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Immunoinformatics-Based Design and In Silico Evaluation of a Multi-Epitope Vaccine Targeting HIV-1 and HIV-2 Envelope Glycoproteins

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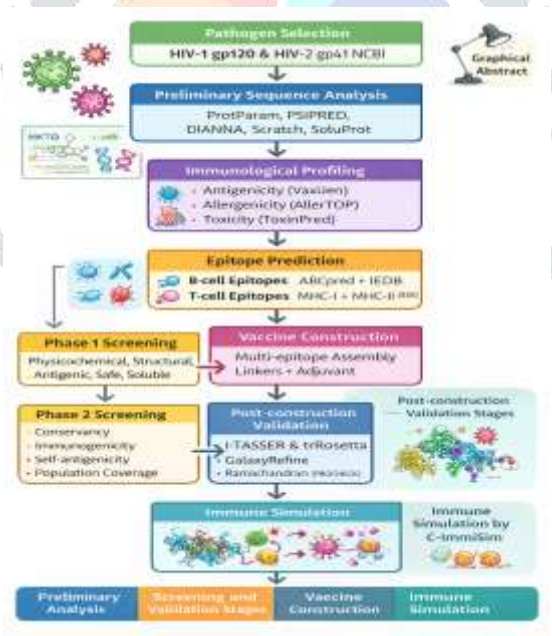
Abstract : Human Immunodeficiency Virus (HIV) continues to pose a significant global health burden, necessitating the development of effective preventive vaccines. In the present study, an immunoinformatics-driven approach is employed to design and evaluate a multi-epitope vaccine construct targeting envelope glycoproteins of HIV-1 and HIV-2. Immunogenic B-cell, cytotoxic T lymphocyte (CTL), and helper T lymphocyte (HTL) epitopes are systematically predicted and screened for antigenicity, allergenicity, toxicity, solubility, and population coverage. Selected epitopes are assembled into a single vaccine construct using appropriate linkers and adjuvant sequences to enhance immunogenicity and structural stability. The tertiary structure of the vaccine construct is predicted using I-TASSER, refined with GalaxyRefine, and validated with PROCHECK, which confirms acceptable stereochemical quality. Global structural reliability is supported by TM-score and RMSD analyses. Furthermore, in silico immune simulation using C-ImmSim predicted robust humoral and cellular immune responses, including antibody class switching, cytokine induction, and immune memory formation. Overall, the computational findings suggest that the proposed multi-epitope vaccine construct possesses favourable immunogenic and structural properties, warranting further experimental validation.



IndexTerms - HIV vaccine; multi-epitope vaccine; immunoinformatics; epitope-based vaccine design; structural modelling; immune simulation

I. INTRODUCTION

Human Immunodeficiency Virus (HIV) remains a major global public health challenge, with millions of individuals affected worldwide despite significant advances in antiretroviral therapy. (Mundlia et al. 2025; Addissouky et al. 2024) The virus primarily targets $CD4^+$ T lymphocytes, leading to progressive immune dysfunction and increased susceptibility to opportunistic infections and malignancies (Vaillant et al., 2019; Karris et al., 2011). Although combination antiretroviral therapy has substantially improved patient survival, it does not eliminate the virus and requires lifelong adherence, underscoring the urgent need for an effective preventive vaccine (Cafaro et al., 2022).

HIV exhibits extensive genetic variability and complex immune evasion mechanisms, which have hindered the development of a universally effective vaccine. In particular, the high mutation rate of viral envelope proteins and the diversity of circulating viral

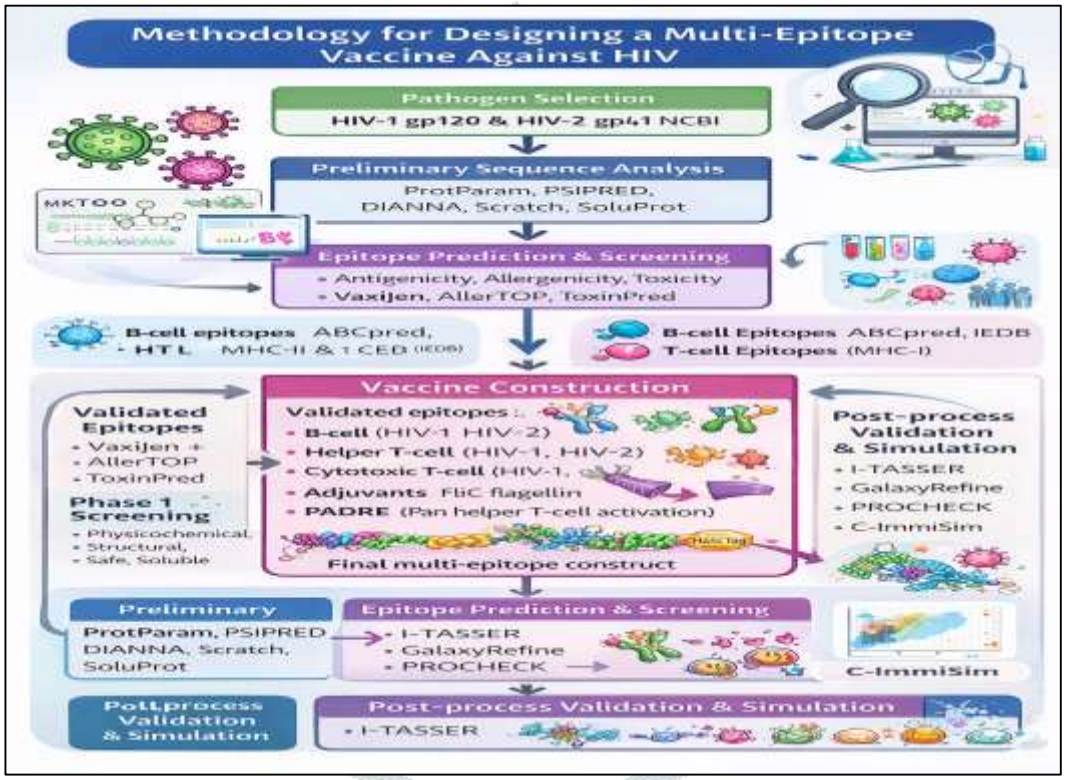
strains complicate traditional vaccine strategies (Fang et al., 2025; Zhong et al., 2022; Collins & Baltimore, 1999). Consequently, contemporary vaccine research has increasingly focused on rational design approaches that target conserved immunogenic regions capable of eliciting both humoral and cellular immune responses (Soleymani et al., 2022; Rao et al., 2025; Bahrami et al.,2019)

Multi-epitope vaccine design has emerged as a promising strategy to overcome these challenges. By integrating carefully selected B-cell, cytotoxic T lymphocyte (CTL), and helper T lymphocyte (HTL) epitopes into a single construct, multi-epitope vaccines aim to induce broad and robust immune responses while minimising unwanted allergenic or toxic effects (Mortazavi et al., 2024; Yu et al., 2024; Parvizpour et al., 2020). Advances in immunoinformatics and computational biology have significantly accelerated this process, enabling systematic epitope screening, vaccine assembly, structural evaluation, and immune response simulation prior to experimental validation(Cernuto et al., 2025; Yurina et al., 2022; Hegde et al., 2017).

Among HIV structural components, envelope glycoproteins play a pivotal role in viral attachment, membrane fusion, and immune recognition. HIV-1 gp120 and HIV-2 gp41, in particular, contain multiple immunogenic regions and represent attractive targets for epitope-based vaccine development (Yingliang et al., 2007; Kim et al., 2008). Incorporating epitopes derived from both HIV-1 and HIV-2 may further enhance global population coverage and immunological relevance.

