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Computational Design and in Silico Evaluation of A Multi-Epitope Vaccine Candidate Targeting Hiv Envelope Proteins

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Abstract

Human Immunodeficiency Virus (HIV) remains a major global health challenge due to its high genetic variability and complex immune evasion mechanisms. In the present study, an immunoinformatics-based strategy was employed to design a multi-epitope vaccine candidate targeting envelope proteins associated with HIV entry pathways. The vaccine construct was evaluated through a series of computational analyses, including protein–protein docking, structural dynamics assessment, codon optimisation, and in silico cloning.

Protein–protein docking was performed to investigate the interaction potential of the vaccine construct with host receptors involved in viral entry, including CD4, CCR5, and CXCR4. The docking results demonstrated favourable binding interactions with progressively stable docking scores across the receptor engagement stages, suggesting structural compatibility between the vaccine construct and host receptor systems. Post-docking interaction analysis identified hydrogen bonds, salt bridges, and hydrophobic contacts contributing to interface stability.

Normal mode analysis was performed to examine the dynamic behaviour of the docked complexes, revealing a stable structural architecture with limited flexible regions primarily located in loop segments. Codon optimisation using the JCat server indicated efficient expression potential in *Escherichia coli* K12 with an optimal Codon Adaptation Index and balanced GC content. In silico cloning further confirmed successful integration of the vaccine gene into the pET-28a(+) expression vector.

Overall, the computational results suggest that the designed multi-epitope construct exhibits favourable structural stability, receptor interaction potential, and expression feasibility, supporting its suitability for further experimental validation as a prospective HIV vaccine candidate.

Keywords: Multi-epitope vaccine, HIV, Immunoinformatics, molecular docking, protein–protein interaction, normal mode analysis, codon optimisation, in silico cloning

1. INTRODUCTION

The virus's high mutation rate and its capacity to evade host immune responses complicate traditional vaccine development strategies (Gayle HD, Hill GL, 2001; WHO, 2022). As a result, alternative methods, particularly immunoinformatics-based vaccine design, have garnered increasing attention for their ability to identify conserved immunogenic regions that can elicit targeted immune responses (Khan et al., 2020). Multi-epitope vaccine design represents a promising strategy that integrates various antigenic

determinants to stimulate both humoral and cellular immunity (Rojas et al., 2021). By incorporating B-cell and T-cell epitopes from viral proteins, these constructs aim to enhance immune recognition while addressing limitations of whole-pathogen or single-antigen vaccines. Modern computational tools facilitate the systematic identification, selection, and structural evaluation of immunogenic epitopes, thereby significantly expediting vaccine development processes (Doytchinova & Flower, 2007).

In HIV infection, the viral envelope proteins are pivotal for host cell entry, as they interact with the CD4 receptor and chemokine co-receptors such as CCR5 and CXCR4 (Liu et al., 2017). Targeting these receptor-binding pathways provides a strategic opportunity to disrupt viral attachment and entry. In silico approaches, including molecular docking and structural dynamics analyses, enable a detailed examination of vaccine–receptor interactions, offering insights into the stability and feasibility of the designed constructs (Zhang et al., 2018).

In this study, a computational framework was employed to evaluate a multi-epitope HIV vaccine construct. Protein–protein docking was performed to assess its interaction potential with key host receptors involved in viral entry. Structural dynamics analysis utilising normal mode analysis was also conducted to investigate the stability of the complex. Additionally, codon optimisation and in silico cloning were performed to assess the feasibility of heterologous expression of the vaccine construct. Together, these analyses provide a comprehensive structural and functional assessment of the designed vaccine candidate, supporting its potential for further experimental validation.

2. METHODOLOGY

2.1 Molecular Docking Analysis

2.1.1 Vaccine–CD4 Docking

Protein–protein docking was initially performed between the vaccine construct and the CD4 receptor to assess binding feasibility. The structure of the CD4 receptor was retrieved from the Protein Data Bank (PDB ID: 2B4C), and heteroatoms along with water molecules were removed to prepare it for docking (Wang et al., 2016). Docking was performed using a rigid-body approach, and the docked complexes were ranked by cluster size and binding energy (Zhang et al., 2019). The top-ranked complex was selected for further interaction analysis.

2.1.2 Vaccine–CCR5 Docking

To simulate sequential receptor engagement, the vaccine–CD4 complex was docked with the CCR5 co-receptor (PDB ID: 4MBS). The CCR5 receptor structure was prepared similarly to the CD4 receptor before docking (Cohen et al., 2018). The best-scoring docked complex was selected based on energetic stability and interface complementarity, reflecting potential receptor–co-receptor interactions (Yasuda et al., 2020).

2.1.3 Vaccine–CXCR4 Docking

In a similar manner, docking was performed with the CXCR4 co-receptor (PDB ID: 3ODU) to evaluate alternative receptor interactions. The prepared CXCR4 structure was docked with the vaccine–receptor complex, and the resulting complexes were assessed for binding affinity, interface stability, and interaction consistency (Liu et al., 2021).

2.2 Protein–Protein Interaction Analysis

Following molecular docking, the selected vaccine–receptor complexes were subjected to detailed interaction analysis to characterise the molecular contacts that stabilise the complexes. The top-ranked docked structures obtained from the docking analysis were used as input for interaction evaluation (Wang et al., 2018). Hydrogen-bond interactions between the vaccine construct and receptor proteins were

identified to determine the specific polar contacts contributing to binding specificity and stabilisation of the complex. Salt bridge interactions formed between oppositely charged residues were also analysed, as these electrostatic interactions often play a crucial role in strengthening protein–protein interfaces (Kumar et al., 2017). Additionally, hydrophobic interactions between non-polar residues were examined to assess their contribution to interface packing and structural stability. Interaction networks and residue-level contacts within the docked complexes were analysed using the PDBsum server (Laskowski et al., 2018), which provides a detailed summary of intermolecular interactions, including hydrogen bonds, salt bridges, and non-bonded contacts. The spatial arrangement and distribution of interacting residues were further inspected using molecular visualisation tools such as PyMOL (Schrödinger, 2020) or Chimaera Visualizer (Pettersen et al., 2004) to confirm the docking interface and evaluate contact regions. The number, type, and distribution of intermolecular interactions served as indicators of binding strength and structural compatibility between the vaccine construct and the host receptors. Complexes exhibiting multiple stabilising interactions at the interface were considered favourable, indicating stable receptor–vaccine binding conformations.

2.3 Normal Mode Analysis and Molecular Dynamics Simulation

To investigate the dynamic behaviour and stability of the docked complexes, normal mode analysis was performed using the iMODS server (López-Blanco et al., 2014). Deformability profiles, B-factor mobility, eigenvalues, covariance matrices, and elastic network models were analysed to assess molecular flexibility and structural stability. Lower eigenvalues were interpreted as indicators of stable collective motions within the complexes.

2.4 Codon Optimisation for Heterologous Expression

Codon optimisation of the vaccine construct was performed using the JCat server (Grote et al., 2005) to enhance expression efficiency in *Escherichia coli* K12. Codon adaptation index (CAI), GC content, and codon usage bias were analysed to ensure compatibility with the host expression system. Prokaryotic ribosome binding sites and restriction sites incompatible with cloning were avoided during optimisation.

2.5 In Silico Cloning

The optimised nucleotide sequence was in silico cloned into the pET28a(+) expression vector using standard restriction enzyme sites. The cloning strategy ensured the correct orientation and reading frame of the insert. The presence of a C-terminal His-tag facilitated potential downstream purification, confirming the feasibility of experimental expression.

3. RESULTS AND DISCUSSIONS

3.1 Molecular Docking Analyses

Docking stage	Receptor	Docking mode	Cluster ID	Cluster size (members)	Lowest weighted score
Stage 1	CD4	Balanced	Cluster 0	51	–1322.3
Stage 2	CCR5	Balanced	Cluster 0	44	–1912.1
Stage 3	CXCR4	Balanced	Cluster 0	83	–2123.0

Table 1. ClusPro-based molecular docking results of the multi-epitope HIV vaccine construct with host receptors. Docked complexes were selected based on the largest cluster size and the lowest weighted energy score within the dominant cluster, as recommended for ClusPro protein–protein docking analysis. Docking was performed using the Balanced mode to account for both shape complementarity and electrostatic interactions.

To evaluate the interaction potential of the designed multi-epitope HIV vaccine construct with host receptors involved in viral entry, multi-stage protein–protein docking was performed using ClusPro in Balanced mode. The Balanced docking strategy integrates shape complementarity and electrostatic contributions, providing a reliable assessment of large protein–protein interactions.

For each docking stage, the top-ranked complex was selected based on the largest cluster size and the lowest weighted energy score, reflecting stable and reproducible binding conformations.

