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In-silico design of a multi-epitope vaccine candidate against *Strongyloides stercoralis*

Yanick Aqua Stong Tangan^{1*}, G. Takop Nchanji^{2,3}, Eric Ndumu¹¹, Abey Blessings Ayuk¹, Kongnyu Brandon Ngala¹¹, Melvis Berinyuy Dulanzev¹, Ntang Emmaculate Yaah¹, Lahngong Methodius Shinyuy^{1,4}, Cabirou Mounchili Shintouo⁵, Bernis Neneyoh Yengo⁵, Andreen Egbe Enowkpen¹¹, Mary Teke Efeti^{1,6,7}, Joan Amban Chick⁸, Ketura Yaje Gwei¹, Arnaud Azonpi Lemoge⁹, Luc Vanhamme¹⁰, Jacob Souopgui¹⁰, Stephen Mbiga Ghogomu^{1*} and Robert Adamu Shey^{1,2*}

Abstract

Background *Strongyloides stercoralis* is a soil-transmitted helminth causing strongyloidiasis, mainly affecting impoverished populations in tropical and subtropical regions. Despite its high global prevalence, affecting an estimated 613.9 million people, it remains neglected in public health initiatives. Current treatments, including albendazole and ivermectin, show variable efficacy and face potential resistance, making vaccine development critical for long-term control and achieving WHO targets.

Methods We employed an immunoinformatics approach to design a multi-epitope vaccine candidate by combining B-cell and T-cell epitopes from key L3-stage proteins involved in *S. stercoralis* infection. In addition, interactions with TLR4 were implemented to assess the vaccine's ability to elicit an innate immune response.

Results The selected proteins exhibited strong conservation, suggesting potential cross-protection across *Strongyloides* strains. The vaccine candidate was predicted to be antigenic, non-allergenic, and immunogenic, with immune simulation demonstrating robust activation of humoral and cellular immune responses. Extensive HLA coverage analysis indicated broad population applicability. Additionally, protein–protein docking and normal-mode analyses indicated efficient binding and stable interaction of the vaccine candidate with TLR4.

Conclusion This study provides a promising approach to designing multi-epitope vaccines against *S. stercoralis* designed through integrative immunoinformatics. Overall, the designed vaccine candidate demonstrated immunogenicity and safety features that warrant further experimental validation in vitro and in vivo. These findings lay important groundwork for future vaccine development aimed at improving the prevention and control of strongyloidiasis globally.

*Correspondence:

Yanick Aqua Stong Tangan
tangan.yanick@ubuea.cm
Stephen Mbiga Ghogomu
stephen.ghogomu@ubuea.cm
Robert Adamu Shey
sheynce@gmail.com

Full list of author information is available at the end of the article



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Keywords *Strongyloides stercoralis*, Multi-epitope vaccine, Immunoinformatics, Computational vaccine design, SS-MEV-1

Introduction

Strongyloides stercoralis (*S. stercoralis*) is an intestinal soil-transmitted helminth that infects people worldwide and causes higher infection rates in the tropics and subtropics, where favorable ecological conditions, low sanitary standards, and poor hygiene persist [1]. It is commonly found in sub-Saharan Africa, Southeast Asia, the southeastern United States, southern Europe, Latin America, South America, Bangladesh, and the West Indies [2, 3]. Despite its widespread occurrence of strongyloidiasis remains largely unknown due to limited surveillance [4, 5]. However, current efforts are focused on updating this outdated estimation. Buonfrate et al. have presented new estimates of *S. stercoralis* prevalence globally, suggesting that the worldwide prevalence of strongyloidiasis could affect as many as 613.9 million individuals [6]. *S. stercoralis* is an important soil-transmitted nematode, a cause of severe morbidity and mortality, but has often been neglected [7]. One in four people globally suffers from soil-transmitted helminthiasis (STH), which affects impoverished communities lacking sufficient water, sanitation facilities, and socio-economic advancement opportunities [8]. The medical community has traditionally concentrated its research on four specific STH species, which include: *A. lumbricoides*, *T. trichiura*, and the two hookworm types *Ancylostoma duodenale* and *Necator americanus*. The disease burden measurement, difficulties, and control intervention monitoring challenges have led to the exclusion of *S. stercoralis* from public health strategies despite it being a standard STH alongside the other four species [9], and it is estimated to infect 30 to 100 million people [3]. The parasite has a very complex life cycle, including direct, auto-infective, and free-living cycles. Infection, resulting in human strongyloidiasis, can occur through skin penetration of infective third-stage larvae (L3) [1]. The World Health Organization (WHO) points out that, with a lack of appropriate therapy, strongyloidiasis cannot be resolved and may persist for life [10]. Also, the infection may be severe and even life-threatening in patients with immunodeficiency [11]. However, chronically infected individuals who become immunosuppressed due to corticosteroid treatment or infection with HTLV-1 can develop hyperinfection syndrome, which can affect several organs, a condition that can be life-threatening [12, 13]. Although treatments such as albendazole or ivermectin are available for *S. stercoralis* infections, efficacy is rarely 100% [14, 15], and there is a potential tendency for resistance towards these drugs [16]. The emergence of resistance to Ivermectin for onchocerciasis treatment in

humans has been reported [17, 18]. Additionally, treating the potentially fatal hyperinfection syndrome poses significant challenges. [19]. Numerous efforts and attempts have been made to develop experimental vaccines against nematode infections using various sources of antigen, formulations, and immunization protocols, with reductions in worm burden induced by these vaccines ranging from 30 to 90% [20, 21]. Among the intestinal helminths, experimental vaccines against hookworm infections have been developed using single antigens derived from larvae [22] and administered with different adjuvants, including alum, in which there was a reduction of approximately 30% in challenge hookworm survival in animals immunized with these vaccines [23]. This relatively low level of protection, however, was sufficient to qualify one of these vaccines for clinical development and human testing [24]. There is a clear necessity for new strategies to address this disease. Designing multi-epitope vaccine candidates would be ideal to improve existing control methods. Multi-epitope vaccines (MEVs) represent an innovative approach that employs immune informatics techniques to develop more effective vaccines against infectious diseases, providing various advantages [25]. Unlike traditional vaccine approaches, which primarily rely on empirical evidence, multi-epitope vaccines present several advantages. These include the capability to select epitopes that can trigger robust cellular and humoral immune responses simultaneously, the elimination of undesirable antigen components that could lead to adverse immune reactions or side effects, and the ability to influence the immune response by incorporating specific agonists for pathogen recognition receptors [26]. Developing an effective multi-epitope vaccine primarily depends on selecting appropriate candidate antigens and their immune-dominant epitopes. Multi-epitope vaccines, capable of activating both B-cell and T-cell responses, offer a promising strategy for attaining long-lasting immunity against pathogens [27]. Beyond their application in infectious diseases, multi-epitope vaccines hold promise for personalized therapy in cancer patients. The effectiveness of multi-epitope vaccines is clear across various pathogens, showing promising outcomes against bacteria like *M. tuberculosis* [28], *Helicobacter pylori* [29], and SARS-CoV [30]. Immunoinformatics pipelines using combined B- and T-cell epitope prediction, structural modeling, docking, and immune simulations have been successfully applied in numerous recent studies across diverse pathogens, supporting the general utility of such computational approaches for vaccine design [31]

This study aims to develop a multi-epitope vaccine candidate against *S. stercoralis* by combining several B-cell and T-cell epitopes from key L3 stage proteins obtained from UniProtKB, along with a TLR4 adjuvant and PADRE and TAT peptides. This approach is designed to enhance antigenicity and immunogenicity, with the goal of eliciting robust humoral and cellular immune responses against *S. stercoralis*. The study also seeks to provide a promising strategy for developing a robust and more efficient protection against *S. stercoralis* and to lay the groundwork for future experimental validation of the vaccine's efficacy and safety.

Material and methods

Protein sequence retrieval

The amino acid sequences of 9 proteins (accession numbers: A0A0K0E6J0, A0A0K0DY51, A0A0K0ECK4, A0A0K0EG68, A0A0K0EMX1, A0A0K0E2F4, A0A0K0DTP5, A0A0K0ELA9, and A0A0K0EKU6) were retrieved from the UniProtKB database (<https://www.uniprot.org/>) (accessed on 29 October 2024) in the Fasta format into a .txt file and were used to design a potential multi-epitope vaccine against *S. stercoralis*. The proteins were selected based on their antigenicity and their ability to interact with the immune system [32, 33].

Preliminary analyses

The proteins were then subjected to several preliminary analyses which include: Signal peptide prediction using SignalP6.0 server (<https://services.healthtech.dtu.dk/services/SignalP-6.0/>) (accessed on 29 October 2024) and TOPCONS server (<https://topcons.cbr.su.se/pred/>) (accessed on 29 October 2024) to differentiate between classical secretory proteins and non-secretory proteins, Sequence localization was performed using the DeepLoc 2.1 server (<https://services.healthtech.dtu.dk/service/s/DeepLoc-2.1/>) (accessed on 30 October 2024) and the WoLF-PSORT server (https://wolfpsort.hgc.jp/aboutWoLF_PSORT.html.en) (accessed on 30 October 2024), and lastly prediction of transmembrane domains was done using DeepTMHMM 1.0 server (<https://services.healthtech.dtu.dk/services/DeepTMHMM-1.0/>) (accessed on 30 October 2024) and TOPCONS server (<https://topcons.cbr.su.se/pred/>) (accessed on 30 October 2024).

Protein conservation analyses

To evaluate the potential for cross-protection against other related nematode infections by the selected proteins, a BLAST search (<https://www.uniprot.org/>) (accessed on 30 October 2024) was performed on the UniProtKB database, and the percentage identities for homologous relatives of the full length of the selected proteins were obtained. This is because a strong degree of conservation across the *Strongyloides* genus implies

the potential for cross-protection [34]. The UniProtKB is a central database that collects and connects information from various large and diverse sources. It is the most thorough catalog of protein sequences and their functional annotations [35].

Linear B-cell epitope prediction

B-cell epitopes are antigenic determinants recognized by the immune system and represent the specific piece of the antigen to which B lymphocytes bind [36]. These are vital in vaccine design [37]. Linear B-cell epitopes were primarily predicted using the BepiPred-3.0 web server (<https://services.healthtech.dtu.dk/services/BepiPred-3.0/>) (accessed on 5 November 2024) and then the ABCpred server (<https://webs.iitd.edu.in/raghava/abcpred/>) (accessed on 5 November 2024). The BepiPred server is a sequence-based tool that utilizes numerical data from protein language model (LM) embedding to significantly enhance the accuracy of predicting linear and conformational B-cell epitopes across various independent test sets [38]. Using a default server threshold, linear B-epitopes of various lengths for the selected proteins were predicted. The ABCpred server employs artificial neural networks for linear B-cell epitope prediction [39].

Cytotoxic T lymphocytes (CTL) epitope prediction

Cytotoxic T-lymphocyte (CTL) epitopes for proteins selected were predicted using the publicly available NetCTL 1.2 server (<https://services.healthtech.dtu.dk/services/NetCTL-1.2/>) (accessed on 5 November 2024). This prediction method integrates prediction of MHC class I binding peptides, proteasomal C-terminal cleavage, and TAP (Transporter Associated with Antigen Processing) transport efficiency. Although the server allows for predictions of CTL epitopes restricted to 12 MHC class I supertypes, only the A2, A3, and B7 supertypes were used for this study since these supertypes can attain up to 90% coverage [40]. MHC class I binding and proteasomal cleavage are conducted using artificial neural networks, while TAP transport efficiency is estimated using a weight matrix [41]. The threshold value for epitope identification was set at the default score of 0.75 for the prediction of CTL epitopes. CTL epitopes were included based on emerging evidence that CD8⁺ T cells may play auxiliary roles in helminth immunity through cytokine production rather than direct cytotoxicity [42].

Helper T-cell (HTL) epitope prediction

The NetMHCII-2.3 server (<https://services.healthtech.dtu.dk/services/NetMHCII-2.3/>) (accessed on 5 November 2024) was used to predict HTL epitopes, which were 15-mer length for human alleles. The NetMHCII 2.3 server predicts binding of peptides to HLA-DR, HLA-DQ, and HLA-DP alleles using artificial neural networks

[43], using the default server parameter for strong binders (SB) and weak binders (WB) [44]. Epitopes for HLA-DR, HLA-DQ, and HLA-DP alleles were predicted for all selected proteins, and only the highly promiscuous epitopes (those that can bind to multiple alleles) and classified as strong binders were chosen for the multi-epitope vaccine design.

Epitope antigenicity prediction

In order to design a multi-epitope vaccine candidate that can elicit a strong protective immune response, the VaxiJen v2.0 server (<https://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html>) (accessed on 5 November 2024) was used to predict the antigenicity scores of all potential epitopes. This server employs an alignment-independent method that relies on the physicochemical properties of peptides and proteins to classify antigens. VaxiJen was selected for its alignment-independent prediction based on auto-cross-covariance transformation, allowing robust performance even with novel or synthetic sequences. [45]. The default threshold for the parasite model was set to 0.4 and was used to predict epitope antigenicity, and only those with scores above 0.5 were selected to construct the chimeric antigen. Figure 1 provides a flowchart showing the steps involved in this study.

Population coverage analysis

The IEDB Population Coverage tool (<http://tools.iedb.org/population/>) was used to estimate the global coverage of CTL and HTL epitopes, including respective HLA alleles, across various geographical regions spanning multiple continents and sub-regions. The IEDB Population Coverage tool estimates population immune coverage using HLA allele frequencies, supporting class I and II T-cell epitopes and reporting combined or separate coverage with average and minimum epitope hits [46]. For this study, our focus was on the A2, A3, and B7 for MHC class I epitopes, and HLA-DR, HLA-DQ, and HLA-DP alleles for MHC class II epitopes as these offer optimal population coverage for strongyloidiasis endemic zones [40, 44].

Design of the multi-epitope vaccine candidate

The multi-epitope vaccine candidate sequence was generated using selected high-scoring CTLs, high-affinity HTL epitopes, and linear B-cell epitopes that were predicted simultaneously by the BepiPred-3.0 and ABCpred servers, and were joined together using AAY, GPGPG, and KK linkers, respectively. Linkers are crucial components in engineering multi-epitope vaccines, enabling effective separation of epitopes while preserving their functionality and accessibility to the immune system [47]. Furthermore, a 50-S ribosomal protein L7/L12 (Locus RL7_MYCTU, UniProt ID: P9WHE3) was selected as a built-in adjuvant (since it is reported to be a TLR4

agonist) [48] to enhance the vaccine's immunogenicity and was incorporated at the N-terminal via an EAAAK linker. TLR4 is essential for effective immune responses against *S. stercoralis* [49]. The sequence of the adjuvant was retrieved from the UniProtKB database (<http://www.uniprot.org/>) (accessed on 29 October 2024). The EAAAK linker has been reported to improve protein expression, stability, and biological activity [50]. GPGPG linkers have been employed to reduce the formation of junctional epitopes in epitope-based vaccines, thereby aiding in antigen processing and presentation [51]. The pan HLA-DR epitope (PADRE) and cell-penetrating TAT peptide sequences were incorporated between the adjuvant and the epitopes using the GGGS linker. The PADRE sequence is a universal helper T cell epitope capable of binding to various HLA-DR molecules, activating CD4+ T cell immune responses, and stimulating the secretion of various cytokines [52]. TAT is a small HIV regulatory protein that enhances viral transcription and spread; its protein transduction domain (PTD) enables cell penetration, thereby improving antigen uptake by APCs and lymph node targeting [53, 54]. The GGGS linker was used to ensure flexibility and effective separation of individual epitopes [55]. AAY linkers were used to join CTL epitopes to enable proteasomal cleavage, ensuring efficient epitope separation, reduced junctional immunogenicity, and enhanced vaccine immunogenicity [56]. For the linear B-epitopes, KK linkers were used. They have been reported to maintain the independent immune reactivity of each epitope [57]. In summary, all the incorporated linkers are crucial for ensuring an extended conformation (flexibility), aiding in folding, separating protein domains, and generally enhancing the stability of the recombinant multi-epitope vaccine structure [58]. Lastly, a 6xHis-tag was added at the C-terminal of the multi-epitope vaccine candidate for application in identification and purification procedures [21].

