

Immunoinformatics-Guided Design and Molecular Dynamics Evaluation of a Multi-Epitope Vaccine Targeting High-Risk HPV-16 and HPV-18

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Author Details	Abstract
Keywords: HPV-16; HPV-18; E5; E7; Multi-Epitope Vaccine; Immunoinformatics; Molecular Docking	HPV types 16 and 18 are oncogenic genotypes responsible for a substantial proportion of cervical cancer cases. Although prophylactic HPV vaccines are available, they do not eliminate established infections. In this study, an immunoinformatics approach was used to design a multi-epitope vaccine derived from the E5 and E7 proteins of HPV-16 and HPV-18. CTL, HTL, and B-cell epitopes were selected according to predicted antigenicity, non-allergenicity, non-toxicity, HLA-binding potential, and population coverage. The selected epitopes were assembled with suitable linkers and an adjuvant to generate 405-amino-acid vaccine construct. The construct had a predicted molecular weight of 45.49 kDa, theoretical pI of 9.37, instability index of 29.00,
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Corresponding E-mail & Author*: Sadia Zahid Department of Biotechnology, Faculty of Life Sciences, University of Okara, Okara 56130 Pakistan Email: sadiazahid@uo.edu.pk	theoretical pI of 9.37, instability index of 29.00,

aliphatic index of 71.65, and GRAVY score of -0.360 . It was predicted to be antigenic and non-allergenic, while combined MHC class I and II epitope analysis indicated greater than 90% global population coverage. The three-dimensional structure was subsequently predicted, refined, and evaluated for structural quality. Molecular docking was used to examine interactions between the vaccine construct and the selected immune receptor, followed by 100-ns molecular dynamics simulation in Desmond. The simulation revealed conformational adjustments, region-specific flexibility, coordinated residue motions, and changes in structural compactness over the trajectory. Overall, the findings provide a computational basis for further

experimental evaluation of HPV-16/18 E5/E7-derived multi-epitope vaccine. Experimental studies are required to confirm its expression, safety, immunogenicity, and potential therapeutic relevance.

Introduction

HPV is small non-enveloped virus with double-stranded circular DNA genome belonging to Papillomaviridae family. The virus comprises more than 200 types of HPV, classified into low and high risk. High risk types of HPV are considered to be primary cause of cervical cancer as well as other types of cancers, including anal and oropharyngeal cancers (Van Den Borst et al., 2025). Although the burden of HPV-related cancers is high, it is even higher in low and middle-income countries where vaccine and screening coverage for cervical cancer prevention is low (Hamid et al., 2025).

Vaccination with prophylactic vaccines, such as Gardasil or Cervarix, prevents the majority of cases of human papillomavirus (HPV) infections by eliciting strong neutralizing antibody responses in immunized individuals using virus-like particles that mimic the HPV virus outer capsid structure made from the virus L1 protein (Balhara et al., 2024). Importantly, however, these vaccines do not remove established HPV infections from an individual and also do not treat HPV-induced precancerous and cancerous lesions that have already developed. Rather, their primary value is prevention of new HPV infections. Therefore, much interest has focused on development of therapeutic vaccines to treat established HPV infections by eliciting strong cell-mediated immune responses against expressed HPV viral and oncogenic antigens in infected and transformed cancer cells. Recent evidence suggests that such vaccine may even be able to treat certain precancerous stages of HPV-associated cancers; more clinical studies are required, however, to test its efficacy (Gonçalves et al., 2024).

HPV early proteins, such as E5 and E7, of high-risk HPV are promising targets for therapeutic approaches, since expression of these proteins is crucial for establishment of viral persistence and for HPV-associated transformation of cells (Wolf et al., 2024). Moreover, viral early proteins are probably recognized by cellular immune system as foreign in cells that express high-risk HPVs. Several experimental studies proved that epitopes from early HPV proteins E5 and E7 elicit cellular immune reactions and can even be tumor-removing in animal models (Namvar et al., 2020). Recently also conserved epitopes from early HPV proteins were used in experimental investigations to induce antigen-specific cellular responses, thus opening up new perspectives for a therapeutic application as a vaccine.

Epitope-based vaccine development faces significant challenges due to extensive polymorphism of human leukocyte antigens (HLA), which are involved in antigen presentation to T cells in individuals and populations. To overcome limitations of single-epitope vaccines, multi-epitope vaccines can be designed that comprise several immunologically relevant epitopes in one single construct. In addition to CTL epitopes that recognized by MHC-I, CTL, HTL epitopes, recognized by MHC class II molecules and presented to helper T-lymphocytes (HTL) can be included in multi-epitope vaccines (Ojha et al., 2022). Furthermore, B-cell epitopes that are recognized by B cells can be included to increase immunological diversity. Some epitopes can be selected that are recognized by several different HLA alleles. Such multi-epitope vaccines have already been modeled in silico for vaccines aimed at treatment of infections caused by HPV-16 and HPV-18, including vaccines based on E5 and E7-derived epitopes (Kumar et al., 2022; Sanami et al., 2022).