In the first docking stage, the vaccine construct bound to the CD4 receptor. The dominant cluster (Cluster 0) comprised 51 members and exhibited the lowest weighted energy score of –1322.3, indicating favourable and stable interaction with the primary HIV attachment receptor.

In the second stage, docking of the vaccine–CD4 complex with the CCR5 co-receptor yielded a dominant cluster of 44 members, with the lowest weighted energy score of –1912.1. The more favourable binding energy compared to the initial docking stage suggests enhanced interaction stability within a multi-receptor environment.

In the third stage, docking to the CXCR4 co-receptor yielded the largest dominant cluster, comprising 83 members, with the lowest weighted energy score of –2123.0. The increased cluster size and highly favourable energy indicate a strong, stable interaction with CXCR4, supporting the vaccine construct's structural adaptability to alternative HIV entry pathways.

Overall, the presence of large dominant clusters and progressively favourable energy scores across all docking stages demonstrates consistent interaction behaviour and structural compatibility of the vaccine construct with CD4 and chemokine co-receptors. These findings support the feasibility of receptor engagement by the designed vaccine construct and provide a structural basis for further analyses of interactions and dynamics.

3.1.1 Structural Representation of Docking Stages

The three-dimensional structures involved in the docking analysis are illustrated in Figures 1–7. These figures represent the vaccine construct, receptor structures, and the resulting docked complexes obtained during the sequential docking procedure.

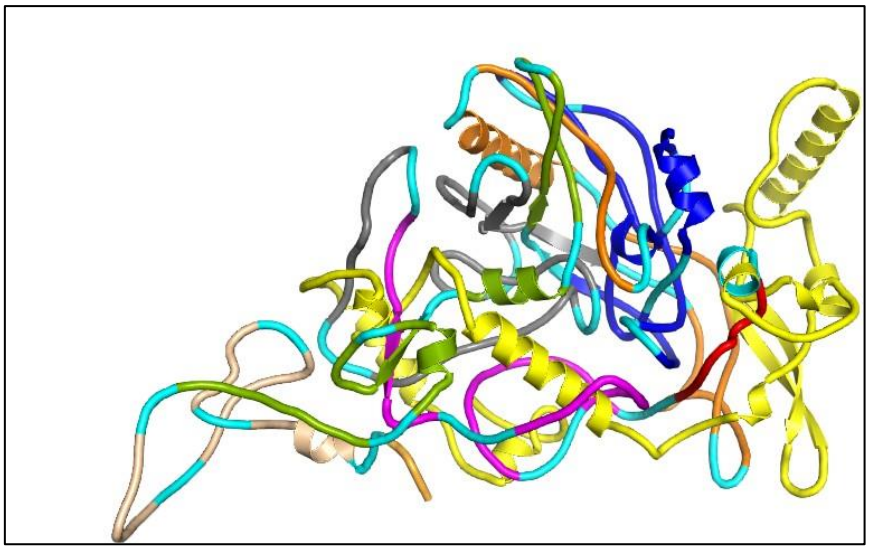


Figure 1: The protein structure of Vaccine. Yellow= Flic Adjuvant, Linkers= Cyan , Padre Linker = Red, MHC2 HIV1 = Magenta, MHC2 HIV2 =Grey, Bcell HIV1= Orange, B cell HIV2=Blue , MHC1 HIV1 = Green , MHC1 HIV2=Light Brown

The figure above shows the predicted tertiary structure of the designed multi-epitope HIV vaccine construct. The ribbon representation highlights the arrangement of α-helices, β-sheets and connecting loop regions forming a compact folded structure. The presence of flexible linker regions and surface-exposed residues suggests that the vaccine construct maintains structural adaptability while preserving epitope accessibility. Such structural organisation is advantageous for receptor binding and antigen presentation.

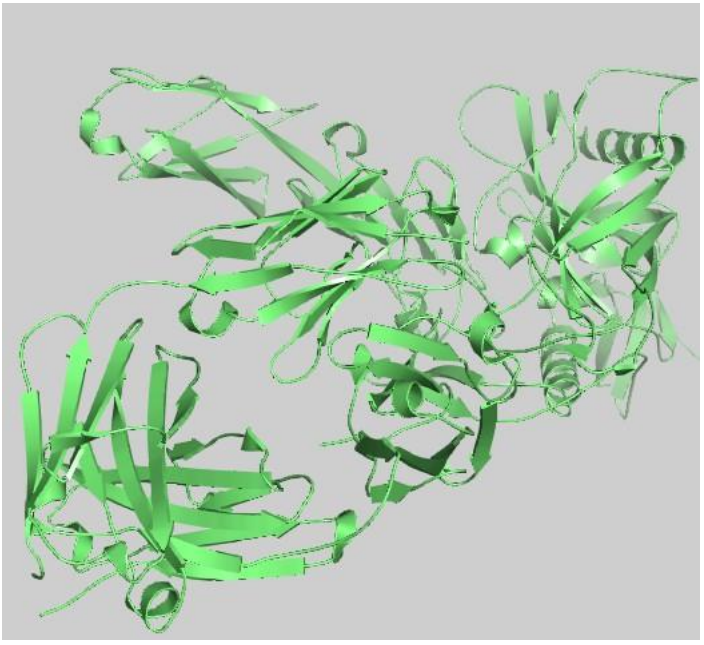
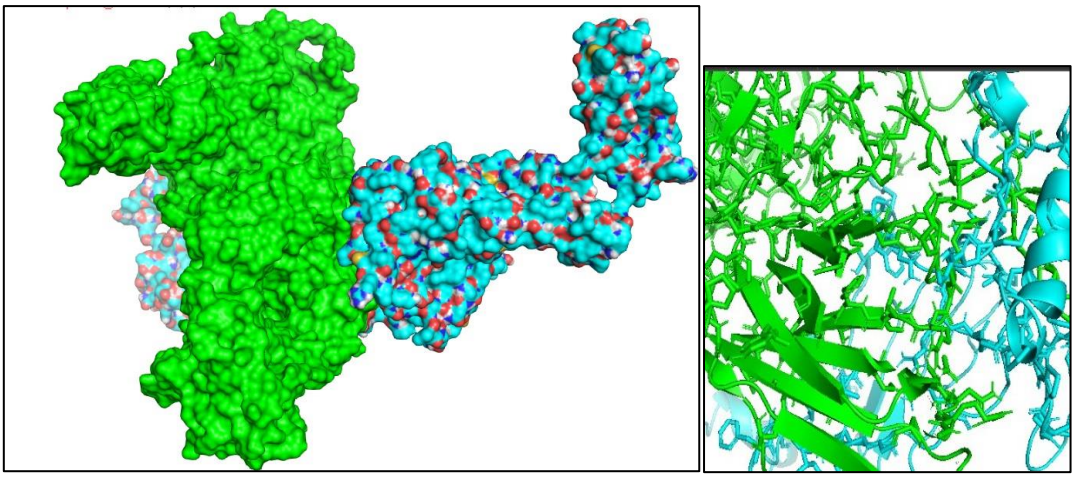


Figure 2: Protein structure of CD4 Receptor (PDB ID: 2B4C)

The figure above illustrates the structure of the CD4 receptor (PDB ID: 2B4C) used for the first-stage docking. The receptor exhibits multiple immunoglobulin-like domains predominantly composed of β-sheet structures. These domains provide the binding interface required for interactions with viral envelope proteins during HIV entry and therefore serve as a suitable target for evaluating vaccine–receptor interactions.

In the first docking stage, the vaccine construct was docked with the CD4 receptor to evaluate its ability to interact with the primary HIV attachment receptor. The resulting docked complex is shown in Figure 3, where the receptor is represented in green (CD4 receptor) and the vaccine construct in cyan. The docking interface demonstrates extensive surface complementarity between the two proteins, indicating favourable geometric and structural compatibility. The interaction region suggests that the vaccine construct can form stable interactions with CD4 receptor domains.

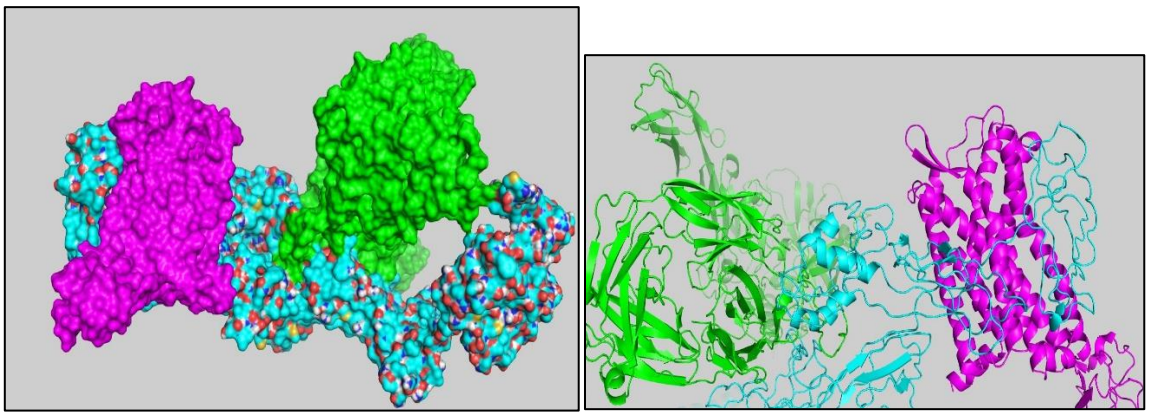


A) **B) Zoomed Structure in cartoon**

Figure 3(A & B): 1st Stage of Protein-protein docking between CD4 Receptor (green, PDB ID : 2B4C) and Ligand (Vaccine construct in cyan)

To simulate the sequential receptor engagement mechanism associated with HIV entry, the vaccine–CD4 complex obtained from the first stage was subsequently docked with the CCR5 co-receptor.