In this study, an immunoinformatics-driven strategy was employed to design and evaluate a multi-epitope vaccine construct targeting the envelope glycoproteins of HIV-1 and HIV-2. The approach integrates epitope prediction and rigorous screening, rational vaccine construction, structural modelling and validation, and in silico immune simulation to assess immunogenic potential. The findings provide a comprehensive computational framework supporting the proposed vaccine construct and lay the groundwork for subsequent experimental validation.

II METHODOLOGY



2.1 Pathogen Selection and Sequence Acquisition

Human Immunodeficiency Virus (HIV) is selected as the target pathogen because it infects $CD4^+$ T lymphocytes, leading to progressive immune dysfunction and increased susceptibility to opportunistic infections. To ensure broad global relevance, envelope glycoproteins from both HIV-1 and HIV-2 are considered. Accordingly, the protein sequences of HIV-1 gp120 and HIV-2 gp41 are retrieved from the NCBI database. These envelope glycoproteins play critical roles in viral attachment, membrane fusion, and immune recognition and therefore serve as suitable targets for immunoinformatics-based vaccine design.

2.2 Initial Sequence Characterisation

The retrieved gp120 and gp41 sequences are subjected to preliminary bioinformatics analyses to evaluate their physicochemical properties, antigenicity, allergenicity, toxicity, solubility, and structural features. Physicochemical profiling is performed using ExPASy ProtParam (Gasteiger et al., 2005), while antigenicity, allergenicity, and toxicity were assessed using VaxiJen (Irini et al., 2007), AllerTOP (Bangov et al., 2024) v2.0, and ToxinPred (Gupta et al.). Secondary-structure prediction, disulfide-bond analysis, and tertiary-structure modelling are performed using PSIPRED (Liam et al., 2000), DIANNA (Ferrè F, Clote P. DiANNA 2005), and I-TASSER (Zheng et al.2025; Zhou et al., 2022; Zheng et al., 2022). Only immunologically safe, antigenic, and structurally feasible regions are retained for downstream epitope prediction.

2.3 Epitopes' Prediction

Linear B-cell epitopes are predicted using ABCpred (Saha et al., 2006; Saha et al., 2007) while cytotoxic (MHC-I) and helper T-cell (MHC-II) epitopes are identified using IEDB)-based prediction tools (Vita et al., 2024) for both HIV-1 and HIV-2. Thus, high-confidence epitopes filtered through Phase-1 and Phase-2 analyses are retained for Multi-epitope vaccine construction…

2.3.1 Multi-epitope Filtering and Validation

Epitope selection is done using 2-phase screening analysis protocols.

Phase-1 screening includes physicochemical suitability, structural feasibility, antigenicity, non-allergenicity, non-toxicity, and solubility, whereas Phase-2 screening evaluates population coverage and self-antigenicity.

Multi-epitope Vaccine Construction

Validated B-cell and T-cell epitopes from HIV-1 and HIV-2 are assembled into a single Multi-epitope vaccine. Linkers appropriate for MHC-I, such as 'AAY'; MHC-II, such as 'GPGPG'; solubility of B-cell epitopes, such as 'KK' along with 'EAAAK'; Flic-adjuvant PADRE, and His6 tag are used. The primary objective of this vaccine design is to maximise immune recognition while preserving structural stability.

2.5 Post-Construction Analyses and Validation of the Vaccine Construct

Following construction, the complete vaccine sequence is re-evaluated for antigenicity, allergenicity, and toxicity to confirm that epitope assembly has not compromised immunological safety. The tertiary structure of the construct is then predicted, refined using GalaxyRefine (Heo et al., 2013) to optimise stereochemical quality, and subsequently validated using PROCHECK (Laskowski et al., 1993; Laskowski et al., 1996).

2.6 In Silico Immune Simulations

The immunogenic potential of the vaccine construct is evaluated using an agent-based immune simulation framework, namely C-immisim (Stolfi et al., 2022; Rapin et al., 2010; Laubenbacher et al.). The simulation has assessed primary, secondary, and tertiary immune responses, antibody isotype switching, cytokine production, and the generation of memory B- and T-cells. These simulations provided insights into the durability and robustness of the predicted immune response.

III. RESULTS AND DISCUSSION

3.1 Preliminary Sequence Analysis of HIV Envelope Proteins

The amino acid sequences of HIV-1 gp120 and HIV-2 gp41 are subjected to extensive preliminary bioinformatics analyses to evaluate their suitability for epitope-based vaccine design. Physicochemical profiling revealed that both envelope glycoproteins possess molecular features compatible with immunogenic proteins, including acceptable molecular weight, stability indices within permissible limits, and hydropathicity values indicative of surface-exposed regions. These properties are essential for effective immune recognition and downstream vaccine formulation. The same is presented in the table below (Table1).

Parameter	HIV-1 gp120	HIV-2 gp41
Sequence length (aa)	483	584
Molecular weight (Da)	54,080.64	66,755.25
Theoretical isoelectric point (pI)	9.05	8.88
Instability index	38.86 (Stable)	37.47 (Stable)
Aliphatic index	75.03	71.63
GRAVY	−0.401	−0.407
Negatively charged residues (Asp + Glu)	42	53
Positively charged residues (Arg + Lys)	57	68
Estimated half-life (E. coli)	>10 h	>10 h
Extinction coefficient (M^{-1} cm^{-1})	72,515	118,270

Table 1: Physicochemical properties of HIV envelope proteins predicted using ProtParam

Secondary-structure prediction has demonstrated a balanced distribution of α-helices, β-sheets, and random coils. Regions enriched in coils and loops were of particular interest, as such flexible segments are more likely to be solvent-accessible and immunologically active. Disulfide bond prediction further indicates the presence of stabilising cysteine bridges, particularly within gp120, consistent with its known structural complexity and functional role in viral attachment.

Solvent accessibility and structural feature prediction highlighted multiple surface-exposed segments across both proteins. These regions are prioritised for epitope mapping, as surface accessibility is a critical determinant of antigen–antibody and antigen–T-cell receptor interactions.

3.2 Antigenicity, Allergenicity, Toxicity, and Solubility Assessment

Antigenicity analysis using alignment-independent prediction approaches confirmed that both gp120 and gp41 exhibit strong antigenic potential, surpassing the defined threshold values. This supports their relevance as immunological targets in HIV vaccine development.

Allergenicity screening has demonstrated that the majority of predicted antigenic regions are non-allergenic, reducing the risk of adverse hypersensitivity reactions. Complementary toxicity analysis further confirmed that the shortlisted peptide regions are non-toxic, thereby enhancing the safety profile of the proposed vaccine components.

Solubility prediction indicated that the selected regions possess favourable solubility characteristics upon recombinant expression, after vaccine construction, an important factor for large-scale production and experimental validation.

3.3 B-Cell Epitope Prediction and Analysis

Linear B-cell epitope prediction using multiple complementary tools identifies several peptide regions with high probability of eliciting humoral immune responses. These epitopes are primarily located in surface-exposed, flexible regions of the envelope proteins, consistent with known antibody-binding sites in HIV.

Only epitopes that are simultaneously antigenic, non-allergenic, non-toxic, and structurally feasible are retained. This stringent filtering ensured that the selected B-cell epitopes possessed both immunogenic potential and safety, thereby increasing the likelihood of effective antibody-mediated neutralisation.

