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Computational Design and Immune Profiling of a Multi-Epitope Subunit Vaccine Targeting the PAC Adhesin of *Streptococcus mutans*

Mahnoor Qaiser*
Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore, Pakistan
Sara Yasmin
Institute of Biochemistry and Biotechnology, University of veterinary and Animal Sciences Lahore, Lahore, Pakistan
Naila Ashraf
Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore, Pakistan
Amna Khalid
Department of Biological Science, Superior University, Lahore, Pakistan
Aqsa Sajid
Institute of Pure and Applied Biology, Bahauddin Zakaria University Multan, Multan, Pakistan
Hafiza Syeda Yusra Tirmizi
Department Of Biomedical Engineering, Hamdard University, Karachi, Pakistan
Azeem Sharif
Institute of Microbiology, University of Veterinary and Animal Sciences, Lahore, Pakistan

Author Details

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Corresponding E-mail & Author*:
Mahnoor Qaiser
Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore, Pakistan
Email: mhnrrao22@gmail.com

Abstract

Streptococci mutans colonization and capacity to form biofilms has been major in the propagation of dental caries, which is one of the most common infectious diseases in the world. A significant pathogenic factor in bacterial adhesion to tooth enamel is the major surface adhesin PAc (antigen I/II), which is a significant therapeutic target of preventive intervention. A multi-epitope subunit vaccine against the PAc protein was constructed computationally in this study by means of an integrated workflow of immunoinformatic. The IEDB platform was used to predict antigenic, non-allergenic, and non-toxic T-cell epitopes and further filtered them based on immunogenicity, binding affinity by MHC, and conservation. The immunologically relevant linkers were used to assemble selected epitopes into a chimeric construct, which was then paired with human beta defensin as an adjuvant. ProtParam and Protein-Sol were used to analyze the physicochemical properties of the constructed vaccine, and the secondary and tertiary structures were analyzed with PSIPRED and AlphaFold and stereochemical validation was done. Molecular docking and molecular dynamics studies revealed that the construct was able to interact with immune receptors in a stable manner with good binding energies. Analysis of population coverage showed that it could be widely used globally and immune simulation showed robust humoral

and cellular responses, including the formation of memory cells. In general, the computational workflow facilitates the possibility of the constructed construct as a safe and immunogenic vaccine against *S. mutans*, which is worth future experimental verification.

Introduction

Streptococcus mutans is a significant etiological pathogen of dental caries since it is among the most widespread infectious diseases of children and adults worldwide. Regardless of the oral hygiene, fluoridation and public health awareness, the burden of dental caries is high in the whole world especially among the undermanned population (1,2). The capabilities to adhere to the tooth enamel surface, develop biofilm, carbohydrate metabolism, and decreased acidic byproducts favor disease development (3,4). The cell-surface adhesin PAc (sometimes also known as antigen I/II or SpaP) is one of the many virulence factors involved in initial binding to salivary glycoproteins, biofilm development and long-term colonization of the oral cavity (3,5).

PAc is a highly immunogenic and conserved protein that would be a desirable vaccine target in the prevention of *S. mutans* colonization and eventual cariogenic activity (6). In any case, however, the conventional vaccine methods, including whole-cell vaccines and purified protein vaccines, have their own inherent limitations, such as safety issues, inconsistency in immunogenicity, and cross-reactivity with human antigens. These issues highlight the necessity of the next-generation vaccines which should be safe, accurate and able to result in broadly protective immune responses (7,8).

Multi-epitope subunit vaccines have also become a promising approach which takes advantage of immunogenic peptide fragments of choice other than whole proteins or pathogens. Current developments in immunoinformatic have made it possible to screen epitopes in large amounts and predict antigenic and structural behavior as well as to perform computational evaluation of immune responses. The method can significantly lower the burden of the experiment and speed up the discovery of vaccines, especially in organisms whose genomes are well-characterized (9,10).

We developed a multi-epitope vaccine candidate against PAc adhesin of *S. mutans* using a complete computational framework in this study. T-cell epitopes were predicted as antigenic, non-toxic and non-allergenic and inserted into a recombinant construct which was complemented with immunostimulatory adjuvant and molecular linkers. We also compared the physicochemical characteristics of the construct, simulated and solved its tertiary structure, measured molecular interactions with immune receptors and simulated immune responses in silico. Coverage analysis on the population also was done so as to achieve global applicability.

Together, this work introduces a rationally designed vaccine construct that has a good predicted immunogenicity, extensive HLA binding ability, and an advantageous safety and biochemical profile. These results provide a groundwork on the basis of future laboratory validation and preclinical development of novel anti-caries vaccine.

Methodology

Retrieval of Target Proteins

Proteomic sequences of the selected viral strains were retrieved in FASTA format from the UniProt database (https://www.uniprot.org). An extensive repository of knowledge regarding the structure and functions of proteins. Only complete and validated sequences were considered in order to maximize accuracy and reliability for subsequent epitope prediction and vaccine formulation evaluations (11).

Selection of Target Proteins

Candidate proteins were selected based on antigenicity, allergenicity, and toxicity. Antigenicity was evaluated using the VaxiJen server (http://www.ddg-

pharmfac.net/vaxijen/), allergenicity was assessed via AllerTOP v2.0 (https://www.ddg-pharmfac.net/AllerTOP/), and toxicity was determined using ToxinPred (https://webs.iiitd.edu.in/raghava/toxinpred/). Transmembrane topology and subcellular localization were analyzed using the TMHMM server (https://services.healthtech.dtu.dk/services/TMHMM-2.0/) (12).

Epitope Prediction and Filtration

MHC class I, and MHC class II epitopes were predicted using the IEDB server (http://tools.iedb.org/main/tcell/). ANN 4.0 was used for MHC class I, and NN-align 2.3 for MHC class II predictions. Epitopes with low IC50 values and high predicted immunogenicity were selected (13).

Vaccine Construct Assembly

In order to preserve structural flexibility and enable effective processing, epitopes were meticulously put together into a multi-epitope vaccination design utilizing the appropriate linkers. While MHC class II epitopes were combined using the AAY linker, MHC class I epitopes were joined using the SSL linker to improve immunogenicity and epitope presentation. Human β-defensin was introduced as an adjuvant at the N-terminus via an EAAAK linker with a MHC class I epitope, which offers structural stiffness and functional isolation from nearby epitopes, in order to further enhance the immune response. A 6xHis tag was added to the C-terminus using a KK linker in order to facilitate downstream expression and purification. Additionally, a VS removal signal was added to enhance build stability and streamline purification (14).