The structure of the CCR5 receptor (PDB ID: 4MBS) used for the second stage docking is shown in Figure 4. CCR5 is a chemokine receptor belonging to the G-protein-coupled receptor (GPCR) family and is characterised by multiple transmembrane α-helical domains. These structural elements form a ligand-binding cavity that mediates viral entry following CD4 attachment.



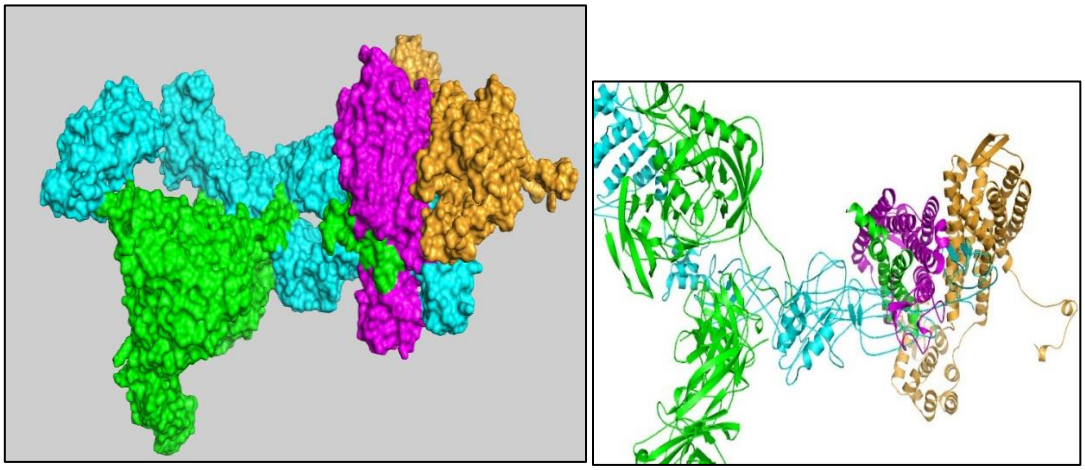
A) **B) Zoomed structure in a cartoon**

Figure 4(A & B): 2nd stage of protein-protein docking between the Receptor CCR5 (Magenta, PDB ID: 4MBS) and the Ligand consisting of 2 parts (Vaccine construct – cyan + CD4 Receptor- 2B4C)

The resulting docked complex from the second docking stage is illustrated in Figure 4. In this representation, the CCR5 receptor is displayed in magenta, while the previously docked vaccine–CD4 complex remains visible in cyan and green. The formation of a multi-component complex indicates that the vaccine construct remains structurally stable while accommodating additional receptor interactions. The docking interface demonstrates favourable spatial positioning of the proteins, supporting the possibility of simultaneous receptor engagement.

To further evaluate alternative HIV entry pathways, the docked complex was subjected to a third docking stage involving the CXCR4 co-receptor.

The structure of the CXCR4 receptor (PDB ID: 3ODU) is presented in Figure 5. Like CCR5, CXCR4 belongs to the GPCR family and has a seven-transmembrane helical architecture. This receptor plays a crucial role in HIV entry for X4-tropic viral strains.



A) **B) Zoomed structure in a cartoon**

Figure 5 (A & B): The 3rd stage of protein-protein docking between Receptor CXCR4 (bright orange, PDB ID: 3ODU) and the ligand consisting of 3 parts (Vaccine construct -cyan, CD4 Receptor-green and CCR5 Receptor-magenta)

The final docked complex obtained from the third stage is shown in **Figure 6**, where the CXCR4 receptor is represented in orange along with the previously docked receptor–vaccine components. The resulting multi-protein assembly demonstrates stable structural integration, indicating that the vaccine construct remains compatible with multiple receptor-binding environments. The complex exhibits extensive interface contacts, suggesting that the vaccine construct can interact with both primary and coreceptors involved in HIV entry.

Overall, the structural visualisation of the docking stages reveals progressive receptor engagement involving CD4, CCR5, and CXCR4. The stable configuration of the vaccine construct within these complexes supports the ClusPro docking results, which showed favourable cluster sizes and binding energies across all stages. These observations suggest that the designed multi-epitope vaccine construct possesses structural adaptability and receptor interaction potential relevant to HIV entry mechanisms.

All three stages of protein-protein docking involving the vaccine construct and the receptors are summarised in the figure below (Figure 7).

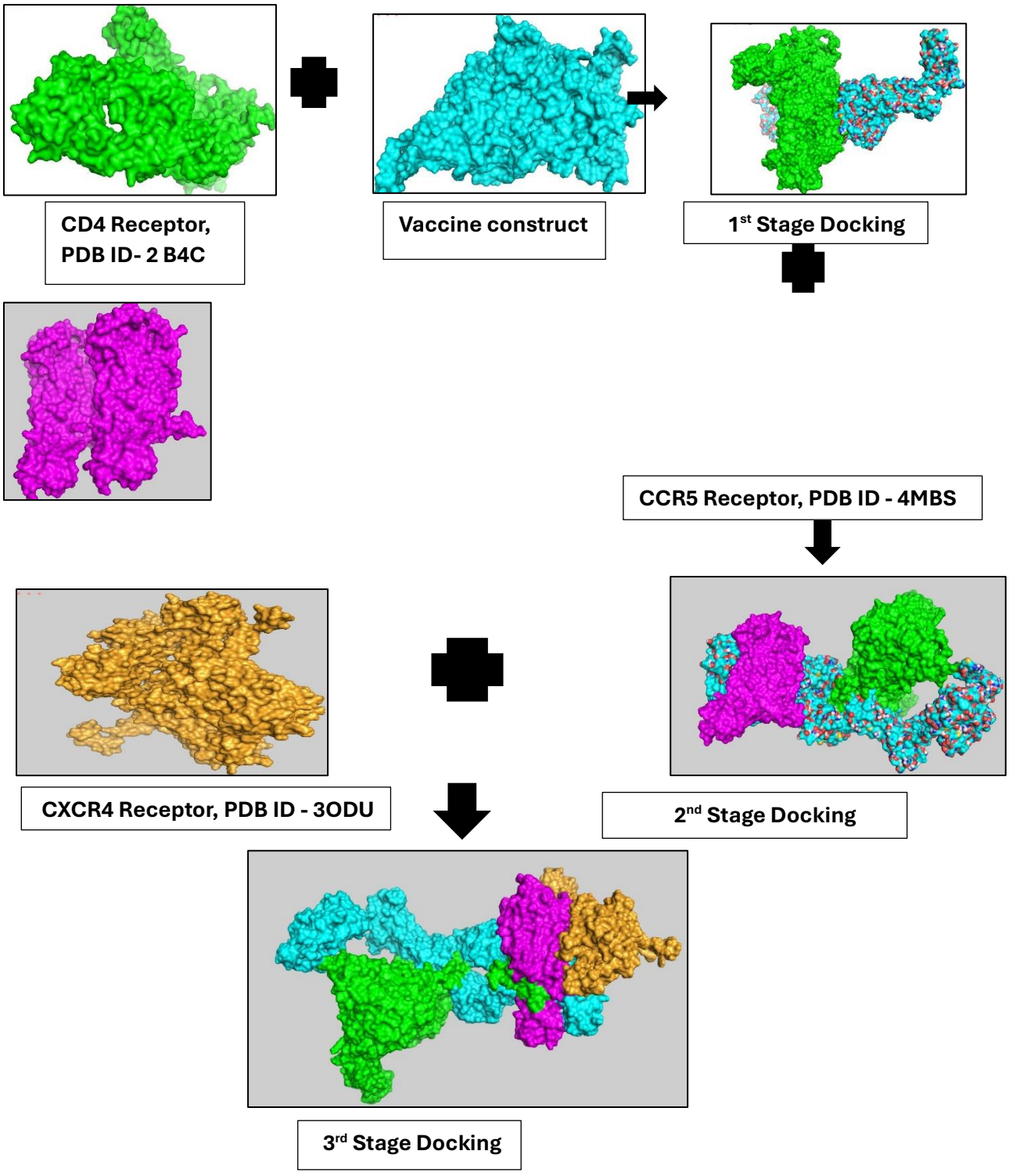


Figure 6: Shows all 3 stages of docking interactions between the receptors and the vaccine construct