IFN- γ inducing epitope prediction

Interferon gamma (IFN- γ), a cytokine essential for both adaptive and innate immune responses, stimulates macrophages and natural killer cells and provides a heightened response to MHC antigens [59]. 15-mer IFN- γ epitopes for the designed vaccine construct were predicted using the IFNepitope server (<https://webs.iiitd.edu.in/raghava/ifnepitope/predict.php>) (accessed on 7 October 2025). Predictions of IFN- γ epitopes were separately done for the adjuvant and the main vaccine construct due to restrictions in the number of residues that can be used for prediction by the server. The prediction was performed by a motif and support vector machine (SVM) hybrid approach. The server is based on a dataset that consists of IFN- γ -inducing and non-inducing MHC class-II binders, which can activate T-helper cells [60].

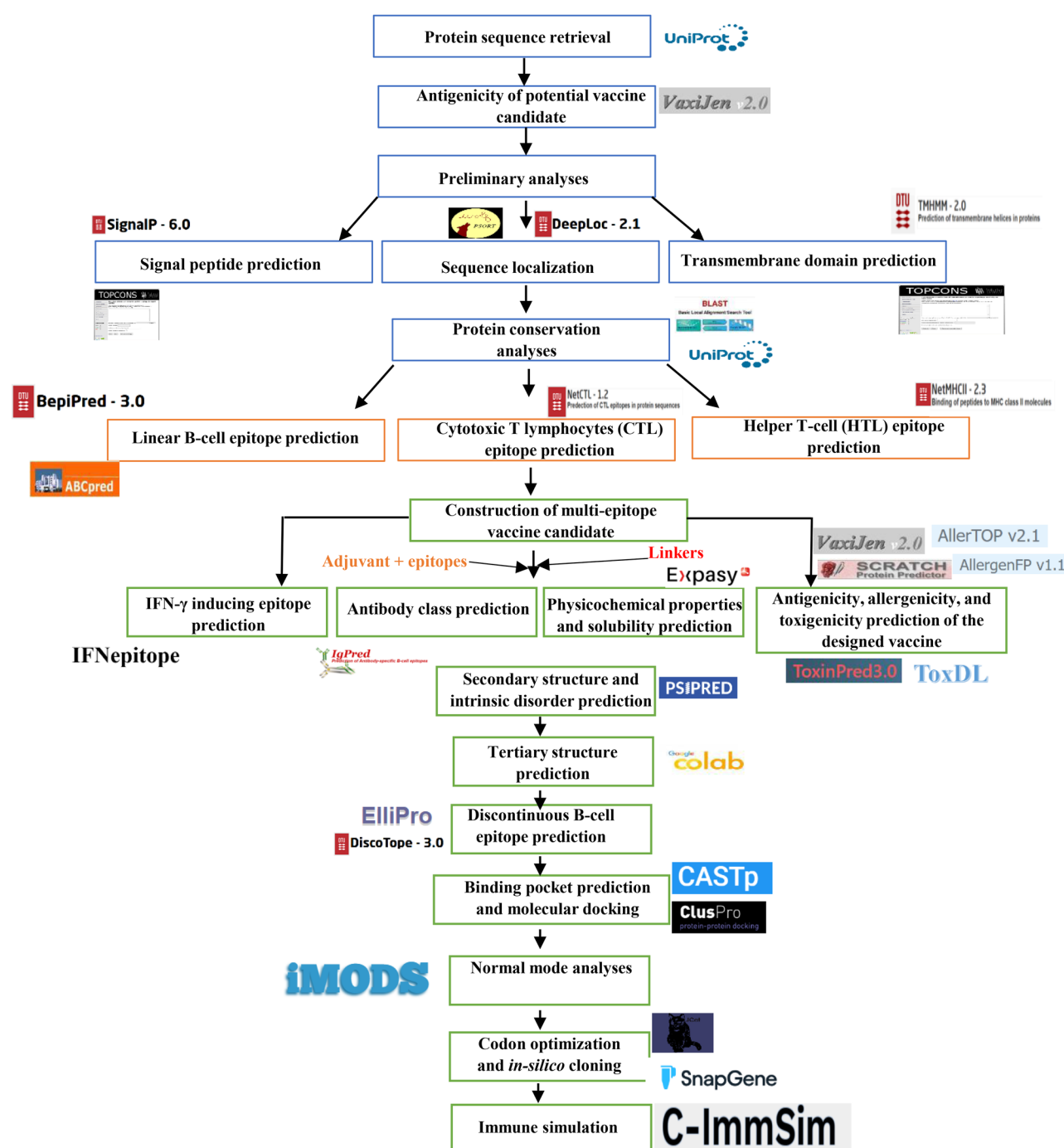


Fig. 1 A flowchart illustrating the key steps of the study design. The entire approach used in the study comprises several phases, which began with the identification and retrieval of the target protein antigens and conservation analysis. Following the preliminary screening for localization, signal peptide, and transmembrane regions, epitope predictions (CTL, HTL, and linear-B cell epitopes) from selected proteins were performed. The vaccine candidate was designed, followed by 3D modeling, refinement, and validation. Molecular docking of the designed vaccine candidate and TLR4, was followed by normal mode analysis. *in-silico* immune simulation to investigate the potential of the vaccine candidate to elicit an immune response was performed. Lastly, reverse translation, codon optimization, and *in-silico* cloning in a suitable plasmid for bacterial expression were performed

Prediction of epitopes for mouse MHC II alleles

To examine the potential of the designed vaccine candidate to stimulate immune responses in preclinical studies, 15-mer MHC II binding peptides for mouse alleles

in the designed vaccine candidate were predicted using the NetMHCII 2.3 server (<https://services.healthtech.dtu.dk/service.php?NetMHCII-2.3>) (accessed on 12 October 2025). This server uses artificial neural networks

to predict the binding affinities to molecules covering human alleles (HLA-DR, HLA-DQ, and HLA-DP) as well as 7 mouse alleles. The operation of this server has been described above.

Antibody class prediction

Specific immunoglobulins, mainly IgG, have been found to play a role in immune protection against *S. stercoralis* [19]. The IgPred server (<https://webs.iitd.edu.in/raghava/igpred/index.html>) (accessed on 7 October 2025) was used to predict specific antibody classes elicited by the predicted linear B-epitope sequences. The IgPred server employs a support vector machine (SVM) to predict B-cell epitopes that can induce IgG, IgA, and IgE, antibodies. The algorithm was developed by analyzing 11,981 IgG, 2341 IgE, and 403 IgA specific B-cell epitopes obtained from the IEDB, and it was observed that each class of antibody has unique features [61].

Physicochemical properties and solubility prediction

The various physicochemical parameters of the multi-epitope vaccine construct were assessed using the online web server ExPASy ProtParam (<https://web.expasy.org/protparam/>) (accessed on 20 November 2024) [62]. These parameters consisted of amino acid composition, theoretical pI, and instability index, in vitro and in vivo half-life upon expression in *Escherichia coli*, yeast, and mammal cells, aliphatic index, molecular weight, and the grand average of hydropathicity (GRAVY). In addition, the solubility of the multi-epitope vaccine was evaluated using the NetSOLP-1.0 server (<https://services.healthtech.dtu.dk/services/NetSOLP-1.0/>) (accessed on 20 November 2024). NetSOLP-1.0 predicts protein solubility and purification suitability in *E. coli* directly from sequence using deep-learning protein language models, enabling the server to achieve state-of-the-art predictive performance [63]. Furthermore, the DeepSoluE tool (<http://39.100.246.211:10505/DeepSoluE/>) (accessed on 20 November 2024) employs a long-short-term memory (LSTM) network with hybrid physicochemical and amino acid features to predict bacterial protein solubility [64].

Antigenicity, allergenicity, and toxigenicity prediction of the designed vaccine

Both ANTIGENpro and VaxiJen v2.0 (described previously) were used to predict the antigenicity of the designed multi-epitope chimeric antigen. ANTIGENpro server from Scratch Protein Predictor (<https://scratch.proteomics.ics.uci.edu/>) (accessed on 30 December 2024), employs machine learning (ML) trained on experimental datasets, offering better generalizability than older motif-based tools [65]. AllerTOP v2.0 and AllergenFP were used to predict multi-epitope vaccine allergenicity. AllerTOP v2.0 (https://www.ddg-pharmfac.net/allertop_test/)

(accessed on 30 December 2024) utilizes amino acid E-descriptors, along with auto- and cross-covariance, and k nearest neighbors (kNN) classification to predict allergens, achieving 85.3% accuracy by 5-fold cross-validation [66]. The AllergenFP server (<https://www.ddg-pharmfac.net/AllergenFP>) (accessed on 30 December 2024), on the other hand, employs an alignment-free, descriptor-based fingerprint approach to classify allergens vs non-allergens, encoding sequence properties with auto- and cross-covariance (ACC), comparing fingerprints by Tanimoto coefficient, and achieving 88% accuracy with a Matthews correlation coefficient (MCC) of 0.759 [67]. Meanwhile, the ToxinPred3 server (<https://webs.iitd.edu.in/raghava/toxinpred3/prediction.php>) (accessed on 30 December 2024) was employed to predict the toxicity of the vaccine construct.

Secondary structure and intrinsic disorder prediction

The PSIPRED v4.0 server was used for secondary structure predictions. PSIPRED, a web-based, freely-accessible online server (<https://bioinf.cs.ucl.ac.uk/psipred/>) (accessed on 20 February 2025) was used to accurately predict the protein secondary structure based on primary amino acid sequences. Position-specific iterated BLAST (Psi-BLAST) was conducted to identify and select sequences with significant homology to the vaccine protein. PSIPRED achieved a normal Q3 score of 81.6% when an exceptionally stringent cross-validation strategy was employed to evaluate its performance. The selected sequences were then used to create a position-specific scoring matrix [68]. Disordered proteins make up an important class of antigens in a wide range of human pathogens, and are usually targets of protective immune responses [69]. Prediction of intrinsically disordered regions (IDRs) in the designed chimeric vaccine candidate was done using the IUPred3 server (<https://iupred3.elte.hu/>) (accessed on 20 February 2025). IUPred predicts intrinsically disordered regions from amino acid sequences or UniProt IDs using an energy-based estimation algorithm [70] and the PrDOS server (<https://prdos.hgc.jp/cgi-bin/top.cgi>) (accessed on 20 February 2025). PrDOS predicts protein disorder using two predictors—one from local amino acid sequence and one from template proteins—combined via SVM and PSI-BLAST [71].

Disulfide bond prediction

Disulfide bridges are covalent bonds between two cysteine residues formed via their reactive thiol (–SH) groups. [72]. Disulfide bonds enhance protein stability and folding; disulfide engineering was used to introduce them into the vaccine model. [73]. Disulfide bond prediction was done using Dipro on Scratch Protein Predictor (<https://scratch.proteomics.ics.uci.edu/>) (accessed on 25 September 2025). Dipro predicts disulfide bonds using

2D recurrent neural networks (RNNs), support vector machines (SVMs), graph matching, and regression, identifying bond presence, number, cysteine states, and pairs with top benchmark accuracy [74].

Tertiary structure prediction

Prediction of the 3D structure of the designed chimeric vaccine was done using the ColabFold tool (<https://colab.research.google.com/github/sokrypton/ColabFold/blob/main/AlphaFold2.ipynb>) (accessed on 20 February 2025), an easy-to-use interface that uses the AlphaFold2 [75] technology within the Google Colab environment. ColabFold offers an enhanced prediction of protein structures and complexes by combining the fast homology search of MMseqs2 with AlphaFold2 or RoseTTAFold [76]. The obtained 3D structure was later refined using the GalaxyRefine server (<http://galaxy.seoklab.org/cgi-bin/submit.cgi?type=REFINE>) (accessed on 20 February 2025). The GalaxyRefine improves protein structures by rebuilding and repacking side chains, followed by molecular dynamics simulation, achieving superior local quality as demonstrated in CASP10 [77]. Assessment of the overall quality score of the refined chimera 3D structure was done using the ProSA-web server (<https://prosa.services.came.sbg.ac.at/prosa.php>) (accessed on 21 February 2025). ProSA is commonly employed to analyze 3D models of protein structures for potential errors and is used for error recognition in experimentally determined structures, theoretical models, and protein engineering [78]. The ERRAT server (<https://saves.mbi.ucla.edu/>) (accessed on 21 February 2025) was also used to validate the 3D structure of the vaccine construct. The ERRAT server analyzes non-bonded atom-atom interactions compared to reliable high-resolution crystallography structures [79].

Discontinuous B-cell epitope prediction

Over 90% of B-cell epitopes are discontinuous, formed by distant protein segments brought together through folding [80], but discontinuous in the linear sequence. Identifying conformational epitopes reveals true antibody-binding sites, offering insights beyond linear epitope mapping [81]. The ElliPro (<http://tools.iedb.org/elliPro/>) and DiscoTope-3.0 (<https://services.healthtech.dtu.dk/services/DiscoTope-3.0/>) servers (accessed on 15 May 2025) were used to predict discontinuous (conformational) B-cell epitopes in the designed chimera. ElliPro predicts and visualizes antibody epitopes using a modified Thornton method, clustering, MODELLER, and Jmol. Tested on conformational epitopes, it outperformed other tools with an AUC of 0.732 [82]. This server predicted epitopes with default parameters (minimum score of 0.5 and maximum score of 0.6). DiscoTope-3.0, on the other hand, accurately predicts discontinuous B-cell epitopes using inverse folding from solved or

AlphaFold structures, outperforming current methods on both linear and non-linear epitopes. [83].

Binding pocket prediction, molecular docking, binding affinity, and interaction analyses

To assess the binding pockets in the designed chimera, the CASTp 3.0 server (<http://sts.bioe.uic.edu/castp/index.html?2was>) (accessed on 30 July 2025) was used. CASTp is a web server that uses the alpha shape method developed in computational geometry to locate, delineate, and measure different geometric and topological properties of protein structures [84]. To evaluate the interaction between the designed chimera and TLR4, protein-protein docking analysis was done using the ClusPro 2.0 server (<https://cluspro.bu.edu/login.php>) (accessed on 30 July 2025). The ClusPro 2.0 server has consistently been ranked among the top docking servers, as shown in the Critical Assessment of Predicted Interactions (CAPRI), offering strong predictive performance for the docking of protein-protein complexes [85]. The server conducts docking through several computational steps: (i) rigid body docking sampling billions of conformations using PIPER with FFT correlation; (ii) clustering the 1000 lowest-energy structures by RMSD to identify the largest clusters as likely models; and (iii) refining selected structures via energy minimization. It also performs similarity-based docking by retrieving templates from experimental databases and applying energy-based optimization for structural flexibility [85]. The binding affinity of the predicted interaction between the vaccine candidate and the TLR4 immune receptor was analyzed using the PROtein binDing enerGY (PRODIGY) prediction server (<https://wenmr.science.uu.nl/prodigy/>) (accessed on 4 August 2025). The server implements a straight forward but very efficient predictive model based on intermolecular contacts and properties derived from the non-interface surface [86]. The docked complexes were then further analyzed on the PDBsum server (<https://www.ebi.ac.uk/thornton-srv/databases/pdbsum/>) (accessed on 4 August 2025), which creates a 2D image of the interactions at the ligand-protein interface from the 3D coordinates.

Normal mode analyses

It is essential to identify flexible areas of protein structures to understand their biological roles [87]. Normal mode analysis (NMA) for the docked vaccine candidate-TLR4 complex was performed using the iMODS server (<https://imods.iqf.csic.es/>) (accessed on 4 August 2025). The analysis was performed to show the internal dihedral coordinates and measure the cooperative functional motions of the docked complex. The essential dynamic simulation program of the iMODS server was deployed to ensure the energy minimization, molecular stability, and atomic mobility of the vaccine candidate in the

docked complex. The server determines the possible motions of the vaccine candidate-TLR4 complex based on specialized parameters, including B-factors, RMSD, eigenvalues, deformability of the complex, covariance values, and elastic models [88].

