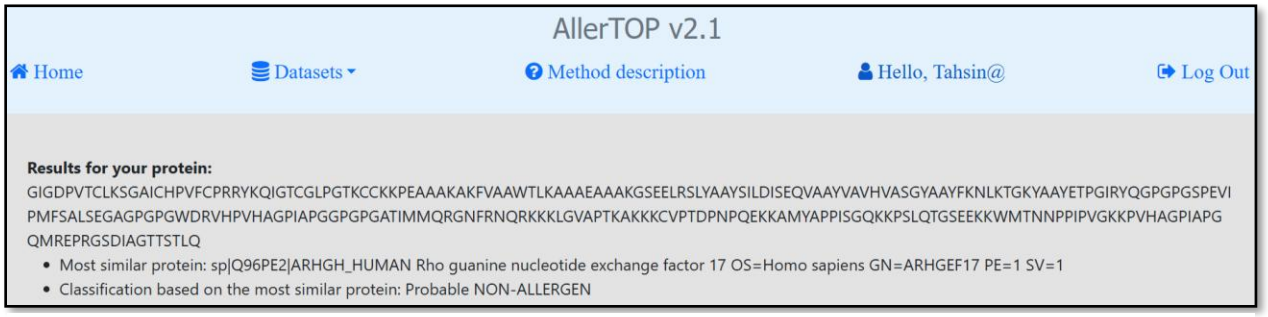


Figure 12: Allergenicity score of the constructed vaccine by AllerTop v2.1

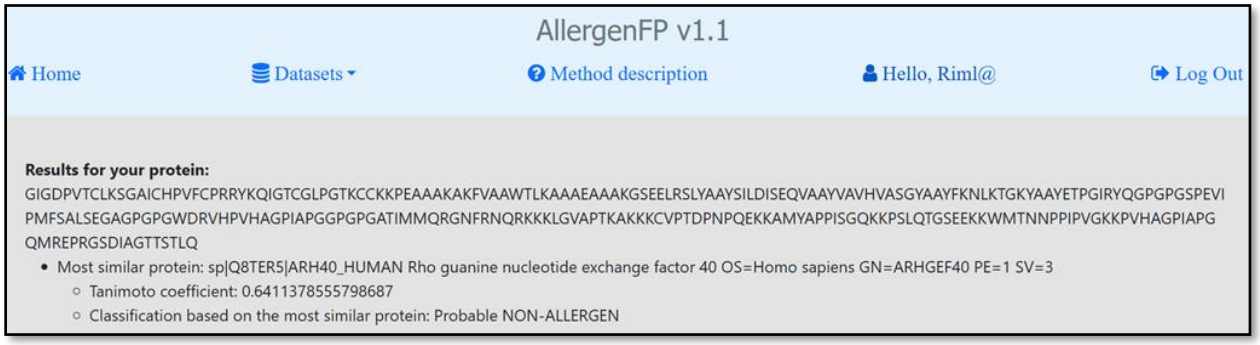


Figure 13: Allergenicity score of the constructed vaccine by AllergenFP v1.1

Figure 14: Allergenicity score of the constructed vaccine by AlgPred 2

The T3DB (Toxin and Toxin Target Database) was used to assess the vaccine's toxicity. The result ‘zero’ toxic finding or harmful hits indicates that the constructed vaccine is non-toxic and potent for safe administration.

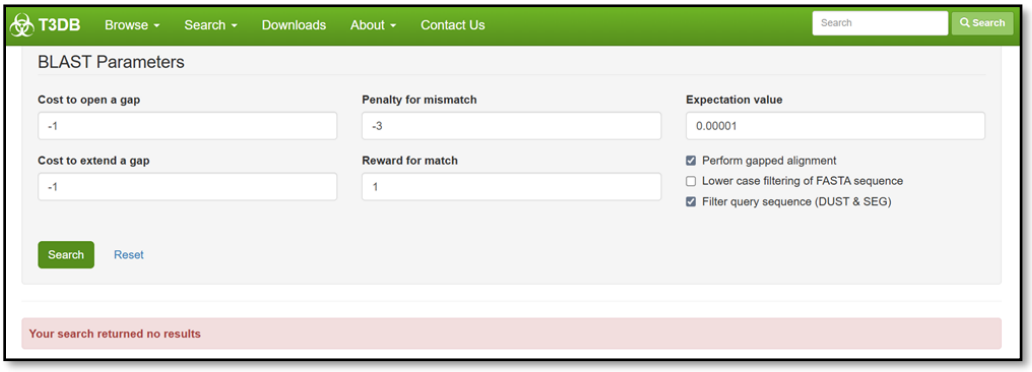


Figure 15: Toxicity identification of constructed vaccine by T3DB (Wishart et al., 2015)

3.7 Evaluation of In-silico Biochemical Features of Constructed Vaccine

The vaccine is modestly sized and basic in character, consisting of 269 amino acids with a molecular weight of around 28.2 kDa and a predicted pI of 9.74. The amino acid content is well-balanced, with the most abundant amino acids being alanine (14.0%), glycine (11.5%), proline (11.5%), and lysine (10.0%), which all contribute to solubility and flexibility.