3.4 T-Cell Epitope Prediction (MHC Class I and II)

T-cell epitope prediction revealed multiple high-affinity binders for both MHC class I and MHC class II alleles. These epitopes are essential for inducing cytotoxic T lymphocyte responses and for activating helper T cells, respectively.

Epitopes exhibiting low predicted IC_{50} values and favorable percentile ranks were prioritized, as these parameters correlate with strong HLA binding and effective antigen presentation. The inclusion of both MHC I and MHC II epitopes ensures the activation of cellular immunity alongside humoral responses.

3.5 Phase-1 Epitope Screening Results

Phase 1 screening applies multiple filters encompassing physicochemical suitability, antigenicity, allergenicity, toxicity, structural stability, surface accessibility, and solubility (Tables 2 & 3). A significant proportion of initially predicted epitopes are excluded during this phase, highlighting the importance of multi-parameter screening.

The retained epitopes demonstrated a favourable balance between immunogenicity and safety. This rigorous filtering minimises the inclusion of unstable or potentially harmful peptide regions and lays a robust foundation for advanced immunological analyses.

3.5.1 Phase 1: Screening Results :

Epitope class	Epitope sequence	Start–End	Length	Allele	Binding score	% Rank	VaxiJen (≥0.4)	Allergenicity	Toxicity	Solubility (Innovagen)
B-cell	SDTITLPCRIKQIINM	383	15	–	ABCpred **0.89 (Rank 5)**	–	0.4941	Non-allergen	Non-toxin	**Poor**
B-cell	LFCASDAKAYDTEVHN	24	16	–	ABCpred **0.89 (Rank 5)**	–	0.5621	Non-allergen	Non-toxin	**Good**
B-cell	RGKVQKEYAFFYKLDI	138	16	–	ABCpred **0.89 (Rank 5)**	–	0.5009	Non-allergen	Non-toxin	**Good**
B-cell	KTIIVQLNTSVEINCT	254	16	–	ABCpred **0.88 (Rank 6)**	–	0.6936	Non-allergen	Non-toxin	**Poor**
CTL (MHC-I)	AYDTEVHNV	32–40	9	HLA-A*02:01	**0.089673**	1.5	0.4509	Non-allergen	Non-toxin	**Good**
CTL (MHC-I)	KVSFEPIPI	19–27	9	HLA-A*02:01	**0.066511**	1.8	2.4609	Non-allergen	Non-toxin	**Good**
CTL (MHC-I)	TLPCRIKQI	67–75	9	HLA-A*02:01	**0.051223**	2.1	1.1073	Non-allergen	Non-toxin	**Good**
CTL (MHC-I)	VLVNVTENF	57–65	9	HLA-A*02:01	**0.046869**	2.2	0.4762	Non-allergen	Non-toxin	**Poor**
CTL (MHC-I)	KEYAFFYKL	63–71	9	HLA-A*02:01	**0.025420**	3.1	0.6017	Non-allergen	Non-toxin	**Good**
CTL (MHC-I)	YCAPAGFAI	29–37	9	HLA-A*02:01	**0.017224**	3.8	1.3004	Non-allergen	Non-toxin	**Good**

HTL (MHC-II)	VKIEPLGVAPTKAKR	61–75	15	HLA-DRB1*01:01	**0.289296**	6.4	1.9737	Non-allergen	Non-toxin	**Good**
HTL (MHC-II)	IKNCSFNISTSIRGK	46–60	15	HLA-DRB1*01:01	**0.181597**	9.4	0.6515	Non-allergen	Non-toxin	**Good**
HTL (MHC-II)	APAGFAILKCNNKTF	31–45	15	HLA-DRB1*01:01	**0.153296**	11.0	0.6107	Non-allergen	Non-toxin	**Poor**

Table2. Shortlisted B-cell, CTL (MHC-I), and HTL (MHC-II) epitopes from HIV-1 gp120 after Phase-I screening

B-cell epitopes are predicted using ABCpred and ranked by prediction score. CTL and HTL epitopes are predicted using IEDB NetMHCpan and NetMHCIIpan tools, respectively, and evaluated based on binding scores and percentile ranks. All epitopes are further screened for antigenicity, allergenicity, toxicity, and solubility prior to inclusion in the multi-epitope vaccine construct.

Epitope class	Epitope sequence	Start–End	Length	Allele	Binding score	% Rank	VaxiJen (≥0.4)	Allergenicity	Toxicity	Solubility (Innovagen)
B-cell	QRRYSSTPVRNKRGVF	498	17	–	ABCpred 0.87 (Rank 6)	–	1.0192	Non-allergen	Non-toxin	Good
B-cell	NWVEDKNQTRRNYCHI	404	16	–	ABCpred 0.84 (Rank 8)	–	0.4869	Non-allergen	Non-toxin	Good
B-cell	LRCNDTNYSGFEPKCT	235	16	–	ABCpred 0.83 (Rank 9)	–	1.4104	Non-allergen	Non-toxin	Good
B-cell	TKPGAGSDPEVAFMWT	373	16	–	ABCpred 0.82 (Rank 10)	–	0.7149	Non-allergen	Non-toxin	Good
B-cell	GKNVYLPPREGELACE	430	17	–	ABCpred 0.80 (Rank 12)	–	1.4204	Non-allergen	Non-toxin	Good
CTL (MHC-I)	ILLTSACLV	12–20	9	HLA-A*02:01	0.279359	0.58	0.6552	Non-allergen	Non-toxin	Moderate
CTL (MHC-I)	FVLGFLGFL	35–43	9	HLA-A*02:01	0.204596	0.77	0.8634	Non-allergen	Non-toxin	Moderate
CTL (MHC-I)	AMGARSLTL	49–57	9	HLA-A*02:01	0.183575	0.87	1.2761	Non-allergen	Non-toxin	Moderate
CTL (MHC-I)	TLSAQSRTL	56–64	9	HLA-A*02:01	0.160337	0.98	1.0394	Non-allergen	Non-toxin	Moderate
HTL (MHC-II)	VTVFYFGIPAWKNASI	26–40	15	HLA-DRB1*01:01	0.635107	2.2	0.5821	Non-allergen	Non-toxin	Moderate
HTL (MHC-II)	QTSTWFGFNGTRAEN	26–40	15	HLA-DRB1*01:01	0.415458	4.2	1.2189	Non-allergen	Non-toxin	Moderate
HTL (MHC-II)	TNITFSAEVAELYRL	66–80	15	HLA-DRB1*01:01	0.255924	7.2	0.7621	Non-allergen	Non-toxin	Moderate

HTL (MHC-II)	ELGDYKLIEITPIGF	1–15	15	HLA-DRB1*01:01	0.236039	7.7	1.8728	Non-allergen	Non-toxin	Good
HTL (MHC-II)	AASIINETSNCIENN	61–75	15	HLA-DRB1*01:01	0.216373	8.2	0.4132	Non-allergen	Non-toxin	Good

Table 3. Shortlisted B-cell, CTL (MHC-I), and HTL (MHC-II) epitopes from HIV-2 after Phase-I screening
B-cell epitopes were predicted using ABCpred and ranked by prediction score. CTL and HTL epitopes were predicted using IEDB NetMHCpan and NetMHCIIpan tools, respectively, and evaluated based on binding scores and percentile ranks. All epitopes were further screened for antigenicity, allergenicity, toxicity, and solubility prior to inclusion in the multi-epitope vaccine construct.