Immunoinformatics solutions provide a systematic approach for identification and assessment of potential epitopes prior to experimental verification by biochemistry or wet lab techniques. Tools are available for assessment of epitope candidate (s) for potential MHC binding, antigenicity, toxicity and population

coverage. Epitopes that pass these assessments can be linked together to form multi-epitope vaccines. Structural information can be generated by structural modeling, molecular docking and MD simulations. The information generated by these approaches can significantly decrease the number of candidates that actually need to be tested in the wet lab for further validation. Despite growing interest in therapeutic HPV vaccination; however, a rational choice and evaluation of multi-epitope based vaccine candidates targeting most prevalent high-risk HPV types is needed. The strategy to develop a vaccine using E5/E7 protein subunits that includes a variety of immune components and presents a broad HLA-profile might help to select candidates for experimental verification. All of the recently computationally designed HPV multi-epitope vaccines (Kumar et al., 2022; Sanami et al., 2022) need to be individually evaluated for their immunological properties as well as for their structural properties, receptor binding and dynamics.

In the present study, we designed a multi-epitope vaccine construct using E5 and E7 proteins from HPV-16 and HPV-18. The possible CTL, HTL, and B-cell epitopes were selected and vaccine was designed to combine them into a single construct (Tomać, 2016). The designed vaccine was then evaluated for predicted population coverage, antigenicity, allergenicity, immunological properties, physicochemical characteristics and fold prediction. The interaction study of the designed vaccine with various receptors was also carried out by molecular docking. The structural stability of designed vaccine and its complex with receptors was evaluated through 100-nanosecond molecular dynamics simulation. The study was conducted by using immunoinformatics approach and designed vaccine can be further tested experimentally to evaluate its potential as therapeutic agent for treatment of infections caused by high-risk HPV-16 and HPV-18 types.

Materials and Methods

Sequence retrieval and epitope prediction

Protein sequences corresponding to E5 and E7 from HPV-16 and HPV-18 were obtained from NCBI and saved in FASTA format. Multiple sequence alignment was carried out using ClustalW prior to epitope screening. CTL and HTL epitopes were identified through Immune Epitope Database (IEDB) Analysis Resource. For selection of T-cell epitopes, MHC class I-II predictions were filtered using percentile-rank thresholds of <1 and <10, respectively. Linear B-cell epitopes were identified using IEDB platform. Further, shortlisted epitopes were further examined for predicted antigenic potential with VaxiJen v2.0, whereas allergenicity and toxicity were evaluated using ALLERDET and ToxinPred, respectively. For HTL epitopes, cytokine-inducing potential was additionally assessed by predicting IFN- γ , IL-4, and IL-10 responses using IFNepitope, IL4Pred, and IL10Pred, respectively.

Population coverage analysis

Global population coverage of shortlisted T-cell epitopes was evaluated with IEDB Population Coverage tool. Coverage was calculated separately for the MHC class I-II associated epitope sets and was also examined after combining both classes of HLA–epitope associations.

Multi-epitope vaccine construction

The final construct was assembled from selected CTL, HTL and B-cell epitopes, producing sequence of 405 amino acids. A 60S ribosomal protein was positioned at N-terminus as an adjuvant. To organize the epitope regions, AAY linkers were placed between CTL epitopes, GPGPG linkers between HTL epitopes and KK linkers between B-cell epitopes. A 6 $\times$ His tag was added at C-terminal end of construct.

Physicochemical and immunological assessment

Physicochemical profiling of completed vaccine sequence was performed with ExPASy ProtParam. The analysis included predicted molecular mass, theoretical pI, instability index, aliphatic index, GRAVY and estimated half-life. The construct was then screened for antigenic potential using VaxiJen v2.0, while possible allergenic behavior was evaluated with AllerTOP v2.0 and ALLERDET.

Structural modelling and validation

Structures were predicted using PSIPRED for secondary structure and AlphaFold for tertiary structure, which was then refined using GalaxyRefine. The resulting model was checked on ProSA-web, checked against a Ramachandran plot using MolProbity/PROCHECK and scored using ERRAT to measure overall structural quality.

Molecular docking and interaction analysis

A refined version of vaccine was then subjected to protein–protein docking with the immune receptor chosen by the user. The resulting docked complex was then analyzed using PDBSum and LigPlot+ for hydrogen bonds, salt links, hydrophobic interactions and for the interacting residues at the vaccine-receptor interface.

Molecular dynamics simulation

The selected vaccine receptor complex was subjected to a 100-ns molecular dynamics simulation in Desmond. Following system preparation, energy minimization, and equilibration, the resulting trajectory was examined for structural deviation, residue-level flexibility, and secondary-structure behavior using RMSD, RMSF, and secondary-structure analyses. Additional conformational assessment was carried out through PCA, cumulative variance analysis, DCCM analysis and radius of gyration measurements to characterize collective motions, residue correlations, flexibility and changes in overall compactness of complex.

Results

Selection of T-cell and B-cell epitopes

Immunoinformatics screening of E5 and E7 proteins of HPV-16 and HPV-18 identified eight MHC class I CTL epitopes, seven MHC class II-restricted HTL epitopes, and three B-cell epitopes for incorporation into multi-epitope vaccine construct.

The selected CTL epitopes were TAVSSTWHW, NTTPIVHLK, TLQDVSLEV, YTAVSSTWHW, NVKHKSAIV, WTLQDVSLEV, KWTLLQDVSL and TAVSSTWHWT. All eight were predicted as non-allergenic and showed antigenicity scores ranging from 1.0614 to 1.8258. KWTLLQDVSL exhibited the highest antigenicity score (1.8258), whereas NTTPIVHLK showed the lowest predicted percentile rank (0.01) among the evaluated MHC-I interactions. The selected epitopes displayed predicted binding across several HLA class I alleles, including HLA-A02:01, HLA-A24:02, HLA-A33:01, HLA-A68:01, HLA-B08:01, HLA-B53:01, HLA-B57:01 and HLA-B58:01.

