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Research Article

Immunoinformatics Design of Multi-Epitope Peptide-Based Vaccine Against *Cyprinid Herpesvirus-3* (CyHV-3) Targeting Thymidine Kinase Proteins

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Abstract

The common carp *Cyprinus carpio* is a freshwater teleost and is among the most economically significant fishes in aquaculture throughout the world. Taxonomically, *C. carpio* are a complex of species including subspecies *Cyprinus carpio carpio. C. carpio* are now threatened by *Cyprinid Herpesvirus*-3 (CyHV-3), the causative agent of Koi Herpesvirus Disease (KHVD), which causes severe morbidity and mortality in ornamental koi and common carp and can infect or be transmitted by other species. Despite these devastating circumstances, effective vaccinations or other medications for the control of KHVD are not readily available. For this reason, the aim of the current study was to formulate a multi-epitope vaccine against *Cyprinid Herpesvirus*-3 (CyHV-3) using an immunoinformatics approach. To assess the immunodominant T- and B-cell epitopes, the CyHV-3 proteomes were employed. Following a thorough evaluation, we constructed a strategy for vaccination employing four possible epitopes selected from among each of the three relevant epitope groups: cytotoxic T-lymphocyte, helper T-lymphocyte and linear B-lymphocyte. Important qualities used in the evaluation of the resultant vaccine are that it will be highly soluble, antigenic, immunogenic and non-allergenic. Among acceptable physicochemical qualities, the anticipated structure of the vaccine bears a close resemblance to that of the original protein. Additional considerations include a robust and sustained predicted binding between the vaccine and the Toll-Like Receptor (TLR9). Simulations of molecular dynamics confirm the likelihood of a strong binding stability and structural tightness. Moreover, the computer-generated immunological simulation revealed that the vaccine, when administered to fish, should induce immune responses comparable to those in real life. Finally, codon optimization based on *Escherichia coli* K12 produced favorable indications of GC content and acceptably high CAI value, as applicable to the cloning vector pET28+ (a). Overall, these results show that the proposed peptide vaccine is a promising option for CyHV-3 prophylaxis.

Keywords: CyHV-3; Cyprinid Herpesvirus; Multi-Epitope Vaccine; Immunoinformatics; Immune Simulation; Vaccine

Introduction

Common carp (including ornamental koi carp) *Cyprinus carpio L. is* commercially valuable and is historically the first domesticated freshwater fish species; it is cultured extensively across the world [1-3]. This is one of the most important aquatic species raised for human consumption as well as for ornamental purposes, currently ranking 4th among cultured fish species, representing 8.6% of the world's finfish harvest [3]. Common carp farming has negligible environmental consequences; in 2020, 4.236 million tonnes of this fish was farmed, an increase of over 200 thousand tonnes compared to five years earlier [3,4]. This substantial increase in production, which was already at a very high level, further increases the chance of infectious diseases spreading in a farmed species [5]. The Cyprinidae family comprises a majority of farmed freshwater fish production worldwide, serving as a critically essential food source for many Asian countries. Seven of the nine fish species most abundantly cultured in 2020 are members of this family [3]. Koi herpes viruses infect common carp and ornamental koi fish, frequently resulting in mass mortality on carp farms [6]. The CyHV-3 variant of these viruses has also been found to be carried asymptomatically by other cyprinids, which could represent a greater threat to common carp and koi fish [7,8]. Moreover, the CyHV-3 strain is more dangerous due to its reactivation ability in response to environmental cues that induce stress; once infected, it persists and can inflict damage throughout the life span.

The emerging agent CyHV-3, which belongs to the family Alloherpesviridae, is the cause of fatal illnesses in koi and common carp fish. Following the dissemination of the virus, numerous symptoms have been observed in fish, including gill and internal organ necrosis, lethargy, epidermal abrasions and excessive mucus production [6]. The virus affects not only the internal organs, such as the brain, kidney tissues, liver and intestines, but also the external body. Moreover, due to a lack of immunity, secondary infections that occur after a first infection with CyHV-3 increase the risk of mortality [9].

The genome of CyHV-3 virions is contained within an icosahedral capsid, consisting of a lipid envelope and a thin, amorphous layer of protein. CyHV-3 residues are located in the transition region between the capsid and envelope. Common and koi carp are susceptible to infection with CyHV-3 transmission through gills or skin [10,11].

The common carp (*Cyprinus carpio carpio*) currently ranks among the most abundantly cultured fish species worldwide [3]. Some Cyprinids and a few other fishes have displayed positive PCR or in-situ hybridization results for CyHV-3, suggesting that they may be capable of transmitting viable virus as either carriers or vectors. Generally, results regarding the susceptibility of species other than *Cyprinus carpio* and its hybrids to CyHV-3 are indeterminate and remain subject to further clarification [12].

Vaccines are desperately needed to safeguard animal welfare and aquaculture. The Kovax company has developed live, attenuated viral vaccines for both common and koi carp, although they have only limited commercial availability [13,14]. One report cites the licensing of the Kovax vaccine as "available for use in Israel" [15]. A second live, attenuated viral vaccine against CyHV-3 has been described recently, although we have been unable to confirm its commercial availability [16]. Regardless, the aquaculture industry is very much in need of the development of effective measures for the prevention of CyHV-3.

Epitopes are critical for clinical and biological studies because they enable the development of vaccines against diverse and rapidly evolving diseases. Multi-epitopes play a significant role in the development of vaccines because they serve as precursors to diverse and rapidly evolving diseases. For in silico vaccine design, both B-cell and T-cell epitopes were utilized to evaluate potential designs for the vaccine. Initially, we chose a suitable receptor and linker for designing a vaccine that would bind to Cytotoxic T Lymphocyte (CTL), High-affinity T Lymphocyte (HTL) and Linear B Lymphocyte (LBL) epitopes [17].

Toll-Like Receptor 9 (TLR9) was used as an adjuvant in the development of a potentially successful vaccination, selected because of its efficiency at recognizing viral proteins; this quality is associated with the ability to prevent infection. TLR9 is involved in boosting the immune response and is also essential for efficient translation and synthesis [18]. Consequently, epitopes can be used to predict the immune reactions of the host, including humoral and cell-mediated immune responses. Moreover, toxicity is among the fundamental obstacles to preparing safe and effective vaccines against infection. As a result, it is necessary to optimize the vaccine before using it as an in vivo therapeutic agent [19]. The vaccine development process can be made more safe, effective

and economically viable through computational design. Peptide vaccines are advantageous in comparison with traditional vaccines and can be prepared and tested for use in the next generation of vaccines. These are constructed specifically to target the immune system, minimizing or eliminating allergic effects. T-cell and B-cell epitopes have been combined to create a multi-epitope vaccine, which has been linked with an adjuvant protein to ensure it is both antigenic and allergen-free [20].

Therefore, the vaccine was docked with the immunological receptors of the vaccine in order to predictively evaluate in vivo vaccine stability. Finally, codon optimization was performed using an in silico approach to assure maximum expression in the chosen host expression system.

Material and Methods

Proteome Collection and Evaluations of Probable Proteome Antigenicity

The NCBI database was used to identify 224 potentially useful CyHV-3 proteomes for the intended vaccine development. This database is freely accessible and is popularly accepted and respected as a source of accurate protein sequence data. Viral proteome sequences were downloaded from the VaxiJen server (v2.0) for predictive assessment of antigenicity; this server uses Auto Cross-Covariance (ACC) transformation techniques and our assessments were made with a 0.525 threshold for antigenicity (VaxiJen v2.0 server [21]). Those protein sequences in this proteome with the highest antigenicity scores were selected for further study.

Predictions of CTL Epitopes and MHC Class I Alleles

Cytotoxic T lymphocytes (CTL) are immunocytes that are capable of directly destroying other infected cells. Through the viral entry process, they contribute significantly to host defensive mechanisms. The selected protein sequences were evaluated using the NetCTLv1.2 server (http://www.cbs.dtu.dk/services/NetCTL/) to predict CTL epitopes. Selected epitopes were subjected to further evaluation using the following three servers: VaxiJen v2.0, MHC class I immunogenicity (http://tools.iedb.org/immunogenicity/), ToxinPred (http://crdd.osdd.net/raghava/toxinpred/) and AllerTop v2.0 (https://ddg-pharmfac.net/3). The default parameters were utilized for all predictions from these servers.

Prediction and Evaluation of Helper T-Lymphocyte Epitopes

Helper T-cells (HTLs) are critical in the generation of adaptive immunity because they identify foreign antigens, activating B and cytotoxic T-cells to eliminate pathogens. The MHC class II binding allele prediction tool (at http://tools.iedb.org/mhcii/) was used to identify the HTL epitopes. The antigenicity and capacity to produce interferon-c (IFNc), interleukin-4 (IL4) and interleukin-10 were used for further investigation of the anticipated epitopes (IL10). VaxiJen v2.0 was used to forecast the Antigens, while the IFNepitope, IL4pred and IL10pred servers were used with default settings to predict the IFNc, IL4 and IL10 characteristics, respectively.

Prediction and Assessment of Linear B Lymphocyte Epitopes

The induction of humoral immunity, also known as antibody-mediated immunity, relies heavily on B-cell epitopes. Antibodies and B-cells, which are a variety of leukocytes, work together to stimulate the immune system and destroy invaders. The iBCE-EL server, accessible at http://www.thegleelab.org/iBCE-EL/, was used with the default settings to predict the linear B lymphocyte epitopes. Evaluating projected LBL epitopes also involved using the VaxiJen v2.0, ToxinPred and AllerTop v2.0 servers.

Estimation of Population Coverage

To gauge a vaccine's global efficacy, computational vaccination design analyzes the frequency of HLA (Human Leukocyte Antigen) alleles that correspond to the target epitope. HLA binding alleles for T-cell epitopes were used to determine population coverage. Selected epitopes and associated allelic data were submitted using the IEDB population coverage tool.

Peptide Modeling and Molecular Docking

We used the PEP-FOLD server v3.0 to submit our chosen CTL and HTL epitopes for modeling. A total of 200 simulations were carried out using a sOPEP sorting algorithm. The HLA-B 15:01 and HLA-C 06:02 alleles were considered for chosen CTL epitopes,

whereas the DRB101:01 and DRB115:01 alleles were evaluated for chosen HTL epitopes. The HLA allele crystal structures were downloaded from the Protein Data Bank (PDB) and analyzed in BIOVIA Discovery Studio. For estimation of molecular docking capabilities, the AutoDock program constructed a grid-box around the functional location of each HLA allele. The docking properties of selected epitopes in their interactions with corresponding HLA alleles were determined using the AutoDock Vina script. The effectiveness of epitope binding was measured using the corresponding co-crystal ligands as positive controls.

The Multi-Epitope Approach to Designing a Potentially Safe, Effective Vaccine

The chosen CTL, HTL and LBL epitopes were linked to the potential vaccine design using an appropriate adjuvant and linkers. Toll-Like Receptor 9 (TLR9) was selected and utilized as an adjuvant because viral proteins recognize this receptor and it is important for the translation and synthesis of the vaccine candidate for structural adaptation. For this reason, the CCL35.2 protein was evaluated as a potential adjuvant for immunogenic improvement of the vaccine candidate. The bi-functional linker EAAAK, which divides two weakly interacting B domains with numerous helix-forming peptides, was used to connect the vaccine's front to an adjuvant. While Gly-Pro-Gly-Pro-Gly (GPGPG) linkers were used to join the CTL of choice, Lys-Lys (KK) linkers were employed to join the LBL and Ala-Ala-Tyr (AAY) linkers were used to join the CTL. Proteasomes utilize AAY cleavage sites to improve protein stability, reduce their immunogenicity and improve epitope presentation. The bi-lysine KK linker assists with the maintenance of the vaccine construct's independent immunogenic activity, while the glycine-proline linker of the GPGPG avoids the generation of junctional epitopes and simplifies immune processing.

Evaluation of the Physicochemical and Immune Systems

A protein's physicochemistry provides considerable insight into its fundamental characteristics. Predictions of physicochemical features of the vaccine were carried out using the ProtParam service, allowing researchers to more fully grasp the vaccine's core nature. We also used the VaxiJen v2.0, MHC-I immunogenicity and SOLPRO servers to assess the vaccine's immunological qualities [22].

Secondary Structure Prediction

PSIPRED v4.0 (PSI-blast-based secondary structure prediction) and SOPMA (Self-Optimized Prediction Method with Alignment) servers were used to determine the construct's 2D structural properties, such as alpha helix, beta turn and random coils [23]. The SOPMA server has a prediction accuracy greater than 80%. Molecular 2D structural characteristics were examined in order to ascertain the vaccine's structural and compositional qualities.