Number of amino acids: 269
Theoretical pI: 9.74
Molecular weight: 28257.70

Amino acid composition: CSV format

Ala (A)	35	13.0%
Arg (R)	10	3.7%
Asn (N)	6	2.2%
Asp (D)	5	1.9%
Cys (C)	7	2.6%
Gln (Q)	10	3.7%
Glu (E)	12	4.5%
Gly (G)	31	11.5%
His (H)	5	1.9%
Ile (I)	13	4.8%
Leu (L)	11	4.1%
Lys (K)	27	10.0%
Met (M)	6	2.2%
Phe (F)	5	1.9%
Pro (P)	31	11.5%
Ser (S)	14	5.2%
Thr (T)	14	5.2%
Trp (W)	3	1.1%
Tyr (Y)	10	3.7%
Val (V)	14	5.2%

Figure 16: Amino acid composition by Protparam server (Gasteiger et al., 2005)

The aliphatic score 62.90 indicates moderate thermostability, whilst the instability index 34.31 indicates stability. A protein with negative GRAVY value (-0.429) is hydrophilic, meaning it is more soluble in water. The anticipated half-life (30 hours in mammalian cells) indicates strong in vivo stability and is appropriate for vaccine expression.

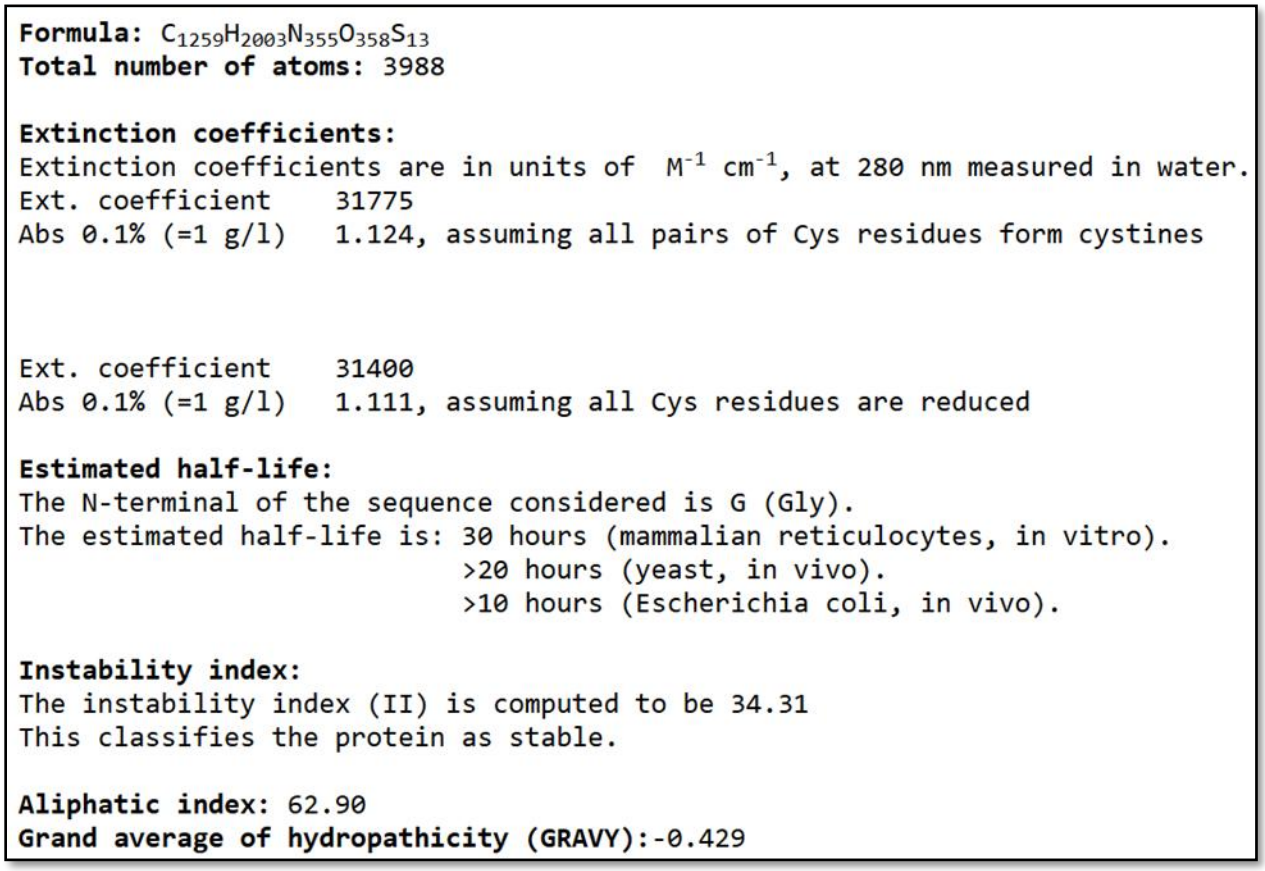


Figure 17: Evaluation of biochemical features of the constructed vaccine through instability index, aliphatic index, GRAVY (Kyte & Doolittle, 1982)

All of these properties work together to ensure that the vaccine design is soluble, stable, and potentially immunogenic, which qualifies it to undergo further experimental validation.

3.8 Generation of 3D Model of Prepared Vaccine

The constructed vaccine sequence was submitted in the Phyre2 tool in FASTA format, the modeling was carried out in normal mode and as output the 3D structure of the constructed multi-epitope vaccine was generated.