Seven HTL epitopes, YWKHMRLECAIYYKA, LECAIYYKAREMGFK, AIYYKAREMGFKHIN, CAIYYKAREMGFKHI, EMGFKHINHQVVPTL, WKHM RLECAIYYKAR and MGFKHINHQVVPTLA, were retained following MHC class II screening. All were predicted as non-allergenic, with antigenicity scores ranging from 1.0480 to 1.4971. AIYYKAREMGFKHIN showed the highest antigenicity score (1.4971) and was predicted to interact with three HLA-DR alleles. The selected HTL epitopes were reported as positive IFN- γ inducers and showed predicted IL-4/IL-10-inducing potential.

Three linear B-cell epitopes, LTHYENDSTDLRDHID, AEKYSK, and FKKHCT, were also selected. Their predicted antigenicity scores were 0.6107, 0.4439, and 0.4780, respectively, and all three were classified as non-allergenic. LTHYENDSTDLRDHID displayed the highest antigenicity among the selected B-cell epitopes.

Population coverage of selected T-cell epitopes

The population coverage analysis was performed to evaluate predicted global representation of selected T-cell epitope–HLA combinations. The MHC class I-restricted epitopes demonstrated approximately 95% predicted global population coverage, indicating broad representation of the selected CTL epitope–HLA combinations.

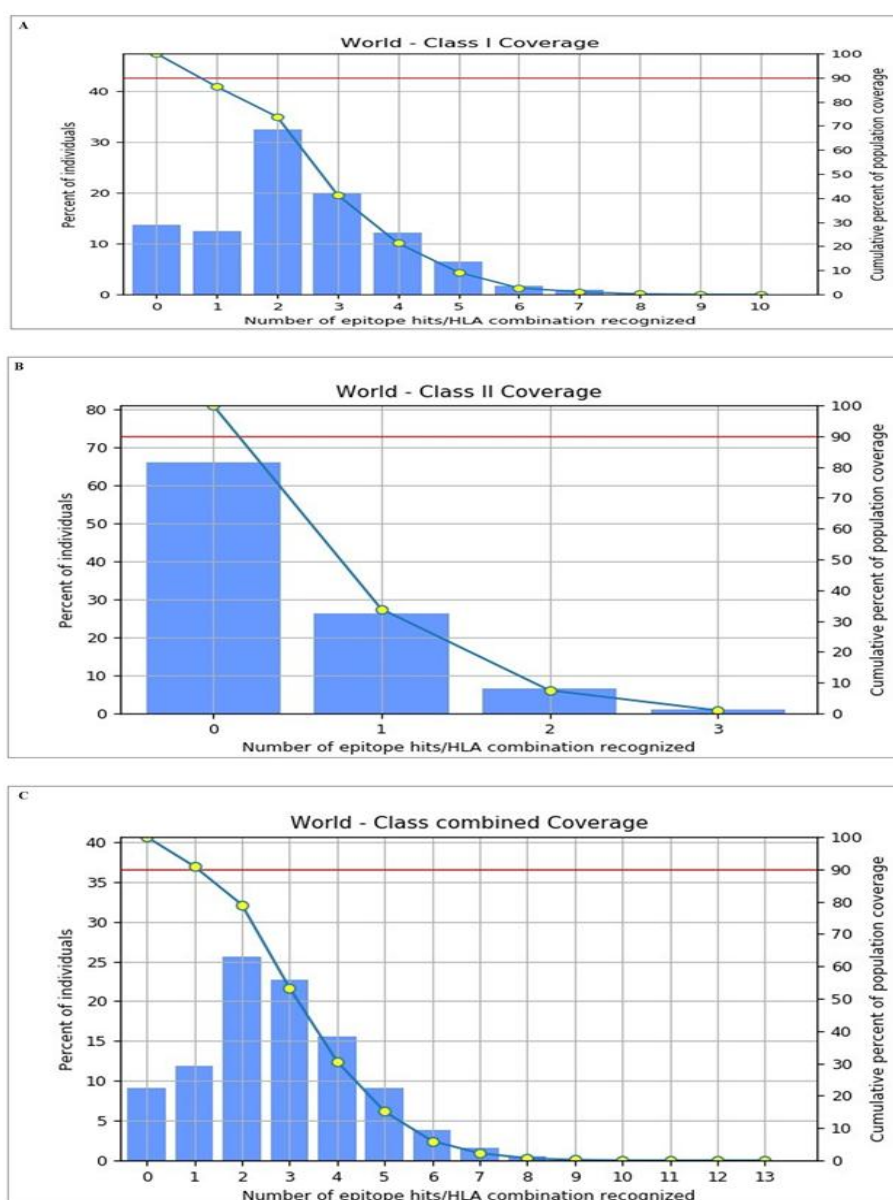


Figure 1. Global population coverage analysis of the selected T-cell epitopes from HPV-16 and HPV-18 showing (A) MHC class I-restricted epitope coverage, (B) MHC class II-restricted epitope coverage, and (C) combined MHC class I and II epitope–HLA population coverage.

In contrast, considerably lower population coverage was identified for the MHC class II-restricted epitopes. Approximately two-thirds of the population showed no recognized class II epitope–HLA combination, while approximately one-third was

represented by at least one predicted combination. Pasted text When the MHC class I-II epitopes were evaluated together, combined analysis showed greater than 90% predicted global population coverage. The combined distribution further demonstrated recognition of multiple epitope–HLA combinations across the represented population. Overall, the analysis indicated broad predicted population representation of selected T-cell epitopes, predominantly supported by the MHC class I component (Fig. 1).