Homology Modelling, Refining and Validating 3D Structures

The popular server I-TASSER was employed for prediction of the vaccine's 3D structure using the constructed amino acid residues. ITASSER web produces 3D structures showing the protein's structure and roles with a high degree of precision utilizing an advanced algorithm. A protein sequence can be predicted and determined using this web server, including the C-score, TM-score value, RMSD and top five models. The generated 3D structure was downloaded in PDB format based on the C-score. Its values range from -5 to 2, where higher values indicate a highly confident protein model. To refine the vaccine structure, the identified 3D structure was submitted to the GalaxyRefine online web-based server. CASP10 refine was used to run this webserver [24]. Vaccine structure validity was ascertained based on the Ramachandran plot score (a measure of the standard deviations from the mean value) and the Z-score value (Z-scores within the native protein range indicate acceptability of the proposed model). The Ramachandran plot was analyzed by the Rampage server which considers allowed and disallowed regions of amino acid and the Z-score plot was analyzed by the ProSA-web [25].

Molecular Docking Studies

Analysis of interactions involved in the binding of modeled proteins to receptor molecules can be accomplished through the use of molecular docking studies. Our refined vaccine model was submitted as the ligand and TLR9 protein as the immunological receptor for molecular docking evaluation using the ClusPro v2.0 server, which is accessible at https://cluspro.bu.edu/. The TLR9 protein was selected and downloaded from the PDB server. PyMOL v2.3.4 software was used to separate the protein from the attached ligand, then to remove water and other chemicals from the receptor.

Normal Mode Analysis

The dynamics and stability of the vaccine-receptor complex was simulated for critical assessment by both software- and server-based tools. The complex was submitted to the iMODS server (http://imods.chaconlab.org) for Normal Mode Analysis (NMA). This process provides estimations of eigenvalues, deformability, B-factors and elastic network models with predictive value for explanation of the likely intracellular movement of aggregate proteins.

Immune Response Simulation

The vaccine construct was uploaded to the C-IMMSIM v10.1 server (http://www.cbs.dtu.dk/services/C-ImmSim-10.1/) and responses were retrieved for careful evaluation. A minimum interval between initial and booster doses was set at 30 days, as previously described. An in silico simulation was conducted with three injections administered at intervals of 1, 84 and 168, where one time step corresponds to one hour in reality. In addition to the simulation step maximum of 300, the stimulation parameters were kept at their default values.

Codon Adaptation and In silico Simulation of Cloning

For foreign gene expression in a host organism, codon optimization must be carried out in consideration of characteristics of the host species. To adapt the codon according to the requirements, the construct was submitted to the JCat server (http://jcat.de/). In this study, we chose the widely used *E. coli* K12 host, in which the entire operation is carried out with avoidance of the following three criteria: (1) restriction enzyme cleavage sites, (2) prokaryotic ribosome binding sites and (3) rho-dependent termination of transcription. The adapted sequence was evaluated based on the Codon Adaptation Index (CAI) value and Guanine-Cytosine (GC) content [26]. Finally, the adapted nucleotide sequence was used for in silico cloning into the pET28a (þ) expression vector. The entire in silico cloning operation was executed in SnapGene v4.2 software.

Results

Selection of Proteins for Maximal Antigenicity

Components of the CyHV-3 proteome were used to develop a vaccine containing 224 amino acids. Proteins displaying the highest antigenicity were chosen, based on evaluation using the widely-used server Vaxijen v2.0. This process provided insights of potential utility in the prediction of appropriate therapeutic actions against the CyHV-3 virus (Table 1). These antigenicity scores were both encouraging and consistent; a Vaxijen prediction for CyHV-3 had an antigenicity score of 0.5820 and an ANTIGENpro prediction had a score of 0.5947. Sequence information for the chosen 224 amino acids (CyHV-3) protein was deposited in GenBank under the ID number AVL28465.1QJD15206.

Potential CTL Epitopes

The selected protein was predicted to contain 137 CTL epitopes, each consisting of nine amino acids (Table S1). There were 29 CTL epitopes that were antigenic, immunogenic, harmless and non-allergenic. Due to the large number of prospective epitopes, four CTL epitopes were selected for final vaccine development based on their antigenicity scores (Table 1). The C-score is the combined score that the NetCTL server provides. The four epitopes with the lowest C-scores were chosen because this is usually indicative of high surface accessibility of the corresponding region in the protein. Regions with low C-scores are more likely to interact with antibodies and immune cells, making them potentially immunogenic. Characteristics other than C-scores, such as antigenicity, conservation and stability, were also considered in the vaccine design process.

CTL epitope	C-Score	Antigenicity	Immunogenicity	Toxicity	Allergenicity
VCMKCKMRD	2.2828	Yes	Yes	Non-toxic	Non-allergenic
KLTAVCMKC	2.2611	Yes	Yes	Non-toxic	Non-allergenic
KMRDAPFTV	2.0278	Yes	Yes	Non-toxic	Non-allergenic
LTAVCMKCK	2.0010	Yes	Yes	Non-toxic	Non-allergenic

Table 1: CTL epitopes chosen for application in the final vaccine formulation.

Potential HTL Epitopes

The IEDB server was used first to identify 260 HTL epitopes, each consisting of 15 amino acids (Table S2). A total of 16 HTL epitopes were capable of triggering the evaluated cytokines, specifically IFNc, IL-4 and IL-10. On the basis of the antigenic score (Table 2), we considered incorporating the top four HTL epitopes into the final vaccine construct.

HTL Epitope	Antigenicity	IFNγ	IL-4 Inducer	IL-10 Inducer	Toxicity	Allergenicity	Allele
KCKMRDAPFTVRISQ	1.3989	Positive	Inducer	Inducer	Non-toxic	Non-allergenic	HLA-DRB3*01:01
ESYQAVCRPCLTGFR	0.4851	Positive	Inducer	Inducer	Non-toxic	Non-allergenic	HLA-DRB3*02:02
AVCMKCKMRDAPFTV	1.8094	Positive	Inducer	Inducer	Non-toxic	Non-allergenic	HLA-DRB1*03:01
FTVRISQGTDLVQVG	0.9261	Positive	Inducer	Inducer	Non-toxic	Non-allergenic	HLA-DRB1*01:01

Table 2: Four HTL epitopes selected for final vaccine construction.

Potential LBL Epitopes

Our preliminary study identified 210 LBL epitopes, each 15 amino acids in length. As a result of advance assessment, fourteen epitopes stood out as having desirable antigenicity, negligible toxicity and non-allergenic characteristics (Table S3). The four most appealing LBL epitopes for vaccine design were selected based on their high antigenicity scores (Table 3).

LBL Epitope	Probability	Antigenicity	Toxicity	Allergenicity	Allele
TVRISQGTDLVQVGG	0.956	0.5655	Non-toxic	Non-allergenic	HLA-DRB1*01:02
AQYELYGPPPPPPAH	0.956	0.4124	Non-toxic	Non-allergenic	HLA-DRB1*01:01
RMAQYELYGPPPPPP	0.956	0.4067	Non-toxic	Non-allergenic	HLA-DRB1*01:01
RRLERLSYSGRRCIA	0.6581	0.7918	Non-toxic	Non-allergenic	HLA-DRB1*01:01

Table 3: The LBL epitopes chosen for the final vaccine formulation.

Vaccine Construct and Basic Properties

Finally, the selected twelve epitopes were combined with linker adjuvants for formulating designated vaccines, such as AAY, GPGPG and KK linkers, respectively. The construct was rendered immunogenic beforehand by adding an adjuvant (Fig. 1). The epitopes included in the separate class are, for example, 4 CTL, 4 HDL and 4 LBL. As a result, the total number of 295 amino acids is predicted for the final vaccine candidate (Fig. 4).

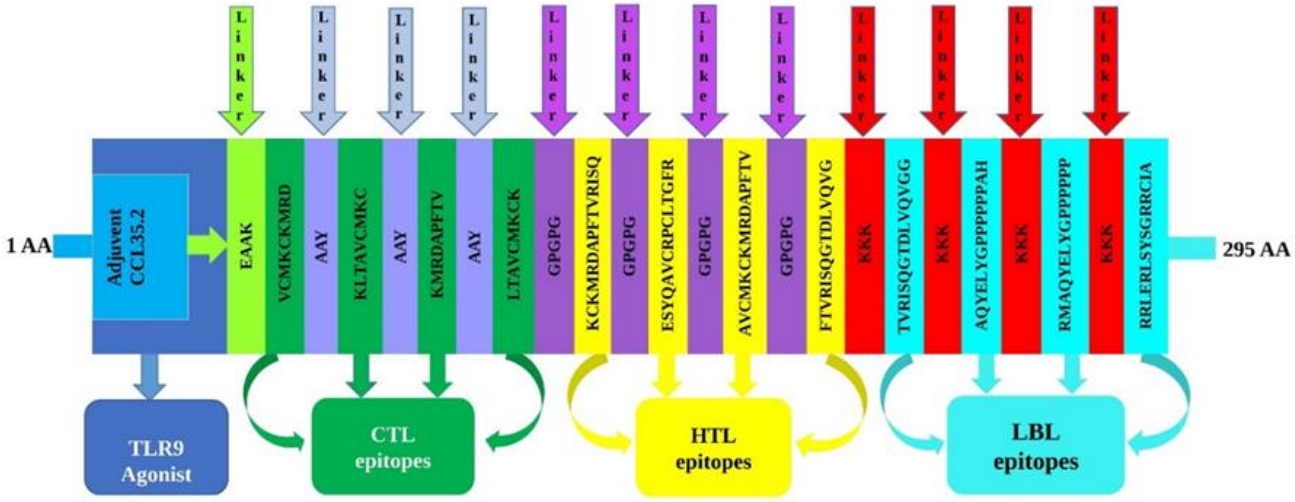

Figure 1: The dark blue, red, green, purple and green rectangle boxes represent the adjuvant, CTL, HTL and LBL epitopes, respectively, that were incorporated in the vaccination designs. EAAAK linkers (green) connected the adjuvant and the first CTL epitope; AYY linkers (light purple) joined CTL epitopes; GPGPG linkers (deep purple); and KK linkers (red) joined HTL epitopes.

Evaluation of Immunological and Physicochemical Characteristics

Physicochemical properties play important roles in the construction of the vaccine included in Table 4. Our analysis revealed that the molecular weight of the vaccine was 32393.28 Da. The isoelectric point is focused on the separation and purification of vaccine, which was compiled at 9.82 in the study and other properties were documented, such as the chemical formula (C1425H2297N409O390S31), instability index (44.72), aliphatic index 61.15 and grand average of hydropathicity -0.297. Moreover, immunological potency expressed the vaccine-related effect on the host, where the antigenicity score was 0.5005. This analysis is not only of value for revealing uses, but also provides information about allergenicity and solubility (Table 4).

Characteristics	Finding	Remarks
Number of amino acids	295	Suitable
Molecular weight	32393.28	Average
Theoretical pI	9.82	Slightly basic
Chemical formula	C1425H2297N409O390S31	
Extinction coefficient (at 280 nm in H_2O)	25870	
Estimated half-life (mammalian reticulocytes, in vitro)	30 hours	
Estimated half-life (yeast-cells, in vivo)	>20 hours	
Estimated half-life (*E. coli*, in vivo)	>10 hours	
Instability index of vaccine	44.72	Stable
Aliphatic index of vaccine	61.15	Thermostable
Grand average of hydropathicity (GRAVY)	-0.297	Hydrophobic
Antigenicity	0.5005	Antigenic
Immunogenicity	1.47358	Immunogenic
Allergenicity	No	Non- allergenic
Solubility		Soluble

Table 4: Antigenic, allergenic and physical-chemical features of the prepared vaccine candidate.

Secondary structural characteristics assessed using two distinct servers include α-helix, β-strand and random coils. In the design, the SOPMA server projected 27.80% α-helix, 21.36% β-strand and 44.75% random coils. The PSIPRED server, on the other hand, predicted 431.04% α-helix, 23.47% β-strand and 45.49% random coils (Table 5).

SOMPA SERVER			**PSIPRED SERVER**		
Featured	Amino Acid	Percentage	Featured	Amino Acid	Percentage
Alpha helix	82	27.80%	Alpha helix	86	31.04%
Extended strand	63	21.36%	Extended strand	65	23.47%
Random coil	132	44.75%	Random coil	126	45.49%

Table 5: The secondary structural features of the vaccine construct.

Tertiary Structure, Refinement and Validation

The popular web-based server I-TASSER was used to generate five models. Firstly, models were selected based on their C-score (-2.96). The lowest C score was chosen for the preparation of the vaccine. Moreover, the model was uploaded to Ramachandra web server for confirming 84.7% regions with GDT-HA s 0.9146, RMSD value 13.2±4.1Å and Estimated TM-score = 0.38±0.13.