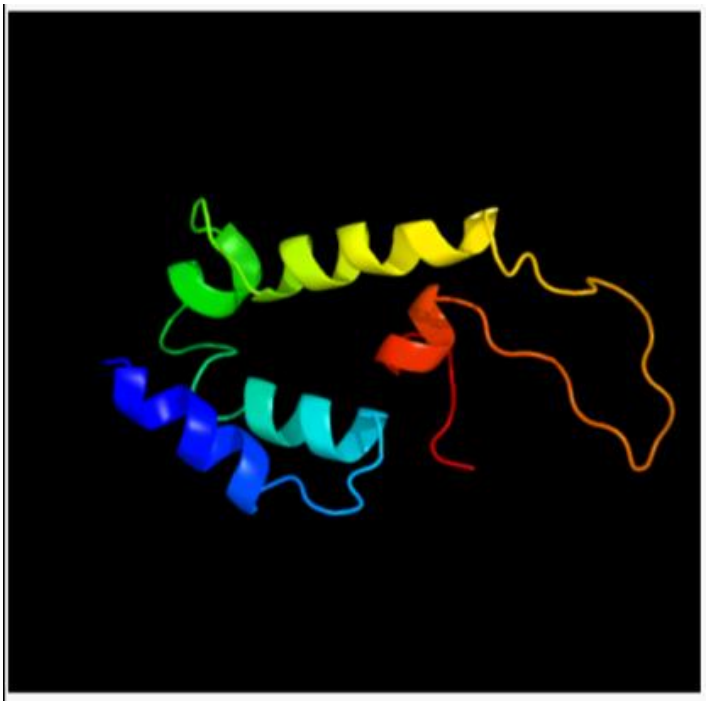


Figure 18: Construction of 3D model (Kelley et al.,2015)

3.9 Coverage Assessment

IEDB population coverage study was done to support the structural evaluation. This assesses functional epitope recognition across global HLA alleles, in contrast to template-based models. The analysis showed a wide worldwide coverage (≈98.9%), assuring that the chosen B-cell, HTL, and CTL epitopes are immunologically relevant to a wide range of populations. Projected coverage remained high across all WHO regions, reflecting the inclusion of epitopes binding to multiple prevalent HLA supertypes. This breadth minimizes population-specific gaps and strengthens confidence that the construct can elicit responses in diverse demographic settings.

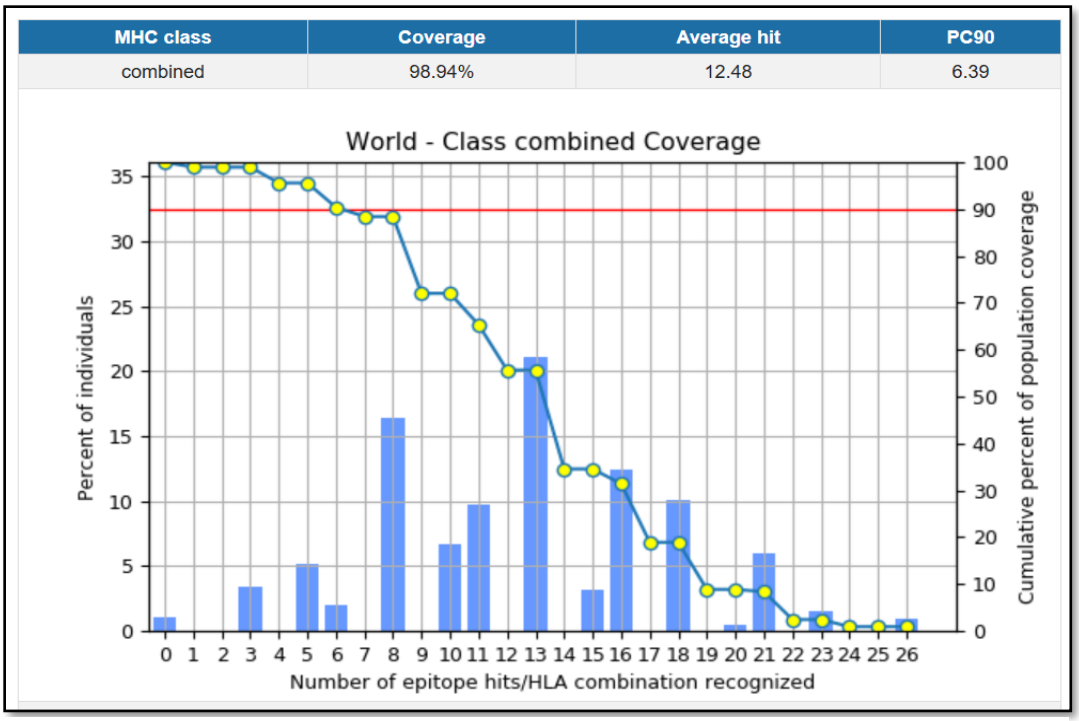


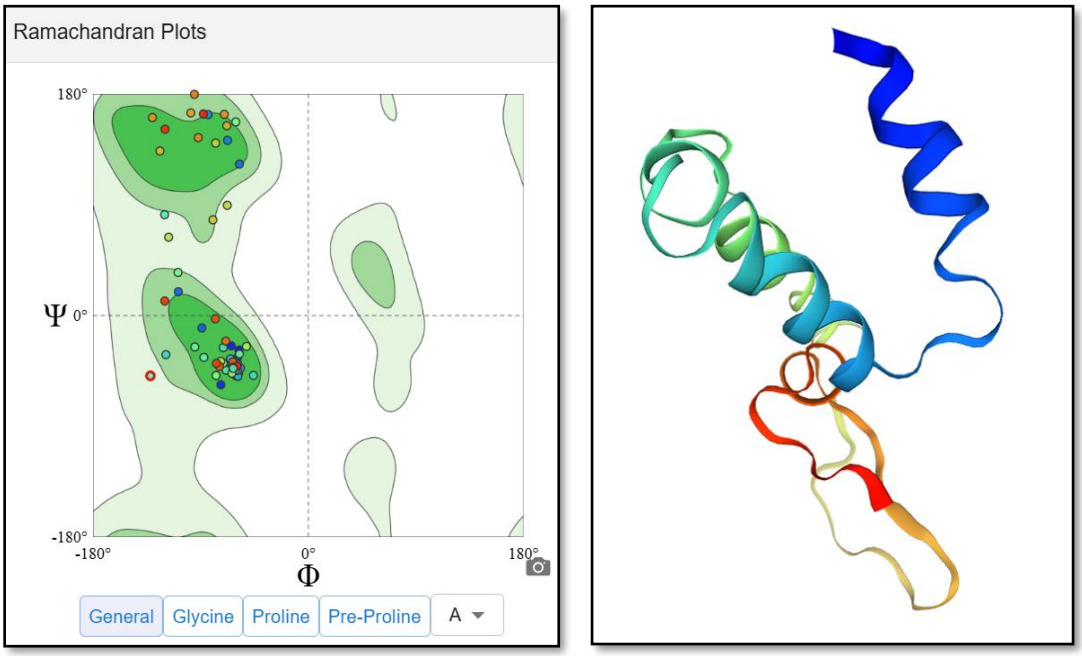
Figure 19: World-class combined coverage by IEDB (Pandey et al., 2018)