Construction of the multi-epitope vaccine

The selected CTL, HTL and B-cell epitopes were assembled into a single 405-amino-acid vaccine construct targeting HPV-16 and HPV-18. A 60S ribosomal protein adjuvant was positioned at N-terminus, whereas polyhistidine (His) tag was added at C-terminal end. Epitope groups were separated using class-specific linkers, with AAY connecting CTL epitopes, GPGPG joining HTL epitopes, and KK linking B-cell epitopes (Fig. 2). This arrangement produced a modular construct containing components intended to support cytotoxic T-cell, helper T-cell, and B-cell immune responses.

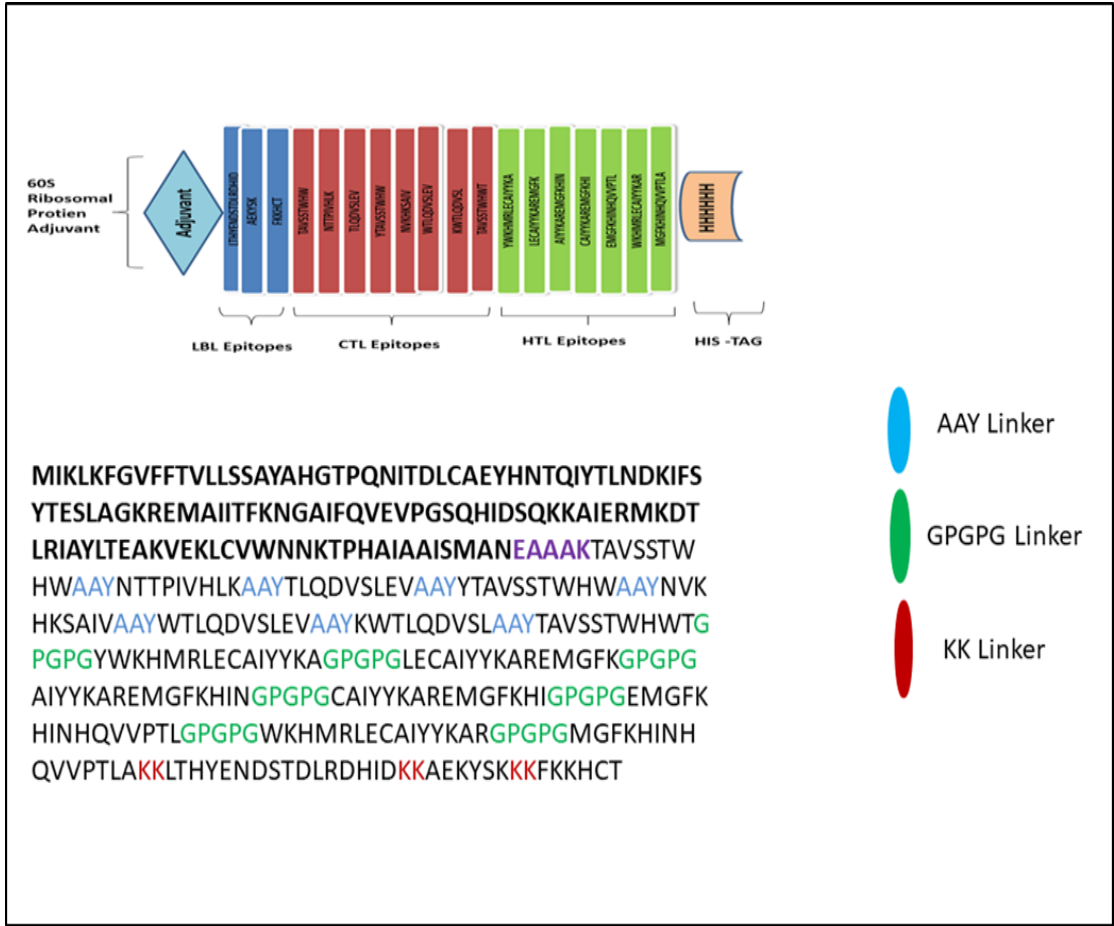


Figure 2. Schematic representation of the HPV-16/18 multi-epitope vaccine construct

Physicochemical and immunological properties of the vaccine construct

The designed multi-epitope vaccine consisted of 405 amino acids with predicted molecular weight of 45.49 kDa and theoretical isoelectric point (pI) of 9.37. The predicted half-life was 30 h in mammalian reticulocytes in vitro, >20 h in yeast in vivo, and >10 h in *Escherichia coli* in vivo. The construct had an instability index of 29.00 (stable), aliphatic index of 71.65. The negative GRAVY value (−0.360) indicated an overall hydrophilic character. The construct contained a predicted total of 6,359 atoms (Table 1). The vaccine construct was further predicted to be antigenic

and non-allergenic, supporting its selection for subsequent structural modelling and evaluation.

Parameter	Predicted value
Sequence length	405 a.a
Molecular weight	45.49 kDa
Theoretical pI	9.37
Instability index	29.00
Aliphatic index	71.65
GRAVY	−0.360
Half-life in mammalian reticulocytes (in vitro)	30 h
Half-life in yeast (in vivo)	>20 h
Half-life in <i>E. coli</i> (in vivo)	>10 h
Total number of atoms	6,359
Antigenicity	Predicted antigenic
Allergenicity	Predicted non-allergenic

Predicted secondary structure of 405-amino-acid vaccine construct is shown in Figure 3. This construct is mostly composed of coil regions that account for more than fifty percent of the entire sequence. Most of these coils are located in the linker regions as well as in the epitope regions. Alpha-helices are largely found in adjuvant regions as well as in other parts of the sequence. In contrast to the two previous secondary structure elements, beta-strands are less abundant in the vaccine construct.