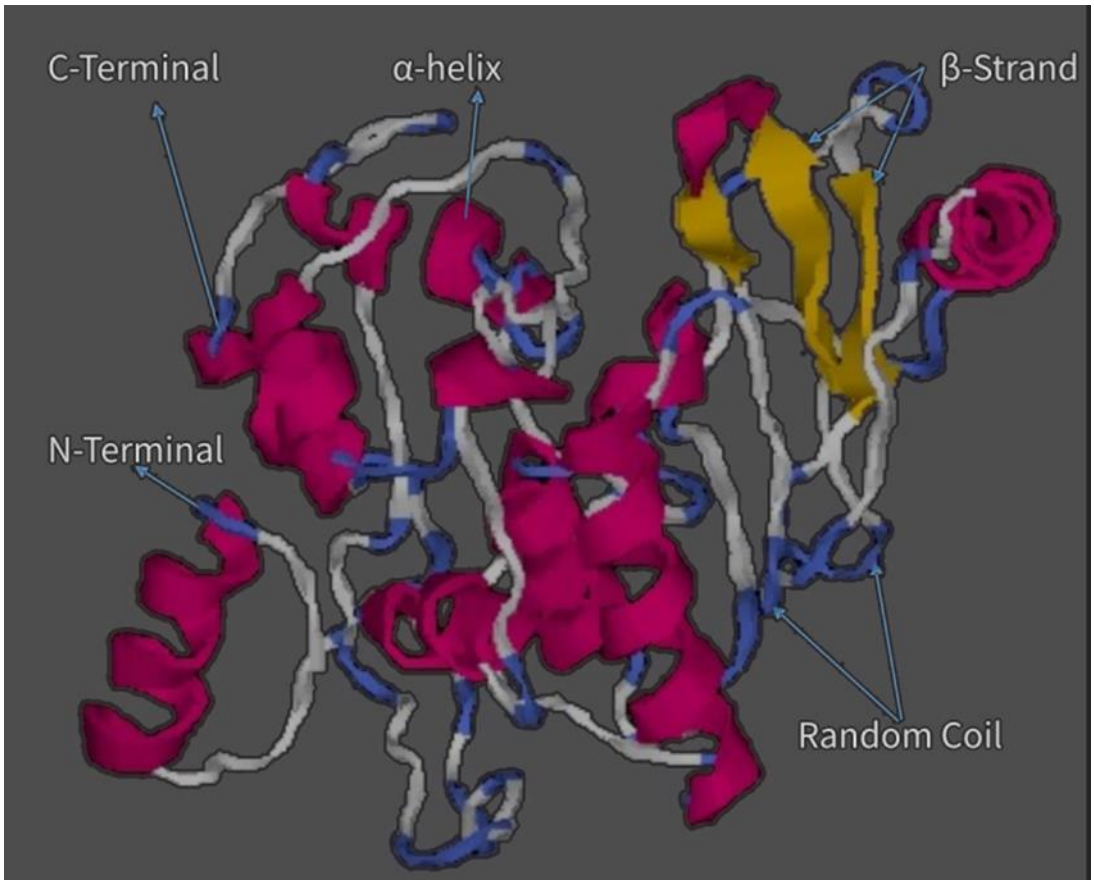

Figure 2: The figure shows the tertiary structure of designated vaccine. The indicated domains are color-coded in red (α-helix), yellow (β-strand) and blue (random coil).

The tertiary model was validated with RAMPAGE servers, whereas ProSA-web servers recognized errors from the vaccine. The refined vaccine model shows the 89.15% allowable regions and the 7.4% disallowable regions (Fig. 3). The crude model had a documented Z-score value of -0.61 from the vaccine and after refinement, the value was -0.54 (Fig. 3).

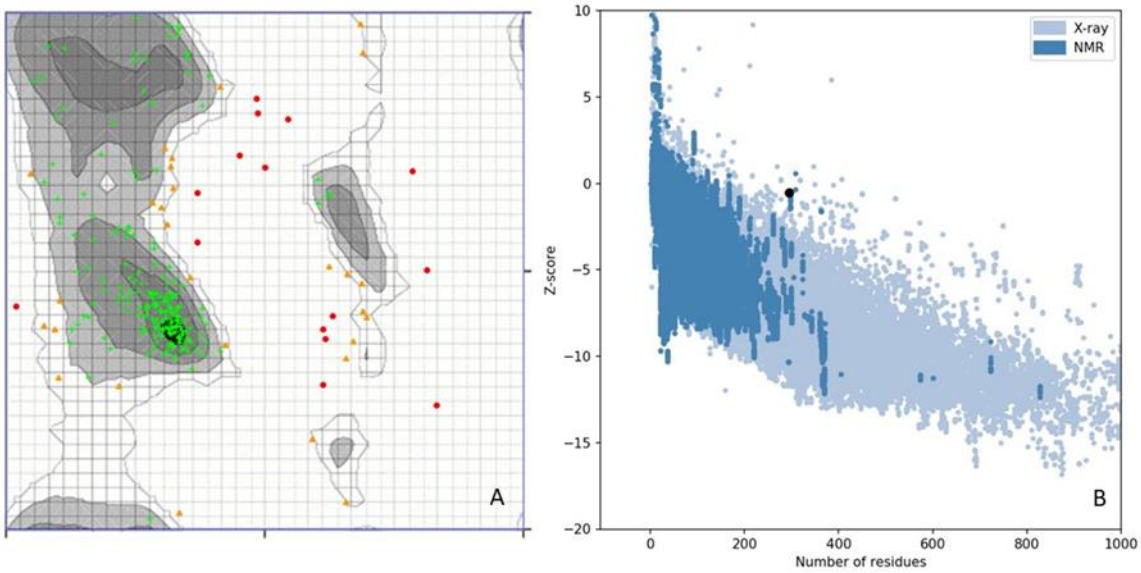

Figure 3: Ramachandra plot shows the allowed (green) and disallowed regions (red) (Fig A) and the z-core was documented from the refined vaccine model.

Molecular Docking Studies

The interaction between the vaccine and TLR9 was documented and is presented in Fig. 4. It was performed through the ClusPro v2.0 server. The lowest energy score was chosen from the 30 docked complexes with various poses. Model 1 was suitable for the designated vaccine by including all criteria. It was consequently selected as the most outstanding candidate vaccine-TLR9 complex, which had an energy score of -1065.1 (Table S4 and Fig. 4), a docking score of -392.94 and a confidence score of 0.992. The receptor and ligand interface residues are listed in the supplementary file (Table S5).

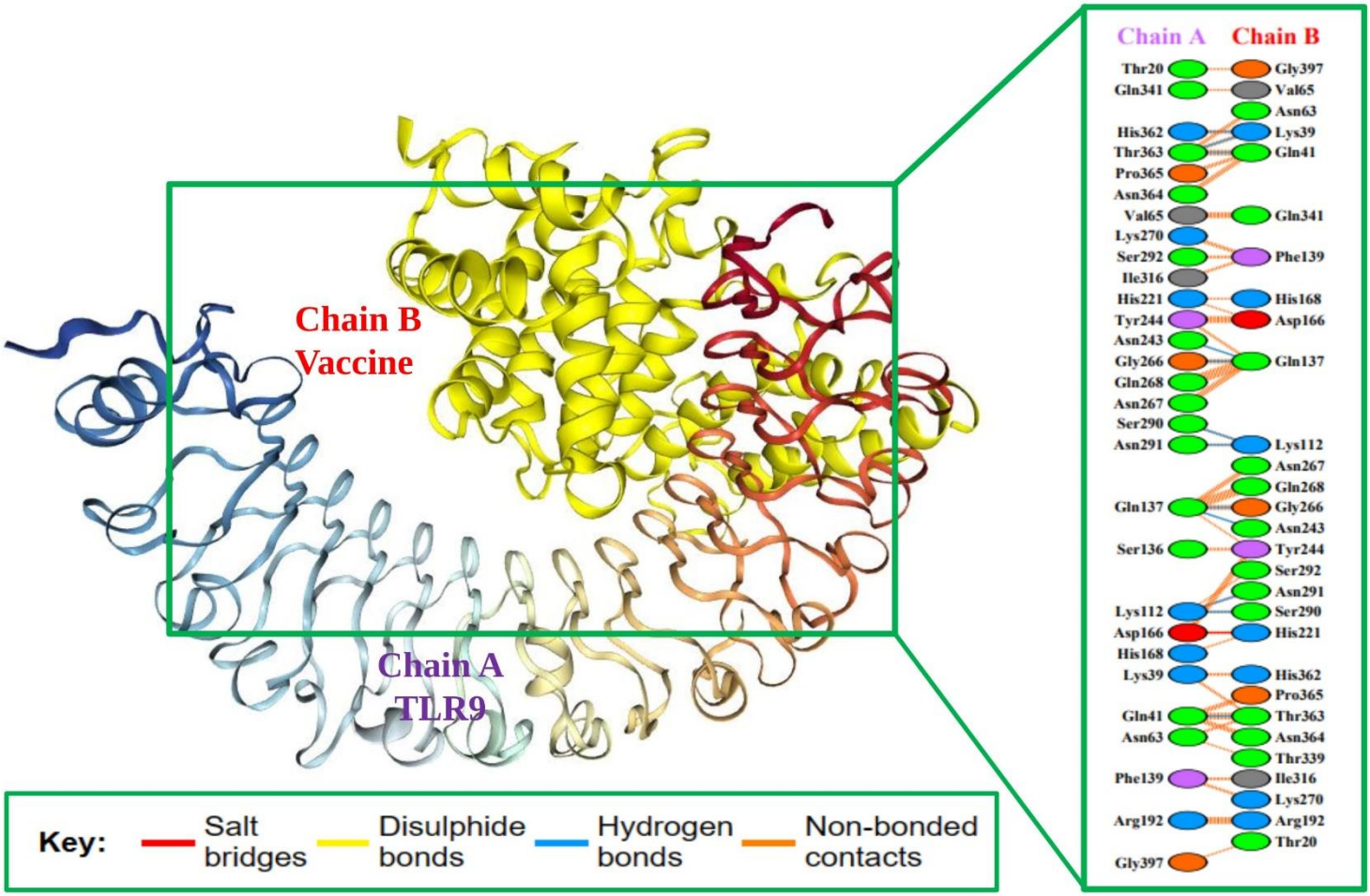

Figure 4: The figure displays the vaccine and receptor interaction.

Molecular Dynamics Simulation

The internal coordinates of the complex were compiled through network meta-analysis. The independent distortion was compiled through the buildup of deformability in residues and it was documented using the chain hinge method (Fig. 5). Receptor structures may be overinterpreted because they are often improved without any limits on the number of atomic B-factors that can be added (Fig. 5). The eigenvalue was estimated (1.539 e-04) from the complex, which shows that each variance was steadily decreasing (Fig. 5). For these reasons, it was concluded that the binding interaction had a compact conformation with minor fluctuations from the vaccine and TLR9 complex.

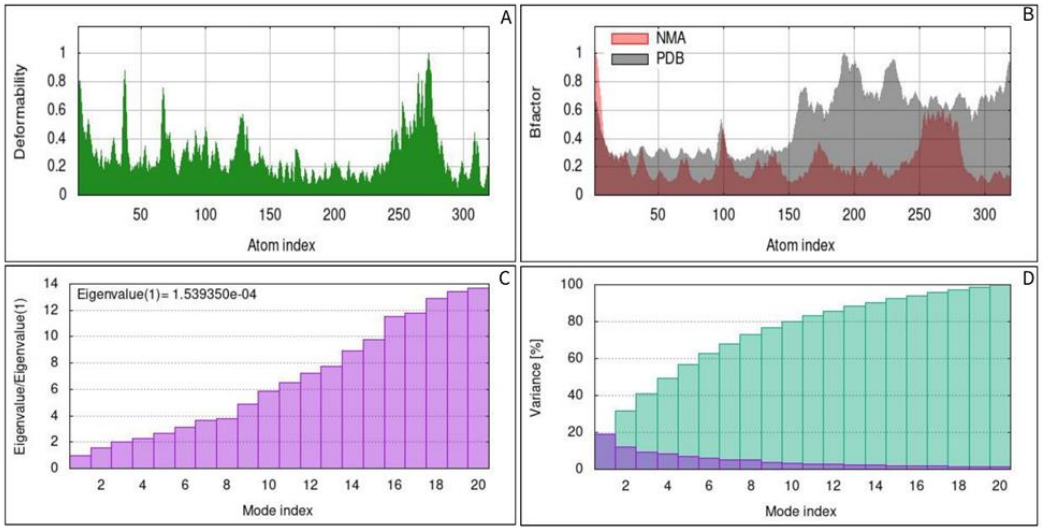

Figure 5: Molecular dynamics simulation of the vaccine-TLR9 complex. Herein, different plots show (A) deformability index (B) atomic B-factors, (C) eigenvalue and (D) NMA variance.