Immunological and Biochemical Profiling of Vaccine Construct

The VaxiJen server was used to evaluate the construct's antigenicity in order to confirm its ability to elicit an immune response. Allergenicity was evaluated using AllerTOP v2.0, which classifies proteins as either allergic or non-allergenic using auto cross-covariance analysis. ToxinPred was used to evaluate toxicity and make sure there were no harmful themes in the design. Protein solubility was predicted using Protein-Sol (https://protein-sol.manchester.ac.uk/), which estimates solubility from amino acid composition to evaluate expression feasibility (15).

Physicochemical Properties

The ProtParam tool (https://web.expasy.org/protparam/) was used to compute the physicochemical properties of the designed vaccine construct. The construct's stability, solubility, and suitability for experimental expression and purification were predicted by parameters such as molecular weight, theoretical isoelectric point (pI), instability index, aliphatic index, and extinction coefficient (16).

Population Coverage Analysis

The population coverage of the predicted T-cell epitopes was assessed using the IEDB Population Coverage tool (http://tools.iedb.org/population/). This method determines the proportion of individuals who should respond to a certain set of epitopes based on the distribution of HLA alleles in different ethnic and geographic groups (17).

Secondary Structure Prediction

Secondary structure prediction of the vaccine construct was performed using the PSIPRED server (http://bioinf.cs.ucl.ac.uk/psipred/). This tool divides residues into random coils, beta-strands, and alpha-helices using position-specific scoring matrices that are obtained from sequence alignments. Assessing the protein's stability and possible immunogenicity requires an understanding of its structural arrangement and folding pattern. The secondary structural profile that was produced clarified this (18).

3D Structure Prediction

The tertiary (3D) structure of the vaccine construct was modeled using AlphaFold 3 (https://alphafoldserver.com/), It predicts protein structures using inter-residue distances and folding patterns based on deep learning. With high confidence, this approach generated a three-dimensional model that provided a structural framework for other analyses like stability assessment, molecular docking, and epitope accessibility (19).

Structural Validation

PROCHECK and the SAVES server v6.0 were used to structurally validate the modeled vaccine design. While ERRAT scores were computed to evaluate the overall model dependability and detect any structural flaws, Ramachandran plots were created to examine the stereochemical quality of the protein backbone. These analyses verified that the predicted three-dimensional structure was accurately represented and that it was suitable for further docking and molecular dynamics research (20).

Molecular Docking

Molecular docking of the vaccine construct with relevant immune receptors was performed using ClusPro 2.0 (https://cluspro.org/tut_dock.php). Interaction energies, cluster scores, and the number and kind of hydrogen bonds formed were used to evaluate the various binding conformations that the docking process produced. This study shed light on the vaccination's longevity, binding affinity, and capacity to activate immune receptors (21).

Interaction Analysis

Interaction analysis of the docked vaccine–receptor complexes was performed using LigPlot+ (https://www.ebi.ac.uk/thornton-srv/software/LigPlus/). The intricate two-dimensional schematics produced by this program provided a clear illustration of the chemical contacts required for immune activation and receptor recognition, including hydrogen bond networks, hydrophobic interactions, and important interacting residues (22).

Codon Optimization and In-Silico Cloning

The amino acid sequence of the vaccine construct was reverse-translated into a nucleotide sequence using EMBOSS Backtranseq. Codon usage was optimized for efficient expression in Escherichia coli using the online Codon Optimization tool (https://www.novoprolabs.com/tools/codon-optimization), ensuring high translational efficiency and stability. The optimized gene sequence was subsequently inserted in silico into the pET28a(+) expression vector using SnapGene software, facilitating downstream cloning, expression, and purification studies (23).

Immune Simulation

The immune response elicited by the vaccine construct was simulated using the C-ImmSim server (https://kraken.iac.rm.cnr.it/C-IMMSIM/index.php). The simulation was used to model the dynamics of B-cell activation, CD4+ and CD8+ T-cell responses, immunoglobulin production, and cytokine secretion, including IL-2 and IFN-γ. Long-term immunological memory development was also evaluated. Comparative simulations with and without the adjuvant were used to assess its impact on immune activation, which provided insight into the potential durability and effectiveness of the vaccine (24).

Results

Retrieval of Target Proteins

The analysis identified the protein Major cell-surface adhesin PAc (UniProt ID**:** P11657; Gene: pac**;** Organism: Streptococcus mutans, Taxonomy ID: 1309). The entry is annotated as protein existence level 1 (PE = 1)**,** corresponding to sequence version 1 (SV = 1). The predicted three-dimensional structure of the PAc protein is presented in Figure 1**,** providing a structural foundation for subsequent computational analyses.

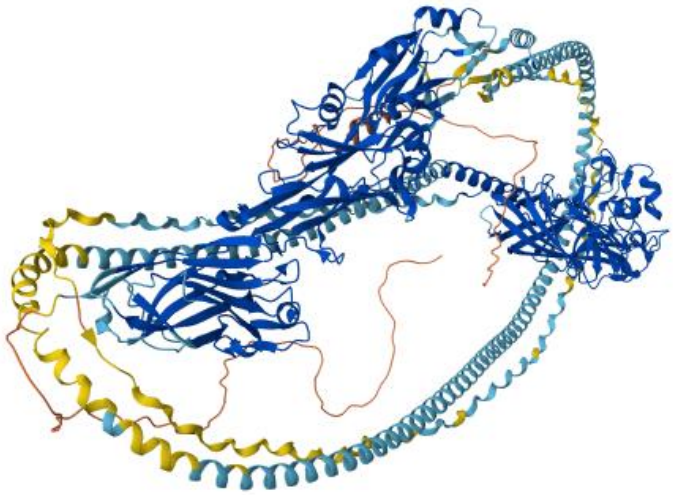

Figure 1. Predicted three-dimensional structure of the selected viral protein

Selection of Target Proteins

VaxiJen analysis assigned the protein a high antigenicity score of 0.6656, strongly suggesting its potential to elicit an immune response. Toxicity and allergenicity screening further confirmed that the protein is non-toxic and non-allergenic, reinforcing its favorable safety profile. Topology prediction using TMHMM 2.0 revealed a single transmembrane helix spanning residues 1541–1560, with the N-terminal region (residues 1–1540) located on the extracellular side**,** and a short C-terminal segment (residues 1561–1565) positioned on the cytoplasmic side**.** This topology highlights substantial extracellular accessibility, making the protein a promising candidate for epitope-based vaccine design (Figure 2).