Figure 3. Predicted secondary structure of HPV-16/18 multi-epitope vaccine construct

The predicted tertiary structure of multi-epitope vaccine was refined and subsequently evaluated through ProSA-web, Ramachandran plot analysis, and ERRAT. Refined model showed an organized three-dimensional conformation. Ramachandran analysis indicated that the majority of residues occupied favored or allowed conformational regions, with only limited residues occurring in disallowed regions. ERRAT analysis further indicated generally acceptable non-bonded atomic interactions across most of the refined structure (Fig. 4)

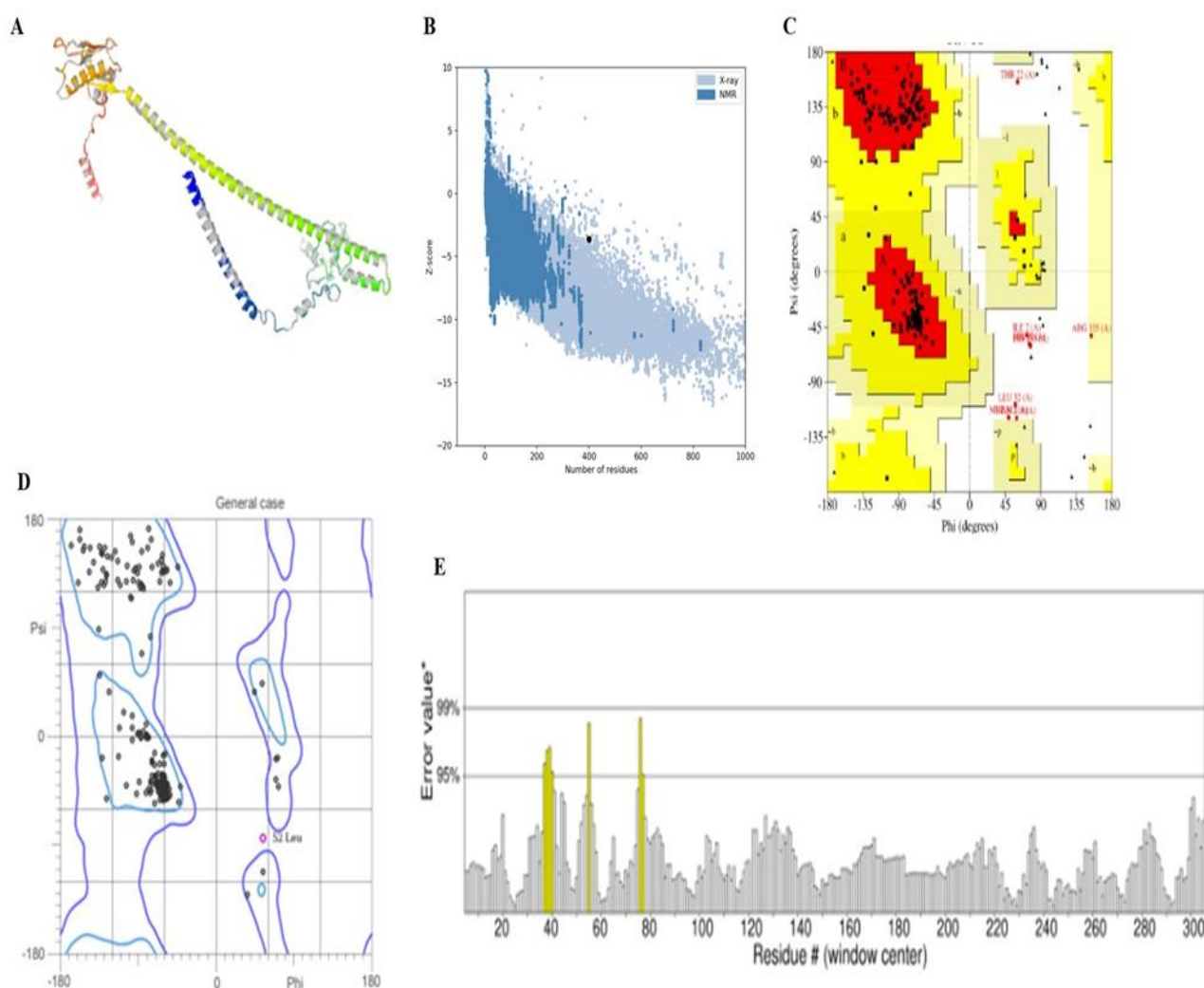


Figure 4. Refined tertiary structure and structural validation of HPV-16/18 multi-epitope vaccine construct

Molecular docking and interaction analysis

Molecular docking was performed to evaluate the interaction between refined HPV-16/18 multi-epitope vaccine construct and selected immune receptor. The docked complex showed extensive contact between vaccine and receptor surfaces. Post-docking analysis revealed multiple non-covalent interactions at the binding interface, including hydrogen bonds, hydrophobic interactions, and salt bridges (Fig.5)