Immune Response Simulation

The simulated immune response to this vaccine bore similarities to established immunological responses to certain infections (Fig. 6). Initial immune responses were shown to be lower than secondary and tertiary immune responses (Fig. 6). Higher levels of antibodies, such as IgG1 + IgG2, IgM and IgG + IgM, are associated with such secondary and tertiary reactions. As a result, antigen extenuation demonstrates the activity of memory cells, leading to accelerated antigen clearance after subsequent exposures. Furthermore, the survival of B-cells, cytotoxic T-cells and helper T-cells (Fig. 6) revealed alterations in immune cells and IgM memory development. Several components (IFN, IL-4 and IL-10) are added to demonstrate higher and lower-level responses (Fig. 6). In terms of proportion (%) and number (cells/mm^3), Th1 reactions were greater than Th0 reactions (6F). Finally, the study predicted dendritic cell movement as well as enhanced and protracted macrophage movement. Dendritic cells are present in tissues that are in direct touch with the external environment, including the mucosa of the lungs, the epithelial cells of the skin and the linings of the nasal cavity and the gastrointestinal tract (Fig. 6).

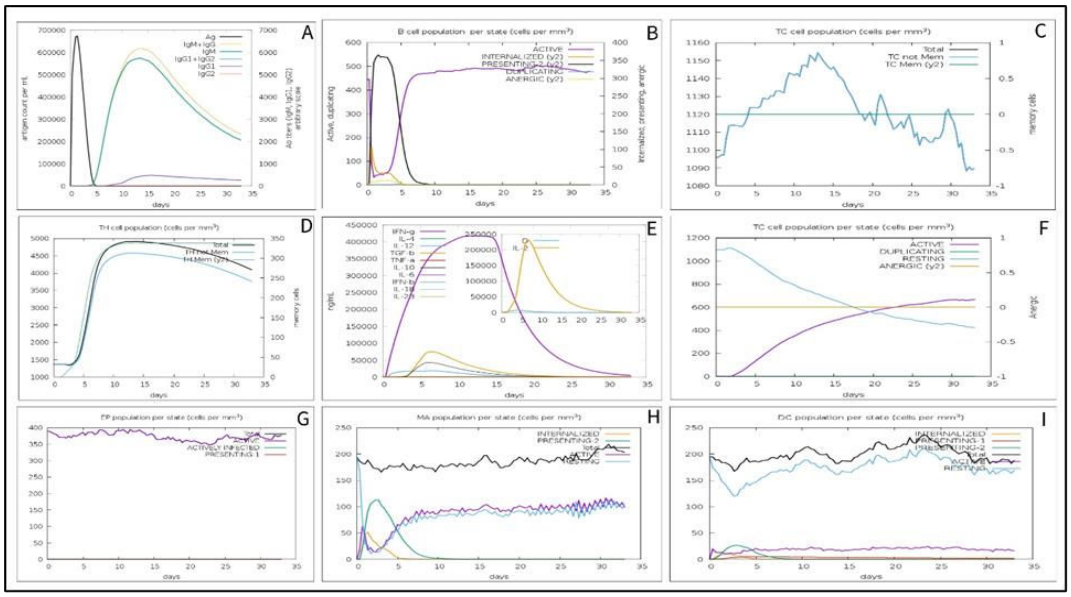

Figure 6: The figure illustrates properties of the proposed vaccine of Cyprinid herpesvirus-3. (A) primary, secondary and tertiary immune responses, (B) B-cell population, (C) cytotoxic T-cell population, (D) helper T-cell population, (E) induction of cytokines and interleukins, (F) Th1 mediated immune response, (G) EP Population per state (H) macrophage population per state and (I) dendritic cell population per state.

Codon Adaptation and In silico Cloning

The translation efficiency was improved for the construction of vaccines through the JCat server, which optimized the codon based on *E. coli* K12. The length of the designated peptide vaccine was 295 AA residues from the 885 lengths of nucleotide sequences. Furthermore, the estimated GC content was 63.50% and the calculated CAI value was 1.0 (Fig. 7). The pET28a (+) vector was used to contract designated vaccines and select restriction sites XhoI and BamHI, respectively, as start and cut points (6C). The SnapGene software was utilized for cloning vector pET28a (+), which helped in the optimization of the proposed vaccine. As a result, the ultimate designated cloning vector size as determined in our study was 5493 nucleotide base pairs (bp) (Fig. 7).

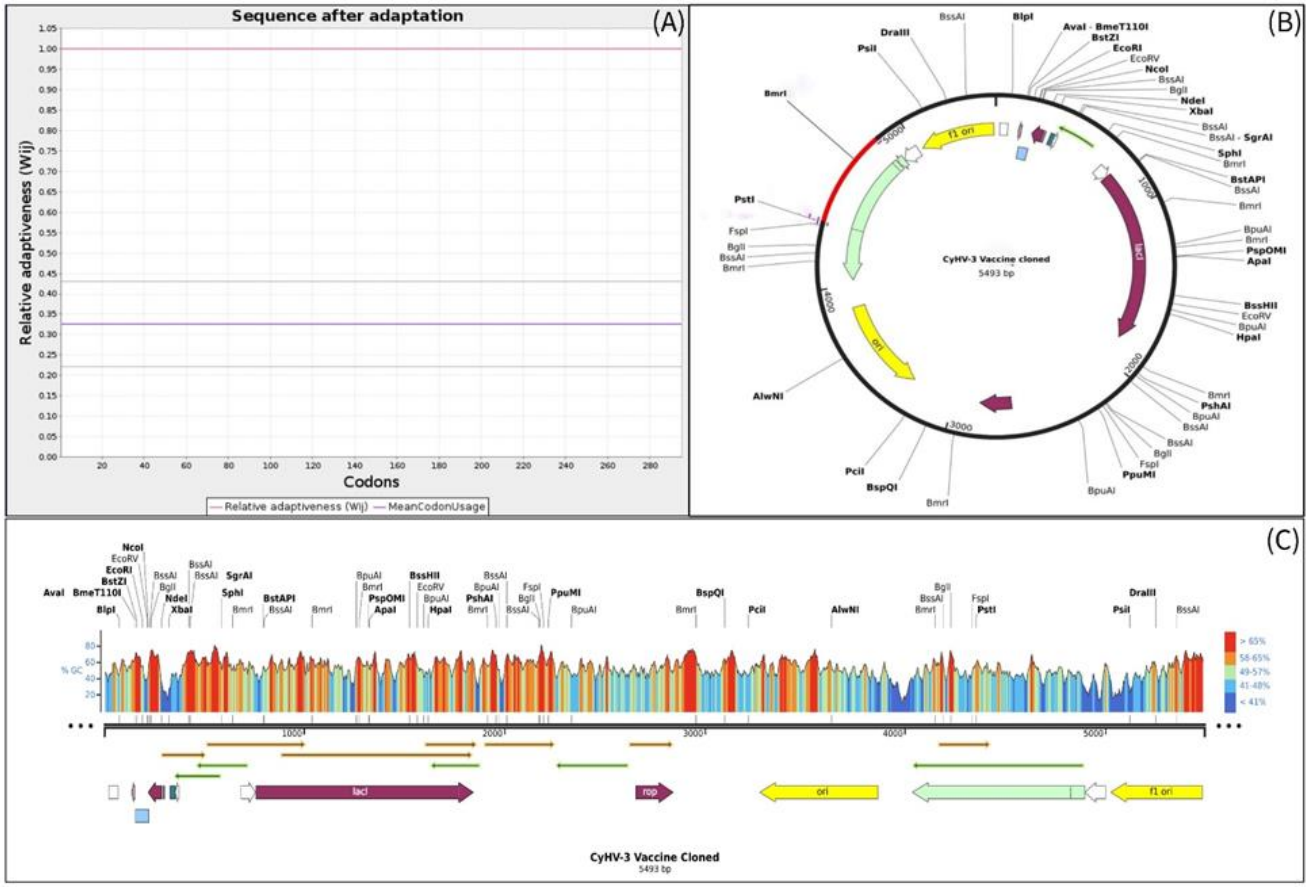

Figure 7: The average GC content (the optimal percentage is between 30 - 70%) of the improved sequence, as shown in Figure 7A. Here, Figures 7 B and C illustrate the in silico cloning of the designed vaccine into the pET-28a (+) vector.

Discussion

The results of this study led to the design of a multi-epitope vaccine against the devastating Cyprinid herpesvirus-3 (CyHV-3), based on an immunoinformatics approach. This pathogen threatens vitally important large-scale *Cyprinus carpio* production. Conclusions based on our analyses were tested and validated using immunoinformatics methods and the vaccine based on the thymidine kinase protein appears to have an appealing assortment of biochemical characteristics with health management potential [27]. When it comes to stopping the spread of illness, only pathogen exclusion approaches the efficacy and safety of vaccination. The protective compound to be developed needs to be effective at inducing fish immunity to illnesses that are easily spread. Here, we seek to deter a CyHV-3 pandemic by developing an epitope-based vaccination that would generate a robust immune response. The use of a strong new vaccine could also stop future pandemics. Without an efficient vaccination, however, *Cyprinid herpesvirus*-3 (CyHV-3) infection and transmission is lethal and notoriously difficult to manage and prevent. Furthermore, no effective immunization to manage the current crisis has been made broadly available. As a result, a novel approach to vaccine development is urgently required to help prevent economic and food-security crises.

Since thymidine kinase protein of Cyprinid herpesvirus-3 (CyHV-3) shows a key role in immune invasion as well as in fish-to-fish transmission, our purpose was to design an epitope vaccine by targeting the thymidine kinase [28]. The thymidine kinase protein was chosen and targeted as an antigenic region by the cellular and humoral immune systems. Moreover, the multiple epitopes such as CTL, HTL and LBL were selected in the design for the proposed vaccine. Four of the highest-scoring antigenic CTL, HTL and LBL epitopes were used to create the vaccine, along with the appropriate linkers. The key feature of the vaccine design was to improve the analysis of the vaccine candidate's stability, folding and expression patterns. The EAAAK linker was used to bind the adjuvant to the CTL epitope, which results in potent cellular and humoral immune responses. It is not only used for the particular antigens but also amplifies the stability and longevity of the vaccine. Finally, it was determined that the vaccine construct contained 295 amino acid residues. In terms of recombinant vaccines, the physicochemical property of solubility is an essential consideration. A solubility evaluating tool projected the vaccine construct's solubility in *E. coli* and the vaccine construct has been shown to be soluble in *E. coli* [29]. The theoretical PI value revealed that the constitution of the vaccine was acidic. The protein's stability index, as proposed by server tools, indicates that it will be stable following synthesis. The vaccine was hydrophilic and thermostable, according to GRAVY and aliphatic index. The vaccine is thought to have an assortment of desirable physical properties and all of the numbers on the different quantifiable factors point to a high degree of likelihood that this vaccine could be an effective defense against CyHV-3.

Furthermore, the 3D receptor structure was validated and refined based on the C-score as well as the lowest energy score. The Z score was -0.54, which appears to be a fully acceptable model for the designated vaccine design. The peptide vaccine showed potential infection-inhibiting activity and a tight interaction between the modelled vaccine as a ligand and the TLR4 protein surface, as suggested by molecular docking between the two, with an energy score of -1065.1. In order to gain insight into the probable physical basis of the protein structure and function of biological macromolecules, molecular dynamics simulation was used. The time-dependent motion of individual atoms in a protein may be modelled using dynamic simulations. A greater degree of variation was seen in the vaccine component, but after 600 ps fluctuations abated, indicating that the modelled vaccine and bound protein are likely to be stable. The research simulated the immune system to determine the most effective immune response to the virus and the optimum behaviour and cell density characteristics for target clearance. An improved immune response, based on higher vaccination doses, activated memory B-cells and T-cells. Increased helper T-cell initiation after vaccination resulted in continued IFN- and IL-2 production. This pattern successfully mimicked the humoral immune response occurring in response to elevated levels of immunoglobulin production [29]. As a result of these efforts, the intended vaccine candidate was able to be cloned in silico into the *E. coli* K12 expression host using the pET28a (+) cloning vector [30]. In summary, this immunoinformatics based strategy produced design parameters for a possible new Cyprinid herpesvirus-3 (CyHV-3) multi-epitope vaccine that could be useful in the management of *Cyprinus carpio* health. A peptide-based vaccine such as this, if successfully developed, could provide a reasonably cost-effective means of helping to prevent the spread of this disease in carp farms. This will help protect and potentially increase the production of carp in Asia, where this is a critically important food fish and protect valuable ornamental koi specimens.