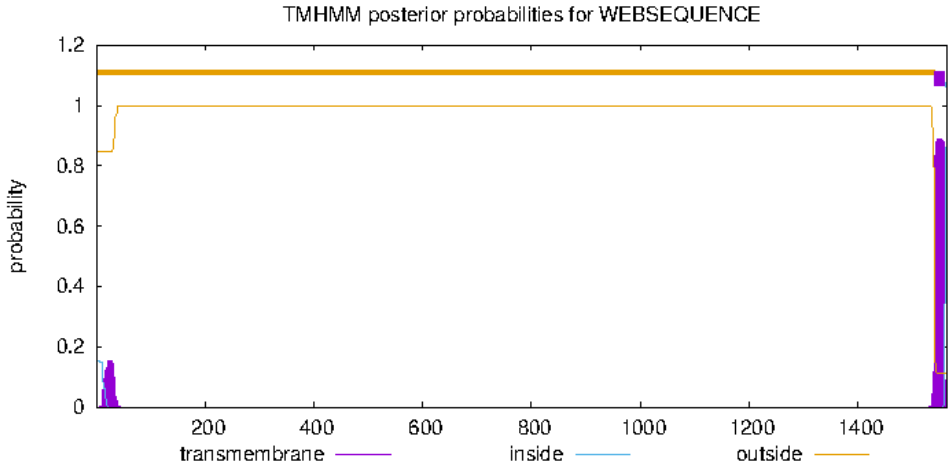

Figure 2. TMHMM prediction of the selected protein showing that all residues located in the extracellular region with transmembrane helices, indicating full surface accessibility for epitope targeting

Prediction of MHC Class I Epitopes

Based on the binding affinity (IC50 value), percentile rank, and immunogenicity, two epitopes produced by MHC class I were chosen. VaxiJen, AllerTOP, and ToxinPred all verified the epitopes and reported that they were non-toxic and antigenic. The epitopes are these, which were anticipated to carry a high affinity to various HLA alleles, as illustrated in Table 2.

Table 2: **Selected MHC Class I Epitopes**

Protein No.	Start	End	Epitope	Length	Netmhcpan_el percentile
1	373	382	ADLPAGRDET	10	17
8	1068	1078	FDVTYDNATN	10	14.28
2	669	680	FIKNEFTFYH	10	39.06

Prediction of MHC Class II Epitopes

The choice of the two MHC class II epitopes was identified to have undergone adequate binding recognition of broad range of alleles, through the assistance of anti-genicity and low values of IC50. Epitopes were screened in the allergenicity and toxicity filters and were suitable to be used in designing the vaccine. Table 3 includes the list of the chosen epitopes.

Table 3: **Selected MHC Class II Epitopes**

Protein No.	Start	End	Epitope	Length	Netmhcpan_el percentile
1	106	117	FQSLVPDIP	12	0.52
1	191	202	TEKPLKPAPVAP	12	12
1	851	863	STVIIYKPQSTAY	13	6.3

Vaccine Construct Assembly

In order to maintain structural flexibility and guarantee effective processing, the multi-epitope vaccine construct was successfully put together utilizing particular linkers. In order to maximize epitope presentation and improve anticipated immunogenicity, MHC class I epitopes were coupled using SSL linkers, whereas MHC class II epitopes were joined using AAY linkers. Through the use of an EAAAK linker, human β-defensin was added as an adjuvant at the N-terminus, offering structural stiffness and functional isolation from nearby epitopes. To aid in downstream purification and enhance construct stability, a 6xHis tag was also inserted at the C-terminus utilizing a KK linker in conjunction with a VS removal signal (Figure 3).

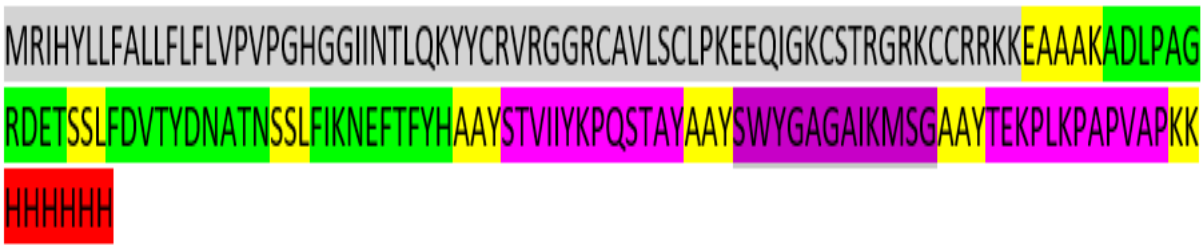

Figure 3. Schematic representation of the multi-epitope vaccine construct. **Gray** represent adjuvant; **Yellow and segments** represent linker sequences. **Green regions** indicate MHC class I epitopes. **Pink regions** represent MHC class II epitopes, and the **red segment** corresponds to the 6x-His tag

Immunological and Biochemical Profiling of Vaccine Construct

Allergenicity and toxicity predictions confirmed that the final multi-epitope vaccine construct is non-allergenic and free of toxic motifs, reinforcing its favorable safety profile. Antigenicity screening with VaxiJen yielded a score of **0.7521**, indicating strong immunogenic potential. Solubility assessment using Protein-Sol produced a predicted scaled solubility score of 0.543, suggesting moderate solubility under physiological conditions (Figure 4). Furthermore, the theoretical isoelectric point (pI) was calculated as 10.290, classifying the construct as a basic protein and supporting its compatibility for heterologous expression in E. coli.

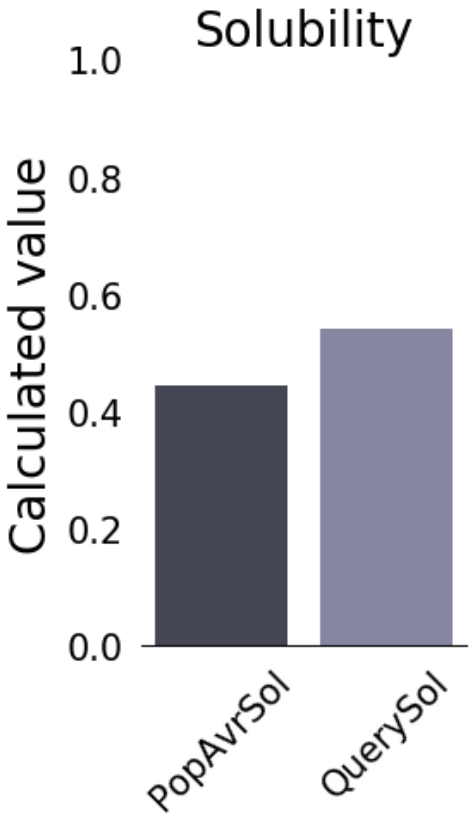

Figure 4. Predicted solubility of the vaccine construct (0.543) compared with E. coli average solubility (0.45), indicating favorable solubility for expression.