3.5.2.1 Phase 1: Secondary Structure Prediction – HIV1

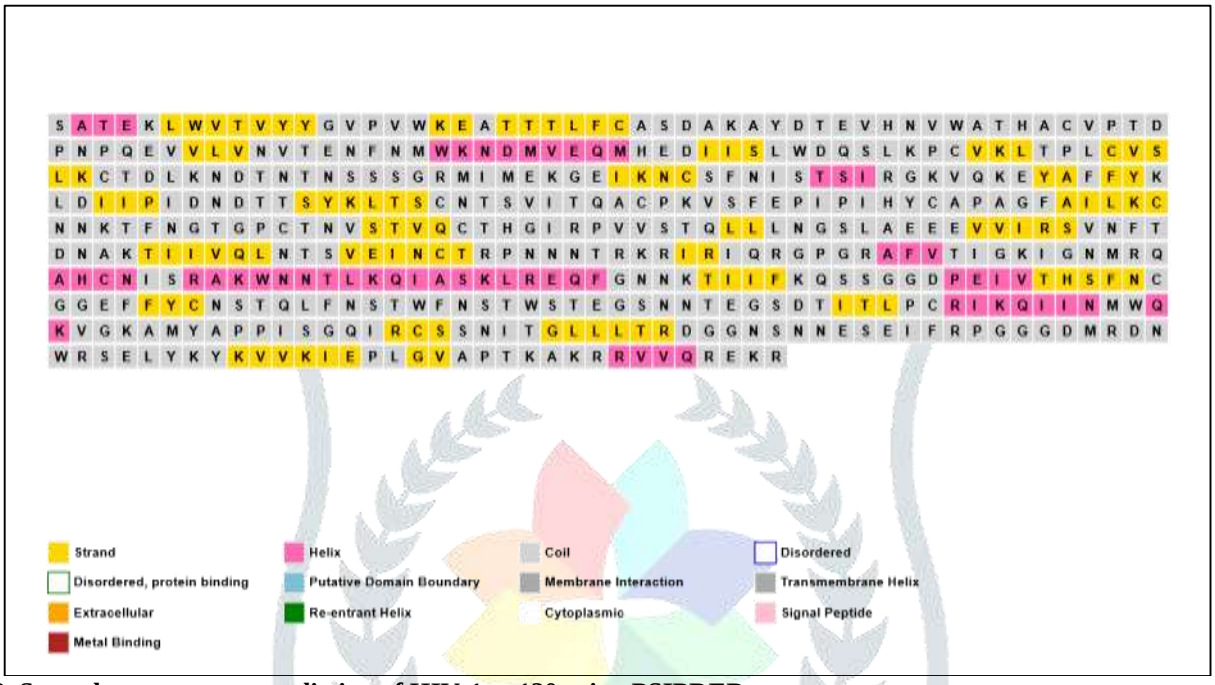


Figure 3. Secondary structure prediction of HIV-1 gp120 using PSIPRED.
The predicted secondary structure shows a heterogeneous distribution of α-helices, β-strands, and coil regions along the gp120 sequence. A substantial proportion of residues are located within flexible coil and loop regions, which are commonly associated with enhanced surface accessibility. Importantly, the majority of shortlisted B-cell and T-cell epitopes overlapped with these coil-dominated regions, supporting their structural feasibility and potential for immune recognition. Overall, this analysis supports the suitability of gp120 as a source protein for epitope-based vaccine design.

3.5.2.2 Phase 1: Secondary Structure Prediction – HIV2

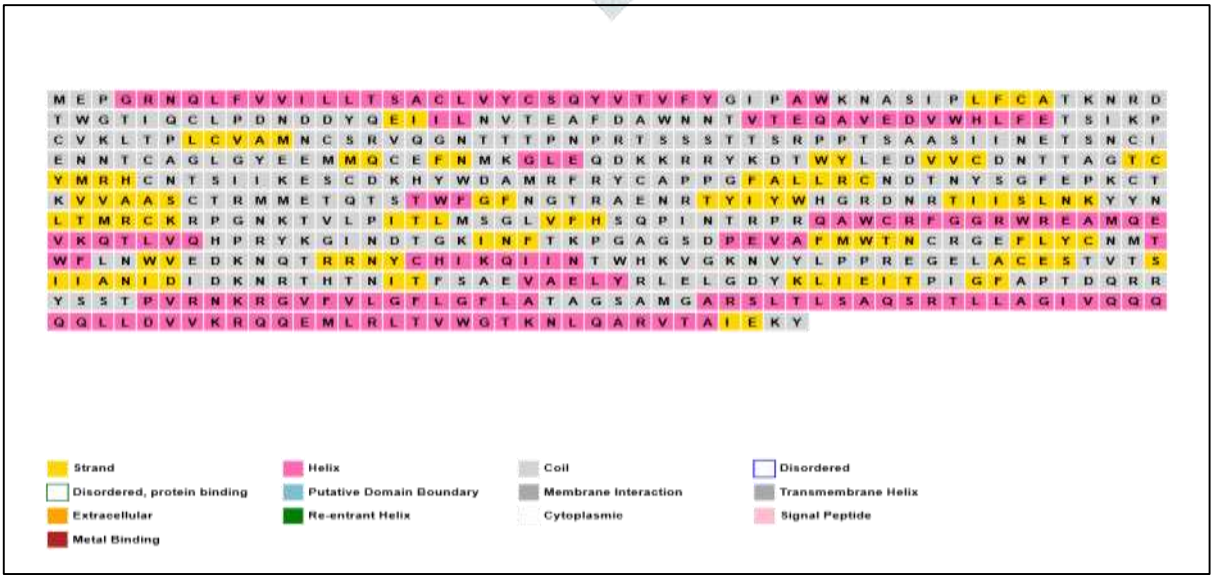


Figure 5. Secondary structure prediction of HIV-2 gp41 using PSIPRED

The PSIPRED-based secondary structure prediction of HIV-2 gp41 revealed a mixed distribution of α-helices, β-strands, and coil regions across the protein sequence. A notable proportion of residues are predicted to adopt coil or loop conformations, which are generally associated with enhanced flexibility and surface exposure. Importantly, the majority of shortlisted B-cell and T-cell epitopes are found to coincide with these flexible regions, supporting their potential accessibility for immune recognition. Overall, the PSIPRED analysis provides structural support for selecting gp41-derived epitopes in the multi-epitope vaccine construct.

3.5.3 Phase 2: Population Coverage Analysis

Population coverage analysis has demonstrated that the selected T-cell epitopes collectively span a broad range of HLA alleles across diverse geographic populations. The combined coverage is substantial, indicating that the designed vaccine has the potential to elicit immune responses in a large proportion of the global population. Figures 6 &7, along with Tables 4 & 5 on Population Coverage, are given below.

3.5.3.1 World Population Coverage for MHC1 & MHC2 OF HIV 1 (GP120)

Figure 6: Global population coverage of HIV-1 gp120–derived epitopes. (A) Cumulative population coverage of selected MHC class I epitopes. (B) Cumulative population coverage of selected MHC class II epitopes.

Population coverage analyses have demonstrated broad global representation of HLA for HIV-1 gp120–derived epitopes. The selected MHC class I epitopes achiev a global population coverage of 90.14%, while MHC class II epitopes showed a comparable coverage of 90.28%. The observed average epitope–HLA hits and PC90 values indicate robust and diverse immune recognition, supporting the suitability of these epitopes for inclusion in a multi-epitope vaccine construct.