3.1.2 Interaction Analysis of Docked Vaccine–Receptor Complex

Overall Interaction Analysis

To investigate the molecular basis of receptor recognition and stability of the docked complexes, detailed interaction analyses were carried out, focusing on key non-covalent interactions, including salt bridges, hydrogen bonds, π-interactions, and electrostatic interactions between the vaccine construct and receptor

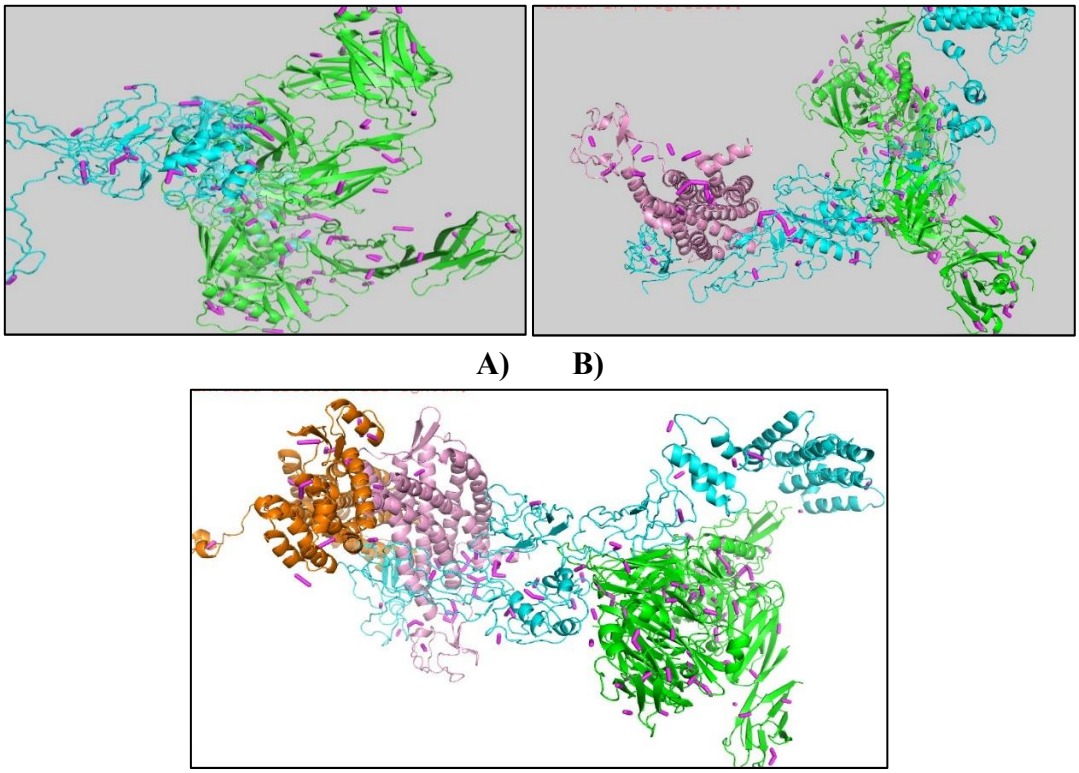
proteins. These interactions play crucial roles in stabilizing protein–protein complexes and determining binding affinity.

The docking analysis revealed that the vaccine construct establishes several stabilising interactions at the receptor binding interface across all three docking stages. The interaction network comprises a combination of polar interactions (salt bridges and hydrogen bonds) and non-polar contacts (π-interactions), together with favourable electrostatic complementarity. These interactions collectively contribute to the structural stability of the docked complexes and suggest that the vaccine construct is appropriately positioned within the receptor-binding region.

3.1.2.1 Salt Bridge Interactions

Salt bridges represent strong electrostatic attractions between oppositely charged amino acid residues and are known to significantly enhance the stability of protein–protein complexes. These interactions typically occur between acidic residues, such as Aspartate (Asp) or Glutamate (Glu), and basic residues, such as Lysine (Lys) or Arginine (Arg).

The docking complexes revealed several salt-bridge interactions at the receptor–vaccine interface, indicating favourable electrostatic complementarity between the charged residues of the interacting proteins. These salt bridges help anchor the vaccine construct within the receptor-binding region and contribute to stabilising the docked complexes throughout the docking stages. The formation of these interactions suggests that the binding interface possesses a stable electrostatic environment supportive of strong receptor–ligand association.



A) **B)**

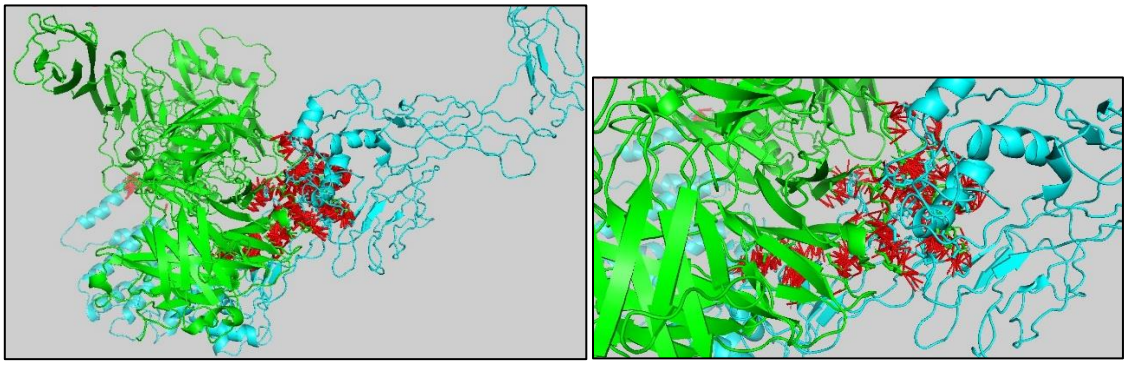
C)

Figure 7: Showing salt bridges (magenta coloured bonds) between oppositely charged residues during the 1st stage (A), 2nd stage (B) and 3rd stage (C) of docking

3.1.2.2 Hydrogen Bond Interactions

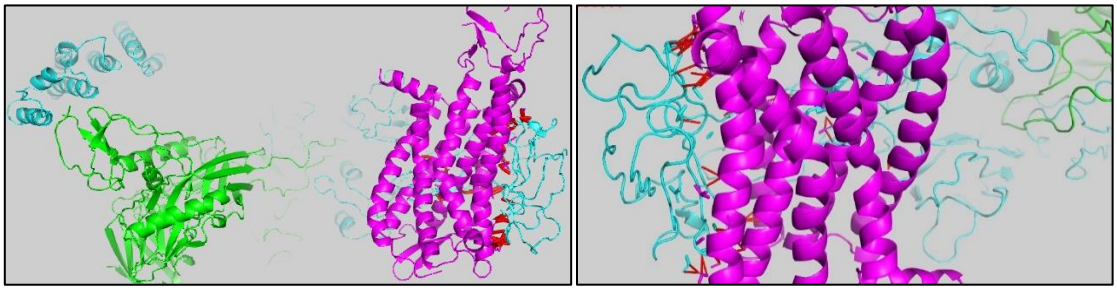
Hydrogen bonding is one of the most important determinants of specificity and stability in protein–protein interactions. The hydrogen bond analysis revealed multiple hydrogen bonds between the vaccine construct and receptor residues across the docking stages. These interactions primarily occur between hydrogen-bond donor and acceptor atoms in polar amino acid residues and backbone groups.

The presence of several hydrogen bonds at the receptor–vaccine interface indicates strong polar interactions that stabilise the docked complexes. These bonds help maintain the proper spatial orientation of the interacting proteins and contribute to the structural integrity of the complex. The distribution of hydrogen bonds observed in the docking complexes suggests that the vaccine construct forms a well-stabilised binding interface with the receptor molecules.