Codon optimization and in-silico cloning

Reverse translation of the protein sequence into a DNA sequence and codon optimization were performed using the Java Codon Adaptation Tool (JCat) server (<https://www.jcat.de/>) (accessed on 16 August 2025). The JCat server allows users to modify the codon usage for any gene to match that of any host organism. The server also provides information on the codon adaptation index (CAI) and GC% content, which have both been reported to affect protein expression in bacteria [89, 90]. To express the designed antigen in *E. coli*, the codon-optimized gene sequence was cloned between the *Nde* I and *Apa* I restriction sites of the pET30a+ vector using the Snapgene tool (<https://www.snapgene.com/>) (accessed on 16 August 2025).

Immune simulation

To further characterize the immunogenicity and immune response profile of the chimeric peptide, in-silico immune simulations were conducted using the C-ImmSim server (<https://kraken.iac.rm.cnr.it/C-IMMSIM/index.php>) (accessed on 18 September 2025) as previously described [91]. The C-ImmSim tool is an agent-based model that employs a position-specific scoring matrix (PSSM) for predicting immune epitopes and machine learning techniques for the prediction of immune interactions. It “simultaneously simulates three compartments that represent three distinct anatomical regions found in mammals: (i) the bone marrow, where hematopoietic stem cells are simulated and generate new lymphoid and myeloid cells; (ii) the thymus, where naive T cells are selected to avoid auto immunity; and (iii) a tertiary lymphatic organ, such as a lymph node [92]. All simulation parameters were set at default, with time steps set at 1, 43, and 85 (each time step is 8 hours, and time

step 1 is injection at time = 0). Therefore, three injections were given two weeks apart.

Results

Protein retrieval

The amino acid sequences of 09 proteins, previously reported to be immunogenic, implicated in pathogenesis or virulence, and identified in the L3 stage of *S. stercoralis* [93–95], (accession numbers: A0A0K0E6J0, A0A0K0DY51, A0A0K0ECK4, A0A0K0EG68, A0A0K0EMX1, A0A0K0E2F4, A0A0K0DTP5, A0A0K0ELA9, and A0A0K0EKU6) were retrieve from UniProtKB database for preliminary analyses, epitope prediction and design of a chimeric antigen.

Preliminary analyses

The SignalP-6.0 server predicted the presence of signal peptides in six of the selected proteins, while the TOPCONS server predicted signal peptides in three of the selected proteins. For TM domain prediction, four proteins (A0A0K0E6J0, A0A0K0EG68, A0A0K0E2F4, and A0A0K0ELA9) were predicted by consensus using TOPCONS to have 1 TM region. The DeepTMHMM 1.0 server, on the other hand, predicted no TM domains in the selected proteins. The DeepLocPro-1.0 server predicted eight of the proteins to be localized in the extracellular space. While one protein was predicted to be localized in the cytoplasm (A0A0K0ECK4). (see Table 1). Following these analyses, eight antigens (A0A0K0E6J0, A0A0K0DY51, A0A0K0EG68, A0A0K0EMX1, A0A0K0E2F4, A0A0K0DTP5, A0A0K0ELA9, and A0A0K0EKU6) were predicted to be localized in the extracellular space by the DeepLocPro-1.0 server. Additionally, the antigens (A0A0K0E6J0, A0A0K0DY51, A0A0K0ECK4, A0A0K0EG68, A0A0K0EMX1, and A0A0K0DTP5) were predicted to possess signal peptides according to the SignalP-6.0 server. Also, WoLF-PSORT predicted three antigens to be localized in the extracellular space (A0A0K0EG68, A0A0K0EMX1, and A0A0K0ELA9) while two antigens (A0A0K0E6J0 and A0A0K0EKU6) were localized in the mitochondrion and

Table 1 Predicted protein localization and signal peptides

Protein	Signal peptide		TM domain		Localization	
	SignalP 6.0	TOPCONS	TOPCONS	DeepTMHMM	DeepLoc 2.1	WoLF PSORT
A0A0K0E6J0	No	No	Yes	No	Extracellular	Mitochondrion and Endoplasmic reticulum
A0A0K0DY51	No	No	No	No	Extracellular	Nucleus, mitochondrion
A0A0K0ECK4	No	No	No	No	Cytoplasm	Cytoplasm and nucleus
A0A0K0EG68	Yes	No	Yes	No	Extracellular	Extracellular
A0A0K0EMX1	Yes	Yes	No	No	Extracellular	Extracellular
A0A0K0E2F4	Yes		Yes	No	Extracellular	Mitochondrion
A0A0K0DTP5	No	No	No	No	Extracellular	Mitochondrion
A0A0K0ELA9	Yes	Yes	Yes	No	Extracellular	Extracellular
A0A0K0EKU6	Yes	Yes	No	No	Extracellular	Mitochondrion, Endoplasmic reticulum

Table 2 Conservation of target proteins in selected *S. stercoralis*

Number	Protein	Percentage identity (%)		
		<i>S. stercoralis</i>	<i>S. ratti</i>	<i>S. venezuelensis</i>
1	A0A0K0EG68	91.1	30.5	32.3
2	A0A0K0EMX1	100	86	75.7
3	A0A0K0E2F4	100	44	38.1
4	A0A0K0ELA9	100	86.4	78.9
5	A0A0K0EKU6	100	50.9	50.5
6	A0A0K0E6J0	100	48.5	45.7
7	A0A0K0DY51	100	57.9	Not found
8	A0A0K0ECK4	100	91.3	86.3
9	A0A0K0DTP5	100	67	52.9

endoplasmic reticulum. A0A0K0E2F4 and A0A0K0DTP5 were predicted to be localized in the mitochondria, and A0A0K0ECK4 was found in the cytoplasm and nucleus. They were subsequently chosen for analyses of epitope prediction (see Table 1). The Resuscitation-promoting factor E (Rpfe, RPFE_MYCTU), O53177, from *Mycobacterium tuberculosis*, known to act as a TLR4 agonist, was retrieved from the UniProtKb database for use as a built-in adjuvant.

Protein conservation in other *Strongyloides* species

A significant degree of conservation among all selected proteins was noted in related species of the *Strongyloides* genus (ranging from 32.3% to 100%) based on a BLAST search conducted against the UniProtKB database and also on a BLASTp search on NCBI. In one instance, no homologs were identified for the selected protein A0A0K0DY51 in the target species *S. venezuelensis*. The analysis of protein conservation compared to their homologs in *S. stercoralis*, *S. ratti*, and *S. venezuelensis* revealed that *S. stercoralis* exhibited the highest level of conservation, with eight out of nine proteins showing 100% conservation, while the remaining protein had a conservation rate of 91.1% (see Table 2).

Cytotoxic T lymphocyte (CTL) epitope prediction

High-scoring CD8+ (9-mer) epitopes were identified from the selected antigens using the NetCTL 1.2 server,

at the default threshold of 0.75. From the predicted epitope sequences, ten CD8+ epitopes were selected for incorporation in the chimera based on their antigenicity (see Table 3).

Helper T lymphocyte (HTL) epitope prediction

High-affinity MHC-II epitopes, assessed through IC50 scores for the HLA-DR, HLA-DQ, and HLA-DP human alleles, were predicted using the NetMHCII 2.3 web server and identified as HTL epitopes. Seven high-affinity HTL epitopes were ultimately chosen for the design of the chimeric vaccine candidate. (see Table 3).

Linear B-lymphocyte (LBL) epitope prediction

Linear B-cell epitopes of different residue lengths were predicted using two servers, and those identified by both servers were chosen for constructing the final chimeric vaccine candidate. In total, eight linear B-cell epitopes were selected to be incorporated into the chimera (see Table 3).

Antibody class prediction

All eight selected linear B-lymphocyte epitopes, initially predicted to be antigenic and chosen for the designed chimeric antigen, were used for antibody class prediction using the IgPred server. All epitopes were predicted to bind to IgG, and none of the epitopes selected was predicted to bind to IgE or IgM, but two epitopes (from proteins A0A0K0E2F4, and A0A0K0DTP5) were predicted to induce both IgG and IgA. The prediction threshold was set to 0.5, with all LBLs exhibiting a score higher than the threshold (see Table 4).

Design of the chimeric multi-epitope vaccine candidate

Predicted epitopes were combined using linkers to form a multi-epitope chimeric antigen (**SS-MEV-1**). As a result, a multi-epitope chimeric vaccine candidate was designed, from of 10 CTL epitopes, 7 HTL epitopes, and 8 linear B-lymphocyte epitopes overall. The chimera was constructed by using the AAY, GPVGP, and KK linkers to connect CD8+ T-epitopes, CD4+ T-epitopes,

Table 3 Epitopes predicted for the design of the multi-epitope vaccine

Protein	LBL	CTL	HTL
A0A0K0EG68	NRPIKPKNNPKPPGLRPKPPGLRP	SATQIGCAV	VNKKQFESLAPAKNYNER
A0A0K0EMX1	ENPGSFSLKDNWKS	KEYLLAGYY	VRDISLRNWIATLTLEL
A0A0K0E2F4	GDEEDGASDTFQGDILL	QPAYVQGI	-
A0A0K0ELA9	EQVFKANNLQVLIPLLGK	NLQVLIPL	-
A0A0K0EKU6	TLKTTKRPVTTTKASVTTKAPKTPAKPSSVATKESTTS	KPTRKTPKT	NFKKPGFSPATGHFTQLVWK
A0A0K0E6J0	RDEYFKSLNFGSPVIYTRYPNIRP	SLYIVIFIL	VGIYMGVKNWSRRKAAALVD
		KPNGWGENL	
A0A0K0DY51	-	DPTTEGAPV	SLYIVIFILGFLSLSLSV
A0A0K0ECK4	IDGKWQHEERTPKGNPFKKEKV	PMHVRIIGK	GYRFPMHVRIIGKCEPK
A0A0K0DTP5	TDFIGRRDRNIRTTTTLKPVKRIKIDTQKL	KHFAMMVWK	RRDRNIRTTTTLKPVKRI

Table 4 Predicted immunoglobulin (ig) classes induced by LBLs

Predicted Linear B-Cell Epitope	Epi-tope score	Predicted Antibody Class		
		IgG	IgE	IgA
NRPIKKPNPKPPGLRPKPPGLRP	1.279	+	-	-
EQVFKANNLQVLIPLLKG	0.533	+	-	-
GDEEDGASDTFQGDILL	0.821	+	-	+
ENPGSFSLKDNWKS	0.793	+	-	-
TLKTTKRPVTTTKSASVTTKAPKTPAKPSSVATKESTTS	1.335	+	-	-
RDEYFKSLNFGPSVIYITRYPNIRP	0.561	+	-	-
IDGKWQHEERTPKGNPFKKEKV	0.791	+	-	-
TDFIGRRDRNIRTTTTLKPKRIKIDTQKL	0.772	+	-	+

and linear B-cell epitopes, respectively. Also, the TLR4 (PDB ID: 4G8A) agonist, RL7_MYCTU from *Mycobacterium tuberculosis*, (RPFE_MYCTU) (accession number P9WHE3) was selected as a built-in adjuvant and integrated at the N-terminal of the designed vaccine candidate using the EAAAK linker to enhance immune-elicited responses. Furthermore, the universal Pan DR Epitope (PADRE) sequence (AKFVAAWTLKAAA), which effectively stimulates CD4+ responses, was incorporated after the *M. tuberculosis* RpfE sequence. Moreover, the TAT sequence (GRKKRRQRRRPQ), which enhances cellular uptake and modulates immune responses [53], was added after the PADRE sequence using the GGGS linker. The 6xHis tag was integrated at the carboxyl terminal of the designed chimeric antigen to facilitate purification and identification methods. The selection of specific linkers

and the built-in adjuvant was guided by their established roles in enhancing epitope processing, structural stability, and immunogenicity in multi-epitope vaccine constructs. The AAY linker was employed between CTL epitopes to facilitate proteasomal cleavage and efficient MHC class I presentation, while the GP GPG linker was used between HTL epitopes to preserve epitope integrity and promote effective MHC class II processing. The KK linker was incorporated between linear B-cell epitopes to maintain epitope flexibility and reduce junctional immunogenicity. The rigid EAAAK linker was used to spatially separate the built-in adjuvant from the epitope-rich region, thereby minimizing structural interference and ensuring independent functional folding. These linkers have been widely validated in immunoinformatics-based vaccine design studies [50, 96, 97]. In summary, the chimeric vaccine candidate, named SS-MEV-1, consisted of 633 amino acid residues, which include 25 peptides from *S. stercoralis* (predicted epitopes), as well as immune enhancers (TLR4 agonist, PADRE, and TAT) and various linkers (Fig. 2).

Physicochemical properties and solubility analysis

The SS-MEV-1 chimeric antigen, composed of 633 amino acid residues, was predicted to have a molecular weight (MW) of 68.7 kDa and a theoretical pI of 10.06, indicating that the protein is basic. The protein was predicted to have a half-life of 30 hours in mammalian reticulocytes in vitro, >20 hours in yeast, and >10 hours in *E. coli* in vivo. Furthermore, physicochemical properties

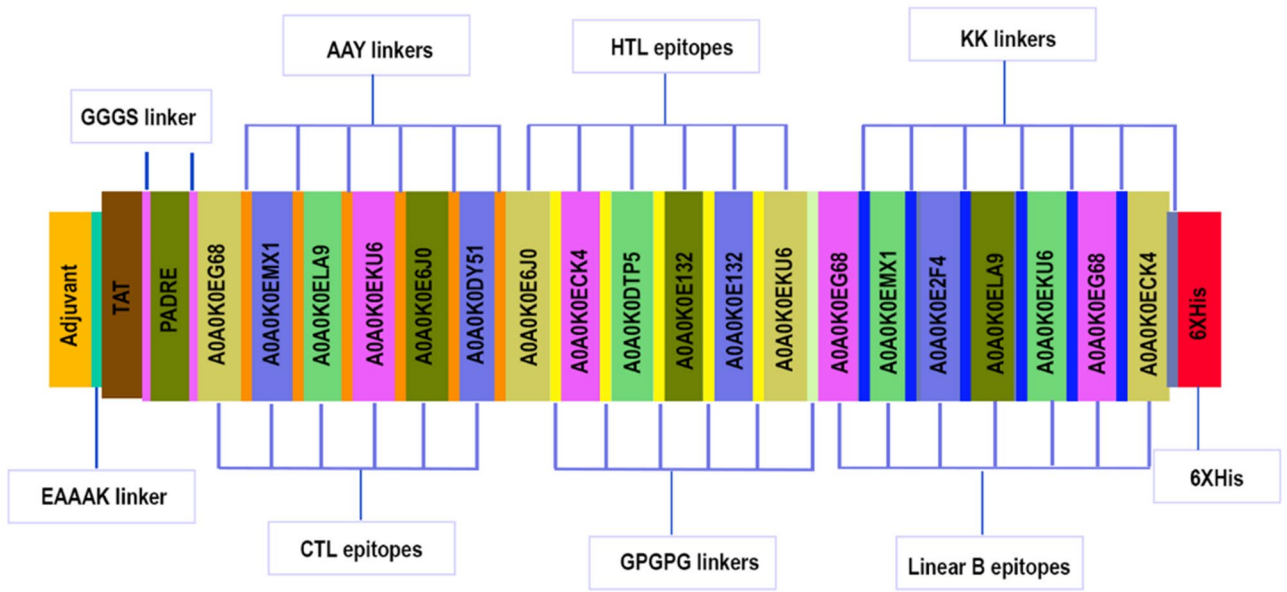


Fig. 2 Schematic illustration of the final multi-epitope vaccine antigen, SS-MEV-1. This 633-amino acid polypeptide sequence includes a built-in adjuvant (yellow) at the N-terminal, linked to the multi-epitope sequence via an EAAAK linker (green), leading to the PADRE and TAT peptides, which are connected through GGGS linkers (pink). The antigenic CTL, HTL, and linear-B cell epitopes are joined by AAY (orange), GP GPG (orange), and KK linkers (blue), respectively. A 6x-his tag is incorporated at the C-terminal for purification and identification, positioned away from the peptide

analyses showed the protein to be rich in Lys (12.5%) and Ala (11.2%). The predicted instability index (II) was calculated to be 32.76. A protein with an instability index < 40 is expected to be stable, while a value > 40 suggests that the protein may be unstable [62]. The predicted GRand Average of hydropathicity (GRAVY) was −0.476, suggesting that the protein is capable of interacting with water molecules [98]. An aliphatic index of 75.29 was predicted, implying that the protein exhibits thermostability [99]. The solubility score was predicted to be 0.4624 by the NetSolP 1.0 server, but 0.4051 on the DeepSoluE server. The protein exhibited varying solubility predictions (0.4624 and 0.4051). This slight discrepancy highlights the inherent variability in solubility predictions and underscores the importance of experimental validation upon expression in bacteria. Furthermore, the NetSolP 1.0 server predicted the protein to have a usability score of 0.4507 (see Table 5).