Population Coverage Analysis

The predicted epitopes were evaluated for their ability to cover diverse HLA alleles across global populations using the IEDB Population Coverage tool. The analysis demonstrated extensive worldwide coverage, with a combined population coverage of 93.63%, indicating that the majority of individuals across different regions are likely to mount an immune response. The average number of epitope hits recognized per individual was 3.92, and 90% of the population was predicted to recognize at least 2.16 **epitopes**, highlighting the construct's broad applicability and immunogenic potential (Table 4) (Figure 5).

Table 4. Predicted global population coverage of the selected epitopes using the IEDB Population Coverage tool

Population/Area	**Class Combined Coverage (%)**	**Average Hit**	**PC90**
World	93.63	3.92	2.16
Average	93.63	3.92	2.16
Standard Deviation	0.0	0.0	0.0

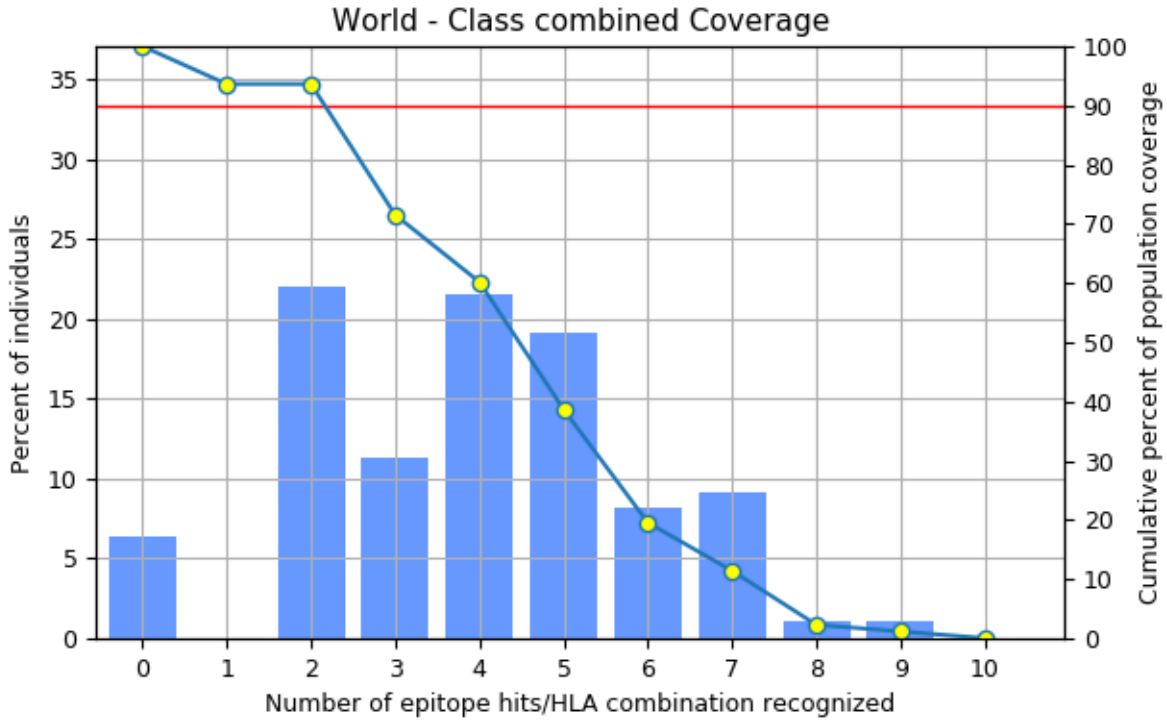

Figure 5. Global population coverage analysis of the predicted epitopes using the IEDB Population Coverage tool, showing 93.63%% combined coverage with an average of 3.92 epitope hits per individual and a PC90 value of 2.16.

Physicochemical Properties

The designed multi-epitope vaccine construct comprised 162 amino acids with a molecular weight of 18,123.99 Da and a theoretical isoelectric point (pI) of 9.60, indicating an overall basic nature. The sequence contained 10 negatively charged residues (Asp + Glu) and 23 positively charged residues (Arg + Lys), confirming a predominance of basic amino acids. The atomic composition of the construct was determined as $C_{821}H_{1269}N_{229}O_{220}S_8$, corresponding to a total of 2,547 atoms. Extinction coefficient analysis at 280 nm predicted values of 22,265 $M^{-1}cm^{-1}$ (absorbance 1.228 at 0.1% concentration) under oxidizing conditions, assuming cystine bond formation, and 21,890 $M^{-1}cm^{-1}$ (absorbance 1.208) under reducing conditions. These results suggest a strong light-absorbing capacity of the protein. The predicted half-life of the construct was 30 hours in mammalian reticulocytes (in vitro), >20 hours in yeast (in vivo), and >10 hours in E. coli (in vivo), indicating a stable expression profile in diverse host systems. The instability index (47.66) classified the construct as unstable; however, the aliphatic index (74.81) reflected acceptable thermostability. Moreover, the GRAVY score (–0.273) indicated an overall hydrophilic nature, suggesting good solubility and compatibility under physiological conditions.

Secondary Structure Prediction

The secondary structure study of the planned vaccine construct revealed a well-organized array of alpha-helices, beta-strands, and coils. Helical areas (highlighted in pink) were found throughout the sequence, primarily at the N-terminus and core portion of the protein. Beta-strands (highlighted in yellow) were placed between helices, which increased the construct's overall stability. The remaining areas were made up of random coils (gray), which provided flexibility while also assuring optimal epitope exposure. Several residues were projected to be extracellular (orange), demonstrating surface accessibility for immune detection, and no transmembrane helices were found, indicating that the design is suitable for recombinant production.

The confidence scores linked with the secondary structure predictions suggested that the modeled conformations were highly reliable, as shown in Figure 6.