IEDB's high global epitope coverage confirms the vaccine's potential for broad population application.

3.10 3D Model Quality Assessment (Ramachandran Plot Prediction)

The Swiss Model Expasy server was used for Ramachandran plotting, and was used to confirm the suggested vaccine construct's stereochemical quality. Only 1.20% of residues were found as outliers, while 91.57% of residues were found in the preferred region. The structural accuracy was further supported by the absence of any rotamer or C-Beta aberrations. For computational models of multi-epitope vaccines, a clash score of 51.30 and a MoiProbity score of 2.69 were shown to be within acceptable range. The significant proportion of residues in advantageous locations suggests that the vaccine construct's backbone dihedral angles (φ and ψ) take on stereochemically reliable conformations. Flexible loop sections are responsible for the minimal outliers, which are

frequently seen in synthetic, multi-epitope complexes due to several linkers and adjuvants (Lovell et al., 2003b).



MolProbity Results

MolProbity Score	2.69
Clash Score	51.30
(A234 PRO-A237 PRO), (A206 ALA-A210 PRO), (A233 ASN-A235 PRO), (A234 PRO-A235 PRO), (A210 PRO-A211 PRO), (A236 ILE-A237 PRO), (A209 ALA-A210 PRO), (A235 PRO-A237 PRO), (A140 ALA-A203 GLU), (A207 MET-A210 PRO), (A233 ASN-A236 ILE), (A217 LYS-A218 PRO), (A233 ASN-A234 PRO), (A231 THR-A235 PRO)	
Ramachandran Favoured	91.57%
Ramachandran Outliers	1.20%
A210 PRO	
Rotamer Outliers	0.00%
C-Beta Deviations	0

Figure 20: Ramachandran plot prediction of the constructed vaccine (Lovell et al., 2003)

3.11 ERRAT and QMEAN Analysis

The ERRAT tool, which assesses the statistics of non-bonded atomic interactions between various atom types, was used to test the stereochemical quality of the planned multi-epitope vaccination

construct. An overall quality factor of 91.667% was obtained from the study, while quality factor above 90% are considered as being of very high-quality models by ERRAT and comparable to crystallographic structures. This outcome validates the construct's structural reliability by exhibiting that most of the non-bonded atomic interactions are within the acceptable range (Colovos & Yeates, 1993b; Mahapatra et al., 2023).

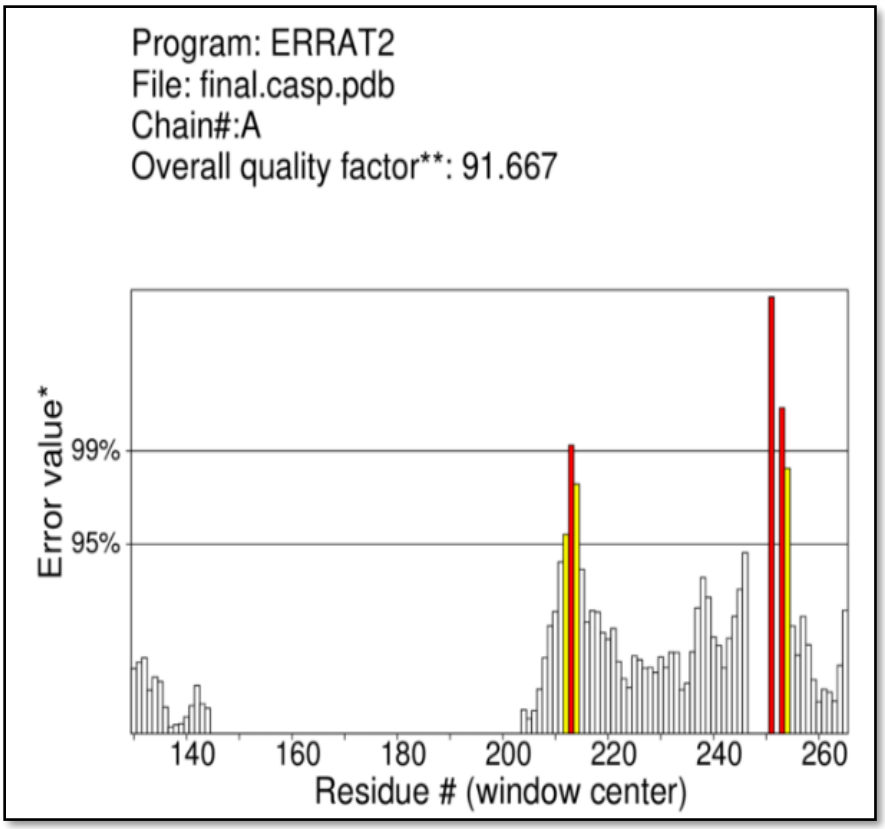


Figure 21: Overall quality factor analysis by ERRAT Mahapatra et al., 2023)