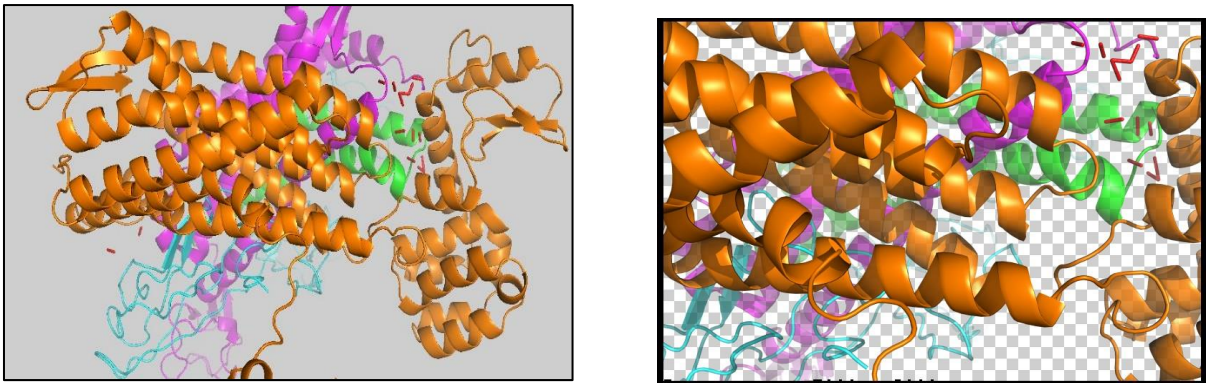
A) H-Bonds = Red, during the 1st stage of docking. B) Zoomed structures showing H-bond in red

Figure 8: (A & B) – Showing H-Bonds during the 1st stage of docking interactions



A) H-Bonds in red during 2nd stage of docking B) Zoomed structures showing H-bonds

Figure 9: (A & B) – Showing H-Bonds during the 2nd stage of docking interactions



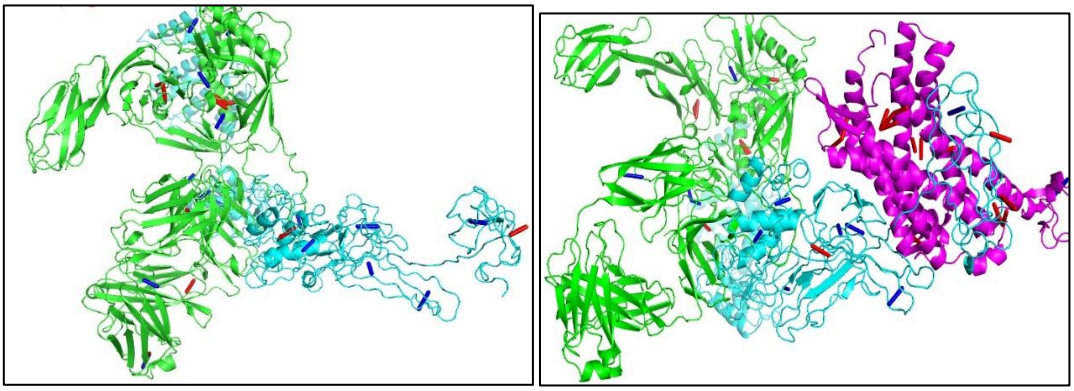
A) B) Zoomed structures showing H-Bond (orange)

Figure 10 (A & B) : H-bonds' interactions during the 3rd stage of docking

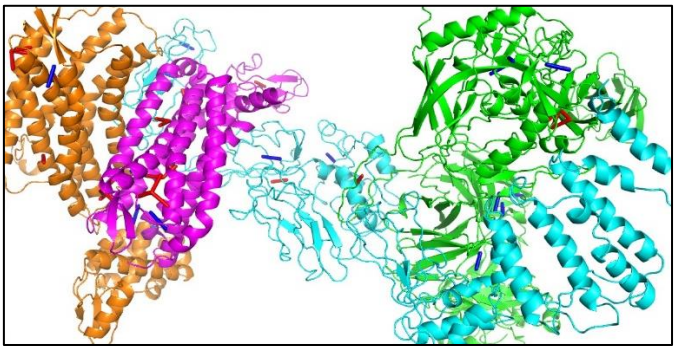
3.1.2.3 π-Interactions

π-mediated interactions, including π–π stacking and π–cation interactions, were also identified within the docked complexes. These interactions arise primarily from aromatic amino acid residues such as phenylalanine, tyrosine, and tryptophan, which contribute to hydrophobic stabilisation and intermolecular packing within protein interfaces.

The interaction analysis demonstrated that aromatic residues in the receptor-binding region form π-mediated contacts with residues of the vaccine construct. These interactions enhance the stability of the complex by strengthening non-polar interactions at the interface and promoting compact packing of the interacting proteins. The presence of π-interactions throughout the docking stages further supports the favourable accommodation of the vaccine construct within the receptor-binding region.



A) **B)**



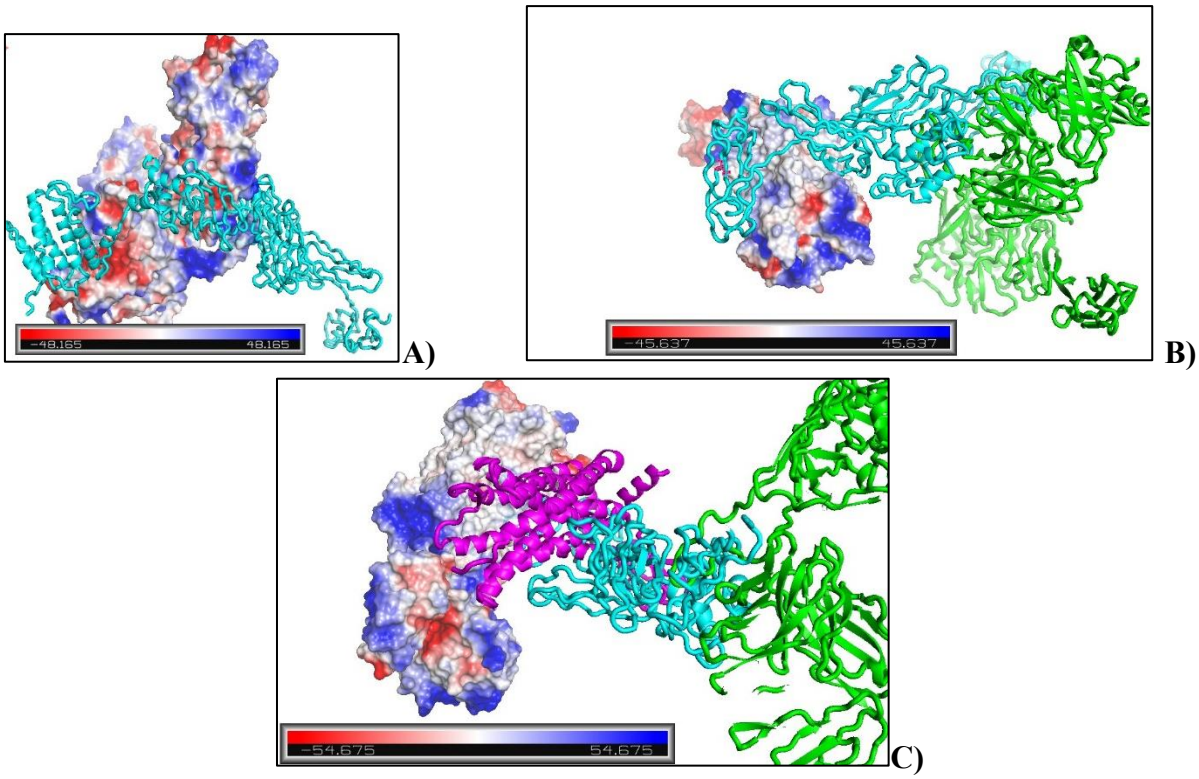
C)

Figure 11(A, B, C): Showing Pi-Pi interactions (red) and Pi-citations (blue) during the 1st, 2nd and 3rd stages of docking, respectively.

3.1.2.4 Electrostatic Interactions

Electrostatic interactions were further analysed to evaluate the charge complementarity between the vaccine construct and receptor proteins. These interactions arise from long-range attractive forces between oppositely charged regions of interacting molecules and play a critical role during protein–protein recognition.

Electrostatic surface analysis revealed complementary charge distribution between the vaccine construct and receptor surfaces, facilitating favourable interaction at the binding interface. Regions of positive and negative electrostatic potential were observed to align in a manner that promotes stable receptor–vaccine association. This electrostatic complementarity contributes to enhanced binding affinity and structural stability of the docked complexes.



Figures 12 (A, B &C): Showing Electrostatic interaction(Red= Negatively charged, White=Neutral and blue= Positively charged) analysis of Receptors at Stage-1 (A), Stage-2 (B) and Stage-3 (C) of docking complexes.

Collectively, the observed network of salt bridges, hydrogen bonds, π-interactions, and electrostatic contacts indicates that the vaccine construct forms a stable and energetically favourable complex with the receptor proteins.

3.1.3 Structural Stability and Binding Affinity of Docked Complexes

To further evaluate the structural stability and binding affinity of the docked vaccine–receptor complexes, the docking clusters generated from the three docking stages were analyzed based on weighted docking scores, cluster size, and energy distribution. These parameters provide insight into the stability of the predicted complexes and the reliability of the docking solutions.