Conclusion

Potential T- and B-cell epitopes in the thymidine kinase protein of CyHV-3 were identified using a number of computational techniques and then used in an in silico design for a multi-epitope vaccine. *Cyprinus carpio* populations may benefit from the proposed vaccine's immunodominant qualities. Importantly, it showed potent binding to the immunological TLR9 enzymatic protein and elicited a powerful immune response in response to CyHV-3 infection. Our results suggest that this vaccine candidate might serve as a foundation upon which to build a safe and effective vaccination program for protection against spread of the pathogenic agent of the CyHV-3 pandemic. Potential epitopes discovered here may be utilized in further research. Follow-up in vitro and in vivo studies are recommended as a next step in the testing and verification of our formulation of the vaccine as a preventative measure against CyHV-3.

Conflict of Interests

The authors have no conflict of interest to declare.

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References
1. Sumon MA, Sumon TA, Hussain MA, Lee SJ, Jang WJ, Sharifuzzaman SM, et al. Single and multi-strain probiotics supplementation in commercially prominent finfish aquaculture: Review of the current knowledge. J Microbiology and Biotechnol. 2022;32(6):681.
2. Jamal MT, Sumon AA, Pugazhendi A, Al Harbi M, Hussain A, Haque F. Use of probiotics in commercially important finfish aquaculture. Int J Probiotics & Prebiotics. 2020;15(1);7-21.
3. FAO 2022. The state of world fisheries and aquaculture 2022. Towards Blue Transformation. Rome, FAO. 2022.
4. Roy K, Vrba J, Kaushik SJ, Mraz J. Nutrient footprint and ecosystem services of carp production in European fishponds in contrast to EU crop and livestock sectors. J Cleaner Production. 2020;270:122268.
5. Waage J, Grace D, Fèvre EM, McDermott J, Lines J, Wieland B, et al. Changing food systems and infectious disease risks in low-income and middle-income countries. The Lancet Planetary Health. 2022;6(9):e760-8.
6. Gotesman M, Kattlun J, Bergmann SM, El-Matbouli M. CyHV-3: the third cyprinid herpesvirus. Diseases of Aquatic Organisms. 2013;105(2):163-74.
7. Bergmann SM, Schütze H, Fischer U, Fichtner D, Riechardt M, Meyer K, et al. Detection of Koi Herpes Virus (KHV) genome in apparently healthy fish. Bulletin of the Euro Assoc Fish Pathologists. 2009;29(5):145-52.
8. El-Matbouli M, Soliman H. Transmission of Cyprinid herpesvirus-3 (CyHV-3) from goldfish to naïve common carp by cohabitation. Research in Veterinary Science. 2011;90(3):536-9.
9. Rakus K, Ouyang P, Boutier M, Ronsmans M, Reschner A, Vancsok C, et al. Cyprinid herpesvirus 3: an interesting virus for applied and fundamental research. Veterinary Res. 2013;44:1-6.
10. Soliman H. *In vivo* replication of cyprinid herpesvirus-3 in goldfish. Assiut Veterinary Medical J. 2019;65(162):71-5.
11. Tolo IE, Bajer PG, Wolf TM, Mor SK, Phelps NB. Investigation of Cyprinid herpesvirus 3 (CyHV-3) disease periods and factors influencing CyHV-3 transmission in a low stocking density infection trial. Animals. 2021;12(1):2.
12. World Organization for Animal Health (WOAH) 2023. Infection with Koi Herpesvirus. Chapter 2.3.6. In: World Organization for Animal Health (OIE) Manual for Diagnostic Tests for Aquatic Animals. 2023. [Last accessed on: March 29, 2024] https://www.woah.org/en/what-we-do/standards/codes-and-manuals/aquatic-manual-online-access/
13. Ronen A, Perelberg A, Abramowitz J, Hutoran M, Tinman S, Bejerano I, et al. Efficient vaccine against the virus causing a lethal disease in cultured *Cyprinus carpio*. Vaccine. 2003;21(32):4677-84.
14. Bondad-Reantaso MG, MacKinnon B, Karunasagar I, Fridman S, Alday-Sanz V, Brun E, et al. Review of alternatives to antibiotic use in aquaculture. Reviews in Aquaculture. 2023;15(4):1421-51.
15. Ma J, Bruce TJ, Jones EM, Cain KD. A review of fish vaccine development strategies: Conventional methods and modern biotechnological approaches. Microorganisms. 2019;7(11):569.
16. Klafack S, Schröder L, Jin Y, Lenk M, Lee PY, Fuchs W, et al. Development of an attenuated vaccine against Koi Herpesvirus

Disease (KHVD) suitable for oral administration and immersion. NPJ Vaccines. 2022;7(1):106.
17. Bibi S, Ullah I, Zhu B, Adnan M, Liaqat R, Kong WB, et al. *In silico* analysis of epitope-based vaccine candidate against tuberculosis using reverse vaccinology. Scientific Reports. 2021;11(1):1249.
18. Yang JX, Tseng JC, Yu GY, Luo Y, Huang CY, Hong YR, et al. Recent advances in the development of toll-like receptor agonist-based vaccine adjuvants for infectious diseases. Pharmaceutics. 2022;14(2):423.
19. Banerjee S, Majumder K, Gutierrez GJ, Gupta D, Mittal B. Immuno-informatics approach for multi-epitope vaccine designing against SARS-CoV-2. BioRxiv. 2020.
20. Hajissa K, Zakaria R, Suppian R, Mohamed Z. Epitope-based vaccine as a universal vaccination strategy against *Toxoplasma gondii* infection: A mini-review. J Adv Vet Anim Res. 2019;6(2):174.
21. Doytchinova IA, Flower DR. VaxiJen: a server for prediction of protective antigens, tumour antigens and subunit vaccines. BMC bioinformatics. 2007;8:1-7.
22. Rowaiye AB, Oli AN, Asala MT, Nwonu EJ, Njoku MO, Asala OO, et al. Design of multiepitope vaccine candidate from a major capsid protein of the African swine fever virus. Veterinary Vaccine. 2023;2(1):100013.
23. McGuffin LJ, Bryson K, Jones DT. The PSIPRED protein structure prediction server. Bioinformatics. 2000;16(4):404-5.
24. Aljahdali MO, Molla MH, Ahammad F. Compounds identified from marine mangrove plant (*Avicennia alba*) as potential antiviral drug candidates against WDSV, an in silico approach. Marine Drugs. 2021;19(5):253.
25. Molla MH, Aljahdali MO. Identification of phytochemical compounds to inhibit the matrix-like linker protein VP26 to block the assembles of White Spot Syndrome Virus (WSSV) envelope and nucleocapsid protein of marine shrimp: In silico approach. J King Saud University-Science. 2022;34(8):102346.
26. Aljahdali MO, Molla MH, Filfilan WM. Whole genome sequence of the newly prescribed subspecies *Oreochromis spilurus saudii*: a valuable genetic resource for aquaculture in saudi arabia. J Mar Sci Eng. 2021;9(5):506.
27. Pyasi S, Sharma V, Dipti K, Jonniya NA, Nayak D. Immunoinformatics approach to design multi-epitope-subunit vaccine against bovine ephemeral fever disease. Vaccines. 2021;9(8):925.
28. Monaghan SJ, Thompson KD, Bron JE, Bergmann SM, Jung TS, Aoki T, et al. Expression of immunogenic structural proteins of cyprinid herpesvirus 3 *in vitro* assessed using immunofluorescence. Veterinary Res. 2016;47:1-4.
29. Maleki A, Russo G, Parasiliti Palumbo GA, Pappalardo F. *In silico* design of recombinant multi-epitope vaccine against influenza A virus. BMC Bioinformatics. 2021;22(Suppl 14):617.
30. Singh A, Thakur M, Sharma LK, Chandra K. Designing a multi-epitope peptide-based vaccine against SARS-CoV-2. Scientific Rep. 2020;10(1):16219.

Supplementary Tables

Epitope	Antigenicity	Score	Immunogenicity	Toxicity	Allergenicity	Molecular weight
RLEEYDAVA	Antigen	1.0955	0.20767	Non-toxic	Non-Allergenic	1065.26
PPRSCNIYP	Antigen	0.7513	-0.0981	Non-toxic	Non-Allergenic	1046.32
VQLLTAGKY	Antigen	0.6865	-0.03753	Non-toxic	Non-Allergenic	992.32
AISAGYLYE	Antigen	0.6677	0.00367	Non-toxic	Non-Allergenic	986.21
AVAVDEGQF	Antigen	0.5519	0.13101	Non-toxic	Non-Allergenic	935.12
PIVSAAPPR	Antigen	0.5369	-0.09398	Non-toxic	Non-Allergenic	907.19
APIVSAAPP	Antigen	0.4908	-0.01299	Non-toxic	Non-Allergenic	822.08
VVQLLTAGK	Antigen	0.4723	0.0298	Non-toxic	Non-Allergenic	928.28
LTAGKYVIV	Antigen	0.4556	-0.05408	Non-toxic	Non-Allergenic	963.32
GPPPPPPAH	Antigen	0.4065	-0.0225	Non-toxic	Non-Allergenic	886.1
DKLTAVCMK	Antigen	1.8632	-0.03568	Non-toxin	Non-Allergenic	1008.38
QVGGAESYQ	Antigen	0.8335	0.03567	Non-toxic	Non-Allergenic	938.09
TALVPMADK	Antigen	0.6717	-0.09218	Non-toxic	Non-Allergenic	945.26
ISQGTDLVQ	Antigen	0.5594	0.06994	Non-toxic	Non-Allergenic	960.19
VCMKCKMRD	Antigen	2.2828	-0.64746	Non-toxic	Non-Allergenic	1113.55
KLTAVCMKC	Antigen	2.2611	-0.23278	Non-toxic	Non-Allergenic	996.43
KMRDAPFTV	Antigen	2.0278	0.18826	Non-toxic	Non-Allergenic	1064.38
LTAVCMKCK	Antigen	2.001	-0.37706	Non-toxic	Non-Allergenic	996.43
KCKMRDAPF	Antigen	1.9994	-0.14888	Non-toxic	Non-Allergenic	1095.45
MRDAPFTVR	Antigen	1.8433	0.20285	Non-toxic	Non-Allergenic	1092.39
PMFAGKSTE	Antigen	1.7574	-0.20957	Non-toxic	Non-Allergenic	967.22
MKCKMRDAP	Antigen	1.757	-0.3152	Non-toxic	Non-Allergenic	1079.47
CKMRDAPFT	Antigen	1.7213	0.11255	Non-toxic	Non-Allergenic	1068.38
AVCMKCKMR	Antigen	1.6361	-0.73955	Non-toxic	Non-Allergenic	1069.54
CMKCKMRDA	Antigen	1.6274	-0.44291	Non-toxic	Non-Allergenic	1085.49
RDAPFTVRI	Antigen	1.5992	0.21716	Non-toxic	Non-Allergenic	1074.36
RLSYSGRRC	Antigen	1.5481	-0.1127	Toxic	Non-Allergenic	1097.37
TAVCMKCKM	Antigen	1.5235	-0.58635	Toxic	Non-Allergenic	1014.46
KSTESCRRL	Antigen	1.4963	-0.02458	Toxic	Non-Allergenic	1079.35
STESCRRLE	Antigen	1.4963	-0.10055	Toxic	Non-Allergenic	1080.29
FAGKSTESC	Antigen	1.4517	-0.34272	Toxic	Non-Allergenic	929.12
IGPMFAGKS	Antigen	1.4409	-0.12687	Toxic	Non-Allergenic	907.22
QGTDLVQVG	Antigen	1.42	-0.01066	Toxic	Non-Allergenic	916.14
ERLSYSGRR	Antigen	1.4091	-0.27056	Toxic	Non-Allergenic	1123.35
NIYPVMVCV	Antigen	1.3399	-0.13412	Toxic	Non-Allergenic	1037.43
RPCLTGFRM	Antigen	1.3229	0.17008	Toxic	Non-Allergenic	1085.41
PCLTGFRMA	Antigen	1.3165	0.11974	Toxic	Non-Allergenic	995.34
IDQRYTEES	Antigen	1.283	0.19042	Toxic	Non-Allergenic	1140.29
DAPFTVRIS	Antigen	1.2643	0.3123	Toxic	Non-Allergenic	1005.25
YPVMVCVEP	Antigen	1.2482	-0.08051	Toxic	Non-Allergenic	1036.39
IYPVMVCVE	Antigen	1.2271	-0.11558	Toxic	Non-Allergenic	1052.44
TESCRRLER	Antigen	1.2266	0.04031	Toxic	Non-Allergenic	1049.4