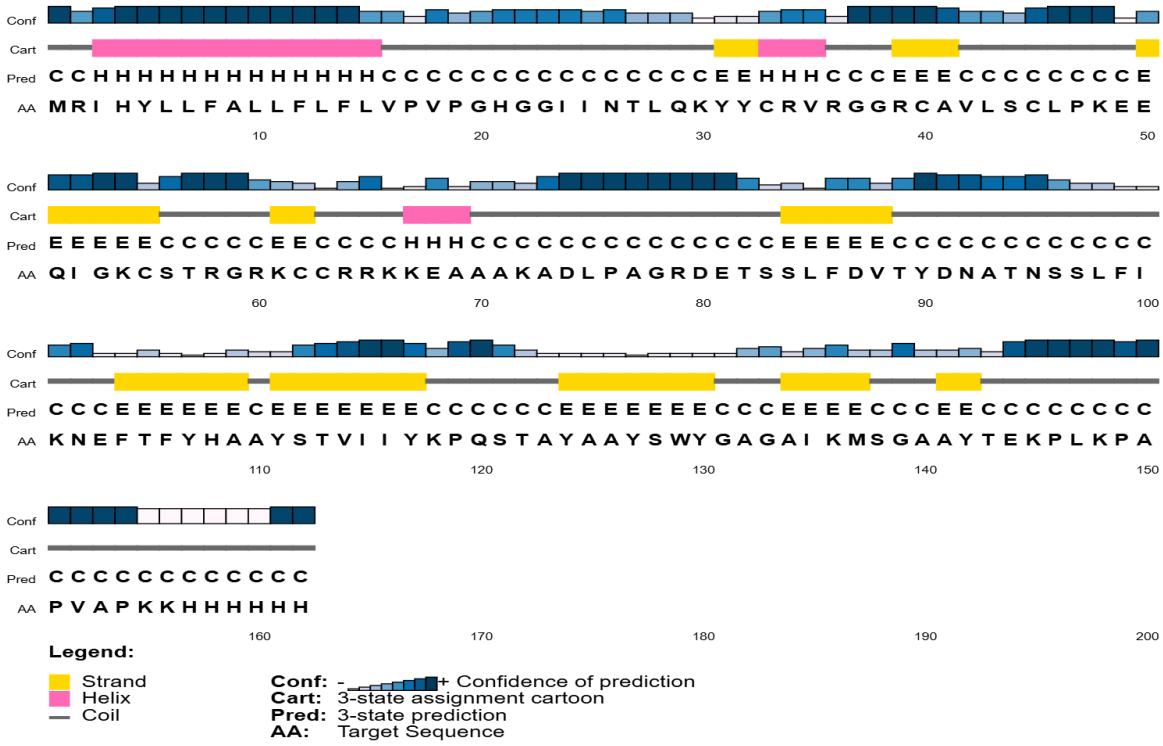

Figure 6. Secondary structure prediction of the target protein generated using PSIPRED. The diagram illustrates predicted α-helices, β-strands, and coil regions along the amino acid sequence, providing insights into the structural organization and potential functional domains.

3D Structure Prediction

AlphaFold 3 was used to model the three-dimensional structure of the planned multi-epitope vaccine construct, which showed a compact and well-organized conformation. Alpha-helices and beta-strands were among the unique secondary structural elements seen in the anticipated structure, with surface-exposed areas matching the chosen epitopes. As seen in Figure 7, this structural configuration facilitates effective epitope accessibility, which is essential for immune recognition, and serves as a basis for further docking and interaction research.

Figure 7. Predicted 3D structure of the designed multi-epitope vaccine construct

Structural Validation

The stereochemical quality of the modeled vaccine construct was evaluated through both 2D and 3D Ramachandran plot analyses. The stereochemical quality of the modeled vaccine construct was validated using both Ramachandran plot and ERRAT analyses. Out of a total of 162 residues**,** 122 residues (84.1%) were located in the most favored regions, while 23 residues (15.9%) occupied the additionally allowed regions**.** Notably, no residues were found in the generously allowed or disallowed regions**,** confirming that the backbone dihedral angles are consistent with acceptable stereochemical geometry. The model comprised 145 non-glycine and non-proline residues, along with 19 glycine and 12 proline residues**,** supporting its structural accuracy. The overall ERRAT score of 88.89 further validated the high reliability and quality of the modeled construct (Figure 8) confirming that the backbone dihedral angles were highly consistent with ideal stereochemical conformations.

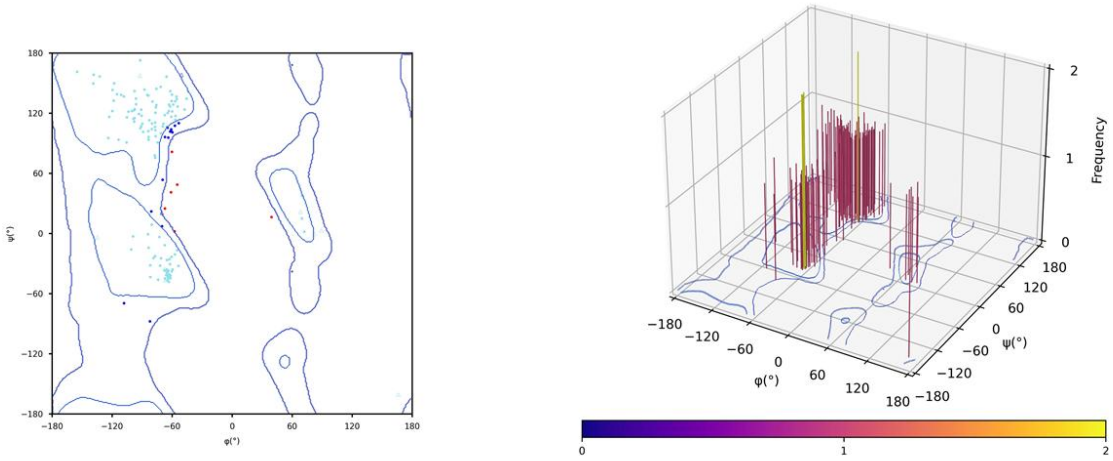

Figure 8. 2D and 3D Ramachandran plot analysis of the modeled vaccine construct

Docking and Interaction Analysis

Docking of the vaccine construct with the receptor yielded a highly favorable binding energy score of –1268 kcal/mol**,** indicating strong binding affinity and structural stability of the complex. The LigPlot+ analysis (Figure 9) highlighted key interactions at the binding interface. Specifically, hydrogen bonds (green dashed lines) were observed between Lys125 of the receptor and residues of the vaccine construct, while hydrophobic contacts (red arcs) involved Leu3, Phe14, and Val16, supporting the stability of the complex.

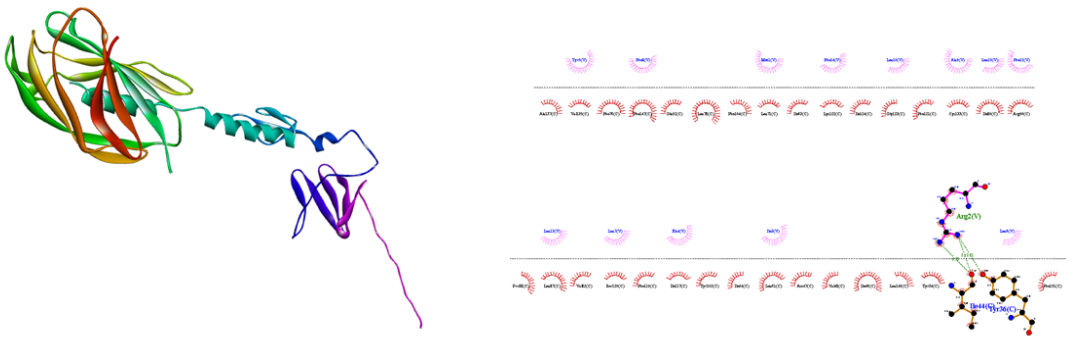

Figure 9. (A) Three-dimensional representation of the protein–ligand docking complex showing the ligand bound within the active site of the receptor. (B) Two-dimensional interaction analysis (LigPlot+) illustrating the hydrogen bonds and hydrophobic contacts between the ligand and key receptor residues.