The docking analysis revealed that all three docking stages produced well-defined clusters with favourable weighted scores, indicating stable interactions between the vaccine construct and receptor proteins. In Stage-1 docking, the dominant cluster comprised 51 members, with the lowest weighted score of approximately −1322.3, suggesting a stable binding conformation. The presence of a relatively large cluster indicates that multiple docking poses converged toward a similar binding orientation, reflecting the reliability of the predicted interaction interface.

In Stage-2 docking, an even stronger interaction profile was observed. The largest cluster contained 83 members, with the lowest weighted score reaching approximately −2123.0. The substantially lower docking score indicates enhanced binding affinity and stronger intermolecular interactions between the vaccine construct and the receptor. The large cluster size further suggests a highly stable docking solution with consistent structural orientation among multiple predicted poses.

Similarly, Stage-3 docking produced a dominant cluster comprising 44 members, with a lowest weighted score of approximately −1912.1. Although slightly higher than the Stage-2 score, the interaction energy

remains highly favorable, indicating strong binding interactions and stable receptor–vaccine complex formation. The clustering pattern observed in this stage also supports the presence of a consistent binding interface.

Overall, the docking results demonstrate balanced and favourable docking scores across all three stages, indicating a stable association between the vaccine construct and receptor molecules. The combination of low energy scores and substantial cluster sizes suggests that the docked complexes are structurally stable and energetically favourable. These findings are further supported by the interaction analyses, which revealed multiple stabilising contacts, including hydrogen bonds, salt bridges, π- π interactions, and electrostatic interactions, at the receptor–vaccine interface.

Collectively, the docking score distribution and clustering behaviour indicate that the designed multi-epitope vaccine construct is capable of forming stable and energetically favourable complexes with the receptor proteins, supporting its potential effectiveness in receptor recognition and immune activation.

The convergence of multiple docking poses into dominant clusters with highly negative weighted scores indicates a reliable and stable binding mode between the vaccine construct and the receptor proteins.

3.1.4 Overall Interpretation of Docking Results

The docking analyses demonstrated that the designed multi-epitope vaccine construct forms stable and energetically favourable complexes with the receptor proteins across all three docking stages. The docking clusters exhibited balanced, highly negative weighted scores, along with substantial cluster sizes, indicating reliable binding conformations and structural stability of the predicted complexes. The convergence of multiple docking poses within dominant clusters suggests a consistent binding orientation of the vaccine construct at the receptor interface.

Interaction analyses further revealed multiple stabilising contacts, including salt bridges, hydrogen bonds, π- π interactions, and electrostatic complementarity, which collectively strengthen the receptor–vaccine association. The complementary charge distribution observed in electrostatic surface analyses also supports favourable intermolecular recognition.

Overall, these findings indicate that the designed vaccine construct exhibits stable receptor-binding characteristics, supporting its potential to effectively engage immune receptors and facilitate downstream immune activation.

3.2 Structural dynamics and stability analysis using Normal Mode Analysis

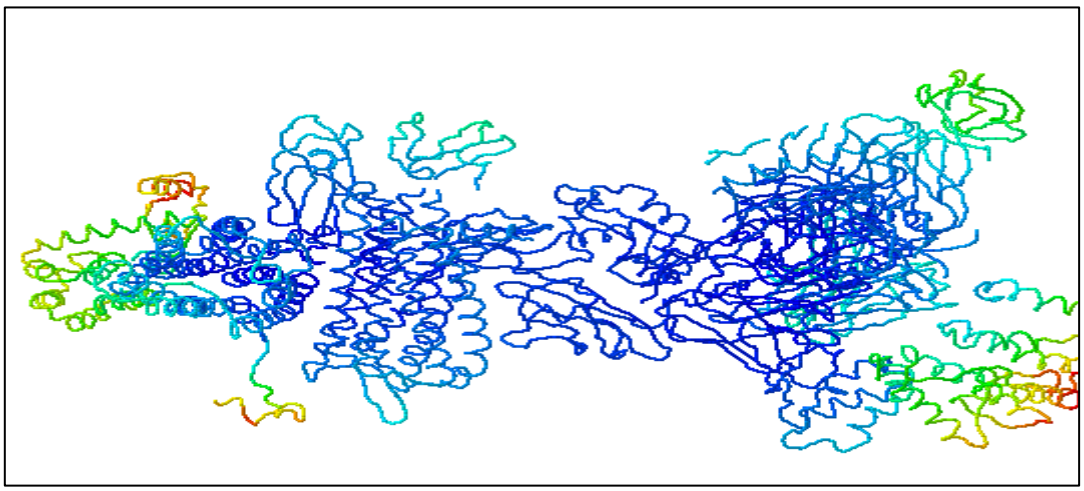


Figure 13. Normal mode analysis of the docked vaccine–receptor complex

Normal mode analysis (NMA) of the selected docked complex was performed using the iMODS server.

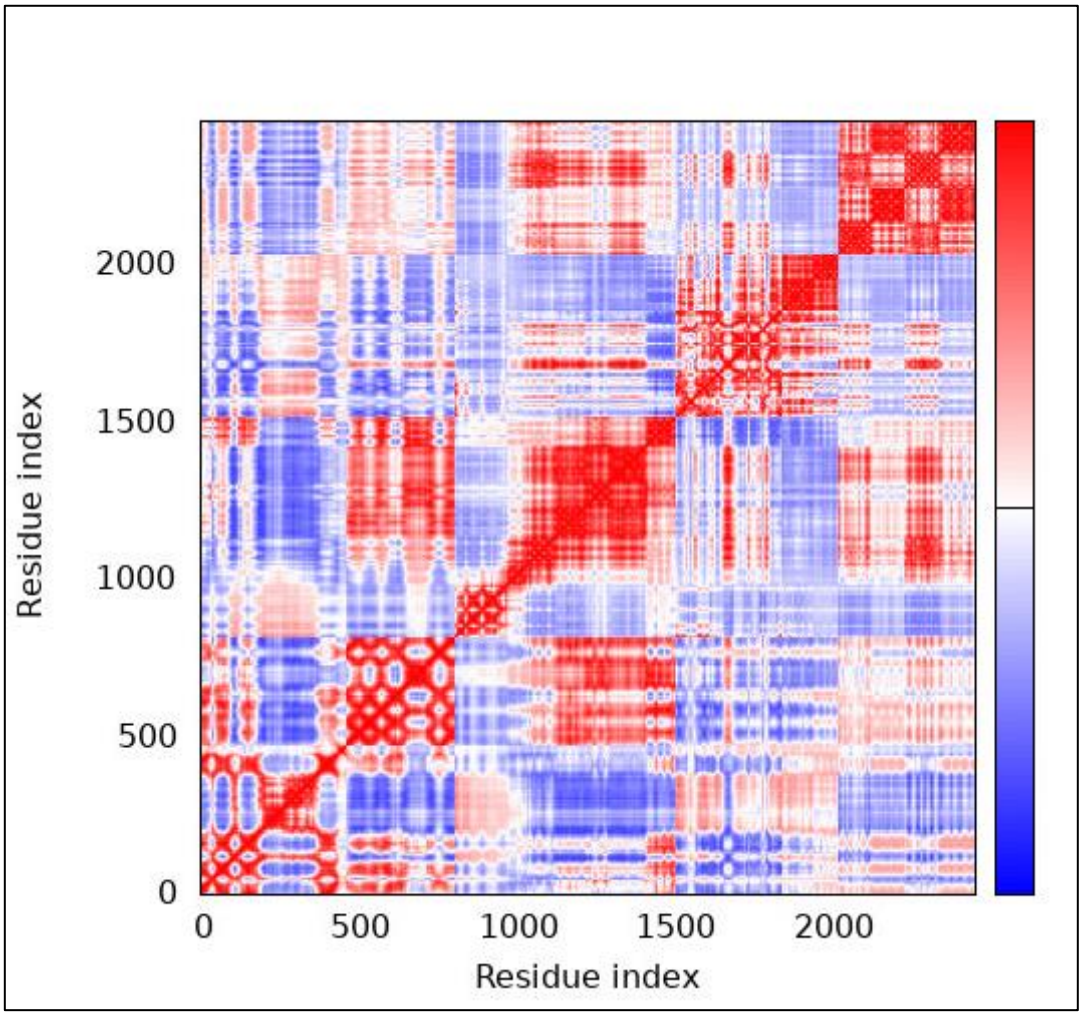


Figure 14: Covariance matrix depicting correlated (red), uncorrelated (white), and anti-correlated (blue) residue motions. The covariance matrix represents correlated (red), uncorrelated (white), and anti-correlated (blue) motions between residue pairs, indicating coordinated collective movements within the docked complex.