Disulfide bond prediction

The disulfide bonds of the final multi-epitope vaccine candidate were predicted using the DIpro server. The multi-epitope vaccine construct consisted of two (02) cysteine molecules at positions 175 and 314 and was predicted to consist of one disulfide bond.

Secondary structure and intrinsic disorder prediction

The secondary structure of the multi-epitope vaccine was predicted using the PSIPRED v4.0 server and was predicted to consist of 36% alpha helices, 8% beta-strands, and 56% coils (Fig. 3A). With regards to solvent accessibility, **SS-MEV-1** was predicted to have 62% of amino acid residues exposed, while 11% fairly exposed, and about 27% were predicted to be buried. A total of 225 amino acid residues, accounting for 38%, were predicted to be found in intrinsically disordered regions (Fig. 3B). Figure 3C presents a visual representation of the secondary structure of the designed vaccine candidate.

Table 5 Physicochemical properties of the designed chimeric vaccine

Property	Measurement
Number of Amino Acids	633
Molecular Weight	68722.79
Formula	C ₂₈₅₅ H ₄₅₃₆ N ₇₉₀ O ₈₀₂ S ₁₀
Theoretical pI	10.06
Instability Index	32.76
Aliphatic Index (AI)	75.29
Grand Average of Hydropathicity (GRAVY)	−0.476
Solubility on expression (DeepSoluE)	0.7682
Solubility on expression (NetSolP 1.0)	0.4624
Usability on expression (NetSolP 1.0)	0.4507

Antigenicity, allergenicity, and toxigenicity prediction

The final sequence had a predicted antigenicity score of 0.7828 on the VaxiJen 2.0 server, using the parasite model with a threshold of 0.4. An antigenic score of 0.928587 was predicted for the designed chimeric construct on the ANTIGENpro server, suggesting it is likely a vaccine candidate. The AllerTOP v0.2 and AllergenFP server's prediction showed that the protein is non-allergenic. A non-allergenic prediction suggests that the vaccine candidate is unlikely to trigger an allergic response in most individuals, making it safer for widespread use [100]. The ToxinPred server predicted that **SS-MEV-1** did not have any toxigenic peptides (see Table 6).

IFN-γ-inducing epitope prediction

Out of the 122 potential IFN-γ-inducing epitopes (15-mers) predicted for the built-in adjuvant, 62 exceeded the epitope prediction threshold. Among the 487 potential epitopes predicted for the main vaccine sequence, 161 IFN-γ-inducing epitopes exceeded the default threshold. The predicted quantity of these IFN-γ-inducing epitopes was correlated with the simulated levels of IFN-γ induction observed after immunization with the chimeric vaccine, as modeled by immune simulation on the C-ImmSim server (Fig. 4).

Prediction of epitopes for mouse MHC II alleles

Out of the 569 potential 9-mer epitopes derived from the antigen for each allele, 124 peptides were predicted to bind to six mouse MHC II alleles for **SS-MEV-1**. Among these, the H-2-IAb allele showed the highest number of epitopes, while the H-2-IAk allele had no binding epitopes (see Table 7)

Prediction of tertiary structure, refinement, and validation

The 3D structure of the multi-epitope vaccine candidate was predicted using AlphaFold2 on the ColabFold interface. 3D structural refinement was done on the GalaxyRefine server, which yielded 5 models. Among these 5 models, “model 2” was selected with parameters GDT-HA (0.7982), RMSD (0.906), MolProbity (1.219), and Rama favored (95.2), which was then used for downstream analyses (Fig. 5B). In addition, the model was evaluated to have a clash score of 1.5, a poor rotamers score of 0, and a Ramachandran plot score of 92.7%. The analysis of the Ramachandran plot of the selected model showed 92.7% of protein residues to be located in favored regions, which is similar to what was obtained from the GalaxyRefine server. Furthermore, it was predicted that 5.2% of residues were predicted to be located in allowed regions, while only 1.2% were predicted in disallowed regions (Fig. 5C). With regards to refinement, the 3D model quality was analyzed on the ERRAT servers and ProSA-web. The selected model has an overall quality

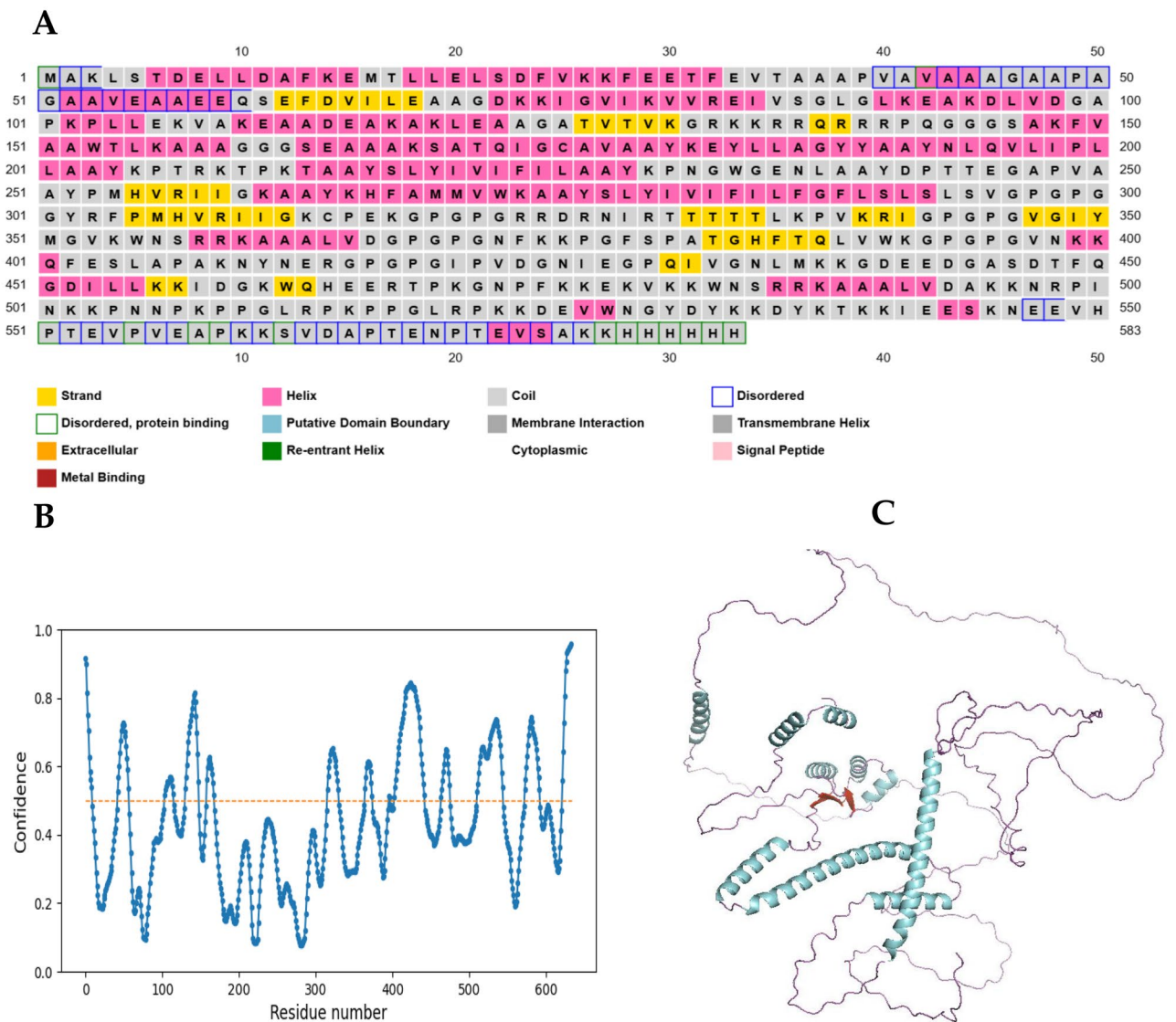


Fig. 3 Graphical representation of the secondary structure of the final subunit vaccine sequence. **(A)** the protein is predicted to consist of alpha-helices (36%), beta strands (8%), and coils (56%). **(B)** according to PrDOS, 31% of positions are expected to be in intrinsically disordered regions. **(C)** the 3D structure of the vaccine candidate showcases the various secondary structure elements, with helices in cyan, strands in red, and coils in pink

Table 6 Antigenicity, allergenicity, and toxigenicity prediction of the designed vaccine

Property	Prediction	Remark
Antigenicity (VaxiJen v2.0)	0.7828	Probable antigen
Antigenicity (ANTIGENpro)	0.928587	Probable antigen
Allergenicity (AllerTOP v2.0)	Probable	Probable
	NON-ALLERGEN	NON-ALLERGEN
Allergenicity (AllergenFP 2.0)	Probable	Probable
	NON-ALLERGEN	NON-ALLERGEN
Toxicity (ToxinPred)	Non-toxin	Non-toxicogenic

factor of 84.4 on the ERRAT server and a Z-score of -3.19 on the ProSA web server (Fig. 5D). A ProSA-web Z-score of -3.19 suggests the protein model is likely within the typical range for native proteins of similar size, indicating

reasonably good quality, but further investigation is recommended to identify potential localized issues [78].

Prediction of discontinuous B-cell epitope

In total, there were 171 residues (27%) predicted by the ElliPro server to be found in 36 conformational B-cell epitopes, with epitope predicted scores in the range of 0.528 to 0.919. The predicted discontinuous epitope residues ranged from 3 to 21 amino acid residues (Supplementary Table 1). The DiscoTope 3.0 server predicted discontinuous B-cell epitopes on the 3D structure generated by the AlphaFold 2 server. These discontinuous B-cell epitopes are highlighted in red on the chimeric vaccine construct (Fig. 5E).

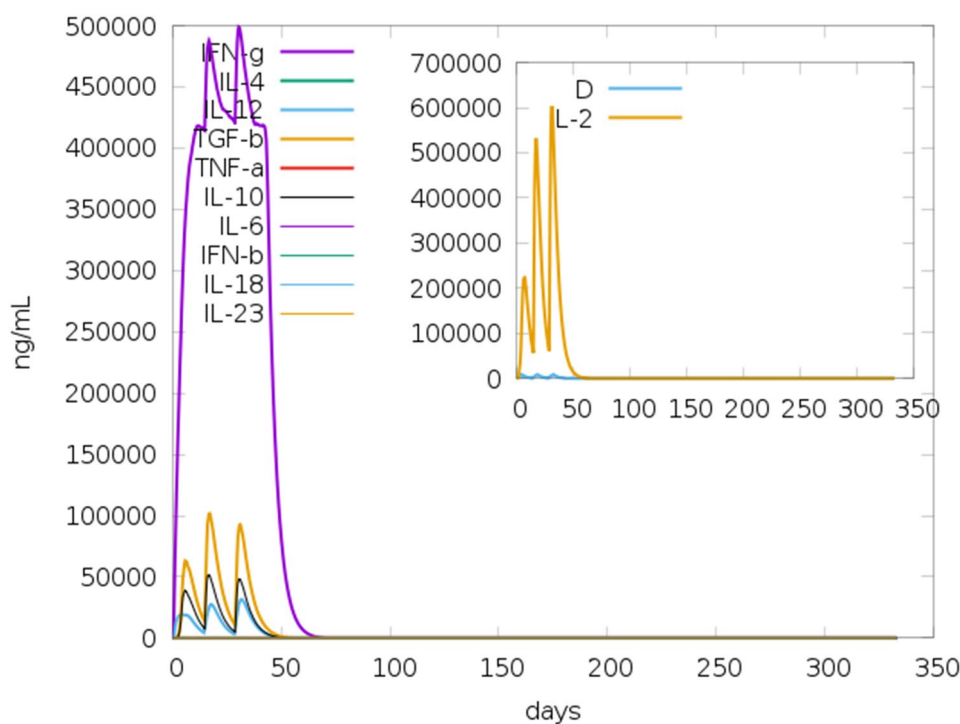


Fig. 4 Immune simulation of the cytokine profile induced by three injections of **SS-MEV-1** administered two weeks apart is presented. The main plot illustrates the varying cytokine levels following the three injections, while the inset plot displays the IL-2 level along with the Simpson index, D, represented by the blue line. The index D measures diversity, with an increase over time indicating the emergence of different epitope-specific dominant T-cell clones. A smaller D value corresponds to lower diversity

Table 7 Predicted peptide counts for binding to mouse H2 class II alleles

Allele	H-2-IAb	H-2-IAAd	H-2-IAk	H-2-IAs	H-2-IAu	H-2-IEd	H-2-IEk
Number of epitopes	40	24	0	18	7	22	13

Molecular docking of subunit vaccine with TLR4

In order to verify the immune response induced by the interaction of the multi-epitope vaccine candidate with TLRs, molecular docking analysis was performed using the ClusPro 2.0 server. TLRs are proteins present in specific immune cells that detect pathogen-associated molecular patterns, leading to the activation of an innate immune response [101]. Some previous studies have reported the involvement of toll-like receptor-4 (TLR-4) in protective immunity against *S. stercoralis* [49, 102]. Molecular docking predicted the binding interaction between the refined 3D structure of the chimeric vaccine candidate and TLR4. Thirty models were generated, visualized, and ranked based on the cluster size obtained from the ClusPro 2.0 server, with the docking complex having the largest cluster size being selected. The **SS-MEV-1**-TLR4 complex with the largest cluster size exhibited a center binding score of −1123.5, and the lowest energy identified at −1423.3.

To further investigate vaccine-receptor interactions, the selected model of the vaccine candidate-TLR4 complex was assessed for interface interactions using PDBsum and binding affinity using the ProDIGY server. The

PDBsum server predicted the formation of 25 hydrogen bonds, 2 salt bridges, and 226 non-bonded contacts between 38 residues from the TLR4 and 32 residues of the chimeric vaccine candidate. Overall, the vaccine candidate demonstrated favorable interactions with TLR4. The relative binding free energy (ΔG) of the multi-epitope vaccine-TLR4 complex was predicted to be −15.9 Kcal/mol, while the predicted K_d was 2.1×10^{-12} . Consequently, the number of contacts made at the interface (IC) per property (ICs charged-charged: 9.0, ICs charged-polar: 31.0, ICs charged-apolar: 33.0, ICs polar-polar: 5.0, polar-apolar: 37.0, ICs apolar-apolar: 26.0) (Fig. 6).