Comparative global population coverage of HIV-1 gp120–derived MHC class I and II epitopes

Virus	MHC class	Population coverage (%)	Average epitope–HLA hits	PC90
HIV-1	Class I	**90.14**	9.51	6.02
HIV-1	Class II	**90.28**	3.51	2.02

Table 4. Global population coverage of HIV-1 –derived MHC class I and II epitopes

Population coverage is calculated using the IEDB population coverage tool based on selected epitope–HLA allele combinations.

3.5.3.2 World Population Coverage for MHC1 & MHC2 OF HIV 2 (GP41)

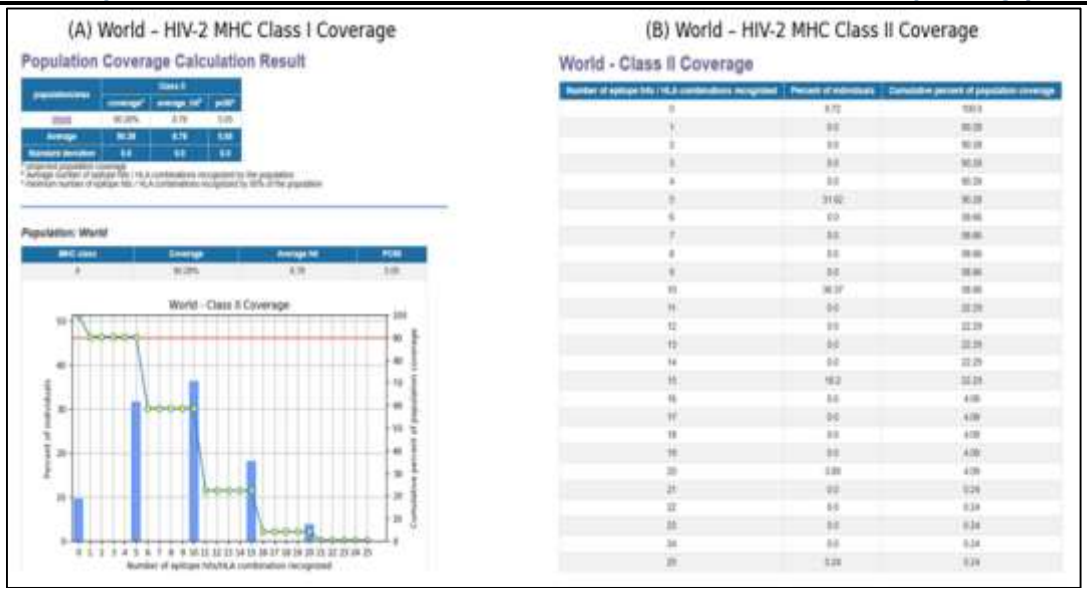


Figure 7. Global population coverage analysis of selected HIV-2–derived epitopes. (A) Cumulative population coverage of MHC class I epitopes. (B) Cumulative population coverage of MHC class II epitopes. Both epitope sets exhibited broad worldwide HLA representation

Population coverage analysis of HIV-2–derived epitopes demonstrated extensive global HLA representation. The selected MHC class I epitopes achieved a global population coverage of 90.14%, while MHC class II epitopes showed a comparable coverage of 90.28%. The observed average epitope–HLA hits and PC90 values indicate robust and redundant immune recognition across diverse populations.

Virus	MHC class	Population coverage (%)	Average epitope–HLA hits	PC90
HIV-2	Class I	**90.14**	7.92	5.02
HIV-2	Class II	**90.28**	8.78	5.05

Table 5. Global population coverage of HIV-2–derived MHC class I and II epitopes

Population coverage, average hits, and PC90 values were calculated using the IEDB population coverage tool based on reported HLA allele frequency distributions.

3.6 Multi-Epitope Vaccine Construction and Validation

Based on the results of Phase 1 and Phase 2 screening, a multi-epitope vaccine construct is assembled using carefully selected B-cell and T-cell epitopes derived from HIV-1 gp120 and HIV-2 gp41. Appropriate linkers are employed to preserve individual epitope immunogenicity and to facilitate efficient antigen processing and presentation. A brief view of the vaccine construct is given below (Figure 8)