The QMEANDisCo global quality assessment approach was used to further evaluate the vaccination model's stereochemical quality. With a QMEANDisCo global score of 0.49 (±0.09), the vaccine design fell within the permitted range of 0 to 1. Values below 0 indicate low-quality models, whereas scores near 1 show a significant resemblance to high-resolution experimental

structures. Some fluctuations are expected due to the presence of synthetic adjuvant sequences, epitope concatenations, and glycine/serine-rich linkers frequently bring inherent disorder and flexibility; they do not indicate model failure. In fact, they increase epitope exposure and immunogenicity (Benkert et al., 2011).

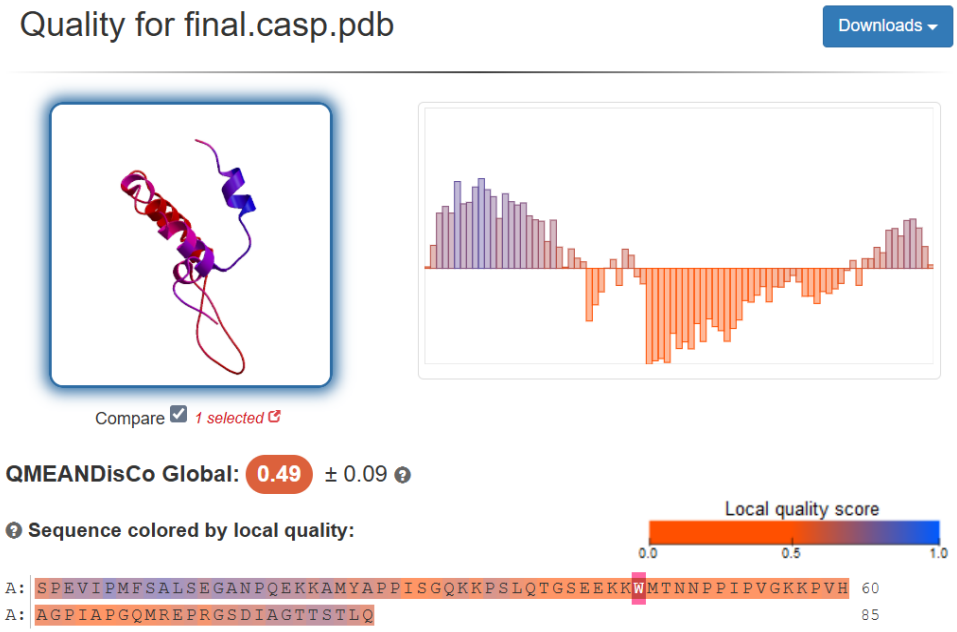
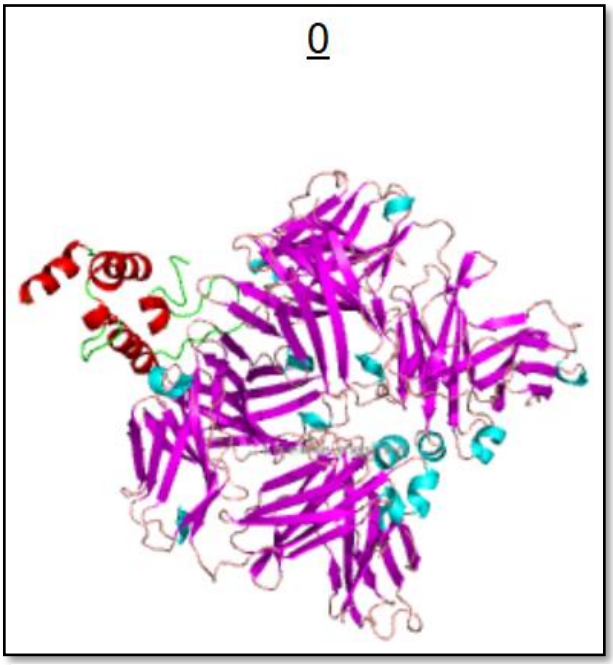


Figure 22: QMEANDisCo Global score (Benkert et al., 2011)

3.12 Molecular Docking with Toll Like Receptor

Molecular docking was done using the ClusPro tool to ensure if the vaccine can effectively bind with the immune receptors like- Toll-Like Receptor 3 (TLR3) or TLR4. In this case TLR3 was the best fit to be docked with. The docking study produced a number of binding clusters, each of which represented a potential vaccine–TLR3 complex configuration. With the lowest energy score of -909.9 kcal/mol, Cluster 4 showed the most advantageous interaction among them, suggesting a very consistent binding affinity because the lower (more negative) the score, the stronger and more stable the predicted interaction. Significant hydrogen bonding and hydrophobic contacts between

the vaccine epitopes and the TLR3 binding groove were shown by the representative docked model, indicating that the developed vaccine may effectively interact with innate immune receptors. It is anticipated that this interaction will trigger downstream signaling pathways, including NF-κB (transcription factor that regulates immune and inflammatory responses) and IRF3 (regulates interferon and antiviral responses), which will increase humoral and cellular



Cluster	Members	Representative	Weighted Score
0	67	Center	-770.8
		Lowest Energy	-841.2
1	65	Center	-900.1
		Lowest Energy	-900.1
2	62	Center	-761.5
		Lowest Energy	-871.9
3	60	Center	-851.7
		Lowest Energy	-856.4
4	55	Center	-909.9
		Lowest Energy	-909.9
5	49	Center	-807.8
		Lowest Energy	-867.9

Figure 23: Molecular docking between TLR-3 and the constructed vaccine (Liu et al., 2008)

immune responses by inducing pro-inflammatory cytokines and type I interferons. The robust clustering consistency and high binding affinity claims that the vaccine is both structurally and functionally capable of triggering TLR3-mediated immunostimulation (Liu et al., 2008).