LDKLTAVCM	Antigen	1.2245	-0.00319	Toxic	Non-Allergenic	993.37
LERLSYSGR	Antigen	1.22	-0.27876	Toxic	Non-Allergenic	1080.33
GPMFAGKST	Antigen	1.1987	-0.14786	Toxic	Non-Allergenic	895.16
AGKSTESCR	Antigen	1.1723	-0.27554	Toxic	Non-Allergenic	938.13
HAIDQRYTE	Antigen	1.1176	0.021	Toxic	Non-Allergenic	1132.32
CRPCLTGFR	Antigen	1.1073	0.06489	Toxic	Non-Allergenic	1052.39
AIDQRYTEE	Antigen	1.1052	0.02882	Toxic	Non-Allergenic	1124.29
RLERLSYSG	Antigen	1.0774	-0.18173	Toxic	Non-Allergenic	1080.33
MAMLELVIG	Antigen	1.0737	0.1315	Toxic	Non-Allergenic	976.4
CNIYPVMVC	Antigen	1.0705	-0.05654	Toxic	Non-Allergenic	1041.43
MLELVIGPM	Antigen	1.0545	0.20894	Toxic	Non-Allergenic	1002.44
GKSTESCRR	Antigen	1.0524	-0.08813	Toxic	Non-Allergenic	1023.24
TYPAISAGY	Antigen	1.0398	0.06246	Toxic	Non-Allergenic	942.15
CLTGFRMAQ	Antigen	1.0339	0.08408	Toxic	Non-Allergenic	1026.36
AMLELVIGP	Antigen	1.0234	0.25733	Toxic	Non-Allergenic	942.32
EEYDAVAVD	Antigen	1.0176	0.15522	Toxic	Non-Allergenic	1010.13
VMVCVEPIK	Antigen	1.0163	0.162	Toxic	Non-Allergenic	1017.44
LELVIGPMF	Antigen	1.011	0.08748	Toxic	Allergenic	1018.42
LEEYDAVAV	Antigen	0.9885	0.14491	Toxic	Allergenic	1008.21
YPAISAGYL	Antigen	0.9813	0.04879	Toxic	Allergenic	954.21
RSCNIYPVM	Antigen	0.977	0.11687	Toxic	Allergenic	1082.42
PVMVCVEPI	Antigen	0.9699	0.04892	Toxic	Allergenic	986.38
MFAGKSTES	Antigen	0.9679	-0.22767	Toxic	Non-Allergenic	957.18
APFTVRISQ	Antigen	0.9677	0.18164	Toxic	Non-Allergenic	1018.3
KLDKLTAVC	Antigen	0.9656	-0.12692	Toxic	Non-Allergenic	990.35
GAESYQAVC	Antigen	0.9552	-0.18947	Toxic	Non-Allergenic	927.11
LSYSGRRCI	Antigen	0.9233	-0.07377	Toxic	Non-Allergenic	1054.35
DQRYTEESK	Antigen	0.918	0.13297	Toxic	Non-Allergenic	1155.3
TGFRMAQYE	Antigen	0.9161	-0.14401	Toxic	Non-Allergenic	1102.35
RRLERLSYS	Antigen	0.9051	-0.00467	Toxic	Non-Allergenic	1179.46
SCNIYPVMV	Antigen	0.9001	0.05002	Toxic	Non-Allergenic	1025.37
PRSCNIYPV	Antigen	0.896	0.00143	Toxic	Non-Allergenic	1048.34
CIAVKHAID	Antigen	0.8909	-0.01453	Toxic	Non-Allergenic	969.29
GFRMAQYEL	Antigen	0.8549	-0.17546	Toxic	Allergenic	1114.41
QRLEEYDAV	Antigen	0.8521	0.23275	Toxic	Allergenic	1122.32
ATYPAISAG	Antigen	0.847	0.03426	Toxic	Allergenic	850.05
VGGAESYQA	Antigen	0.8363	-0.07866	Toxic	Allergenic	881.03
SQGTDLVQV	Antigen	0.8252	0.02838	Toxic	Allergenic	946.16
VIGPMFAGK	Antigen	0.8177	-0.00814	Toxic	Allergenic	917.28
EYDAVAVDE	Antigen	0.7848	0.1714	Toxic	Allergenic	1010.13
VMQRLEEYD	Antigen	0.7778	0.18027	Toxic	Allergenic	1182.44
VQVGGAESY	Antigen	0.7665	0.10517	Toxic	Allergenic	909.09
ELVIGPMFA	Antigen	0.7552	0.09008	Toxic	Allergenic	976.33
RYTEESKVA	Antigen	0.7406	-0.10276	Toxic	Allergenic	1082.29
LVPMADKLD	Antigen	0.7351	-0.3098	Toxic	Non-Allergenic	1001.33

QVTALVPMA	Antigen	0.7242	-0.03193	Toxic	Non-Allergenic	929.27
YDAVAVDEG	Antigen	0.714	0.20842	Toxic	Non-Allergenic	938.07
PFTVRISQG	Antigen	0.6989	0.02252	Toxic	Non-Allergenic	1004.28
LTGFRMAQY	Antigen	0.6934	-0.02076	Toxic	Non-Allergenic	1086.4
LVIGPMFAG	Antigen	0.6916	0.02286	Toxic	Non-Allergenic	904.27
MQRLEEYDA	Antigen	0.6741	0.20723	Toxic	Non-Allergenic	1154.38
GGAESYQAV	Antigen	0.6659	-0.12603	Toxic	Non-Allergenic	881.03
PAISAGYLY	Antigen	0.6626	-0.06287	Toxic	Non-Allergenic	954.21
ISAGYLYEV	Antigen	0.6524	0.08814	Toxic	Non-Allergenic	1014.27
DAVAVDEGQ	Antigen	0.6147	0.21815	Toxic	Non-Allergenic	903.03
DLVQVGGAE	Antigen	0.6136	0.0204	Toxic	Non-Allergenic	887.09
VRISQGTDL	Antigen	0.6135	-0.15845	Toxic	Non-Allergenic	988.24
SCRRLERLS	Antigen	0.6008	0.18953	Toxic	Non-Allergenic	1119.42
YTEESKVAM	Antigen	0.5862	-0.17315	Toxic	Non-Allergenic	1057.3
YGPPPPPPA	Antigen	0.5848	-0.05184	Toxic	Non-Allergenic	892.13
GRRCIAVKH	Antigen	0.5838	0.03782	Toxic	Non-Allergenic	1039.39
AAPPRSCNI	Antigen	0.5829	-0.16937	Toxic	Non-Allergenic	928.18
FRMAQYELY	Antigen	0.5712	-0.05589	Toxic	Non-Allergenic	1220.53
GTDLVQVGG	Antigen	0.5621	-0.01816	Toxic	Non-Allergenic	845.06
TEESKVAMH	Antigen	0.5616	-0.37469	Toxic	Non-Allergenic	1031.27
CRRLERLSY	Antigen	0.5612	0.04584	Toxic	Non-Allergenic	1195.52
KHAIDQRYT	Antigen	0.5579	0.1007	Toxic	Non-Allergenic	1131.38
KQVTALVPM	Antigen	0.5564	0.10848	Toxic	Non-Allergenic	986.37
YSGRRCIAV	Antigen	0.5435	0.19791	Toxic	Non-Allergenic	1024.32
FTVRISQGT	Antigen	0.5296	-0.03861	Toxic	Non-Allergenic	1008.27
GATYPAISA	Antigen	0.5274	0.05057	Toxic	Non-Allergenic	850.05
PPPPPPAHN	Antigen	0.5255	0.01592	Toxic	Non-Allergenic	923.15
QLLTAGKYV	Antigen	0.5211	-0.0787	Toxic	Non-Allergenic	992.32
ESCRRLERL	Antigen	0.5106	0.18928	Toxic	Non-Allergenic	1161.46
TDLVQVGGA	Antigen	0.499	0.0124	Toxic	Non-Allergenic	859.08
AVDEGQFFP	Antigen	0.4964	0.19911	Toxic	Non-Allergenic	1009.2
PPPPPAHNL	Antigen	0.4824	0.03479	Toxic	Non-Allergenic	939.2
ALVPMADKL	Antigen	0.4718	-0.23921	Toxic	Non-Allergenic	957.32
LVQVGGAES	Antigen	0.4709	0.16036	Toxic	Non-Allergenic	859.08
GAPIVSAAP	Antigen	0.4589	-0.0225	Toxic	Non-Allergenic	782.02
PFKQVTALV	Antigen	0.4547	-0.08328	Toxic	Non-Allergenic	1002.35
SYSGRRCIA	Antigen	0.448	0.11178	Toxic	Non-Allergenic	1012.26
IVSAAPPRS	Antigen	0.4319	0.03421	Toxic	Non-Allergenic	897.15
AQYELYGPP	Antigen	0.4292	0.10739	Toxic	Non-Allergenic	1037.26
ESKVAMHSG	Antigen	0.4277	-0.22502	Toxic	Non-Allergenic	945.18
RRCIAVKHA	Antigen	0.4178	0.03028	Toxic	Non-Allergenic	1053.41
FKQVTALVP	Antigen	0.4176	0.09333	Toxic	Non-Allergenic	1002.35
QYELYGPPP	Antigen	0.4171	0.0338	Toxic	Non-Allergenic	1063.3
RMAQYELYG	Antigen	0.4156	-0.02473	Toxic	Non-Allergenic	1130.41
EESKVAMHS	Antigen	0.4152	-0.32297	Toxic	Non-Allergenic	1017.24

SAAPPRSCN	Antigen	0.404	-0.13166	Toxic	Non-Allergenic	902.09
VSAAPPRSC	Antigen	0.4039	-0.02215	Toxic	Non-Allergenic	887.12
TVRISQGTD	Antigen	0.3368	-0.06814	Toxic	Non-Allergenic	976.18
MAQYELYGP	Antigen	0.2096	0.06242	Toxic	Non-Allergenic	1071.34
EGQFFPDLY	Antigen	0.173	0.196	Toxic	Non-Allergenic	1115.33

Table S1: CTL epitopes for the vaccine construction.

Peptide	Antigenicity	Score	IFNγ	IL4	IL10	Toxicity	Allergenicity
KCKMRDAPFTVRISQ	Antigen	1.3989	POSITIVE	Inducer	Inducer	Non-toxic	Non-allergenic
LVIGPMFAGKSTESC	Antigen	1.1855	NEGATIVE	Inducer	Inducer	Non-toxic	Non-allergenic
AGKSTESCRRLERLS	Antigen	1.1015	NEGATIVE	Inducer	Inducer	Non-toxic	Non-allergenic
PPRSCNIYPVMVCVE	Antigen	0.9455	NEGATIVE	Inducer	Inducer	Non-toxic	Non-allergenic
LVPMADKLDKLTAVC	Antigen	0.7553	NEGATIVE	Inducer	Inducer	Non-toxic	Non-allergenic
VMQRLEEYDAVAVDE	Antigen	0.6274	POSITIVE	Inducer	Inducer	Non-toxic	Non-allergenic
VAMHSGATYPAISAG	Antigen	0.5088	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
ATYPAISAGYLYEVM	Antigen	0.4983	POSITIVE	Inducer	Inducer	Non-toxic	Non-allergenic
ATYPAISAGYLYEVM	Antigen	0.4983	POSITIVE	Inducer	Inducer	Non-toxic	Non-allergenic
GRRCIAVKHAIDQRY	Antigen	0.4926	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
YDAVAVDEGQFFPDL	Antigen	0.4831	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
SYSGRRCIAVKHAID	Antigen	0.4275	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
RMAQYELYGPPPPPP	Antigen	0.4067	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
MKCKMRDAPFTVRIS	Antigen	1.7305	POSITIVE	Inducer	Inducer	Non-toxic	Non-allergenic
CMKCKMRDAPFTVRI	Antigen	1.711	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
GKSTESCRRLERLSY	Antigen	1.2185	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
CRRLERLSYSGRRCI	Antigen	1.1665	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
VIGPMFAGKSTESCR	Antigen	0.9742	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
VIGPMFAGKSTESCR	Antigen	0.9742	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
STESCRRLERLSYSG	Antigen	0.9301	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
FTVRISQGTDLVQVG	Antigen	0.9261	POSITIVE	Inducer	Inducer	Non-toxic	Non-allergenic
GPMFAGKSTESCRRL	Antigen	0.8563	NEGATIVE	Non-Inducer	Inducer	Non-toxic	Non-allergenic
HAIDQRYTEESKVAM	Antigen	0.8447	NEGATIVE	Non-Inducer	Inducer	Non-toxic	Non-allergenic
VPMADKLDKLTAVCM	Antigen	0.8357	NEGATIVE	Non-Inducer	Inducer	Non-toxic	Non-allergenic
CIAVKHAIDQRYTEE	Antigen	0.8293	POSITIVE	Inducer	Inducer	Non-toxic	Non-allergenic
IDQRYTEESKVAMHS	Antigen	0.8067	NEGATIVE	Inducer	Inducer	Non-toxic	Non-allergenic
APPRSCNIYPVMVCV	Antigen	0.7793	NEGATIVE	Inducer	Inducer	Non-toxic	Non-allergenic
YEVMQRLEEYDAVAV	Antigen	0.6218	POSITIVE	Inducer	Inducer	Non-toxic	Non-allergenic
CLTGFRMAQYELYGP	Antigen	0.5975	NEGATIVE	Inducer	Inducer	Non-toxic	Non-allergenic
GAESYQAVCRPCLTG	Antigen	0.5457	NEGATIVE	Inducer	Inducer	Non-toxic	Non-allergenic
GAPIVSAAPPRSCNI	Antigen	0.5186	NEGATIVE	Inducer	Inducer	Non-toxic	Non-allergenic
VAMHSGATYPAISAG	Antigen	0.5088	NEGATIVE	Inducer	Inducer	Non-toxic	Non-allergenic
GRRCIAVKHAIDQRY	Antigen	0.4926	NEGATIVE	Inducer	Inducer	Non-toxic	Non-allergenic
ESYQAVCRPCLTGFR	Antigen	0.4851	POSITIVE	Inducer	Inducer	Non-toxic	Non-allergenic
YDAVAVDEGQFFPDL	Antigen	0.4831	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
HSGATYPAISAGYLY	Antigen	0.4478	POSITIVE	Inducer	Inducer	Non-toxic	Non-allergenic
DFMQQPFKQVTALVP	Antigen	0.1737	NEGATIVE	Inducer	Non-Inducer	Non-toxic	Non-allergenic