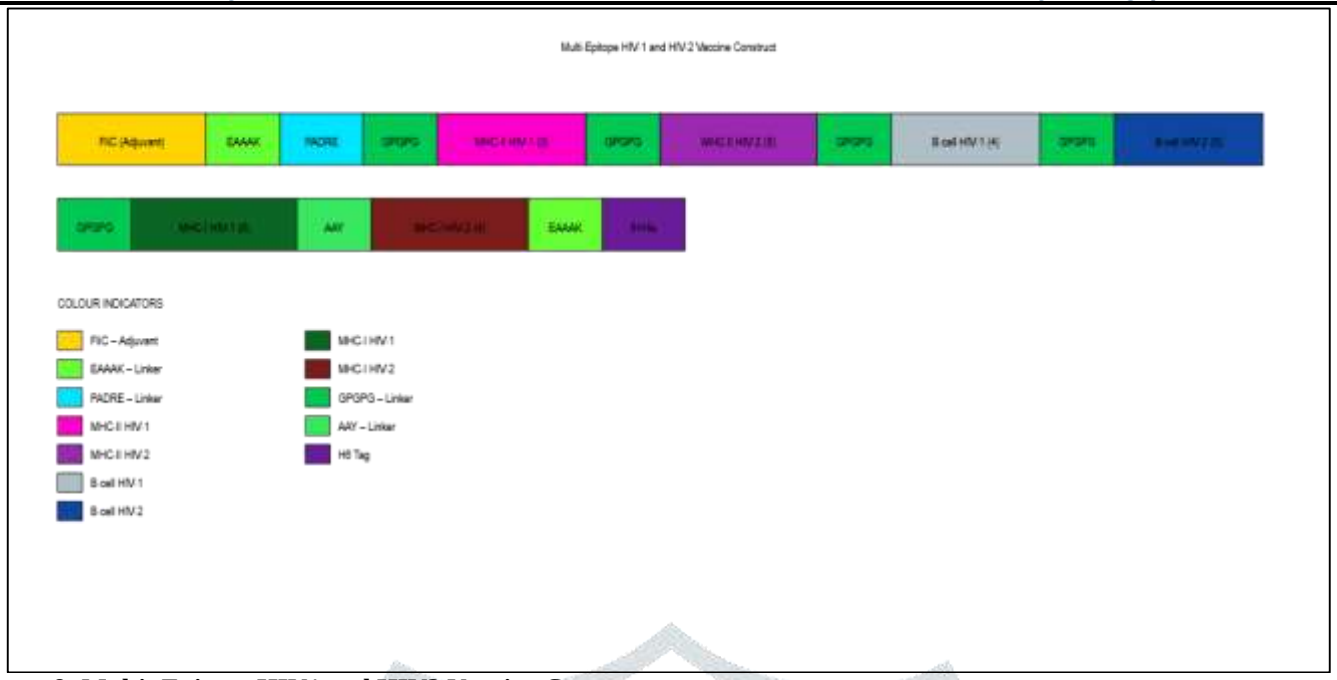


Figure 8: Multi- Epitope HIV1 and HIV2 Vaccine Construct

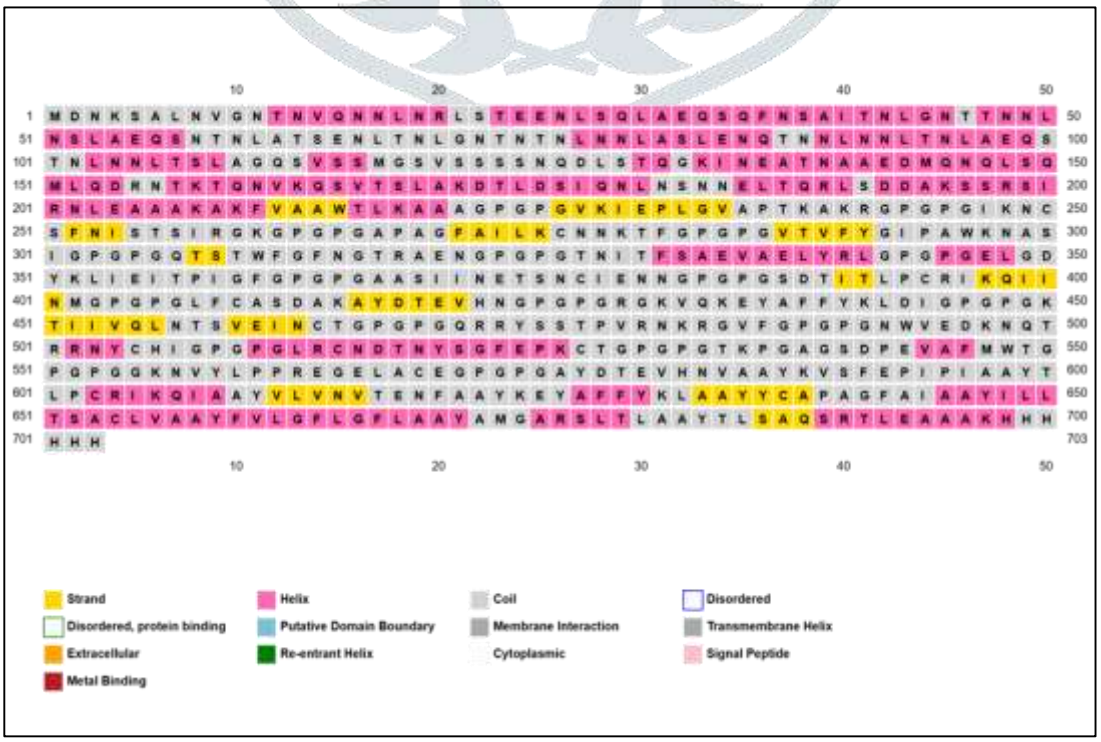
A multi-epitope vaccine construct targeting HIV-1 and HIV-2 is rationally designed by integrating experimentally and computationally validated CTL (MHC-I), HTL (MHC-II), and B-cell epitopes. To enhance immunogenicity, flagellin (FliC) is incorporated at the N-terminus as an adjuvant, followed by the PADRE sequence to ensure broad coverage of the HLA-DR population. Appropriate linkers are strategically employed to preserve epitope integrity and facilitate efficient antigen processing: EAAAK linkers are used to separate major functional domains, GPGPG linkers are used between HTL and B-cell epitopes, and AAY linkers are used between CTL epitopes. A C-terminal 6×His tag was added to facilitate purification and downstream experimental validation. The rational organisation of epitopes and linkers is intended to maximise immune response while minimising junctional immunogenicity.

3.7 Post -Vaccine Construct Analyses

The three-dimensional structure of the designed multi-epitope vaccine construct is predicted using the I-TASSER server. Among the generated models, the top-ranked model with the highest confidence score is selected for further processing. To improve structural quality, the selected model is refined using GalaxyRefine, which optimises side-chain conformations and overall geometry. The refined structure is subsequently validated using PROCHECK to assess stereochemical quality through Ramachandran plot analysis.

3.7.1 Secondary Structure Prediction

Secondary-structure prediction reveals a predominance of random coils interspersed with α-helical regions, indicating structural flexibility that is favourable for epitope exposure and immune recognition. The same is depicted in Psipred figure 9, as below –



3.7.2 I-TASSER Model Quality

The quality of the predicted three-dimensional models generated by I-TASSER was evaluated to identify the most reliable structure for subsequent refinement and validation.

Model	C-score	Estimated TM-score	Estimated RMSD (Å)
Model 1	–2.23	0.45 ± 0.15	13.6 ± 4.0
Model 2	–2.31	Not reported	Not reported
Model 3	–3.12	Not reported	Not reported
Model 4	–3.09	Not reported	Not reported
Model 5	–3.64	Not reported	Not reported

Table 1. I-TASSER Predicted Model Quality Parameters

C-score indicates the model's confidence and typically ranges from -5 to +2, with higher values indicating greater reliability. TM-score assesses global structural similarity; values >0.5 indicate correct topology. RMSD represents the average deviation between predicted and native structures. TM-score and RMSD are provided by I-TASSER only for the top-ranked model.

3.7.3 Tertiary structure prediction and model selection (I-TASSER)

The tertiary structure of the vaccine construct is predicted using the I-TASSER server, which generated multiple structural models. Among these, the top-ranked model is selected based on the highest confidence score, indicating greater reliability and structural convergence than the remaining models. Model1 from I-Tasser is depicted in figure 10a &b, below.

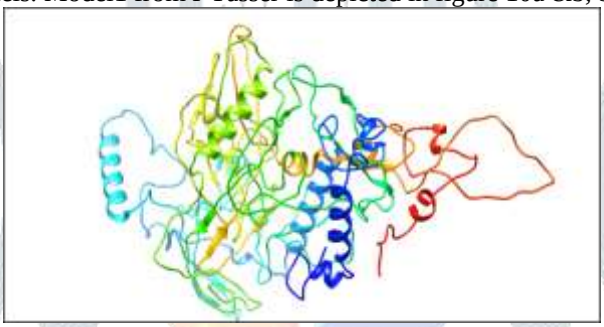


Figure 10a. Rainbow-colored cartoon representation of the I-TASSER-predicted tertiary structure (Model 1) of the vaccine construct. The structure is colored from the N-terminus (blue) to the C-terminus (red), highlighting the overall fold and secondary structure organization.

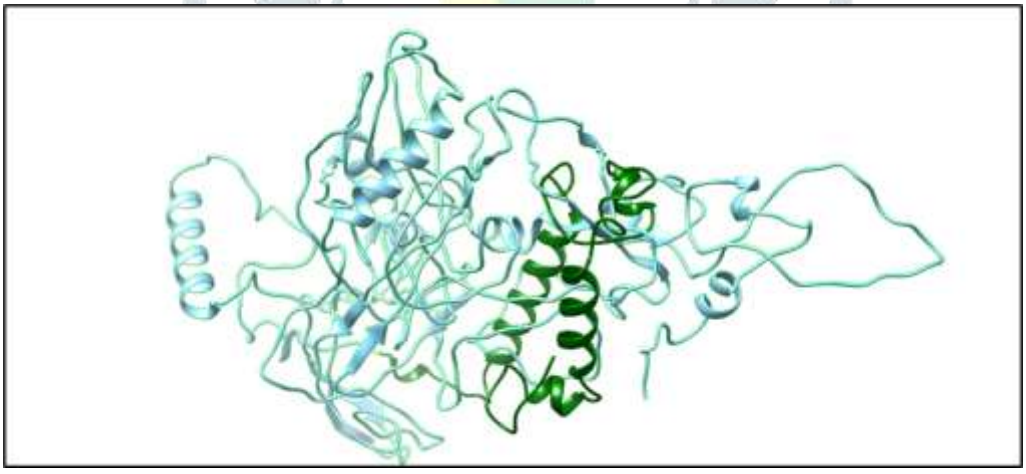


Figure 10b. Cartoon representation of the I-TASSER-predicted vaccine construct (Model 1) showing the spatial distribution of the adjuvant and epitope regions. The adjuvant region is highlighted in a distinct green colour, while the epitope-rich regions are shown in a contrasting sky-blue colour, illustrating their relative positioning within the overall structure.