Codon Optimization and In-Silico Cloning

The sequence of the designed vaccine construct was optimized to enhance its expression efficiency in a prokaryotic host system. the Codon Adaptation Index (CAI) increased from 0.59 before optimization to 0.82 after optimization, indicating strong compatibility with the codon usage preferences of E. coli and suggesting an enhanced potential for efficient translation. Simultaneously, the GC content decreased from 65.23% to 54.12%, placing the construct within the optimal range for bacterial expression and reducing the likelihood of transcriptional inefficiency or mRNA instability. The optimized gene was predicted to encode a polypeptide of 162 amino acids with an estimated molecular weight of 18,107.73 Da**,** a size considered suitable for soluble expression and downstream purification in a prokaryotic system.The optimized gene was subsequently inserted into the pET28a(+) expression vector, confirming its preparedness for downstream experimental validation (Figure 10).

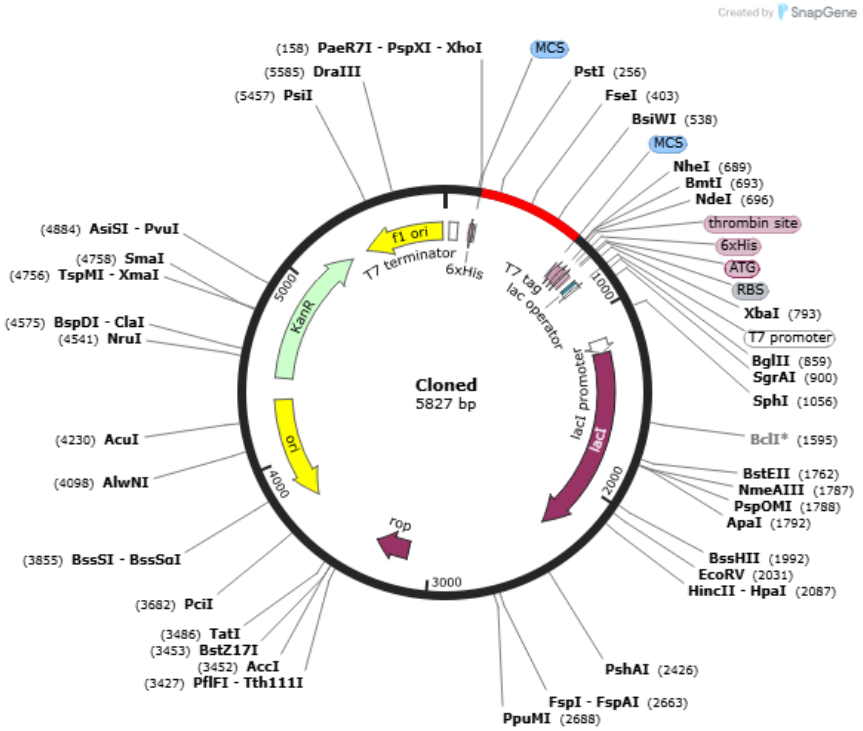

Figure 10. In-silico cloning of the optimized vaccine gene into the E. coli expression vector pET28a (+). The construct is shown inserted in the correct orientation and reading frame, confirming its suitability for downstream expression and experimental validation.

Immune Simulation

Additional evidence of the vaccine efficacy is the humoral and cytokine profiles that were illustrated in Figures 11. The humoral arm had progressive increases in immunoglobulin titers with a higher level of IgM during the initial reaction followed by a class-switch to IgG isotypes, which are visuals of affinity maturation and develops long-term B-cell responsesThe formation of immune complexes coincided with viral clearance and demonstrated functional antibody-mediated neutralization. Cytokine indicators have shown a short-lived but significant increase in levels of interleukin-2 (IL-2), which is associated with clonal proliferation of T cells, as well as

the activation of other pro-inflammatory cytokines that encourage Th1 immunity. The profile of the danger signal that arrived demonstrated the successful activity of the leukocytes and the activation of the innate immune system. All of these findings suggest that the created multi-epitope vaccine architecture will likely elicit a balanced and durable immune response by combining cell-mediated, humoral, and cytokine-mediated disease pathways.

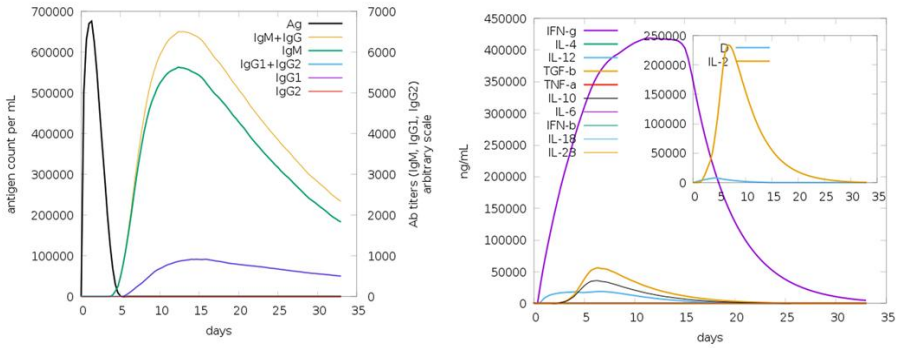

Fig 11: Humoral immune response and cytokine profiling; depicts viral particles, immunoglobulins, and immune complex formation, while cytokine and interleukin concentrations, with IL-2 and danger signals highlighted in the inset.

Figure 12 depicts the kinetics of cellular immunological responses to the vaccination design. After antigen exposure, the C-IMMSIM simulation revealed a significant increase in antigen presentation cells (APCs), which are lymphocyte subsets. Internalized antigen-bearing cells continued to process and present antigens on MHC class II molecules, whereas activated cells increased in number. The low fraction of anergic and resting cells indicated efficient immune activation and the absence of tolerance, whereas the steady percentage of mitotically active cells indicated clonal proliferation. The majority of active and MHC II-presenting populations enable effective antigen capture and presentation to T helper cells, which is required for future B and T cell activation.