3.13 Immune Simulation

The C-ImmSim service, which predicts the immunological profile based on epitope sequence and immune system factors, was used to assess the immune response of the developed multi-epitope vaccine construct. The significant drop in antigen levels (black peaks) after repeated doses in the

simulation results showed a strong antigen clearance after each injection. Antibody titers increased significantly, with IgM showing up initially during the primary reaction and IgG1 and IgG2 subtypes showing a high and prolonged rise during the secondary and tertiary responses. Following booster shots, the combined IgG + IgM response increased significantly, indicating successful memory formation. A sustained humoral response, which is essential for long-lasting immunity, is indicated by the preponderance of IgG. These data imply that the vaccine design has the ability to trigger both primary and memory immune responses, assuring accurate detection and clearance of the antigen upon recurrent exposure. The vaccine's ability to produce long-term immunological memory which is crucial for protective efficacy is further supported by the balanced production of IgM and IgG antibodies (Rapin et al., 2010).

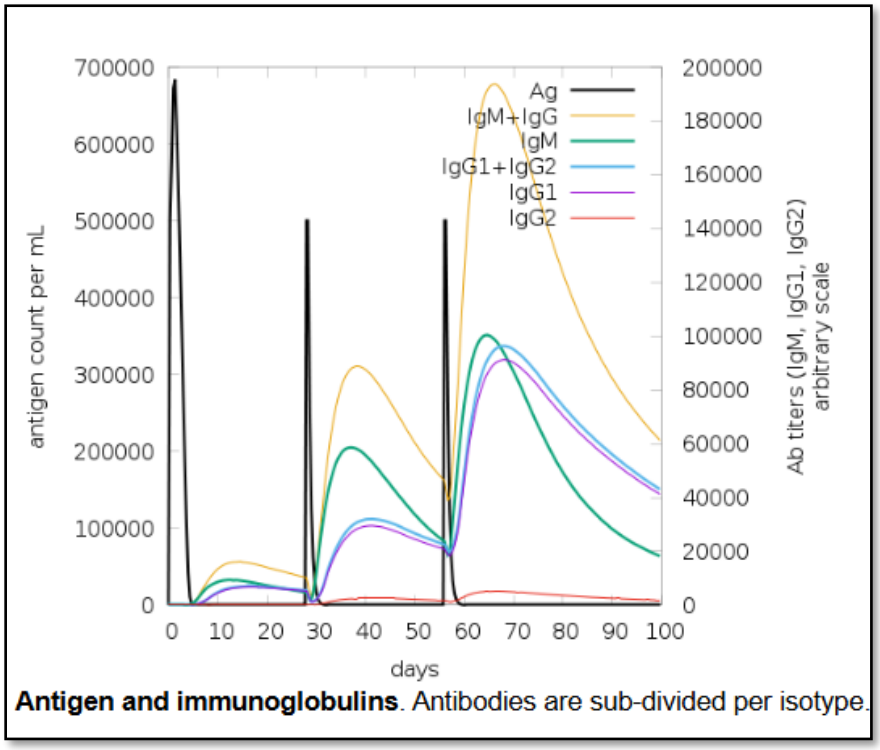
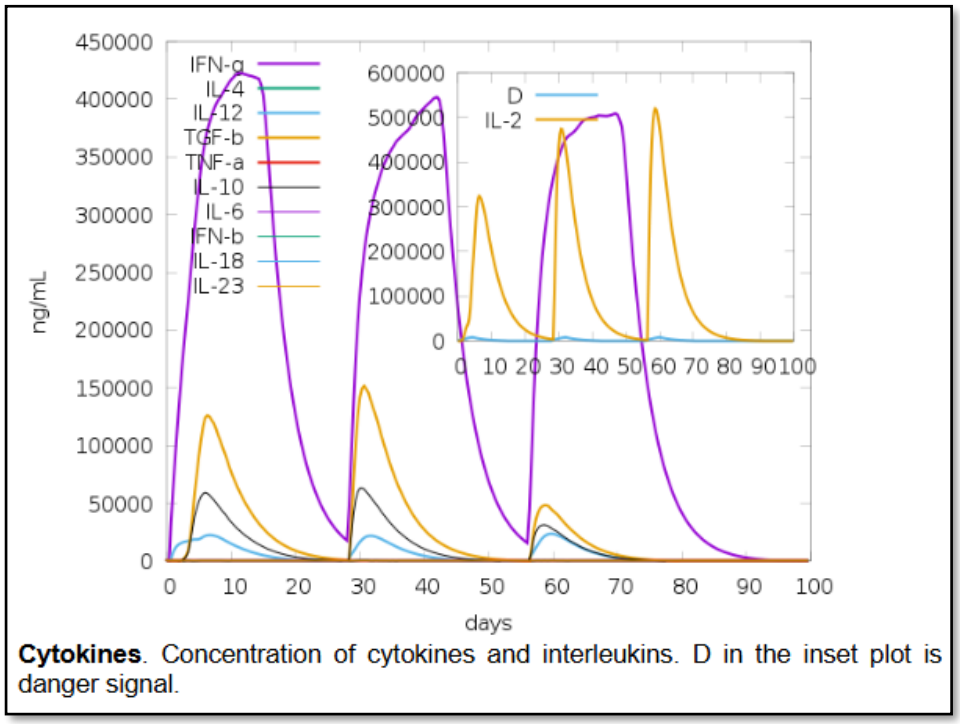


Figure 24: Immune simulation by C-IMMSIM (Rapin et al., 2010)

The immune simulation's cytokine profile exhibited significantly increased levels of IFN-γ and IL-2, supporting robust Th1 and cytotoxic T-cell responses. A balanced immune regulation with adequate helper T-cell activation is further demonstrated by the moderate raised levels of IL-10 and IL-12. The results demonstrate the vaccine's capacity to trigger humorous responses in addition to strong cellular immunity.