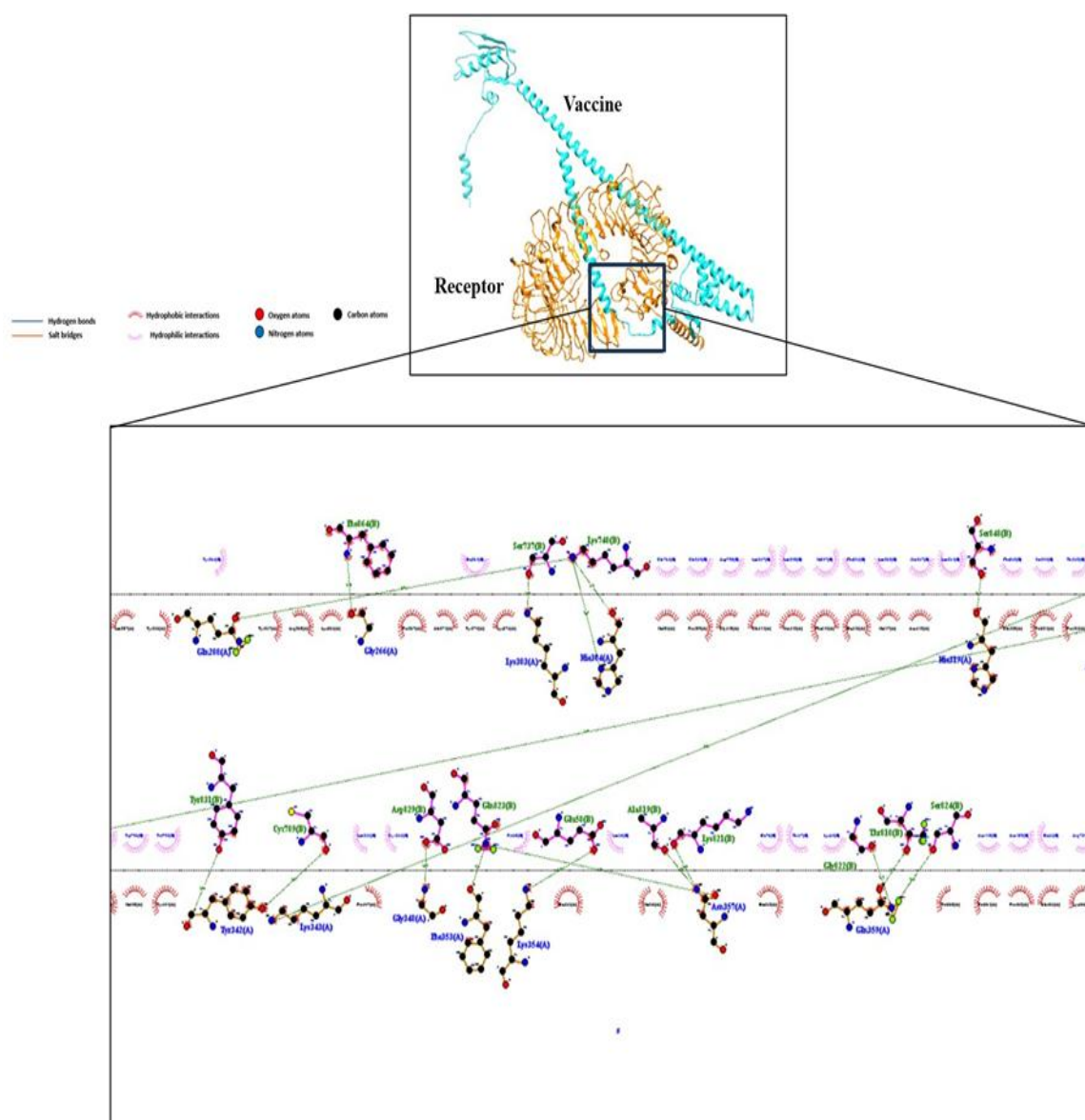


Figure 5. Molecular interaction analysis of HPV-16/18 multi-epitope vaccine–receptor complex

Several residues of the vaccine construct, including **Glu241**, **Lys254**, **Asp353**, and **Arg376**, were identified at the interaction interface, while **Gln214**, **Glu216**, and **Ser248** were reported among the interacting receptor residues. Together, the interaction map demonstrated multiple residue-level contacts between vaccine construct and receptor.

Molecular dynamics simulation

Molecular dynamics (MD) simulation was performed for 100 ns to investigate conformational behavior of HPV-16/18 multi-epitope vaccine–receptor complex. The $C\alpha$ RMSD showed an overall increasing trend during the simulation, reaching approximately 15–20 Å during the later stages, with fluctuations throughout the trajectory (Fig. 6A). RMSF analysis revealed heterogeneous residue-level flexibility across the complex, with several regions exhibiting comparatively higher fluctuations (Fig. 6B). Secondary structure analysis demonstrated the persistence and dynamic variation of α -helical and β -strand elements across different regions of the complex during the simulation (Fig. 6C–D).