3.7.4 Global structural reliability assessment

The selected I-TASSER model exhibited an estimated TM-score of 0.45 ± 0.15, indicating a reasonable global fold and correct overall topology of the vaccine construct. The corresponding estimated RMSD value of 13.6 ± 4.0 Å reflects acceptable structural deviation, which is expected for a designed multi-epitope protein comprising flexible linkers and heterogeneous sequence segments. Together, these metrics support the structural reliability of the predicted model prior to refinement.

3.7.5 Solvent accessibility analysis

Solvent accessibility analysis indicates that a considerable proportion of residues in the vaccine construct are predicted to be surface-exposed. This exposure is advantageous for immune recognition, as accessible regions are more likely to interact with host immune receptors and antibodies, thereby supporting the immunogenic potential of the designed vaccine.

3.7.6 Structural flexibility and Stability Analysis (B-factor)

The normalised B-factor analysis reveals moderate flexibility across the vaccine construct, with no extreme fluctuations, indicating an overall stable structure. Such balanced flexibility is desirable for multi-epitope vaccines, as it supports structural stability while maintaining epitope accessibility.

3.7.7 Structure Refinement & Validation

The selected I-TASSER model is refined using GalaxyRefine to improve stereochemical quality prior to validation.
Ramachandran plot analysis of the refined vaccine construct reveals that 77.5% of residues are located in the most favoured regions, 17.0% in the additionally allowed regions, and only 3.5% in the disallowed regions. This distribution indicates acceptable stereochemical quality for a designed multi-epitope vaccine construct containing flexible linker regions.

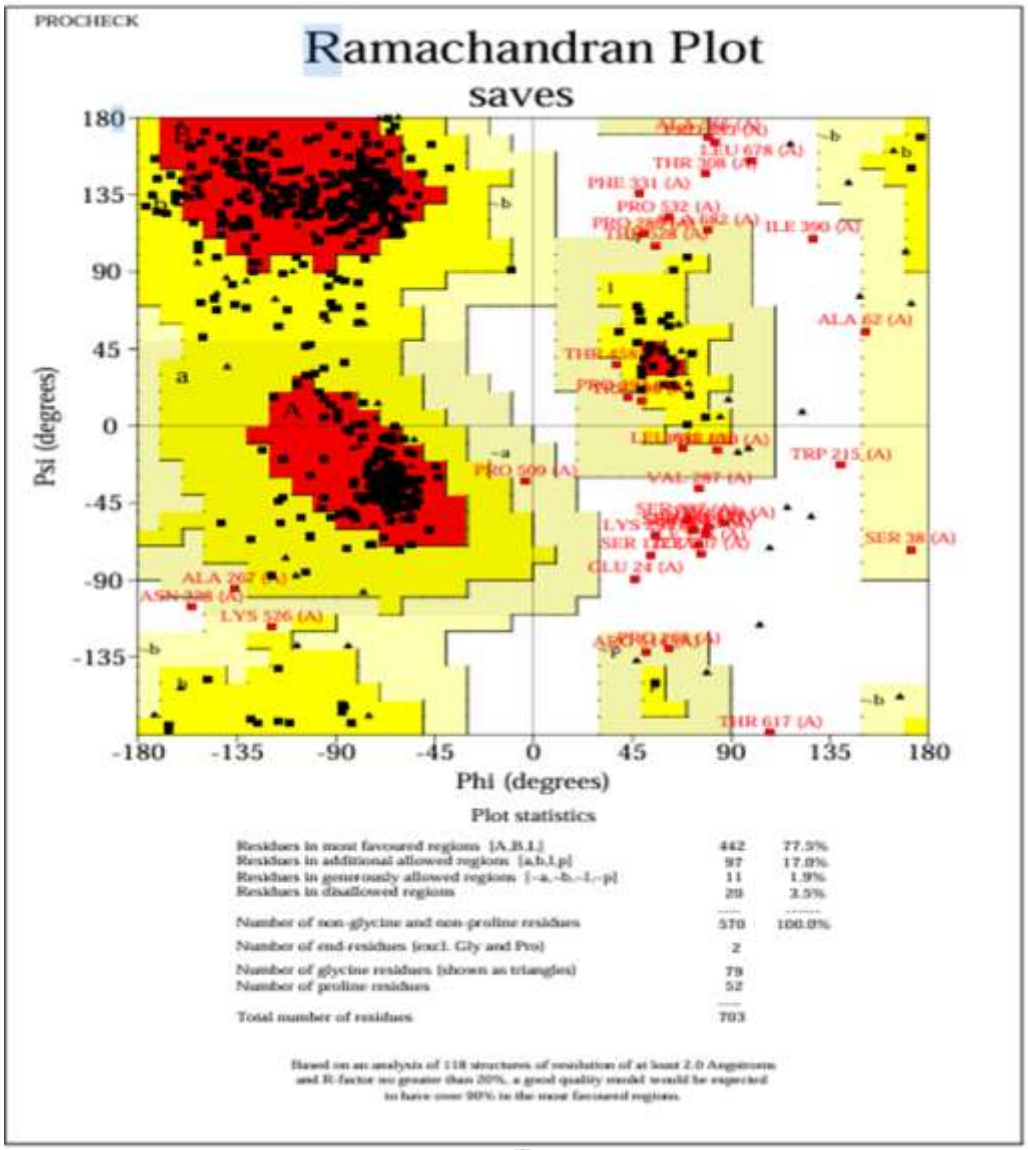


Figure11. Ramachandran plot of the GalaxyRefine-refined vaccine construct generated using PROCHECK, showing the distribution of residues in favoured, allowed, and disallowed regions.

The refined three-dimensional structure of the multi-epitope vaccine construct demonstrates acceptable stereochemical quality as assessed by PROCHECK. Although the proportion of residues in the most favoured regions (77.5%) is lower than that typically expected for compact globular proteins, this observation is consistent with the chimeric nature of the designed vaccine, which incorporates multiple epitopes connected by flexible linker sequences. Such linker-rich architectures inherently introduce conformational flexibility, leading to minor deviations in backbone dihedral angles. Importantly, only a small fraction of residues (3.5%) is located in disallowed regions, indicating the absence of major steric conflicts and supporting the overall structural reliability of the refined model. Similar stereochemical distributions have been reported for other in silico–designed multi-epitope vaccine constructs, where functional flexibility rather than rigid folding is required for effective immune recognition.

3.8 Immune simulation analysis (C-ImmSim)

The immune response induced by the vaccine construct is evaluated using the C-ImmSim server. The simulation demonstrates a rapid primary immune response followed by a pronounced secondary response characterised by elevated immunoglobulin levels. A strong IgM response is observed during the initial phase, followed by sustained IgG production, indicating effective class switching and immunological memory formation. Concurrently, antigen levels decline markedly, suggesting efficient immune-mediated antigen clearance.
In addition, the simulation reveals robust activation of T-cell populations, accompanied by increased cytokine production. Notably, elevated levels of IFN-γ and IL-2 are observed, indicating induction of a Th1-biased immune response and supporting the vaccine construct's potential to elicit effective cellular immunity. Figures 12a & 12 b illustrate the concept pictorially.