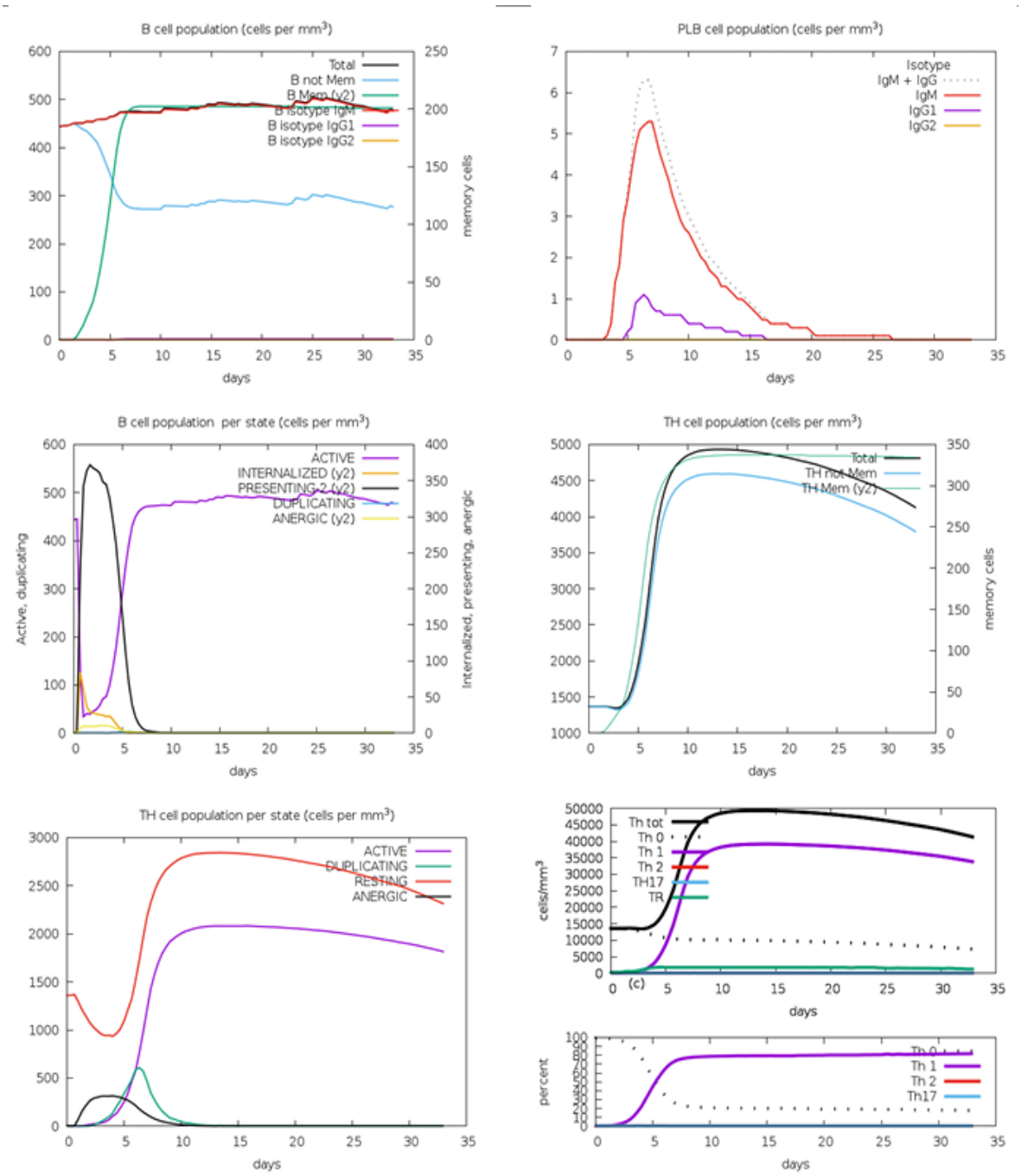

Fig 12: Dynamics of immune cell populations, including activated, internalized, MHC class II–presenting, mitotic, anergic, and resting cells, throughout the simulation.

Figure 13 depicts only sequential waves of activation and regulation, revealing more about the dynamics of immune cell states. Antigen-specific clonal proliferation was demonstrated by the increased production of activated CD4+ and CD8+ T cells, as well as the differentiation of naive lymphocytes into effector phenotypes. Homeostasis and the avoidance of hyper-activation were instances of regulatory changes seen in following cycles. The simulation's capacity to retrieve memory subsets demonstrates that the vaccine design is good at instilling long-term immunological memory, which is a critical component of protective efficacy. The balance of effector expansion and regulatory constriction demonstrates the simulated immune system's ability to respond forcefully while maintaining immunological balance.