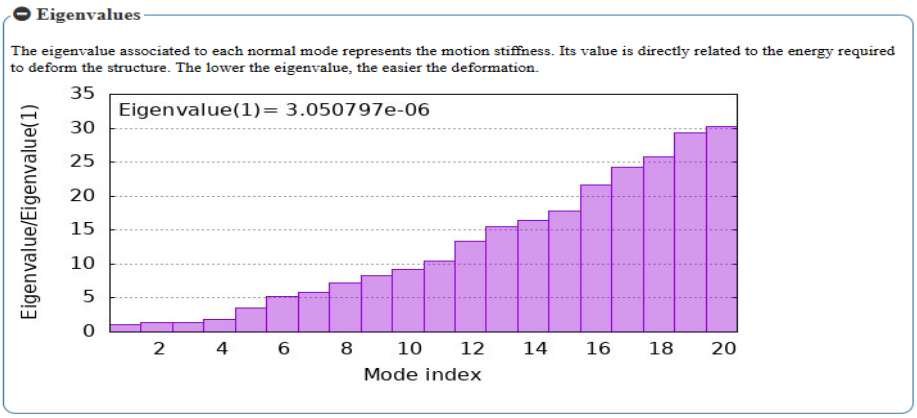


Figure 15: Eigenvalue distribution showing the relative stiffness of the structure, with a low eigenvalue indicating ease of deformation. The eigenvalue distribution of the first 20 normal modes is

shown; low eigenvalues indicate reduced energetic requirements for structural deformation and indicate intrinsic flexibility of the complex.

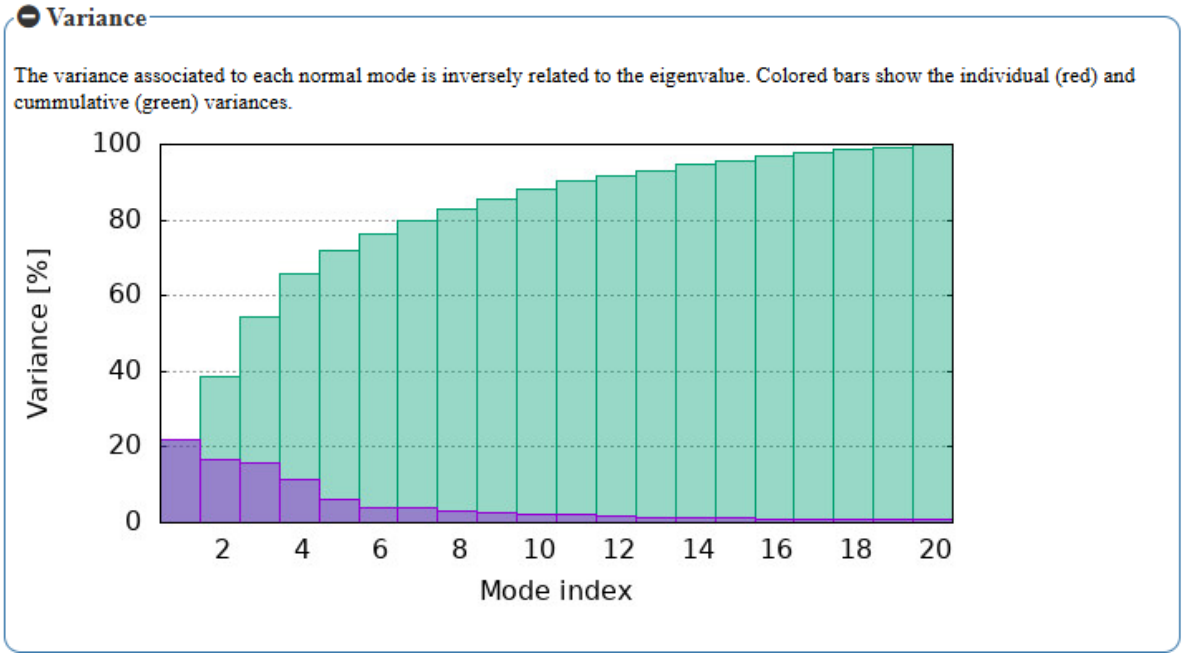


Figure 16:) Variance plot illustrating individual (purple) and cumulative (green) contributions of normal modes Individual (purple) and cumulative (green) variances associated with normal modes are depicted, demonstrating that the first few low-frequency modes contribute predominantly to the overall motion of the complex.

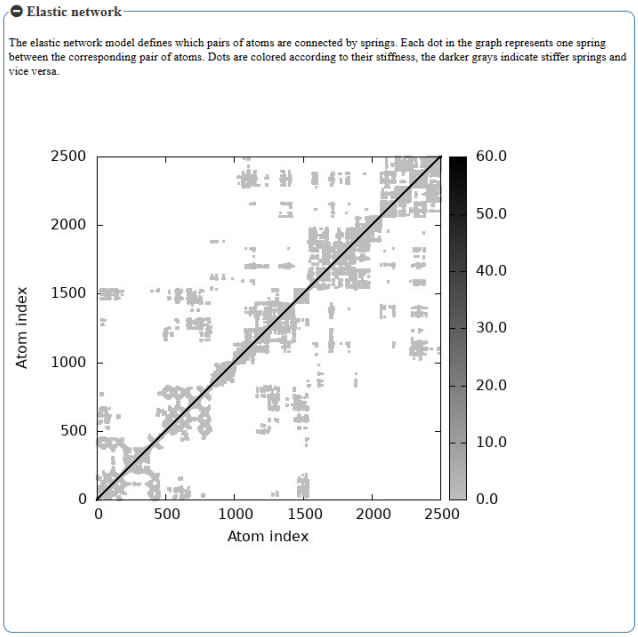


Figure 17: Elastic network model representing inter-residue stiffness, where darker regions indicate stronger interactions. The elastic network model illustrates inter-residue connectivity and stiffness, with darker regions indicating stiffer interactions, reflecting a stable structural framework of the docked complex.

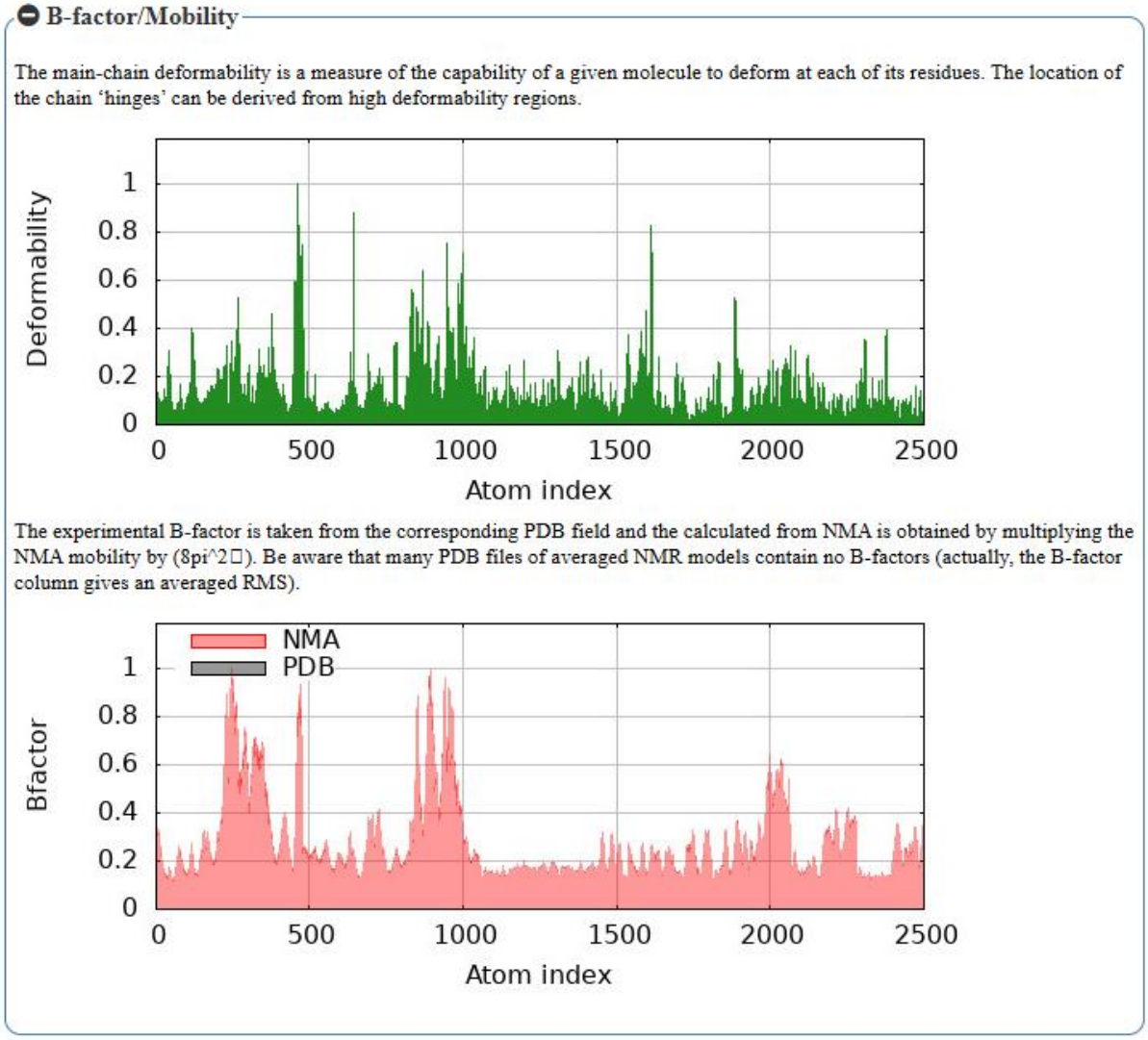


Figure 18: Deformability and B-factor profiles indicating flexible and rigid regions of the complex. Deformability and NMA-derived B-factor profiles indicate localised flexibility primarily in loop and linker regions, while core interaction regions exhibit reduced mobility, supporting structural stability of the complex.