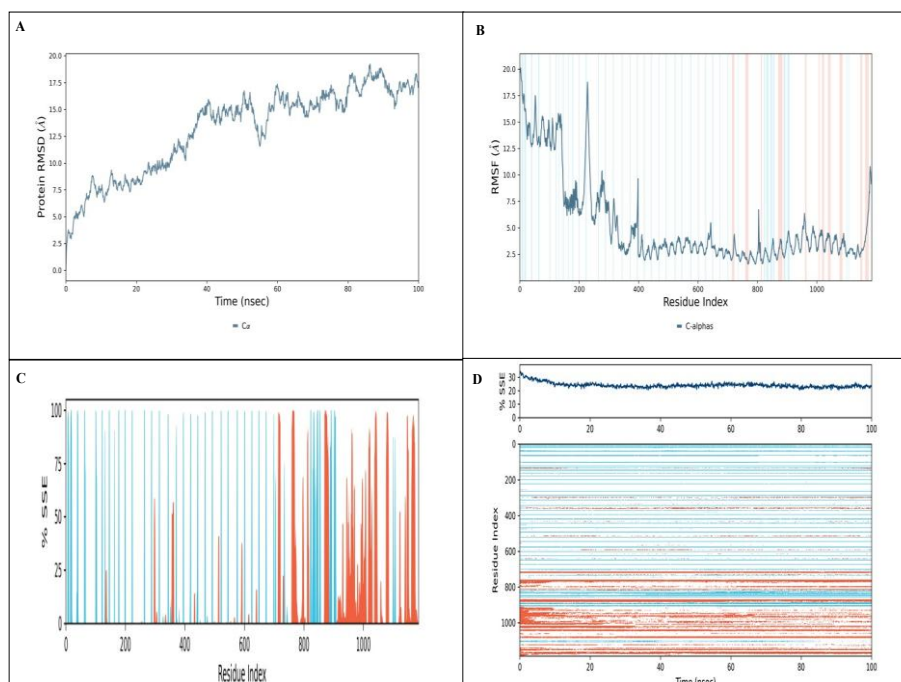


Figure 6. Molecular dynamics analysis of HPV-16/18 vaccine-receptor complex showing RMSD, RMSF, and secondary structure dynamics

Principal component analysis (PCA) showed complex sampled multiple conformational states (Fig. 7A), while cumulative variance profile indicated that the major principal components accounted for a substantial proportion of the overall conformational variance (Fig. 7B). DCCM analysis revealed regions of both positively and negatively correlated residue motions within the complex (Fig. 7C). Radius of gyration analysis showed an overall reduction in Rg across the displayed trajectory, from approximately 54 Å to 45 Å, with intermediate fluctuations, indicating increased compactness of the complex during the analyzed interval (Figure 7D).

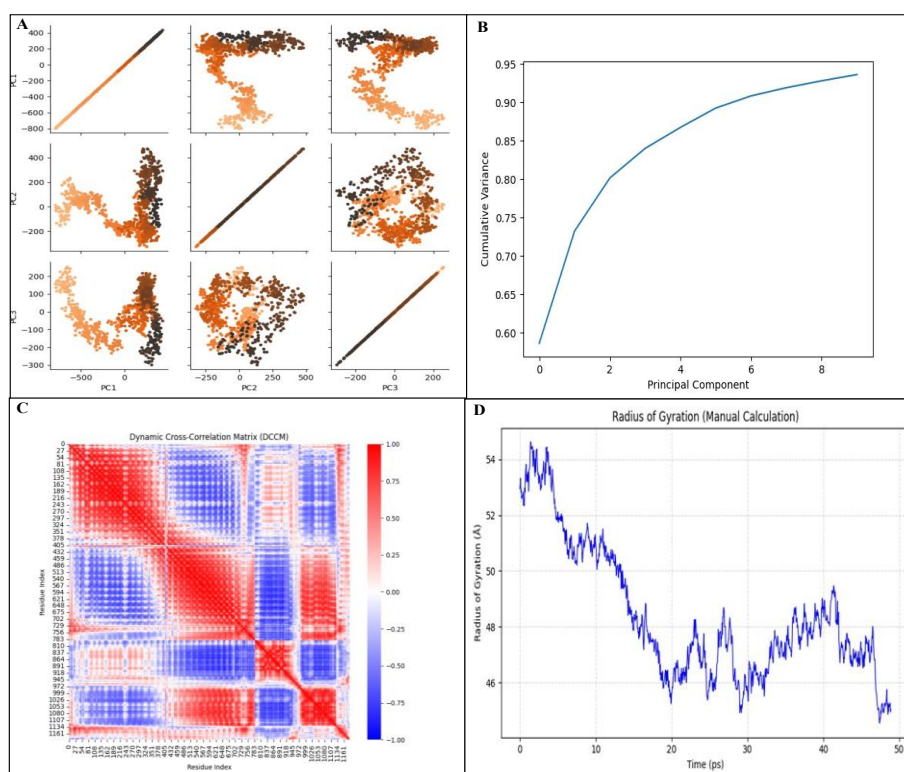


Figure 7. Advanced molecular dynamics analysis of HPV-16/18 vaccine-receptor complex showing PCA, cumulative variance, DCCM, and radius of gyration

Discussion

The persistent infection of HPV-16 and HPV-18, is strongly associated with cervical and other HPV-related malignancies. Although licensed HPV vaccines provide effective prophylactic protection, they have limited therapeutic value against established infection or transformed cells. This has encouraged the development of therapeutic approaches targeting early viral proteins expressed in infected cells (Ramakrishnan et al., 2015). In the present study, E5 and E7 proteins of HPV-16 and HPV-18 were used to develop a multi-epitope vaccine incorporating CTL, HTL and B-cell epitopes. The selection of these early proteins is relevant to a therapeutic strategy because of their involvement in HPV persistence and cellular transformation. A comparable approach was reported by Sanami et al. (2022), who also explored E5- and E7-derived epitopes from HPV-16 and HPV-18 for therapeutic vaccine design. Experimental studies using early HPV proteins have further shown that such epitopes can generate antigen-specific cellular responses and antitumor activity in animal models (Namvar et al., 2020; Zhu et al., 2024).