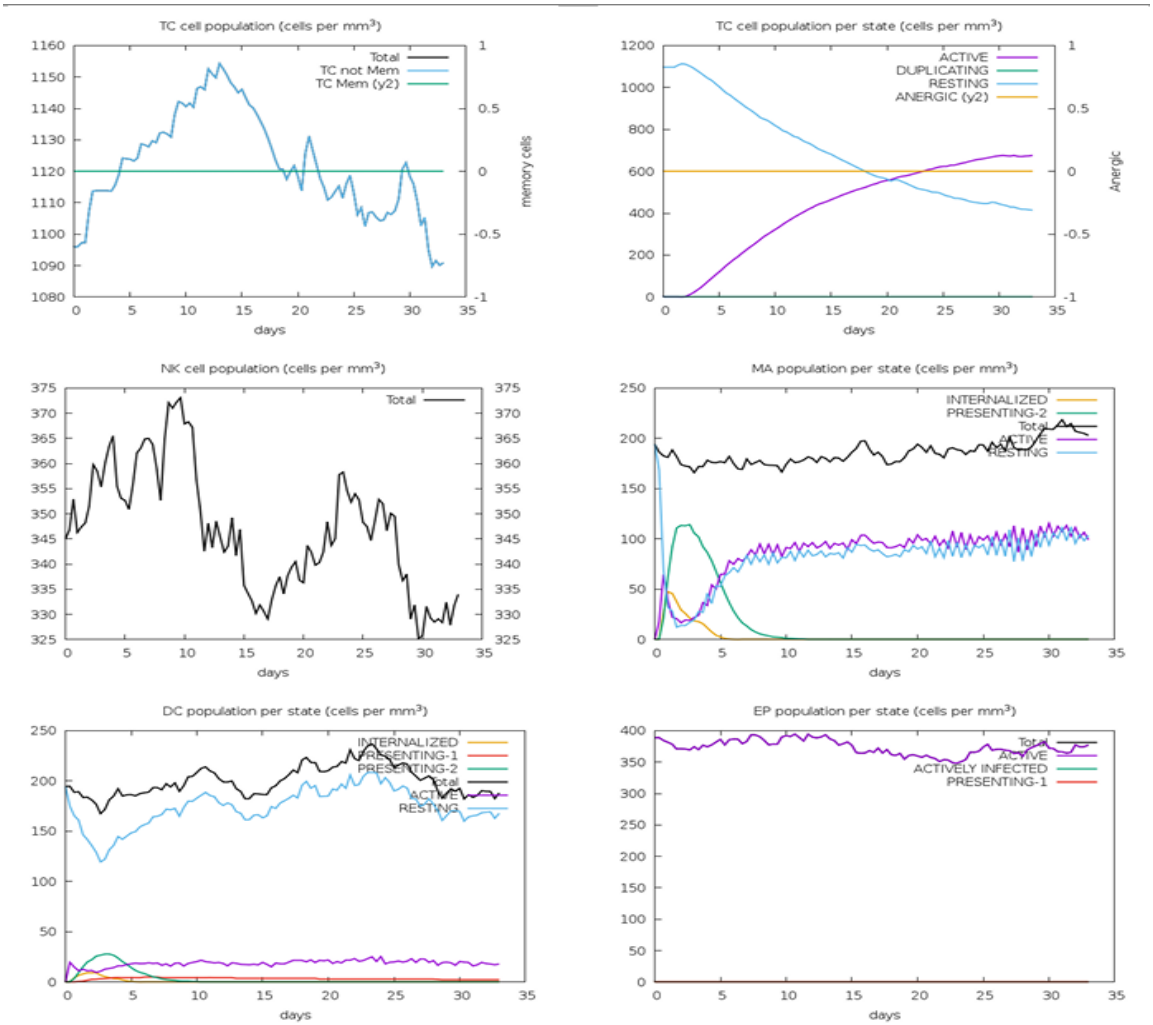

Fig 13: Changes in immune cell states over time, illustrating progression of activation and regulation during antigen exposure.

Discussion

This paper provides an in-silico platform of rational design of a multi-epitope subunit vaccine that targets the PAc (antigen I/II) adhesin of *Streptococcus mutans*, one of the primary virulence factors of bacterial adherence and early biofilm development in dental cavity (25). The workflow based on immunodominance and immunoinformatic was able to identify, screen, and assemble immunodominant epitopes with good immunogenic potential, which were substantiated by significant structural and functional evidence (26). Topology prediction confirmed the high antigenicity score (0.6656) and widespread extracellular exposure of PAc, which was the basis of the choice of this vaccine target. The lack of allergenic and toxic markers dictated the biological compatibility, which showed positive features of vaccine development. It is worth mentioning that the presence of a long extracellular domain implies wide accessibility of host immunity proteins, which adds to its utility as an accessible antigenic substrate (25).

Screening Epitope screening stage was able to specifically recognize strong T-cell stimulating domains in both MHC I and II alleles. These epitopes showed high predicted binding affinities and extensive allele coverage between several human leukocyte antigen (HLA) variants. Construct safety was further narrowed down by the successful elimination of allergenic and toxic candidates. Notably, cumulative IEDB coverage indicated that 93.63% of the world population was covered by the cumulative coverage: this means that the selected epitopes would be effective in

generating immune responses in genetically diverse populations and the construct is broadly applicable (27).

The epitopes were assembled in a chimeric vaccine into which immunomodulatory 6xHis purification tag (at the C-terminus) and immunomodulatory 5-hydrotryptophan 8 (immunomodulatory, beta-defensin) (at the N-terminus) was included to generate a stable and rationally designed construct. The linkers were critical in the separation of epitopes, maximalization of processing pathways, and enhancing antigen presentation. Physicochemical profiling showed that the protein was moderately hydrophilic with a basic character and good solubility predictions and a good half-life prediction and thermostability indices which are compatible with heterologous expression despite the fact that the index of instability is slightly above the stability threshold. These properties are typical of short recombinant vaccine Constructs and they do not rule out successful production and immunogenicity (28).

The secondary and tertiary conformation predictions also justified construct viability. A combination of α-helices, β-strands, and coils was found in an equal proportion by PSIPRED, indicating that the protein was structurally stable and had conformational flexibility to expose epitopes. The stereochemical validity based on the AlphaFold-derived 3D model exhibited excellent Ramachandran assessment and ERRAT reliability scores indicating that the protein folds correctly and is structurally fit to proceed with downstream molecular docking and simulation. Docking interactions showed effective interactions between the vaccine construct and immune receptors, which are typical of possible T-cell pathway activation. Complex stability was evaluated in detail through the use of molecular dynamics simulations. Altogether, this body of evidence suggests that the construct may be successfully recognized by the immune system and maintain stable interactions that are required to present antigens (29).

The immuno-simulation also provided evidence on the ability of the construct to induce humoral and cellular immunity. Recurrent exposure to antigen caused significant alterations in IgM, IgG subclasses, CD4+ and CD8+ activation and memory cell development. These tendencies were in line with immunological forecasts on subunit vaccines. Existence of the beta-defensin adjuvant significantly increased the release of cytokines (such as IL-2 and IFN-7) which highlights its role in enhancing immunity.

Altogether, broad HLA interaction, high antigenicity, favorable structural aspect, and high predicted immune response support the potential of this multi-epitope-based design as a possible vaccination approach against *S. mutans*. Though the computational prediction cannot replace the biological validation, high-throughput method, which is used in the given research, helps to speed up prioritizing the targets and minimizes the risk of early development. In future studies, the expression of constructs, in vitro immunogenicity analyses and preclinical dental caries model analyses will be done to verify safety, antibody levels, and protective efficacy.

Conclusion

In this study, we adopted a structure-guided immunoinformatic strategy to design a novel multi-epitope subunit vaccine targeting the PAc adhesin of *Streptococcus mutans*, a key virulence determinant in dental caries pathogenesis. B-cell and T-cell epitopes were systematically screened based on antigenicity, surface accessibility, conservancy, and non-allergenicity, allowing the rational assembly of a chimeric vaccine construct supported by appropriate adjuvant and linker elements. Molecular docking confirmed strong and stable interactions between the vaccine and human immune receptors, particularly TLR2 and TLR4, suggesting efficient immune activation. Molecular dynamics simulations further validated the structural stability of the docked complexes, exhibiting low RMSD fluctuations and consistent hydrogen-bond formation throughout simulation trajectories. Codon optimization and in silico

cloning demonstrated the feasibility of expressing the construct in E. coli K12, supporting future experimental production. Collectively, the computational findings indicate that the proposed multi-epitope vaccine is a promising candidate for preventing S. mutans colonization and biofilm establishment. While these results provide compelling theoretical support, further experimental validation including in vitro expression, immunogenicity assays, and in vivo challenge studies is essential to establish its true efficacy and translational potential.

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