To further evaluate the structural stability and dynamic behaviour of the docked vaccine–receptor complex, normal mode analysis (NMA) was performed using the iMODS server. NMA provides insight into the collective motions of protein complexes and is commonly employed to assess structural flexibility and stability following docking.

The eigenvalue associated with the first normal mode was calculated to be 4.69×10^{-7}, indicating low energetic requirements for deformation. Such a low eigenvalue suggests that the docked complex can undergo conformational adjustments without compromising overall structural integrity, a desirable property for biologically relevant protein–protein interactions.

The deformability and B-factor profiles revealed limited regions of higher flexibility, primarily in loop and linker segments, whereas the majority of the complex exhibited relatively low mobility. This distribution indicates a stable core architecture with localised flexibility, which may facilitate adaptive binding while maintaining structural stability.

Covariance analysis revealed correlated and anticorrelated residue motions, reflecting coordinated

collective movements within the complex. These correlated motions suggest effective communication between interacting regions of the vaccine construct and the receptor, supporting stable interface dynamics.

The elastic network model further confirmed structural robustness, with dense networks of stiffer springs observed across the core regions of the complex. Collectively, the NMA results indicate that the docked vaccine–receptor complex exhibits favourable dynamic stability with controlled flexibility, supporting the reliability of the predicted interactions and complementing the molecular docking results.

3.3 Expression Analyses (Jcat)

Parameter	Value
Expression host	*Escherichia coli* (K12)
Codon Adaptation Index (CAI)	**1.00**
GC content (%)	**53.26**
Host GC content (%)	50.73
Protein length (aa)	~700

Figure 19: Showing Expression analyses parameters for the vaccine with respective values, expressed in E.coli as host

Codon optimization was performed using the JCat server for *E. coli* K12. A CAI value of 1.00 indicates optimal codon usage compatibility, while the GC content lies within the preferred range for efficient bacterial transcription and translation.

3.4 Codon optimization and in silico cloning

To evaluate the feasibility of heterologous expression, the amino acid sequence of the designed multi-epitope vaccine construct was codon optimised for expression in *Escherichia coli* K12 using the JCat server. Codon optimisation resulted in a Codon Adaptation Index (CAI) of 1.00, indicating optimal compatibility with the host translational machinery.

The GC content of the optimised nucleotide sequence was 53.26%, closely matching the genomic GC content of *E. coli* K12 (~50.73%) and falling within the recommended range for stable transcription and efficient translation. Importantly, codon optimisation did not alter the amino acid sequence of the vaccine construct, thereby preserving its designed structural and immunological features.

These results collectively suggest that the optimised vaccine gene possesses favourable expression characteristics in the selected bacterial host and is suitable for downstream cloning and experimental validation.

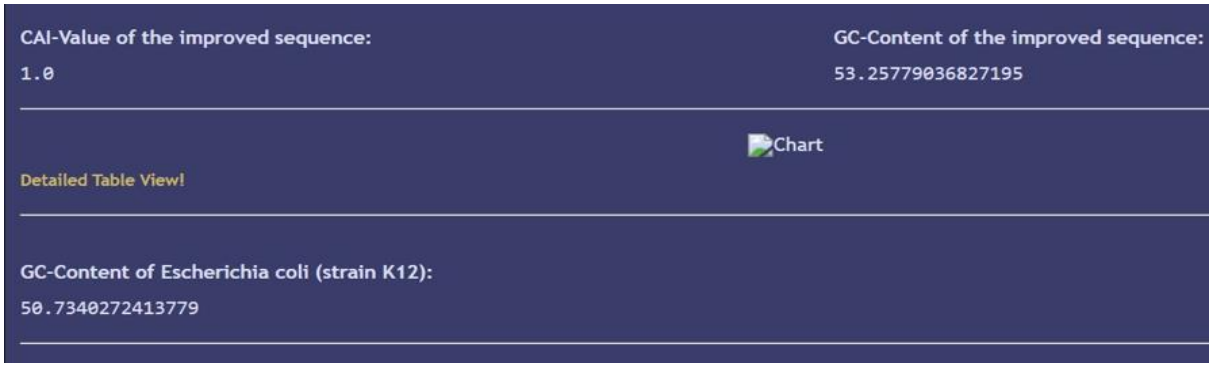


Figure 20. Codon optimization summary of the vaccine construct using JCat

The figure displays the Codon Adaptation Index (CAI) and GC content of the codon-optimized vaccine sequence for expression in Escherichia coli K12, indicating optimal codon usage and balanced nucleotide composition.

3.5 In silico plasmid construction

Following codon optimization, in silico plasmid construction was performed to assess the feasibility of heterologous expression of the designed multi-epitope HIV vaccine construct. The codon-optimised nucleotide sequence was virtually cloned into the pET-28a(+) expression vector, a widely used bacterial expression system for high-level protein production in *Escherichia coli*.

The recombinant plasmid architecture included essential functional elements such as the T7 promoter for transcriptional control, a 6×His tag to facilitate affinity-based purification, and a kanamycin resistance (KanR) gene for selection of transformed cells. The optimised vaccine gene was positioned downstream of the T7 promoter in the correct reading frame, ensuring proper translational initiation and elongation. The presence of a compatible origin of replication (Ori) supports stable plasmid maintenance within the bacterial host.

In silico cloning confirmed the correct orientation and seamless integration of the vaccine gene without introducing frame shifts or premature stop codons. The incorporation of the His-tag enables efficient purification using nickel-affinity chromatography, while the antibiotic resistance marker ensures reliable selection. Overall, the virtual plasmid construction validates the structural and translational feasibility of expressing the optimised vaccine construct in *E. coli*, supporting its suitability for downstream experimental validation.

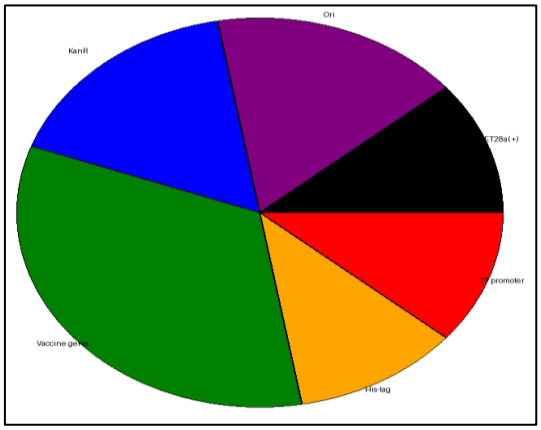


Figure 21. In silico plasmid map of the codon-optimised vaccine construct

Schematic representation of the recombinant pET-28a(+) plasmid showing the insertion of the codon-optimised multi-epitope vaccine gene under the control of the T7 promoter. Key vector elements, including the His-tag, kanamycin resistance gene (KanR), and origin of replication (Ori), are indicated.

4. CONCLUSION

The findings of this study reinforce the potential of using a computationally designed multi-epitope vaccine candidate for targeting HIV envelope proteins. The favourable protein–protein docking interactions with primary host receptors, such as CD4, and chemokine co-receptors, CCR5 and CXCR4, highlight the strategic advantage of targeting these critical pathways to disrupt HIV entry (Liu et al., 2017). Furthermore, the normal mode analysis results confirmed the structural stability of the vaccine construct,

indicating a robust architecture suitable for immunogenic responses (Zhang et al., 2018).
The successful codon optimisation suggests that the vaccine can be efficiently expressed in *Escherichia coli*, which is crucial for large-scale production and facilitates downstream applications (Doytchinova & Flower, 2007). Together, the conducted in silico analyses present a compelling case for advancing this multi-epitope vaccine candidate into experimental validation phases. The adoption of immunoinformatics in vaccine development not only accelerates the identification of effective immunogenic targets but also enhances the feasibility of innovative vaccine strategies against highly variable pathogens such as HIV (Khan et al., 2020; Rojas et al., 2021). Thus, our work lays the groundwork for future studies aiming to combat HIV threats effectively through targeted, computationally designed approaches.

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