Molecular dynamics simulation

The normal mode analysis (NMA) was performed to predict the molecular stability and functional motions of the multi-epitope vaccine candidate-TLR4 complex (Fig. 7). The deformability graph revealed peak points that signify the deformed regions of the main chain residues in the vaccine candidate-TLR4 complex. The areas identified as hinges correspond to regions of high deformability (Fig. 7A). The B-factor plot illustrates the relationship between NMA mobility and the **SS-MEV-1**-TLR4 complex,

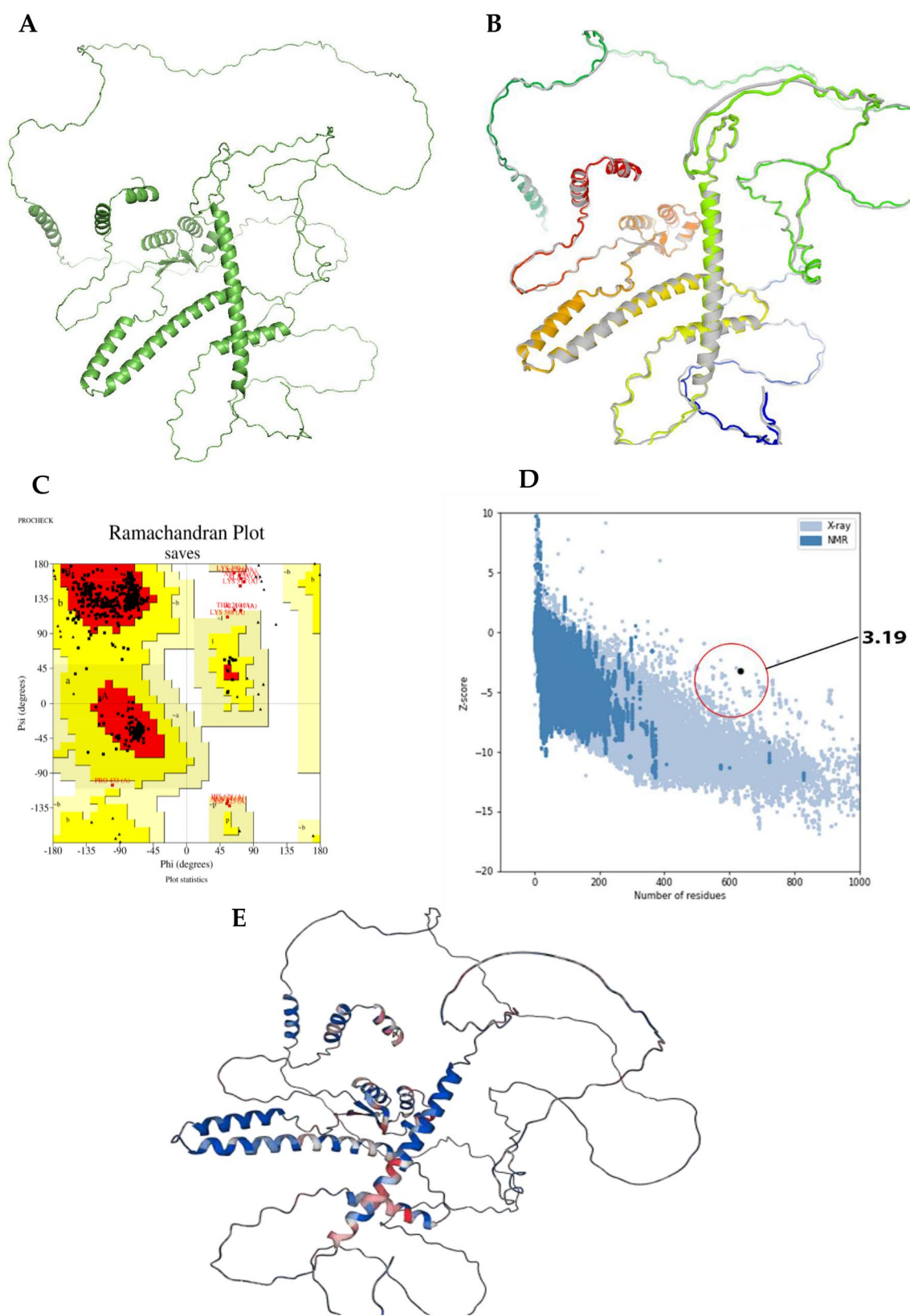


Fig. 5 (See legend on next page.)

(See figure on previous page.)

Fig. 5 Protein modeling, refinement, and validation: **(A)** the initial 3D model of the multi-epitope vaccine candidate was predicted using the ColabFold server. **(B)** the refined 3D structure (colored) was superimposed on the initial “crude model” (gray) using the GalaxyRefine server. Validation of the refined model revealed **(C)** a Ramachandran plot analysis showing that 92.7% of protein residues (green crosses) are in favored regions, 5.2% (orange triangles) in allowed regions, and 1.2% (red circles) in disallowed (outlier) regions. Additionally, **(D)** ProSA-web provided a Z-score of -3.19 , **(E)** discontinuous B cell epitopes are indicated in red on the chimeric vaccine construct

showing the average RMSD values of the docked complex (Fig. 7B). B-factor values indicate the uncertainty associated with each atom. The computed eigenvalue for the multi-epitope vaccine candidate-TLR4 complex was $1.142149\text{e-}08$ which reflects motion stiffness with respect to each normal mode (Fig. 7C). The eigenvalue estimates the energy needed to deform the structure. Each normal mode of the complex is depicted by individual (purple) and cumulative (green) variance in the variance bar. It is worth noting that there is a negative correlation between the variance and the eigenvalue (Fig. 7D). Furthermore, the interactions between the multi-epitope vaccine candidate and TLR4 in the complex are depicted using a covariance matrix. The related motions among various pairs of residues are indicated by correlated (red), uncorrelated (white), and anti-correlated (blue) atomic movements within the multi-epitope vaccine candidate-TLR4 complex (Fig. 7E). An elastic network map was also created to show atom pairs linked by springs in the multi-epitope vaccine candidate-TLR4 complex. Each dot in the graph denotes a spring connecting the corresponding pair of atoms. The color of the dots indicates their stiffness; darker shades of grey represent stiffer regions, while lighter dots signify more flexible areas (Fig. 7F). All the findings from the normal mode analyses suggest the favourable interaction and stability within the multi-epitope vaccine candidate-TLR4 complex.

Immune simulations

C-ImmSim considers the successive and successful immune responses of the state of the cell and models the memory of immune cells by a mechanism that increases their half-life [103]. C-ImmSim server’s immune simulation produced results that align with real immune responses, as demonstrated by a significant increase in the generation of secondary responses. The primary response was characterized by high levels of IgM. The secondary and tertiary responses exhibited significant increases in B-cell populations and in the levels of IgG1, IgG2, IgM, and the combined IgG and IgM antibodies, accompanied by a corresponding decrease in antigen concentration (Fig. 8A, B). This profile suggests the development of immune memory, leading to enhanced clearance of the antigen during subsequent exposures (Fig. 8C). A similarly strong response was observed in the TH (helper) and TC (cytotoxic) cell populations, accompanied by corresponding memory development (Fig. 8C, D). Repeated exposure with 03 injections (administered

two-weeks apart) triggered increasing levels of IgG1 and decreasing levels of IgM, while IFN- γ concentration and TH cell population were maintained at high levels throughout the duration of exposure. In summary, the developed vaccine candidate induced both cellular and humoral immune responses, which are recognized as crucial for protection against *S. stercoralis*.

Population coverage analysis

To assess the potential global applicability of the designed multi-epitope vaccine, a population coverage analysis was performed using the IEDB population coverage calculation tool. This tool is used to assess the predicted global response to a specific set of MHC-restricted epitopes. The population coverage analysis, illustrated in Fig. 9, aimed to determine the likelihood that the selected CTL and HTL epitopes would bind to MHC molecules within the global population. The overall coverage for MHC class I CTL epitopes was 68.27%, while MHC class II HTL epitopes showed a coverage of 84.74%. For specific regions, the predicted coverage for MHC class I CTL epitopes was: Central Africa (56.88%), East Africa (52.73%), South Africa (48.77%), West Africa (53.07%), South America (68.2%), and Southeast Asia (47.54%). The highest percentage for MHC class I was found in South America at 68.2%, while Southeast Asia recorded the lowest with 47.54%. Similarly, for MHC class II HTL epitopes, the coverage estimates were: Central Africa (70.45%), East Africa (79.28%), South Africa (51.56%), West Africa, South America (81.29%), and Southeast Asia (70.92%). Here, West Africa had the highest coverage at 86.73%, while South Africa had the lowest at 51.56% (Supplementary tables 2 and 3). This detailed regional analysis allows for a better understanding of the vaccine’s potential efficacy in diverse populations across these areas, highlighting the importance of targeting regions with higher predicted coverage. This information is vital for guiding future clinical trial designs and vaccine deployment strategies.

In-silico cloning

The gene encoding the designed multi-epitope vaccine candidate was codon-optimized using the Java Codon Adaptation Tool (JCat) to enhance protein expression levels in *E. coli*. The total gene sequence comprised 1700 nucleotides, featuring a Codon Adaptation Index (CAI) of 1.0 and an average GC content of 50.48%. These findings indicate a favorable potential for expression in the *E. coli*

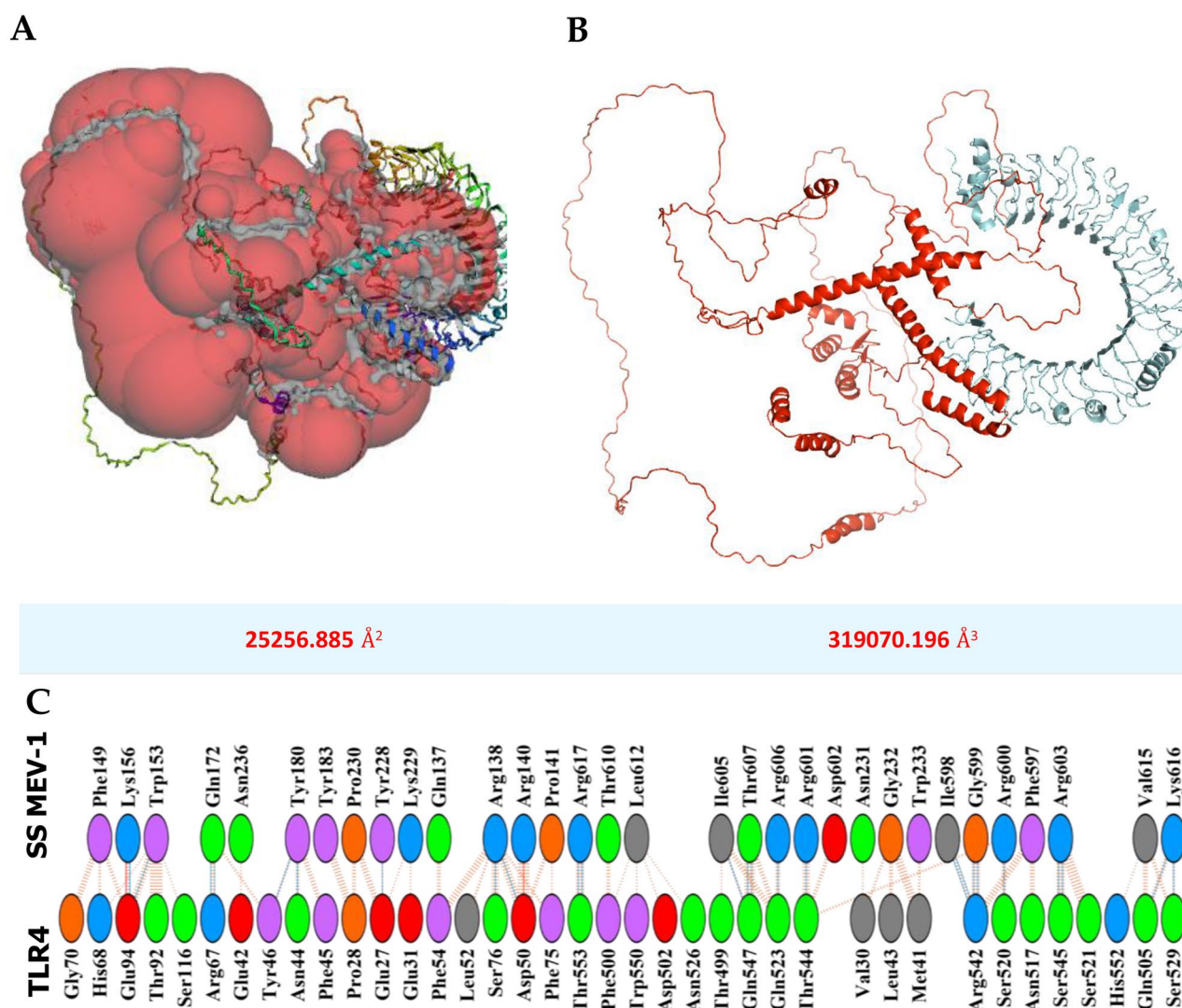


Fig. 6 Protein-protein interaction pocket prediction and molecular docking of the vaccine candidate with the TLR4 receptor were conducted. **(A)** the largest predicted PPI pocket is located at the interface of the designed chimeric vaccine candidate (in red), with a surface area of 25,256.885 Å² and a surface volume of 319,070.196 Å³. **(B)** The docked complex of the designed vaccine candidate (red) and the Toll-like receptor 4 chain (cyan) is shown. **(C)** The interaction network between the vaccine candidate and TLR4 is illustrated, with hydrogen bonding interactions depicted in blue. Non-bonded interactions and salt bridges are represented in orange. Charged residues are color-coded: positive in blue, negative in red, neutral in green, aliphatic in grey, and aromatic in purple

bacterial host. Typically, a GC content between 30 and 70% is considered optimal for effective protein expression [104, 105]. Finally, the adapted codon sequence was inserted into the appropriate sites of the pET30a (+) vector using SnapGene software v3.2.1 to create a recombinant plasmid in-silico (Fig. 10).

Discussions

Neglected tropical diseases (NTDs) are infectious diseases prevalent in tropical and subtropical regions, primarily affecting marginalized communities with limited access to healthcare, clean water, and sanitation [106] and include; several infections from helminths (e.g., soil-transmitted helminths [STHs], schistosomiasis),

protozoa (e.g., leishmaniasis, Chagas disease), bacteria (e.g., trachoma, leprosy), and viruses (e.g., dengue, chikungunya, and rabies) [107]. Strongyloidiasis remains a neglected parasitic disease with limited vaccine research, with few studies identifying potential vaccine antigens from L3-stage proteins in mouse models [108]. While there are chemotherapeutic treatments available for many diseases, they are not sustainable on their own due to high rates of reinfection, risk of drug resistance, and inconsistent management of drug treatment programs. Therefore, Preventive and/or therapeutic NTD vaccines are essential for long-term solutions [109, 110]. Vaccines and passive immunization strategies aimed at preventing or controlling *Strongyloides spp.* infections have been

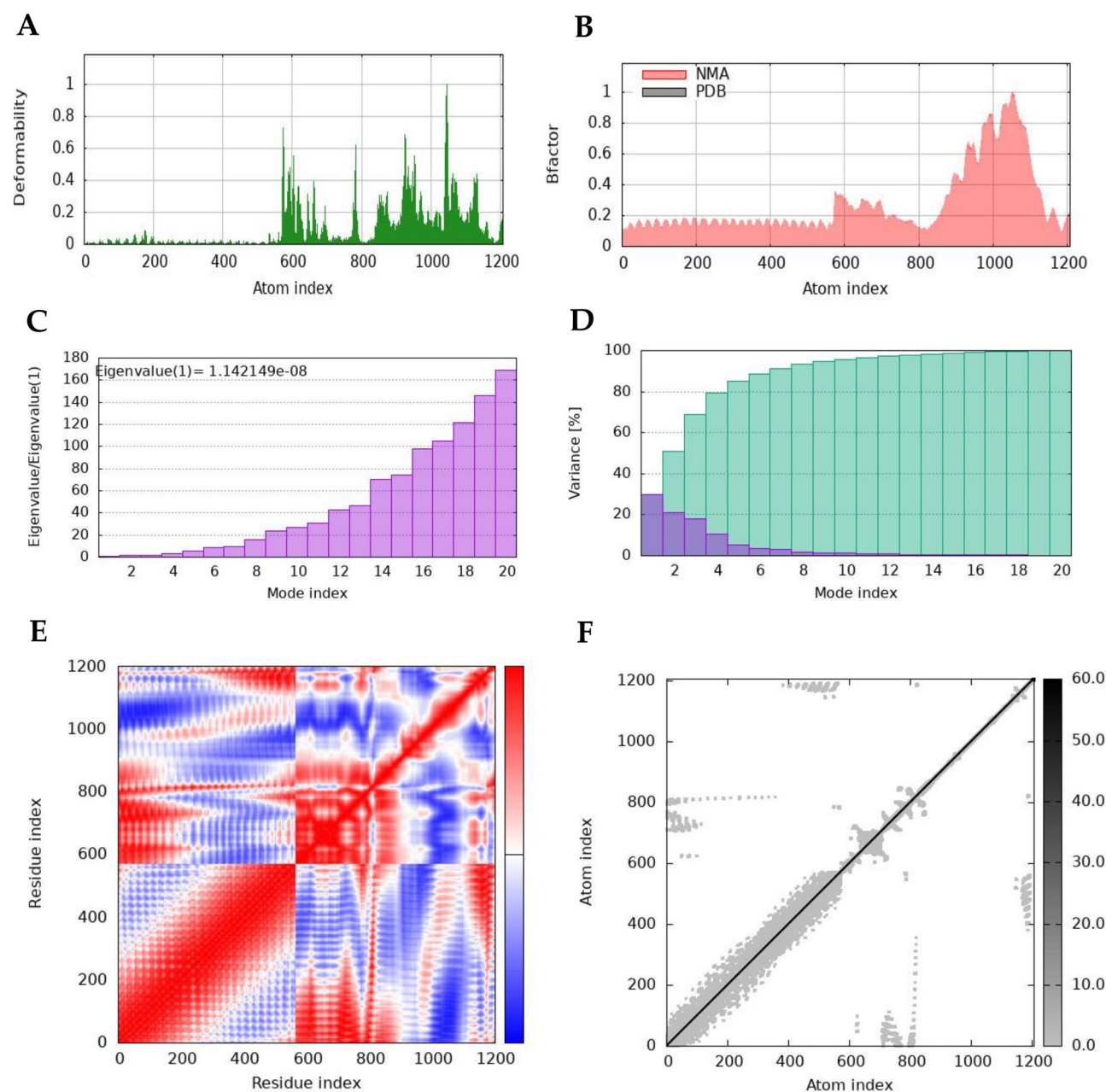


Fig. 7 Molecular dynamics simulation of the vaccine candidate-TLR4 docked complex. **(A)** Displays the main-chain deformability of the SS-MEV-1-TLR4 complex. **(B)** The B-factor quantifies the uncertainty associated with each atom. **(C)** The eigenvalue indicates the motion stiffness related to each normal mode. **(D)** The variance map shows individual (red) and cumulative (green) variances. **(E)** The covariance graph illustrates the mobility of residue pairs, indicating correlated (red), uncorrelated (white), or anti-correlated (blue) movements. **(F)** The elastic network model represents pairs of atoms connected by springs, with darker greys indicating greater stiffness of the springs