The selected epitopes showed favorable predicted antigenicity, non-allergenicity, and recognition by multiple MHC class I-II alleles. Combining both classes of T-cell epitopes is particularly important for a therapeutic construct, as CTL epitopes are intended to support recognition by CD8⁺ T cells, while HTL epitopes may contribute to the coordination of cellular immune responses. Similar combinations of epitopes have been incorporated into previous HPV multi-epitope vaccine designs (Kumar et al., 2022; Sanami et al., 2022). The combined MHC class I-II epitopes in present study provided greater than 90% predicted global population coverage, suggesting broad representation across different HLA backgrounds. This is relevant because HLA polymorphism can considerably influence epitope presentation among populations. Nevertheless, population coverage reflects predicted HLA representation rather than expected vaccine efficacy.

The selected epitopes were assembled into a 405-amino-acid construct using AAY, GPGPG and KK linkers, together with an N-terminal 60S ribosomal protein adjuvant and C-terminal His tag. Such modular organization is widely used in multi-epitope vaccine design to separate different epitope classes within a single sequence (Kumar et al., 2022). The resulting construct showed favorable predicted physicochemical characteristics, including an instability index of 29.00 and GRAVY value of −0.360, indicating predicted stability and an overall hydrophilic nature. It was also predicted to be antigenic and non-allergenic. These characteristics are broadly consistent with previous computational HPV vaccine designs (Carse et al., 2021), although experimental expression, solubility, and safety cannot be inferred from these predictions alone.

Structural assessment provided further support for the computational plausibility of the construct. Secondary structure analysis showed a mixture of α -helices, β -strands and coil regions, while evaluation of refined tertiary structure using ProSA-web, Ramachandran analysis, whereas, ERRAT indicated generally acceptable structural geometry. Similar refinement and validation approaches have been used in previous HPV multi-epitope vaccine studies (Sanami et al., 2022). These findings support the quality of predicted structural model but do not establish the conformation that the recombinant protein would adopt experimentally.

Molecular docking further showed that the refined construct formed an extensive interaction interface with the selected immune receptor. The interface involved hydrogen bonds, hydrophobic contacts, and salt bridges, with vaccine residues Glu241, Lys254, Asp353, and Arg376 contributing to the predicted interactions. These contacts support structural compatibility between the modeled vaccine and receptor and are consistent with the use of receptor docking as a screening step in other HPV vaccine studies. However, docking alone cannot demonstrate receptor activation or downstream immune signaling.

The 100-ns MD simulation provided a more detailed view of the behavior of the docked complex. RMSD increased during the trajectory and reached approximately 15–20 Å during the later stages, indicating considerable conformational rearrangement rather than complete equilibration. RMSF analysis showed region-dependent flexibility, while secondary-structure analysis indicated persistence and local variation of α -helical and β -strand elements. Pasted markdown PCA further showed that complex sampled multiple conformational states, and DCCM identified both correlated and anti-correlated residue motions. The radius of gyration decreased from approximately 54 Å to 45 Å across the displayed trajectory segment, suggesting increased compactness during that interval. Pasted markdown Together, these analyses describe a dynamic vaccine–receptor complex undergoing substantial conformational adjustment, rather than providing evidence of complete structural stabilization or biological activity.

The findings are generally consistent with previous immunoinformatics studies of HPV vaccines. Sanami et al. (2022) reported an E5/E7-based HPV-16/18 construct with favorable predicted immunological and structural properties, while Kumar et al. (2022) similarly used multiple HPV antigens in a computational multi-epitope design. Importantly, experimental studies have also provided biological support for targeting early HPV proteins, with E5/E7-containing constructs producing cellular immune responses and antitumor effects in animal models (Namvar et al., 2020; Zhu et al., 2024). These findings strengthen rationale for experimental evaluation of the present construct, but they do not validate this specific vaccine sequence.

The main limitation of this study is its computational nature. Predicted antigenicity, HLA binding, population coverage, structural quality, docking interactions, and molecular dynamics do not establish natural epitope processing, immune activation, safety, or therapeutic efficacy. Experimental work should therefore focus on recombinant expression and purification, HLA-binding and antigen-presentation studies, and assessment of epitope-specific cellular responses, followed by appropriate in-vivo evaluation.

Overall, the present study provides an E5/E7-based multi-epitope vaccine design targeting HPV-16 and HPV-18 with broad predicted HLA population representation and favorable physicochemical and structural characteristics. Docking identified a defined vaccine–receptor interface, while MD simulation revealed a dynamic complex with region-specific flexibility, coordinated motions, and changes in compactness. These findings provide rational computational basis for experimental evaluation of construct as a potential therapeutic HPV vaccine.

Conclusion

This study presents immunoinformatics-based multi-epitope vaccine targeting the E5 and E7 proteins of HPV-16 and HPV-18. The designed 405-amino-acid construct incorporated selected epitopes showed broad predicted HLA population coverage, favorable physicochemical properties, antigenicity, non-allergenicity and acceptable structural characteristics. Molecular docking identified multiple interactions at the vaccine–receptor interface, while molecular dynamics simulations revealed a conformationally dynamic complex with region-specific flexibility, coordinated residue motions, and changes in compactness. Collectively, these findings support construct as a potential therapeutic HPV vaccine for further investigation. Experimental studies are now required to confirm its expression, safety, immunogenicity and biological activity before its therapeutic potential can be established.

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