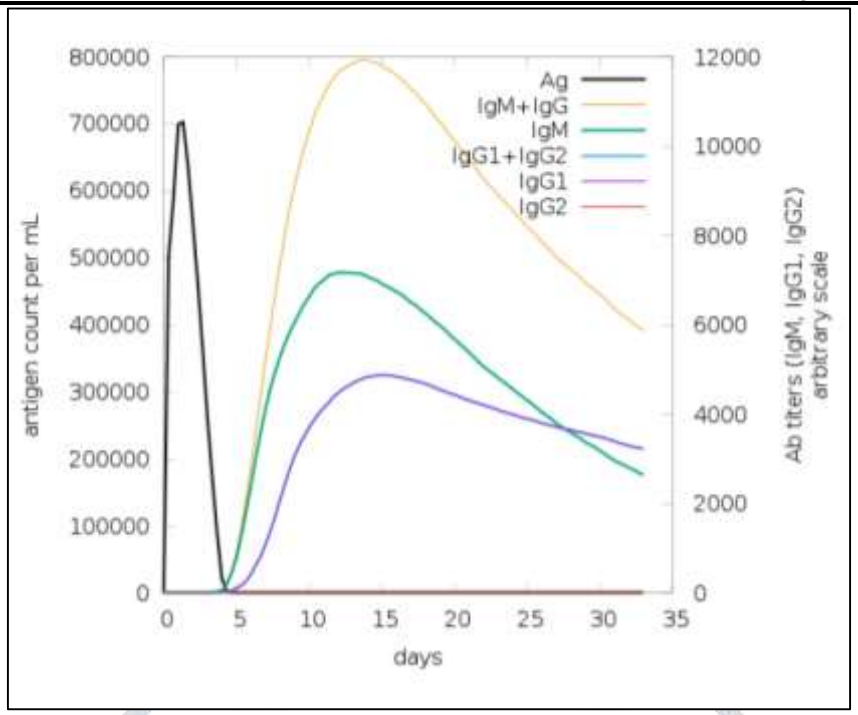


Figure 12 a. Antibody and antigen dynamics

In silico immune simulation of the vaccine construct using C-ImmSim showing antigen clearance and antibody responses (IgM and IgG subclasses) over the simulation period.

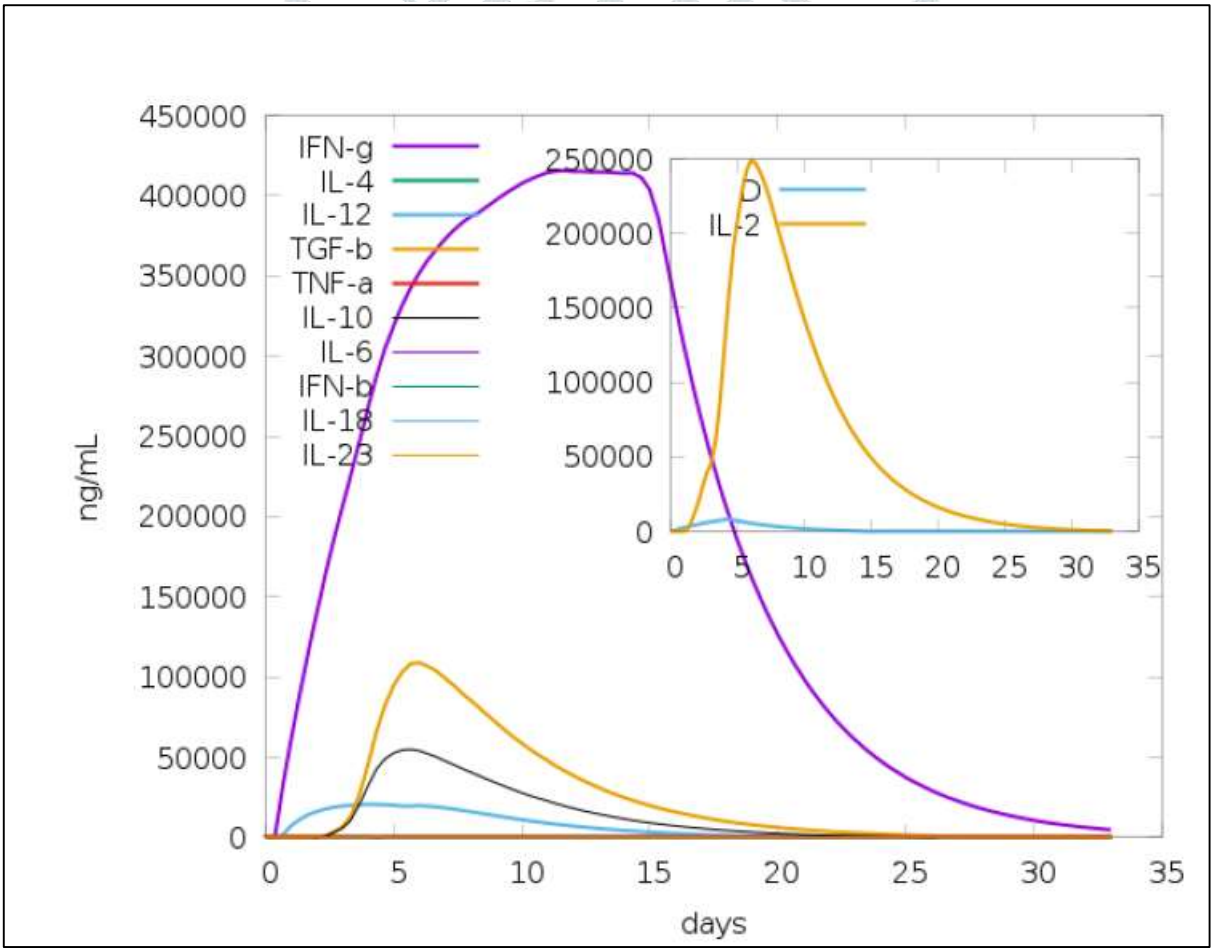


Figure 12b. Cytokine and interleukin profile

Cytokine and interleukin response profile generated by C-ImmSim, illustrating increased levels of IFN-γ and IL-2 following vaccination, indicative of a Th1-type immune response.

The C-ImmSim simulation indicates that the designed vaccine construct can elicit both humoral and cellular immune responses. The observed switch from IgM to IgG dominance, together with the persistence of antibody titres, indicates the establishment of immunological memory. Furthermore, the increased production of IFN-γ and IL-2 supports the activation of cell-mediated immunity, which is critical for effective antiviral defence. Collectively, these findings highlight the immunogenic potential of the proposed multi-epitope vaccine and support its further experimental evaluation.

IV CONCLUSION

In the present study, an immunoinformatics-based approach was employed to design and evaluate a multi-epitope vaccine construct targeting the envelope glycoproteins of HIV-1 and HIV-2. The rational selection and integration of B-cell, cytotoxic T lymphocyte, and helper T lymphocyte epitopes resulted in a construct with favourable antigenic and safety profiles. Structural modelling, refinement, and validation demonstrated acceptable stereochemical quality and structural stability, supporting the feasibility of the designed vaccine at the molecular level. Furthermore, in silico immune simulation predicted robust humoral and cellular immune responses, characterised by immunoglobulin class switching, cytokine induction, and immune memory formation. Collectively, these findings highlight the immunogenic potential of the proposed vaccine construct and provide a computational framework for its further experimental evaluation and development.

V Conflict of Interest

The author declares no conflict of interest

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