Cytokines. Concentration of cytokines and interleukins. D in the inset plot is danger signal.

Figure 25: Levels of cytokines including IFN, γ, IL4, IL6 (Rapin et al., 2010)

B cell population analysis confirms that the vaccine design could generate long-lasting memory responses, which are essential for prolonged immunity. Additionally, it exhibits effective isotype switching (IgM → IgG), demonstrating the vaccine's capacity to produce humoral immunity instantly and over time. In addition to active IgM, IgG1, and IgG2 isotype responses, the

immunological simulation demonstrated a long-term increase in B cell memory formation, suggesting successful antigen presentation and long-term humoral immunity.

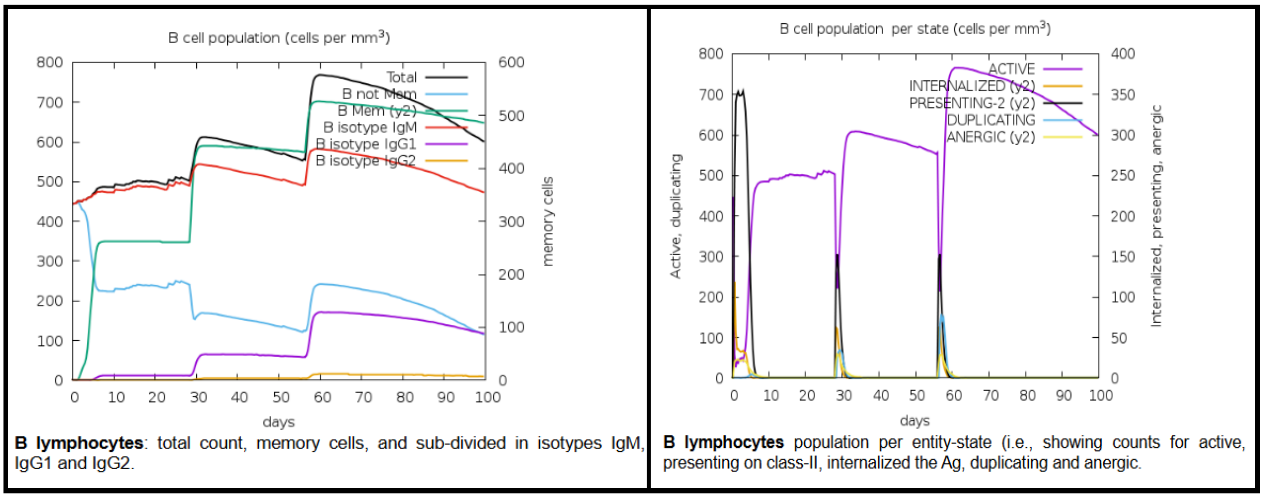


Figure 26: Total count of B lymphocytes (Rapin et al., 2010)

The formation of immunological memory is evident, which displays the total $CD4^+$ T-helper cells and memory cells. Total $CD4^+$ T-helper cells, memory $CD4^+$ T-helper cells significantly increase following each vaccination dose, which is a good sign of long-term immune protection.

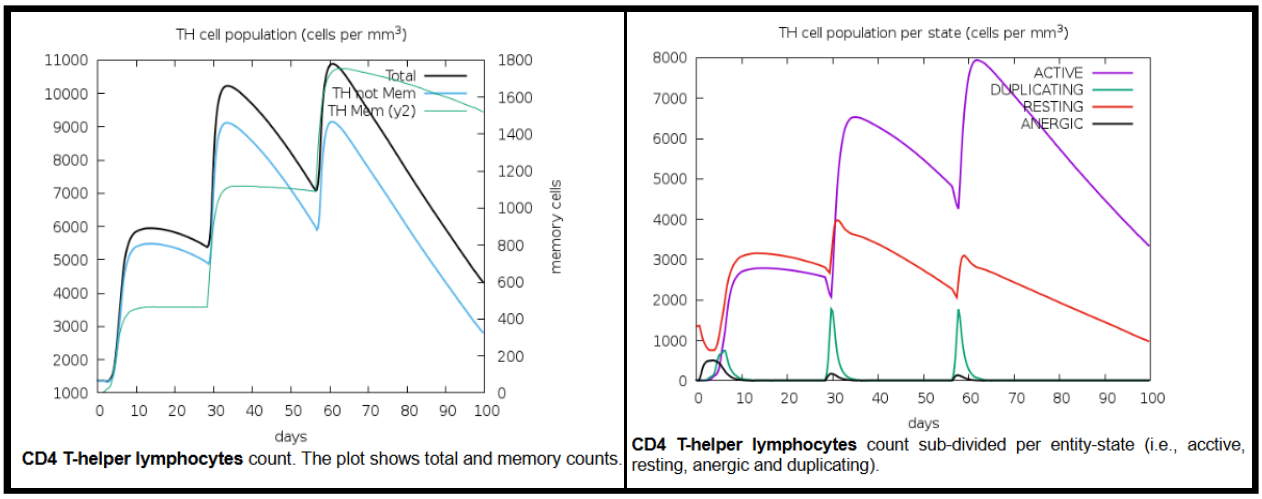


Figure 27: Number of CD4 T-helper lymphocytes (Rapin et al., 2010)