developed and tested in animal models. Vaccine efforts against strongyloidiasis include recombinant protein and DNA vaccines, as well as monoclonal antibody-based passive immunization; however, few human anthelmintic vaccines, including two for hookworm [111], have reached clinical trials, and there is no approved vaccine available for human *S. stercoralis* infection [112]. Herbert et al. (2002) reported most vaccine studies for

the control of *Strongyloides spp.* infection used DOC-Ag from *S. stercoralis* L3i in a mouse model, but none has been translated for use in humans. Considering the global prevalence, impact on quality of life, and severity in immunosuppressed individuals, advancing immunotherapeutic strategies for preventing and controlling strongyloidiasis is urgently needed [113]. Developing innovative immunotherapies for *S. stercoralis* is critical

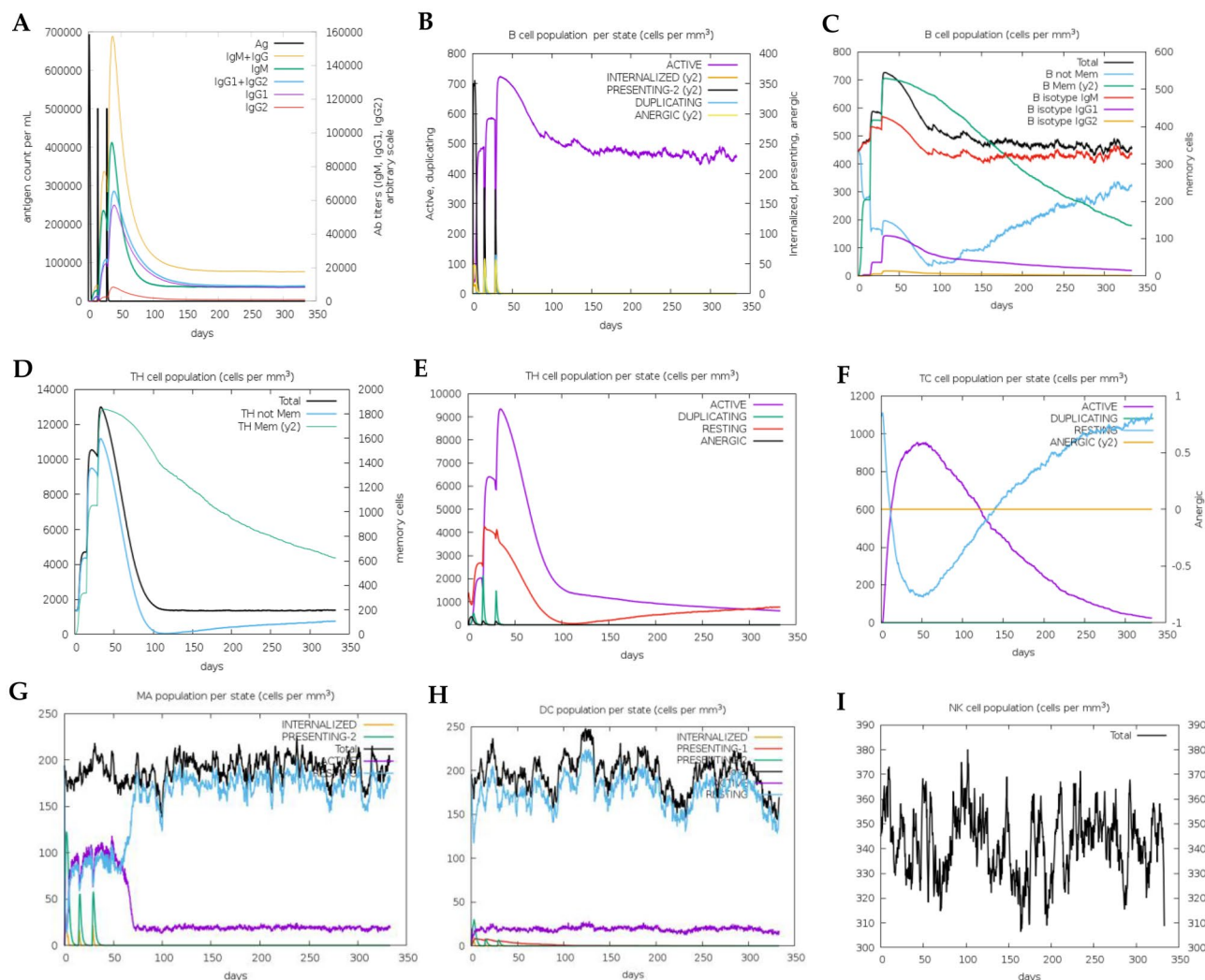


Fig. 8 In-silico simulation of the immune response triggered by the designed vaccine candidate: **(A)** Antigen and immunoglobulins, **(B)** B cell population by state, **(C)** Total B cell population, **(D)** TH cell population, **(E)** TH cell population by state, **(F)** TC cell population by state, **(G)** MA population by state, **(H)** DC population by state, and **(I)** NK cell population

due to the parasite's ability to persist long-term, posing fatal risks in immunosuppressed patients [114, 115]. Epitope-based vaccines provide a new strategy for eliciting targeted immune response while preventing reactions to unwanted epitopes (like epitopes that may drive immune-pathogenic or immunomodulatory responses) in the entire antigen [116]. Potential benefits of epitope-based vaccines include enhanced safety, the ability to systematically engineer the epitopes for greater potency, and the ability to concentrate immune responses on conserved epitopes [29].

Thus, this work aimed at the in-silico design and development of a potential multi-epitope vaccine candidate against *S. stercoralis* using 09 proteins found to be involved in one of the parasite stages of development (L3 larval stage). This stage is primarily targeted in vaccine development approaches, as any vaccine produced may

have both preventive and therapeutic potentials, as previously reported. Selected proteins and epitopes for the vaccine candidate show significant conservation across related strains, suggesting potential for cross-protection. Initially, we predicted B-cell and T-cell epitopes from the selected proteins and fused these using appropriate linkers in order to generate a multi-epitope peptide. This design represents a significant advancement over previous approaches by being the first multi-epitope vaccine designed for *S. stercoralis* that combines both B-cell and T-cell epitopes, along with the inclusion of multiple immunostimulatory components such as PADRE and TAT. [93, 117]. It has been reported that immunity to strongyloidiasis is dependent on both B- and T-cells [118–120]. The role for antibody-dependent cell-mediated cytotoxicity [120] and the TLR4 pathway in immunity to strongyloidiasis has also been well documented

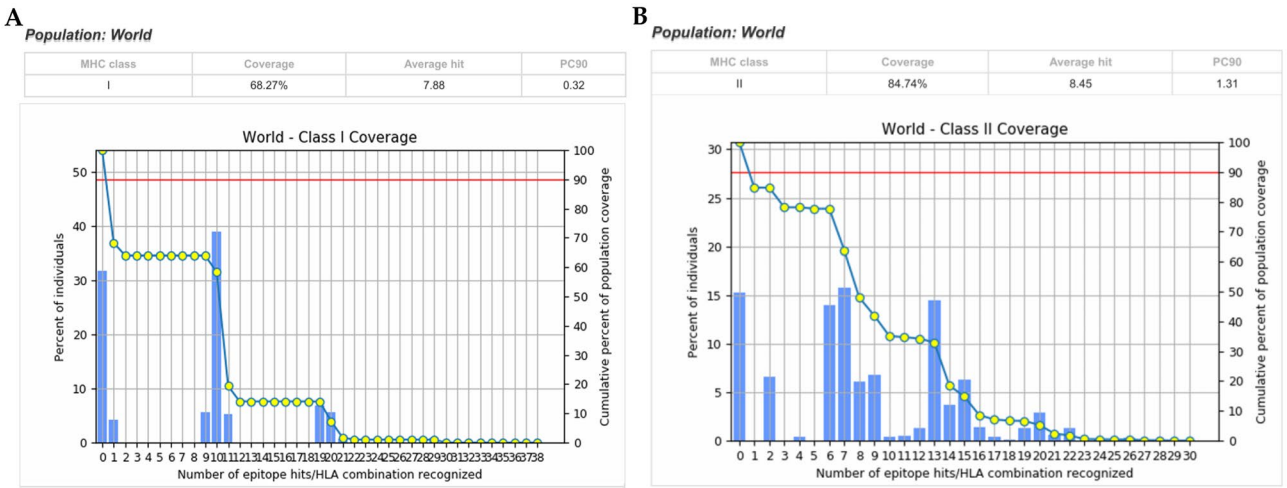


Fig. 9 Population coverage analysis of predicted T-cell epitopes across the global population is presented as follows: **(A)** MHC class I epitope coverage demonstrates a global population coverage of 68.27%, with average hit and PC90 values suggesting extensive HLA representation. **(B)** MHC class II epitope coverage reflects a global population coverage of 84.74%, highlighting the distribution of HLA class II alleles across various populations. The histograms and cumulative curves illustrate the percentage of individuals recognizing a specific number of epitopes-HLA combinations

[49]. Toll-like receptors (TLRs) play a critical role in facilitating interactions between helminth parasites and the host immune system by initiating innate immune responses that drive appropriate adaptive immunity [121, 122]. Several reports have noted that B cells promote and support Th2 type-immune responses during helminth infections, in order to control opposing Th1-type responses [123]. Reports show that C3H/HeJ mice with defective TLR4 signaling fail to develop adaptive immunity against *S. stercoralis* and *Brugia malayi* larvae, highlighting the critical role of TLR4 in protective immunity against nematodes [121, 124]. Additionally, C3H/HeJ mice did not develop protective immunity against larval stages of *Onchocerca volvulus* following immunization with irradiated L3 [125]. The incorporation of the *M. tuberculosis* resuscitation-promoting factor E (RpfE) as a built-in adjuvant was a deliberate strategy to enhance innate immune activation through Toll-like receptor 4 (TLR4) signaling. RpfE has been reported to function as a potent TLR4 agonist, promoting dendritic cell maturation and pro-inflammatory cytokine production, which are critical for the initiation of robust adaptive immune responses. Vaccine design is improved through the use of specialized spacer sequences [97]. As previously reported, AAY, GP GPG, and KK linkers [96, 126] were included between the predicted epitopes to create sequences with reduced junctional immunogenicity, therefore facilitating the rational design and construction of an effective multi-epitope vaccine [97]. The EAAAK linker, which has been previously reported to be valuable in the design of bifunctional proteins [50], was added between the built-in adjuvant sequence and the fused epitopes in order to reach a high level of expression and improved bioactivity of the fusion protein. In addition,

the TAT sequence, a cell-penetrating peptide (CPP), along with the Pan DR Epitope (PADRE), was incorporated into the design to enhance the immune response elicited by **SS-MEV-1**. Cell-penetrating peptides effectively deliver diverse cargoes and enhance peptide vaccine accumulation in lymph nodes, boosting immunogenicity [127, 128]. Conversely, PADRE is a strong activator of CD4+ responses, which has been reported to be critical for the control of *S. stercoralis* infection [120]. Immunoinformatics analyses of the designed vaccine construct revealed a significant presence of high-affinity MHC Class I and II, as well as linear B-cell epitopes. Additionally, IFN- γ epitopes were predicted in the final vaccine. It has been reported that IFN- γ secretion may help to control *S. stercoralis* infection [119]. The lack of allergenic, toxigenic properties, and the ability to cause an immune response (antigenicity) of the designed protein chimera further strengthens its potential as a vaccine candidate. These characterizations are crucial because efficacy and safety are essential components of the vaccine research and development pipeline [129]. The molecular weight of the designed vaccine candidate was 68.7 kDa, and it was predicted to be soluble upon expression; this is true with its simulated immunogenicity. The solubility of the overexpressed recombinant protein in the *E. coli* host is one of the crucial requirements of many biochemical and functional investigations [126]. The theoretical pI is estimated to be 10.06, indicating that the protein is basic in nature. Also, the predicted instability index indicates that the protein will be stable upon expression, thus further strengthening its potential for use. The aliphatic index showed that the protein contains aliphatic side chains, indicating potential hydrophobicity. All these parameters indicate