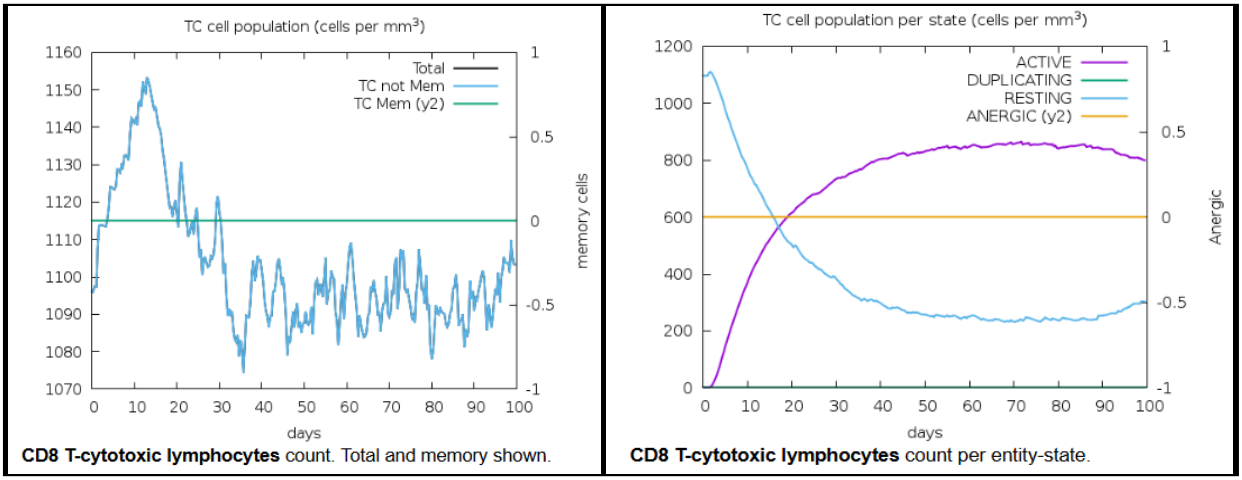


Figure 28: Number of CD8 T-cytotoxic lymphocytes (Rapin et al., 2010)

After vaccination, CD8 T-cytotoxic cells proliferate, with many of them activating early to combat abnormal or pathogenic cells. Meanwhile, some cells develop into memory CTLs, which remain in the body for protection for an extended period of time. The vaccination produces a potent but carefully controlled cytotoxic immune response because the increase in anergic cells helps avoid overreacting.

Chapter 04

Discussion

The in-silico development of an HIV-targeting multi-epitope subunit vaccine combines B-cell, HTL, and CTL epitopes with appropriate spacer sequences to improve processing and presentation. The inclusion of immunostimulatory components such human β-defensin-2 (hBD-2) and PADRE produced dual adjuvanting effects by encouraging both Toll-like receptor activation and MHC class II binding. The goal of this combination strategy was to combat HIV's immune evasion mechanisms and inherent mutability.

The chosen epitopes were safe and immunogenic, as verified through antigenicity, allergenicity, and toxicity tests, guaranteeing their eligibility for vaccine development. A good instability index, aliphatic index, and negative GRAVY score supported the vaccine's solubility and thermostability, according to the physicochemical evaluation. Significantly, quality metrics above acceptable levels were found in ERRAT, QMEANDisCo, and Ramachandran plot studies, which validated the vaccination model's stereochemical reliability and credibility. Strong binding affinity was found when docking with TLR-3, which is consistent with this receptor's function in antiviral immune responses. The construct's capacity to initiate downstream innate immune signaling is supported by the anticipated interactions. Furthermore, immunological simulations (C-ImmSim service) showed strong responses with enhanced cytotoxic T-cell activation, memory B-cells, $CD4^+$ helper T-cells, and IgG and IgM antibody titers, all of which are essential for HIV control.

These results correspond with recent vaccine designs based on immunoinformatics, where multi-epitope design concepts showed potent in-silico immunogenicity against cancer and viral targets

(Naz et al., 2020). The vaccine framework solves important drawbacks of traditional single-epitope or protein-based vaccinations by integrating multiple epitopes, adjuvants, and linkers.

Chapter 05

Conclusion and Future Considerations

To sum up, this study presents the effective in-silico development of a multi-epitope HIV vaccine candidate. The construct is antigenic, non-allergic, stable, and able to elicit humoral and cellular immunological responses because of the addition of precisely screened epitopes, immunoadjuvants (PADRE, hBD-2), and optimized linkers. The model's reliability has been confirmed by structural validation, and molecular docking validated that it may interact with TLR-3, therefore strengthening its capacity to trigger innate immunity. Immune simulations also showed cytotoxic T-cell activation, memory formation, and persistent antibody production.

Despite the encouraging results, it's essential to recognize the limits of in-silico approaches. The complex nature of immunological responses in vivo is not entirely captured by predictions. Thus, in order to evaluate the vaccine's actual effectiveness, experimental validation through in-vitro and in-vivo research would be essential. If validated, this design would be a significant breakthrough in the construction of an HIV vaccine, establishing the groundwork for adjuvant-enhanced, epitope-based immunotherapies that might potentially solve the challenges of immune evasion and variability.

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