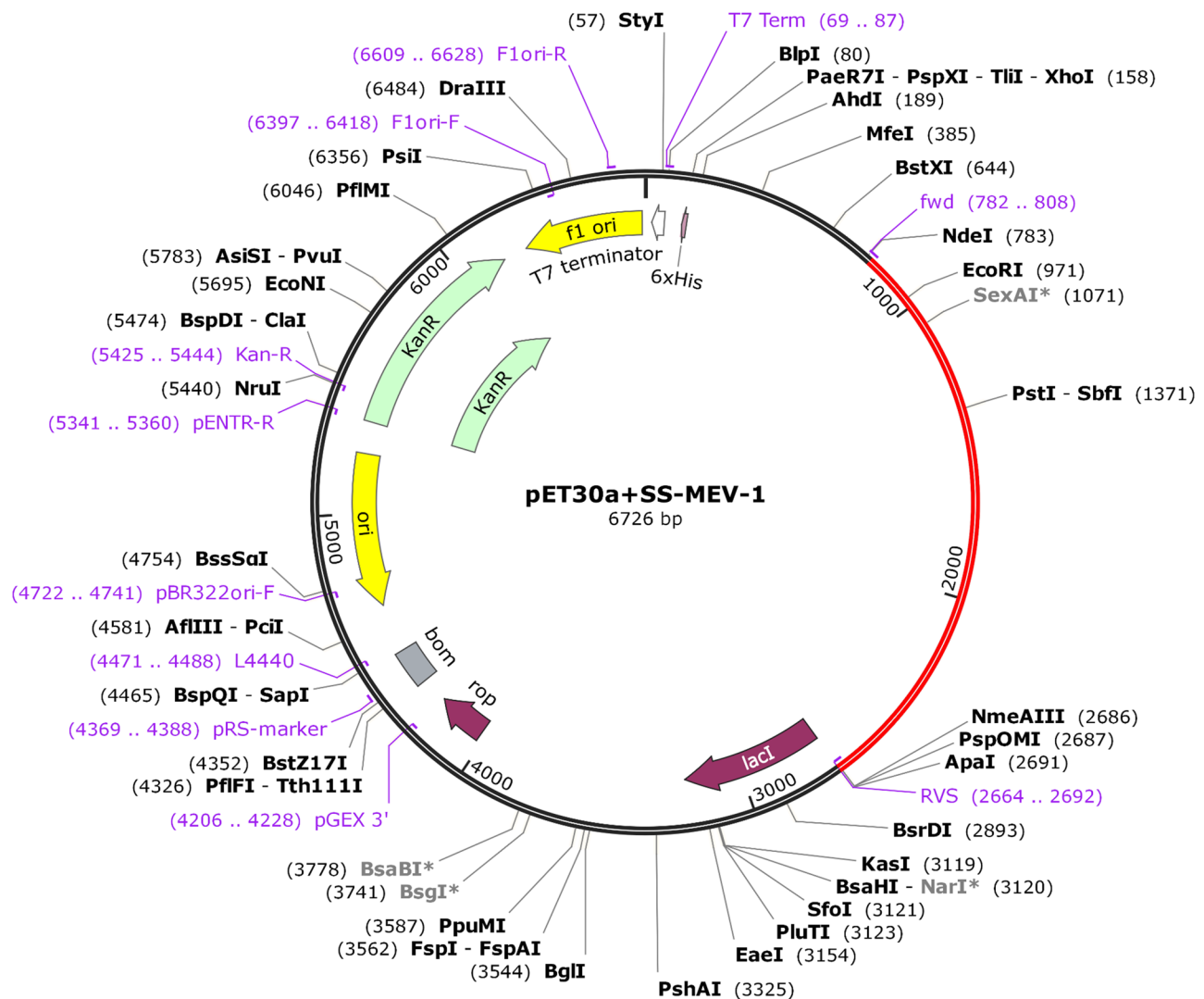


Fig. 10 In-silico restriction cloning of the final vaccine sequence into the pET30a (+) expression vector is shown, with the red section indicating the gene coding for the vaccine and the black circle representing the vector backbone

the recombinant protein is thermally stable and therefore suited for use in endemic areas, most of which are found in sub-Saharan Africa. Understanding the secondary and tertiary structures of the target protein is crucial in vaccine design [97]. Analyses of secondary structure predicted that the protein consisted mainly of coils (56%), with 31% of residues disordered. Regions of natively unfolded proteins and alpha-helical coiled-coil peptides have been noted as significant types of “structural antigens”. When evaluated in synthetic peptides, these two structural forms can fold into their native configurations, allowing them to be recognized by antibodies that are naturally induced in response to infection [130]. The 3D structure of the vaccine candidate showed significant improvement following refinement and exhibited desirable properties based on Ramachandran plot predictions. The Ramachandran plot indicated that the majority of

the residues are located in the favored and allowed regions (92.7%) with very few residues in the outlier region (Fig. 5); this indicates that the quality of the overall model is satisfactory. The findings reveal the presence of a large pocket in SS-MEV-1 and favorable interactions between TLR4 obtained with the presence of different bond types (Fig. 6). As the docking and NMA analyses indicated a stable interaction, normal mode analysis provides insights into collective motions, but full-scale molecular dynamics simulations (100+ ns) with detailed RMSD, RMSE, and radius of gyration analyses should be performed in future studies to comprehensively validate the structural stability of the vaccine-TLR4 complex. The chimeric antigen is anticipated to interact with the TLR4 on professional antigen-presenting cells like dendritic cells to elicit a protective immune response. This potential was also evident in the immune simulation analyses,

which predicted an overall rise in the elicited immune responses triggered by the administration of primer and booster doses. The immune simulation analyses also revealed the development of memory B-cells and T-cells, which persisted for several months. These memory cell populations are crucial to the swift elimination of the pathogen when the host is exposed to *S. stercoralis* [120, 131]. Immune simulation predicted outcomes aligned with typical immune responses. Following repeated exposure to the antigen, there was a general increase in the generated immune responses. In strongyloidiasis, IgG, IgM, and IgE responses to parasite antigens are implicated in disease protection [119, 132]. A population coverage analysis revealed that the selected epitopes could potentially cover 68.27% of the global population for MHC class I alleles and 84.74% for MHC class II alleles, ensuring broad immunogenic applicability (Fig. 9). All the findings discussed here are derived from computational studies. It is crucial to validate this data through both in vitro and in vivo investigations. We are focused on performing MM-PBSA calculations as a priority for the next phase of validation to strengthen the study. The next step, projected at the moment, is to conduct a comprehensive proteome-wide analysis, followed by the expression of this peptide in a bacterial system. Initial expression trials may require optimization, focusing on factors such as codon usage, expression strain, and tags. If challenges occur in expressing the construct in *E. coli*, alternative options include using mammalian or insect cells, which may offer a more conducive environment for proper protein folding and solubility. After successful expression, in-vitro immunogenicity testing will be conducted, taking into account the potential formation of inclusion bodies, which can be solubilized if needed. Following this, mouse immunization studies with challenge experiments will be conducted, along with optimization based on experimental data to validate the results obtained through immunoinformatics analyses. Results obtained from both in-vitro and in-vivo studies of **SS-MEV-1** will supply the necessary pilot data to determine whether the vaccine candidate can be advanced to clinical trials.

Limitations

Despite the in-silico demonstration of advantageous receptor binding, extensive HLA coverage, strong antigenicity, and structural stability of the multi-epitope vaccine construct developed to target *S. stercoralis*, several limitations persist. Notably, the absence of molecular dynamics (MD) simulations and MM-PBSA calculations limits the assessment of long-term stability and binding affinity. Furthermore, the computational predictions, including molecular docking with TLR4, normal mode analysis, codon optimization for *E. coli* production, and

epitope predictions, necessitate empirical substantiation, as no experimental validation has been performed. The simplified immune simulation parameters used may not fully capture the complexity of the immune response. Also, our approach does not incorporate a comprehensive proteome-wide analysis, which could provide insights into a broader array of potential antigenic targets. A more extensive analysis might reveal previously unconsidered proteins with high antigenic potential that are essential for an effective vaccine. Finally, potential expression and folding challenges in *E. coli* could hinder the production of a functional vaccine candidate. While this study lays a solid computational groundwork, experimental validation is essential to demonstrate the vaccine's safety and efficacy in real-world scenarios.

Conclusion

The elimination of *Strongyloides stercoralis* will require novel control strategies, including improved diagnostics, chemotherapeutics, and effective vaccines [17, 18, 25]. In this study, comprehensive immunoinformatics approaches were used to design a multi-epitope vaccine candidate incorporating B-cell and T-cell (HTL and CTL) epitopes derived from proteins expressed in the parasite's infective stages. This chimeric vaccine, uniquely combining PADRE and TAT sequences, demonstrates strong potential to elicit both prophylactic and therapeutic immune responses. Computational analyses predict structural stability, immunogenicity, and broad cross-protection, suggesting that this vaccine could complement existing control measures against strongyloidiasis. Moreover, the anticipated cross-protective effects may provide additional benefits for control programs targeting related neglected tropical diseases. These findings support further experimental evaluation of this multi-epitope vaccine as a promising immunotherapeutic candidate.

Abbreviations

BLAST	Basic Local Alignment Search Tool
CAI	Codon adaptation index
CASP	Critical Assessment of protein Structure Prediction
DNA	Deoxyribonucleic acid
GDT-HA	Global distance test – high accuracy
GRAVY	Grand Average of Hydropathy
HMM	Hidden Markov Models
IDRs	Intrinsically disordered regions
II	Instability index
Ig	Immunoglobulin
JCat	Java Codon Adaptation Tool
Kd	Dissociation constant
MEV	Multi epitope vaccine
SS-MEV-1	<i>Strongyloides stercoralis</i> multi epitope vaccine
MW	Molecular weight
NMA	Normal-mode analysis
PDB	Protein Data Bank
pI	Isoelectric pH
pLDDT	predicted Local Distance Difference Test
PRODIGY	PROtein binDing enerGY

RMSD	Root Mean Square Deviation
SP	Signal peptide
SS	<i>Strongyloides stercoralis</i>
TM	Transmembrane
WHO	World Health Organization

Supplementary Information

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Supplementary material 1

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Data availability

All the data generated or analyzed during this study are included in this published article (and its supplementary information files).

Declarations

Ethics

Not applicable.

Institutional review board

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Informed consent

Not applicable.

Conflicts of interest

The authors declare no conflicts of interest.

Competing interests

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Author details

¹Department of Biochemistry and Molecular Biology, Faculty of Science, University of Buea, Buea, Cameroon

²Tropical Disease Interventions, Diagnostics, Vaccines, and Therapeutics (TroDDIVaT) Initiative, Frontline Association, Tiko, Cameroon

³Department of Microbiology and Parasitology, Faculty of Science, University of Buea, Buea, Cameroon

⁴Laboratory of Pharmacognosy, Center for Interdisciplinary Research on Medicine (CIRM), University of Liège, Liège 4000, Belgium

⁵Department of Microbiology and Immunology, College of Medicine, Drexel University, Philadelphia, PA, USA

⁶Frailty in Ageing Research Group, Vrije Universiteit Brussel, Laarbeeklaan 103, Brussels B-1090, Belgium

⁷Department of Gerontology, Faculty of Medicine and Pharmacy, Vrije Universiteit Brussel, Laarbeeklaan 103, Brussels B-1090, Belgium

⁸Department of Computer and Information Sciences, College of Science and Technology, Covenant University, Ota, Nigeria

⁹Ngonpong Therapeutics, 3640 Concord Pike #1145, Delaware 19803, Wilmington, USA

¹⁰Department of Molecular Biology, Institute of Biology and Molecular Medicine, IBMM, Université Libre de Bruxelles, Gosselies, Charleroi, Belgium

¹¹Centre for Research in Infectious Diseases, CRID-CAM, Yaounde, Cameroon

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References

1. Schar F, et al. *Strongyloides stercoralis*: global distribution and risk factors. *PLoS Negl Trop Dis*. 2013;7(7):e 2288.
2. Agrawal V, Agarwal T, Ghoshal UC. Intestinal strongyloidiasis: a diagnosis frequently missed in the tropics. *Trans R Soc Trop Med Hyg*. 2009;103(3):242–46.
3. Puthiyakunnon S, et al. Strongyloidiasis—an insight into its global prevalence and management. *PLoS Negl Trop Dis*. 2014;8(8):e 3018.
4. Bethony J, et al. Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. *Lancet*. 2006;367(9521):1521–32.
5. Gordon CA, et al. Strongyloidiasis. *Nat Rev Dis Primers*. 2024;10(1):6.
6. Buonfrate D, et al. The global prevalence of *Strongyloides stercoralis* infection. *Pathogens*. 2020;9(6).
7. Jourdan PM, et al. Soil-transmitted helminth infections. *Lancet*. 2018;391(10117):252–65.
8. Albonico M, et al. Controlling soil-transmitted helminthiasis in pre-school-age children through preventive chemotherapy. *PLoS Negl Trop Dis*. 2008;2(3):e 126.
9. Olsen A, et al. Strongyloidiasis—the most neglected of the neglected tropical diseases? *Trans R Soc Trop Med Hyg*. 2009;103(10):967–72.
10. Tiberti N, et al. Systemic profile of immune factors in an elderly Italian population affected by chronic strongyloidiasis. *Parasit Vectors*. 2020. 13(1):515.
11. Luvira V, et al. *Strongyloides stercoralis*: a neglected but fatal parasite. *Trop Med Infect Dis*. 2022;7(10).
12. Keiser PB, Nutman TB. *Strongyloides stercoralis* in the immunocompromised population. *Clin Microbiol Rev*. 2004;17(1):208–17.
13. Montes M, et al. Regulatory T cell expansion in HTLV-1 and strongyloidiasis co-infection is associated with reduced IL-5 responses to *Strongyloides stercoralis* antigen. *PLoS Negl Trop Dis*. 2009;3(6):e456.
14. Horton J. Albendazole: a review of anthelmintic efficacy and safety in humans. *Parasitology*. 2000;121 Suppl: p. :S113–32.
15. Ikeda T. Pharmacological effects of ivermectin, an antiparasitic agent for intestinal strongyloidiasis: its mode of action and clinical efficacy. *Nihon Yakurigaku Zasshi*. 2003;122(6):527–38.
16. Geary TG, et al. Unresolved issues in anthelmintic pharmacology for helminthiases of humans. *Int J Parasitol*. 2010;40(1):1–13.
17. Osei-Atweneboana MY, et al. Phenotypic evidence of emerging ivermectin resistance in *Onchocerca volvulus*. *PLoS Negl Trop Dis*. 2011;5(3):e998.
18. Eng JK, et al. Ivermectin selection on beta-tubulin: evidence in *Onchocerca volvulus* and *haemonchus contortus*. *Mol Biochem Parasitol*. 2006;150(2):229–35.
19. Abraham D, et al. Immunization with the recombinant antigen ss-IR induces protective immunity to infection with *Strongyloides stercoralis* in mice. *Vaccine*. 2011;29(45):8134–40.
20. Bergquist R, Lustigman S. Control of important helminthic infections vaccine development as part of the solution. *Adv Parasitol*. 2010;73:297–326.
21. Shey RA, et al. Computational design and preliminary serological analysis of a novel multi-epitope vaccine candidate against onchocerciasis and related filarial diseases. *Pathogens*. 2021;10(2).
22. Bethony J, et al. Antibodies against a secreted protein from hookworm larvae reduce the intensity of hookworm infection in humans and vaccinated laboratory animals. *Faseb J*. 2005;19(12):1743–45.
23. Zhan B, et al. Molecular cloning, biochemical characterization, and partial protective immunity of the heme-binding glutathione S-transferases from the human hookworm *Necator americanus*. *Infect Immun*. 2010. 78(4):1552–63.
24. Bethony JM, et al. Randomized, placebo-controlled, double-blind trial of the Na-ASP-2 hookworm vaccine in unexposed adults. *Vaccine*. 2008;26(19):2408–17.
25. Maleki A, et al. In silico design of recombinant multi-epitope vaccine against influenza A virus. *BMC Bioinf*. 2022;22(Suppl 14):617.

26. Sanches RCO, et al. Immunoinformatics design of multi-epitope peptide-based vaccine against *Schistosoma mansoni* using transmembrane proteins as a target. *Front Immunol*. 2021;12:621706.
27. Pandey RK, et al. Immunoinformatics approaches to design a novel multi-epitope subunit vaccine against HIV infection. *Vaccine*. 2018;36(17):2262–72.
28. Lei Y, et al. Enhanced efficacy of a multi-epitope vaccine for type A and O foot-and-mouth disease virus by fusing multiple epitopes with *Mycobacterium tuberculosis* heparin-binding hemagglutinin (HBHA), a novel TLR4 agonist. Vol. 121. *Mol Immunol*; 2020. p. 118–26.
29. Zhou WY, et al. Therapeutic efficacy of a multi-epitope vaccine against *Helicobacter pylori* infection in BALB/c mice model. *Vaccine*. 2009;27(36):5013–19.
30. Sanami S, et al. Design of a multi-epitope vaccine against SARS-CoV-2 using immunoinformatics approach. *Int J Biol Macromol*. 2020;164:871–83.
31. Naderian R, et al. Rational design of multi-epitope vaccine for chandipura virus using an immunoinformatics approach. *PLoS One*. 2025;20(10):e0335147.
32. Culma MF. *Strongyloides stercoralis* proteome: a reverse approach to the identification of potential immunogenic candidates. Vol. 152. *Microb Pathog*; 2021. p. 104545.
33. Sanchez-Palencia LF, et al. Evaluation of the immunomodulatory effect of the recombinant 14-3-3 and Major antigen proteins of *Strongyloides stercoralis* against an infection by *S. venezuelensis*. *Vaccines (Basel)*. 2022;10(8).
34. Tarke A, et al. Targets and cross-reactivity of human T cell recognition of common cold coronaviruses. *Cell Rep. Med*. 2023;4(6):101088.
35. Pundir S, et al. UniProt tools. *Curr Protoc Bioinf*. 2016;53: 1 29 1–1 29 15.
36. Sanchez-Trincado JL, Gomez-Perosanz M, Reche PA. Fundamentals and methods for T- and B-Cell epitope prediction. *J Immunol Res* 2017. 2017;2680160.
37. Luo W, Yin Q. B cell response to vaccination. *Immunol Invest*, 2021. 50(7):780–801.
38. Clifford JN, et al. BepiPred-3.0: improved B-cell epitope prediction using protein language models. *Protein Sci*. 2022;31(12):e 4497.
39. Saha S, Raghava GP. Prediction of continuous B-cell epitopes in an antigen using recurrent neural network. *Proteins*. 2006;65(1):40–48.
40. Sette A, et al. The development of multi-epitope vaccines: epitope identification, vaccine design and clinical evaluation. *Biologicals*. 2001;29(3–4):271–76.
41. Peters B, et al. Identifying MHC class I epitopes by predicting the TAP transport efficiency of epitope precursors. *J Immunol*. 2003;171(4):1741–49.
42. Pedras-Vasconcelos JA, Pearce EJ. Type 1 CD8+ T cell responses during infection with the helminth *Schistosoma mansoni*. *J Immunol*. 1996;157(7):3046–53.
43. Nielsen M, Lund O. NN-align. An artificial neural network-based alignment algorithm for MHC class II peptide binding prediction. *BMC Bioinf*. 2009;10:296.
44. Reynisson B, et al. NetMHCpan-4.1 and NetMHCIIpan-4.0: improved predictions of MHC antigen presentation by concurrent motif deconvolution and integration of MS MHC eluted ligand data. *Nucleic Acids Res*. 2020;48(W1):W449–54.
45. Doytchinova IA, Flower DR. VaxiJen: a server for prediction of protective antigens, tumour antigens and subunit vaccines. *BMC Bioinf*. 2007;8:4.
46. Bui HH, et al. Predicting population coverage of T-cell epitope-based diagnostics and vaccines. *BMC Bioinf*. 2006;7:153.
47. Dey J, et al. Designing a novel multi-epitope vaccine to evoke a robust immune response against pathogenic multidrug-resistant *Enterococcus faecium* bacterium. *Gut Pathog*. 2022. 14(1):21.
48. Lee SJ, et al. A potential protein adjuvant derived from *Mycobacterium tuberculosis* Rv0652 enhances dendritic cells-based tumor immunotherapy. *PLoS One*. 2014;9(8):e104351.
49. Kerepesi LA, et al. Toll-like receptor 4 (TLR4) is required for protective immunity to larval *Strongyloides stercoralis* in mice. *Microbes Infect*. 2007;9(1):28–34.
50. Arai R, et al. Design of the linkers which effectively separate domains of a bifunctional fusion protein. *Protein Eng*. 2001;14(8):529–32.
51. Livingston B, et al. A rational strategy to design multi-epitope immunogens based on multiple Th lymphocyte epitopes. *J Immunol*. 2002;168(11):5499–506.
52. Alexander J, et al. Linear PADRE T helper epitope and carbohydrate B cell epitope conjugates induce specific high titer IgG antibody responses. *J Immunol*. 2000;164(3):1625–33.
53. Clark E, Nava B, Caputi M. Tat is a multifunctional viral protein that modulates cellular gene expression and functions. *Oncotarget*, 2017. 8(16):27569–81.
54. Vives E, et al. TAT peptide internalization: seeking the mechanism of entry. *Curr Protein Pept Sci*. 2003;4(2):125–32.
55. Reddy Chichili VP, Kumar V, Sivaraman J. Linkers in the structural biology of protein-protein interactions. *Protein Sci*. 2013;22(2):153–67.
56. Yang Y, et al. In silico design of a DNA-based HIV-1 multi-epitope vaccine for Chinese populations. *Hum Vaccin Immunother*, 2015. 11(3):795–805.
57. Yano A, et al. An ingenious design for peptide vaccines. *Vaccine*. 2005;23(17–18):2322–26.
58. Chen X, Zaro JL, Shen WC. Fusion protein linkers: property, design and functionality. *Adv Drug Deliv Rev*. 2013;65(10):1357–69.
59. Schoenborn JR, Wilson CB. Regulation of interferon-gamma during innate and adaptive immune responses. *Adv Immunol*. 2007;96:41–101.
60. Dhanda SK, Vir P, Raghava GP. Designing of interferon-gamma inducing MHC class-II binders. *Biol Direct*. 2013;8:30.
61. Gupta S, et al. Identification of B-cell epitopes in an antigen for inducing specific class of antibodies. *Biol Direct*. 2013;8:27.
62. Wilkins MR, et al. Protein identification and analysis tools in the ExPASy server. *Methods Mol Biol*. 1999;112:531–52.
63. Rosano GL, Ceccarelli EA. Recombinant protein expression in *Escherichia coli*: advances and challenges. *Front Microbiol*. 2014;5:172.
64. Wang C, Zou Q. Prediction of protein solubility based on sequence physicochemical patterns and distributed representation information with DeepSoluE. *BMC Biol*, 2023. 21(1):p. 12.
65. Magnan CN, et al. High-throughput prediction of protein antigenicity using protein microarray data. *Bioinformatics*. 2010;26(23):2936–43.
66. Dimitrov I, et al. AllerTOP v.2-a server for in silico prediction of allergens. *J Mol Model*. 2014;20(6):2278.
67. Dimitrov I, et al. AllergenFP: allergenicity prediction by descriptor fingerprints. *Bioinformatics*. 2014;30(6):846–51.
68. Buchan DWA, Jones DT. The PSIPRED protein analysis workbench: 20 years on. *Nucleic Acids Res*. 2019;47(W1):W402–07.
69. MacRaid CA, et al. Disordered epitopes as peptide vaccines. *Pept Sci (Hoboken)*. 2018;110(3):e24067.
70. Erdos G, Pajkos M, Dosztanyi Z. IUPred3: prediction of protein disorder enhanced with unambiguous experimental annotation and visualization of evolutionary conservation. *Nucleic Acids Res*. 2021;49(W1):W297–303.
71. Ishida T, Kinoshita K. PrDOS: prediction of disordered protein regions from amino acid sequence. *Nucleic Acids Res*. 2007;35(Web Server issue):W460–4.
72. Patil NA, et al. Cellular disulfide bond formation in bioactive peptides and proteins. *Int J Mol Sci*. 2015;16(1):1791–805.
73. Ryan N, et al. Immunoinformatics approach for design novel multi-epitope prophylactic and therapeutic vaccine based on capsid proteins L1 and L2 and oncoproteins E6 and E7 of human papillomavirus 16 and human papillomavirus 18 against cervical cancer. *Osong Public Health Res Perspect*. 2024;15(4):307–28.
74. Cheng J, Saigo H, Baldi P. Large-scale prediction of disulphide bridges using kernel methods, two-dimensional recursive neural networks, and weighted graph matching. *Proteins*. 2006;62(3):617–29.
75. Jumper J, et al. Highly accurate protein structure prediction with AlphaFold. *Nature*. 2021;596(7873):583–89.
76. Mirdita M, et al. ColabFold: making protein folding accessible to all. *Nat Methods*. 2022;19(6):679–82.
77. Heo L, Park H, Seok C. GalaxyRefine: protein structure refinement driven by side-chain repacking. *Nucleic Acids Res*. 2013;41(Web Server issue):W384–8.
78. Wiederstein M, Sippl MJ. ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins. *Nucleic Acids Res*. 2007;35(Web Server issue):W407–10.
79. Colovos C, Yeates TO. Verification of protein structures: patterns of non-bonded atomic interactions. *Protein Sci*. 1993;2(9):1511–19.
80. Barlow DJ, Edwards MS, Thornton JM. Continuous and discontinuous protein antigenic determinants. *Nature*. 1986;322(6081):747–48.
81. Sivalingam GN, Shepherd AJ. An analysis of B-cell epitope discontinuity. *Mol Immunol*. 2012. 51(3–4):304–09.
82. Ponomarenko J, et al. ElliPro: a new structure-based tool for the prediction of antibody epitopes. *BMC Bioinf*. 2008;9:514.
83. Hoie MH, et al. DiscoTope-3.0: improved B-cell epitope prediction using inverse folding latent representations. *Front Immunol*. 2024;15:1322712.
84. Tian W, et al. Castp 3.0: computed atlas of surface topography of proteins. *Nucleic Acids Res*. 2018;46(W1):W363–67.
85. Kozakov D, et al. The ClusPro web server for protein-protein docking. *Nat Protoc*. 2017. 12(2):255–78.

86. Larsen MV, et al. Large-scale validation of methods for cytotoxic T-lymphocyte epitope prediction. *BMC Bioinf.* 2007;8:424.
87. Teilum K, Olsen JG, Kragelund BB. Functional aspects of protein flexibility. *Cell Mol Life Sci.* 2009;66(14):2231–47.
88. Lopez-Blanco JR, et al. iMODS: internal coordinates normal mode analysis server. *Nucleic Acids Res.* 2014;42(Web Server issue):W271–6.
89. Reis M, Wernisch L, Savva R. Unexpected correlations between gene expression and codon usage bias from microarray data for the whole *Escherichia coli* K-12 genome. *Nucleic Acids Res.* 2003;31(23):6976–85.
90. Nussinov R. Eukaryotic dinucleotide preference rules and their implications for degenerate codon usage. *J Mol Biol.* 1981;149(1):125–31.
91. Shey RA, et al. In-silico design of a multi-epitope vaccine candidate against onchocerciasis and related filarial diseases. *Sci Rep.* 2019;9(1):4409.
92. Rapin N, et al. Computational immunology meets bioinformatics: the use of prediction tools for molecular binding in the simulation of the immune system. *PLoS One.* 2010;5(4):e 9862.
93. Dishnica K, et al. Novel insights into the somatic proteome of *Strongyloides stercoralis* infective third-stage larvae. *Parasit Vectors.* 2023;16(1):45.
94. Marcilla A, et al. Proteomic analysis of *Strongyloides stercoralis* L3 larvae. *Parasitology.* 2010;137(10):1577–83.
95. Brindley PJ, et al. Antigens from the surface and excretions/secretions of the filariform larva of *Strongyloides stercoralis*. *Mol Biochem Parasitol.* 1988;28(3):171–80.
96. Ali M, et al. Exploring dengue genome to construct a multi-epitope based subunit vaccine by utilizing immunoinformatics approach to battle against dengue infection. *Sci Rep.* 2017;7(1):9232.
97. Meza B, et al. A novel design of a multi-antigenic, multistage and multi-epitope vaccine against *Helicobacter pylori*: an in silico approach. Vol. 49. *Infect Genet Evol.* 2017. p. 309–17.
98. Kyte J, Doolittle RF. A simple method for displaying the hydropathic character of a protein. *J Mol Biol.* 1982;157(1):105–32.
99. Ikai A. Thermostability and aliphatic index of globular proteins. *J Biochem.* 1980;88(6):1895–98.
100. Chung EH. *Vaccine allergies*. *Clin Exp vaccine Res.* 2014;3(1):50–57.
101. Duan T, et al. Toll-like receptor signaling and its role in cell-mediated immunity. *Front Immunol.* 2022;13:812774.
102. Venugopal PG, Nutman TB, Semnani RT. Activation and regulation of toll-like receptors (TLRs) by helminth parasites. *Immunol Res.* 2009. 43(1–3):252–63.
103. Castiglione F, et al. Simulating Epstein-Barr virus infection with C-ImmSim. *Bioinformatics.* 2007;23(11):1371–77.
104. Akhtar H, et al. Over expression of a synthetic gene encoding interferon lambda using relative synonymous codon usage bias in *Escherichia coli*. *Pak J Pharm Sci.* 2013;26(6):1181–88.
105. Hia F, Takeuchi O. The effects of codon bias and optimality on mRNA and protein regulation. *Cell Mol Life Sci.* 2021;78(5):1909–28.
106. WHO. World Health Organization. Ending NTDs together towards 2030. 2021.
107. Molyneux DH, Savioli L, Engels D. Neglected tropical diseases: progress towards addressing the chronic pandemic. *Lancet.* 2017;389(10066):312–25.
108. Herbert DR, et al. Immunoaffinity-isolated antigens induce protective immunity against larval *Strongyloides stercoralis* in mice. *Exp Parasitol.* 2002;100(2):112–20.
109. Hotez PJ, et al. Eliminating the neglected tropical diseases: translational Science and New technologies. *PLoS Negl Trop Dis.* 2016;10(3):e0003895.
110. Beaumier CM, et al. New vaccines for neglected parasitic diseases and dengue. *Transl Res.* 2013. 162(3):144–55.
111. Hotez PJ, et al. Human anthelmintic vaccines: rationale and challenges. *Vaccine.* 2016;34(30):3549–55.
112. Wong MTJ, et al. Soil-transmitted helminth vaccines: where are we now? *Acta Trop.* 2023;239:106796.
113. Levenhagen MA, Conte H, Costa-Cruz JM. Current progress toward vaccine and passive immunization approaches for *Strongyloides* spp. Vol. 180. *Immunol Lett.* 2016. p. 17–23.
114. Ramanathan R, Nutman T. *Strongyloides stercoralis* infection in the immunocompromised host. *Curr Infect Dis Rep.* 2008;10(2):105–10.
115. Romero-Cabello R, et al. Hyperinfection with *Strongyloides stercoralis*. *BMJ Case Rep.* 2012. 2012.
116. Vartak A, Suchek SJ. Recent advances in subunit vaccine carriers. *Vaccines (Basel).* 2016;4(2).
117. Huaman MC, et al. Gelatin particle indirect agglutination and enzyme-linked immunosorbent assay for diagnosis of strongyloidiasis using *Strongyloides venezuelensis* antigen. *Trans R Soc Trop Med Hyg.* 2003;97(5):535–38.
118. Mackenzie JR, et al. Eosinophils promote allergic disease of the lung by regulating CD4(+) Th2 lymphocyte function. *J Immunol.* 2001;167(6):3146–55.
119. Kerepesi LA, et al. Human immunoglobulin G mediates protective immunity and identifies protective antigens against larval *Strongyloides stercoralis* in mice. *The J Infect Dis.* 2004;189(7):1282–90.
120. Rajamanickam A, et al. Altered levels of memory T cell subsets and common gamma cytokines in *Strongyloides stercoralis* infection and partial reversal following anthelmintic treatment. *PLoS Negl Trop Dis.* 2018;12(5):e0006481.
121. Rajasekaran S, Anuradha R, Bethunaickan R. TLR specific immune responses against helminth infections. *J Parasitol Res.* 2017. 2017;6865789.
122. Ludwig-Portugall I, Layland LE. Tlrs, Treg, and B cells, an interplay of regulation during helminth infection. *Front Immunol.* 2012;3:8.
123. Hernandez HJ, Wang Y, Staderer MJ. In infection with *Schistosoma mansoni*, B cells are required for T helper type 2 cell responses but not for granuloma formation. *J Immunol.* 1997;158(10):4832–37.
124. Hayashi Y, et al. Protective immunity against *Brugia malayi* infective larvae in mice. I. Parameters of active and passive immunity. *Am J Trop Med Hyg.* 1989;41(6):650–56.
125. Kerepesi LA, et al. Protective immunity to the larval stages of onchocerca volvulus is dependent on Toll-like receptor 4. *Infect Immun.* 2005. 73(12):8291–97.
126. Khatoun N, Pandey RK, Prajapati VK. Exploring leishmania secretory proteins to design B and T cell multi-epitope subunit vaccine using immunoinformatics approach. *Sci Rep.* 2017;7(1):8285.
127. Patel SG, et al. Cell-penetrating peptide sequence and modification dependent uptake and subcellular distribution of green fluorescent protein in different cell lines. *Sci Rep.* 2019;9(1):6298.
128. Backlund CM, et al. Cell-penetrating peptides enhance peptide vaccine accumulation and persistence in lymph nodes to drive immunogenicity. *Proc. Natl. Acad. Sci. U. S. A.* 2022;119(32):e2204078119.
129. Curlin G, et al. Integrating safety and efficacy evaluation throughout vaccine research and development. *Pediatrics.* 2011;127 Suppl 1(Suppl 1):p:S9–15.
130. Wu Y, Collier JH. Alpha-helical coiled-coil peptide materials for biomedical applications. *Wiley Interdiscip Rev Nanomed Nanobiotechnol.* 2017;9(2).
131. Herbert DR, et al. The role of B cells in immunity against larval *Strongyloides stercoralis* in mice. *Parasite Immunol.* 2002. 24(2):95–101.
132. Bonne-Annee S, Hess JA, Abraham D. Innate and adaptive immunity to the nematode *Strongyloides stercoralis* in a mouse model. *Immunol Res.* 2011;51(2–3):205–14.

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