VCMKCKMRDAPFTVR	Antigen	2.039	NEGATIVE	Inducer	Non-Inducer	Non-toxic	Non-allergenic
LTAVCMKCKMRDAPF	Antigen	1.9397	NEGATIVE	Inducer	Non-Inducer	Non-toxic	Non-allergenic
LTAVCMKCKMRDAPF	Antigen	1.9397	NEGATIVE	Inducer	Non-Inducer	Non-toxic	Non-allergenic
KLTAVCMKCKMRDAP	Antigen	1.9143	NEGATIVE	Inducer	Non-Inducer	Non-toxic	Non-allergenic
KLTAVCMKCKMRDAP	Antigen	1.9143	NEGATIVE	Inducer	Non-Inducer	Non-toxic	Non-allergenic
TAVCMKCKMRDAPFT	Antigen	1.829	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
TAVCMKCKMRDAPFT	Antigen	1.829	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
AVCMKCKMRDAPFTV	Antigen	1.8094	POSITIVE	Inducer	Inducer	Non-toxic	Non-allergenic
AVCMKCKMRDAPFTV	Antigen	1.8094	POSITIVE	Inducer	Inducer	Non-toxic	Non-allergenic
MKCKMRDAPFTVRIS	Antigen	1.7305	POSITIVE	Inducer	Inducer	Non-toxic	Non-allergenic
CMKCKMRDAPFTVRI	Antigen	1.7118	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
DKLTAVCMKCKMRDA	Antigen	1.6097	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
DKLTAVCMKCKMRDA	Antigen	1.6097	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
LDKLTAVCMKCKMRD	Antigen	1.5243	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
LDKLTAVCMKCKMRD	Antigen	1.5243	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
KCKMRDAPFTVRISQ	Antigen	1.3989	POSITIVE	Inducer	Inducer	Non-toxic	Non-allergenic
KMRDAPFTVRISQGT	Antigen	1.3035	POSITIVE	Inducer	Inducer	Non-toxic	Non-allergenic
KMRDAPFTVRISQGT	Antigen	1.3035	POSITIVE	Inducer	Inducer	Non-toxic	Non-allergenic
LELVIGPMFAGKSTE	Antigen	1.2806	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
LELVIGPMFAGKSTE	Antigen	1.2806	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
SCRRLERLSYSGRRC	Antigen	1.2771	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
SCRRLERLSYSGRRC	Antigen	1.2771	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
PMFAGKSTESCRRLE	Antigen	1.266	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
PMFAGKSTESCRRLE	Antigen	1.266	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
KLDKLTAVCMKCKMR	Antigen	1.2252	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
GKSTESCRRLERLSY	Antigen	1.2185	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
CKMRDAPFTVRISQG	Antigen	1.2128	POSITIVE	Inducer	Inducer	Non-toxic	Non-allergenic
CKMRDAPFTVRISQG	Antigen	1.2128	POSITIVE	Inducer	Inducer	Non-toxic	Non-allergenic
IGPMFAGKSTESCRR	Antigen	1.2006	NEGATIVE	Inducer	Inducer	Non-toxic	Non-allergenic
IGPMFAGKSTESCRR	Antigen	1.2006	NEGATIVE	Inducer	Inducer	Non-toxic	Non-allergenic
LVIGPMFAGKSTESC	Antigen	1.1855	NEGATIVE	Inducer	Inducer	Non-toxic	Non-allergenic
KSTESCRRLERLSYS	Antigen	1.1703	NEGATIVE	Inducer	Inducer	Non-toxic	Non-allergenic
KSTESCRRLERLSYS	Antigen	1.1703	NEGATIVE	Inducer	Inducer	Non-toxic	Non-allergenic
CRRLERLSYSGRRCI	Antigen	1.1665	NEGATIVE	Inducer	Inducer	Non-toxic	Non-allergenic
ADKLDKLTAVCMKCK	Antigen	1.1234	POSITIVE	Inducer	Inducer	Non-toxic	Non-allergenic
ADKLDKLTAVCMKCK	Antigen	1.1234	POSITIVE	Inducer	Inducer	Non-toxic	Non-allergenic
TESCRRLERLSYSGR	Antigen	1.1128	NEGATIVE	Inducer	Inducer	Non-toxic	Non-allergenic
TESCRRLERLSYSGR	Antigen	1.1128	NEGATIVE	Inducer	Inducer	Non-toxic	Non-allergenic
AGKSTESCRRLERLS	Antigen	1.1015	NEGATIVE	Inducer	Inducer	Non-toxic	Non-allergenic
MLELVIGPMFAGKST	Antigen	1.0939	POSITIVE	Inducer	Inducer	Non-toxic	Non-allergenic
MLELVIGPMFAGKST	Antigen	1.0939	POSITIVE	Inducer	Inducer	Non-toxic	Non-allergenic
ELVIGPMFAGKSTES	Antigen	1.0699	NEGATIVE	Non-Inducer	Inducer	Non-toxic	Non-allergenic
ELVIGPMFAGKSTES	Antigen	1.0699	NEGATIVE	Non-Inducer	Inducer	Non-toxic	Non-allergenic
RDAPFTVRISQGTDL	Antigen	1.0586	POSITIVE	Inducer	Inducer	Non-toxic	Non-allergenic
RDAPFTVRISQGTDL	Antigen	1.0586	POSITIVE	Inducer	Inducer	Non-toxic	Non-allergenic

SCNIYPVMVCVEPIK	Antigen	1.0556	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
SCNIYPVMVCVEPIK	Antigen	1.0556	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
FAGKSTESCRRLERL	Antigen	1.0499	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
FAGKSTESCRRLERL	Antigen	1.0499	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
PRSCNIYPVMVCVEP	Antigen	1.0417	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
PRSCNIYPVMVCVEP	Antigen	1.0417	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
RSCNIYPVMVCVEPI	Antigen	1.0368	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
RSCNIYPVMVCVEPI	Antigen	1.0368	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
DKLDKLTAVCMKCKM	Antigen	1.0224	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
ESCRRLERLSYSGRR	Antigen	1.0122	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
ESCRRLERLSYSGRR	Antigen	1.0122	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
MAMLELVIGPMFAGK	Antigen	1.0084	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
MAMLELVIGPMFAGK	Antigen	1.0084	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
MRDAPFTVRISQGTD	Antigen	0.9967	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
MRDAPFTVRISQGTD	Antigen	0.9967	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
AMLELVIGPMFAGKS	Antigen	0.9833	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
AMLELVIGPMFAGKS	Antigen	0.9833	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
MFAGKSTESCRRLER	Antigen	0.9825	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
MFAGKSTESCRRLER	Antigen	0.9825	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
RLERLSYSGRRCIAV	Antigen	0.9684	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
RLERLSYSGRRCIAV	Antigen	0.9684	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
RLSYSGRRCIAVKHA	Antigen	0.9607	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
RLSYSGRRCIAVKHA	Antigen	0.9607	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
MADKLDKLTAVCMKC	Antigen	0.9534	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
MADKLDKLTAVCMKC	Antigen	0.9534	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
PPRSCNIYPVMVCVE	Antigen	0.9455	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
STESCRRLERLSYSG	Antigen	0.9301	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
FTVRISQGTDLVQVG	Antigen	0.9261	POSITIVE	Non-Inducer	Inducer	Non-toxic	Non-allergenic
ERLSYSGRRCIAVKH	Antigen	0.9178	NEGATIVE	Non-Inducer	Inducer	Non-toxic	Non-allergenic
ERLSYSGRRCIAVKH	Antigen	0.9178	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
RPCLTGFRMAQYELY	Antigen	0.8958	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
RPCLTGFRMAQYELY	Antigen	0.8958	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
PCLTGFRMAQYELYG	Antigen	0.8955	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
PCLTGFRMAQYELYG	Antigen	0.8955	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
AIDQRYTEESKVAMH	Antigen	0.8838	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
AIDQRYTEESKVAMH	Antigen	0.8838	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
CRPCLTGFRMAQYEL	Antigen	0.8756	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
CRPCLTGFRMAQYEL	Antigen	0.8756	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
PMADKLDKLTAVCMK	Antigen	0.8666	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
PMADKLDKLTAVCMK	Antigen	0.8666	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
GPMFAGKSTESCRRL	Antigen	0.8563	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
RLEEYDAVAVDEGQF	Antigen	0.8498	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
RLEEYDAVAVDEGQF	Antigen	0.8498	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
HAIDQRYTEESKVAM	Antigen	0.8447	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
VPMADKLDKLTAVCM	Antigen	0.8357	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic

CIAVKHAIDQRYTEE	Antigen	0.8293	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
VCMKCKMRDAPFTVR	Antigen	0.8213	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
IDQRYTEESKVAMHS	Antigen	0.8067	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
IAVKHAIDQRYTEES	Antigen	0.7987	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
IAVKHAIDQRYTEES	Antigen	0.7987	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
QGTDLVQVGGAESYQ	Antigen	0.7967	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
QGTDLVQVGGAESYQ	Antigen	0.7967	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
RRLERLSYSGRRCIA	Antigen	0.7918	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
RRLERLSYSGRRCIA	Antigen	0.7918	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
DLVQVGGAESYQAVC	Antigen	0.7897	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
DLVQVGGAESYQAVC	Antigen	0.7897	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
VSAAPPRSCNIYPVM	Antigen	0.7867	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
VSAAPPRSCNIYPVM	Antigen	0.7867	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
QRLEEYDAVAVDEGQ	Antigen	0.7808	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
QRLEEYDAVAVDEGQ	Antigen	0.7808	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
APPRSCNIYPVMVCV	Antigen	0.7793	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
SAAPPRSCNIYPVMV	Antigen	0.7726	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
SAAPPRSCNIYPVMV	Antigen	0.7726	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
DAPFTVRISQGTDLV	Antigen	0.7576	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
DAPFTVRISQGTDLV	Antigen	0.7576	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
LVPMADKLDKLTAVC	Antigen	0.7553	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
AAPPRSCNIYPVMVC	Antigen	0.7531	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
AAPPRSCNIYPVMVC	Antigen	0.7531	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
LERLSYSGRRCIAVK	Antigen	0.7431	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
LERLSYSGRRCIAVK	Antigen	0.7431	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
IVSAAPPRSCNIYPV	Antigen	0.7191	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
IVSAAPPRSCNIYPV	Antigen	0.7191	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
PFTVRISQGTDLVQV	Antigen	0.709	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
PFTVRISQGTDLVQV	Antigen	0.709	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
MQRLEEYDAVAVDEG	Antigen	0.6982	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
MQRLEEYDAVAVDEG	Antigen	0.6982	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
EEYDAVAVDEGQFFP	Antigen	0.6813	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
EEYDAVAVDEGQFFP	Antigen	0.6813	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
FRMAQYELYGPPPPP	Antigen	0.6765	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
APFTVRISQGTDLVQ	Antigen	0.6734	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
APFTVRISQGTDLVQ	Antigen	0.6734	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
SQGTDLVQVGGAESY	Antigen	0.6723	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
SQGTDLVQVGGAESY	Antigen	0.6723	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
ISQGTDLVQVGGAES	Antigen	0.668	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
ISQGTDLVQVGGAES	Antigen	0.668	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
VRISQGTDLVQVGGA	Antigen	0.643	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
VRISQGTDLVQVGGA	Antigen	0.643	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
SYQAVCRPCLTGFRM	Antigen	0.6387	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
SYQAVCRPCLTGFRM	Antigen	0.6387	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
DQRYTEESKVAMHSG	Antigen	0.6377	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic

DQRYTEESKVAMHSG	Antigen	0.6377	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
EVMQRLEEYDAVAVD	Antigen	0.6371	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
EVMQRLEEYDAVAVD	Antigen	0.6371	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
GTDLVQVGGAESYQA	Antigen	0.634	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
GTDLVQVGGAESYQA	Antigen	0.634	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
VMQRLEEYDAVAVDE	Antigen	0.6274	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
YEVMQRLEEYDAVAV	Antigen	0.6218	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
TDLVQVGGAESYQAV	Antigen	0.6181	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
TDLVQVGGAESYQAV	Antigen	0.6181	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
LSYSGRRCIAVKHAI	Antigen	0.618	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
LSYSGRRCIAVKHAI	Antigen	0.618	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
PIVSAAPPRSCNIYP	Antigen	0.6174	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
PIVSAAPPRSCNIYP	Antigen	0.6174	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
RCIAVKHAIDQRYTE	Antigen	0.6154	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
RCIAVKHAIDQRYTE	Antigen	0.6154	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
QAVCRPCLTGFRMAQ	Antigen	0.6107	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
QAVCRPCLTGFRMAQ	Antigen	0.6107	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
CLTGFRMAQYELYGP	Antigen	0.5975	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
VCRPCLTGFRMAQYE	Antigen	0.5967	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
VCRPCLTGFRMAQYE	Antigen	0.5967	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
YQAVCRPCLTGFRMA	Antigen	0.5952	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
YQAVCRPCLTGFRMA	Antigen	0.5952	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
MHSGATYPAISAGYL	Antigen	0.5898	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
MHSGATYPAISAGYL	Antigen	0.5898	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
GFRMAQYELYGPPPP	Antigen	0.589	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
GFRMAQYELYGPPPP	Antigen	0.589	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
KHAIDQRYTEESKVA	Antigen	0.5825	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
KHAIDQRYTEESKVA	Antigen	0.5825	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
AMHSGATYPAISAGY	Antigen	0.5806	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
AMHSGATYPAISAGY	Antigen	0.5806	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
TGFRMAQYELYGPPP	Antigen	0.5767	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
TGFRMAQYELYGPPP	Antigen	0.5767	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
TVRISQGTDLVQVGG	Antigen	0.5655	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
TVRISQGTDLVQVGG	Antigen	0.5655	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
GAESYQAVCRPCLTG	Antigen	0.5457	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
RISQGTDLVQVGGAE	Antigen	0.5397	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
RISQGTDLVQVGGAE	Antigen	0.5397	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
AVCRPCLTGFRMAQY	Antigen	0.5345	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
AVCRPCLTGFRMAQY	Antigen	0.5345	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
RYTEESKVAMHSGAT	Antigen	0.5301	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
RYTEESKVAMHSGAT	Antigen	0.5301	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
VQVGGAESYQAVCRP	Antigen	0.529	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
VQVGGAESYQAVCRP	Antigen	0.529	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
AVKHAIDQRYTEESK	Antigen	0.5205	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
AVKHAIDQRYTEESK	Antigen	0.5205	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic

GAPIVSAAPPRSCNI	Antigen	0.5186	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
VKHAIDQRYTEESKV	Antigen	0.5052	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
VKHAIDQRYTEESKV	Antigen	0.5052	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
FKQVTALVPMADKLD	Antigen	0.5018	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
FKQVTALVPMADKLD	Antigen	0.5018	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
RRCIAVKHAIDQRYT	Antigen	0.5	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
RRCIAVKHAIDQRYT	Antigen	0.5	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
LVQVGGAESYQAVCR	Antigen	0.491	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
LVQVGGAESYQAVCR	Antigen	0.491	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
FRMAQYELYGPPPPP	Antigen	0.4894	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
EYDAVAVDEGQFFPD	Antigen	0.4869	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
EYDAVAVDEGQFFPD	Antigen	0.4869	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
ESYQAVCRPCLTGFR	Antigen	0.4851	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
KLDKLTAVCMKCKMR	Antigen	0.4838	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
GATYPAISAGYLYEV	Antigen	0.4719	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
GATYPAISAGYLYEV	Antigen	0.4719	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
LTGFRMAQYELYGPP	Antigen	0.4583	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
LTGFRMAQYELYGPP	Antigen	0.4583	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
LEEYDAVAVDEGQFF	Antigen	0.4503	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
LEEYDAVAVDEGQFF	Antigen	0.4503	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
TYPAISAGYLYEVMQ	Antigen	0.4497	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
TYPAISAGYLYEVMQ	Antigen	0.4497	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
HSGATYPAISAGYLY	Antigen	0.4478	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
AISAGYLYEVMQRLE	Antigen	0.4379	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
AISAGYLYEVMQRLE	Antigen	0.4379	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
VQLLTAGKYVIVAAL	Antigen	0.4353	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
VQLLTAGKYVIVAAL	Antigen	0.4353	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
QLLTAGKYVIVAALD	Antigen	0.4303	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
QLLTAGKYVIVAALD	Antigen	0.4303	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
SYSGRRCIAVKHAID	Antigen	0.4275	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
MAQYELYGPPPPPPA	Antigen	0.423	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
MAQYELYGPPPPPPA	Antigen	0.423	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
ALVPMADKLDKLTAV	Antigen	0.4204	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
ALVPMADKLDKLTAV	Antigen	0.4204	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
AQYELYGPPPPPPAH	Antigen	0.4124	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
AQYELYGPPPPPPAH	Antigen	0.4124	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
RMAQYELYGPPPPPP	Antigen	0.4067	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
PFKQVTALVPMADKL	Antigen	0.4035	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
PFKQVTALVPMADKL	Antigen	0.4035	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
APIVSAAPPRSCNIY	Antigen	0.402	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
APIVSAAPPRSCNIY	Antigen	0.402	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
GYLYEVMQRLEEYDA	Antigen	0.1978	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic
VIVAALDGDFMQQPF	Antigen	0.0468	NEGATIVE	Non-Inducer	Non-Inducer	Non-toxic	Non-allergenic

Table S2: HTL epitopes for the vaccine construction.

Peptides	Antigenicity	Score	Toxicity	Allergenicity
AGKSTESCRRLERLS	Antigen	1.1015	Non-toxic	Non-allergenic
EYDAVAVDEGQFFPD	Antigen	0.4869	Non-toxic	Non-allergenic
YDAVAVDEGQFFPDL	Antigen	0.4831	Non-toxic	Non-allergenic
AVKHAIDQRYTEESK	Antigen	0.5205	Non-toxic	Non-allergenic
KLTAVCMKCKMRDAP	Antigen	1.9143	Non-toxic	Non-allergenic
VKHAIDQRYTEESKV	Antigen	0.5052	Non-toxic	Non-allergenic
IAVKHAIDQRYTEES	Antigen	0.7987	Non-toxic	Non-allergenic
DKLTAVCMKCKMRDA	Antigen	1.6097	Non-toxic	Non-allergenic
GKSTESCRRLERLSY	Antigen	1.2185	Non-toxic	Non-allergenic
KHAIDQRYTEESKVA	Antigen	0.5825	Non-toxic	Non-allergenic
FAGKSTESCRRLERL	Antigen	1.0499	Non-toxic	Non-allergenic
RISQGTDLVQVGGAE	Antigen	0.5397	Non-toxic	Non-allergenic
EEYDAVAVDEGQFFP	Antigen	0.6813	Non-toxic	Non-allergenic
VSAAPPRSCNIYPVM	Antigen	0.7867	Non-toxic	Non-allergenic
LTAVCMKCKMRDAPF	Antigen	1.9397	Non-toxic	Non-allergenic
TAVCMKCKMRDAPFT	Antigen	1.829	Non-toxic	Non-allergenic
KSTESCRRLERLSYS	Antigen	1.1703	Non-toxic	Non-allergenic
LVPMADKLDKLTAVC	Antigen	0.7553	Non-toxic	Non-allergenic
CIAVKHAIDQRYTEE	Antigen	0.8293	Non-toxic	Non-allergenic
STESCRRLERLSYSG	Antigen	0.9301	Non-toxic	Non-allergenic
AAPPRSCNIYPVMVC	Antigen	0.7531	Non-toxic	Non-allergenic
SAAPPRSCNIYPVMV	Antigen	0.7726	Non-toxic	Non-allergenic
AIDQRYTEESKVAMH	Antigen	0.8838	Non-toxic	Non-allergenic
HAIDQRYTEESKVAM	Antigen	0.8447	Non-toxic	Non-allergenic
MFAGKSTESCRRLER	Antigen	0.9825	Non-toxic	Non-allergenic
APPRSCNIYPVMVCV	Antigen	0.7793	Non-toxic	Non-allergenic
DQRYTEESKVAMHSG	Antigen	0.6377	Non-toxic	Non-allergenic
IDQRYTEESKVAMHS	Antigen	0.8067	Non-toxic	Non-allergenic
RLEEYDAVAVDEGQF	Antigen	0.8498	Non-toxic	Non-allergenic
VQVGGAESYQAVCRP	Antigen	0.529	Non-toxic	Non-allergenic
LEEYDAVAVDEGQFF	Antigen	0.4503	Non-toxic	Non-allergenic
PPRSCNIYPVMVCVE	Antigen	0.9455	Non-toxic	Non-allergenic
RCIAVKHAIDQRYTE	Antigen	0.6154	Non-toxic	Non-allergenic
YQAVCRPCLTGFRMA	Antigen	0.5952	Non-toxic	Non-allergenic
ALVPMADKLDKLTAV	Antigen	0.4204	Non-toxic	Non-allergenic
PMFAGKSTESCRRLE	Antigen	1.266	Non-toxic	Non-allergenic
GPMFAGKSTESCRRL	Antigen	0.8563	Non-toxic	Non-allergenic
SYQAVCRPCLTGFRM	Antigen	0.6387	Non-toxic	Non-allergenic
VRISQGTDLVQVGGA	Antigen	0.643	Non-toxic	Non-allergenic
PRSCNIYPVMVCVEP	Antigen	1.0417	Non-toxic	Non-allergenic
RSCNIYPVMVCVEPI	Antigen	1.0368	Non-toxic	Non-allergenic
TESCRRLERLSYSGR	Antigen	1.1128	Non-toxic	Non-allergenic
SCNIYPVMVCVEPIK	Antigen	1.0556	Non-toxic	Non-allergenic
GRRCIAVKHAIDQRY	Antigen	0.4926	Non-toxic	Non-allergenic

IGPMFAGKSTESCRR	Antigen	1.2006	Non-toxic	Non-allergenic
RRCIAVKHAIDQRYT	Antigen	0.5	Non-toxic	Non-allergenic
YSGRRCIAVKHAIDQ	Antigen	0.0431	Non-toxic	Non-allergenic
RMAQYELYGPPPPPP	Antigen	0.4067	Non-toxic	Non-allergenic
SYSGRRCIAVKHAID	Antigen	0.4275	Non-toxic	Non-allergenic
TVRISQGTDLVQVGG	Antigen	0.5655	Non-toxic	Non-allergenic
AVCMKCKMRDAPFTV	Antigen	1.8094	Non-toxic	Non-allergenic
ESCRRLERLSYSGRR	Antigen	1.0122	Non-toxic	Non-allergenic
EVMQRLEEYDAVAVD	Antigen	0.6371	Non-toxic	Non-allergenic
MQRLEEYDAVAVDEG	Antigen	0.6982	Non-toxic	Non-allergenic
QRLEEYDAVAVDEGQ	Antigen	0.7808	Non-toxic	Non-allergenic
RRLERLSYSGRRCIA	Antigen	0.7918	Non-toxic	Non-allergenic
SCRRLERLSYSGRRC	Antigen	1.2771	Non-toxic	Non-allergenic
VMQRLEEYDAVAVDE	Antigen	0.6274	Non-toxic	Non-allergenic
YEVMQRLEEYDAVAV	Antigen	0.6218	Non-toxic	Non-allergenic
AQYELYGPPPPPPAH	Antigen	0.4124	Non-toxic	Non-allergenic
ERLSYSGRRCIAVKH	Antigen	0.9178	Non-toxic	Non-allergenic
CKMRDAPFTVRISQG	Antigen	1.2128	Non-toxic	Non-allergenic
CRRLERLSYSGRRCI	Antigen	1.1665	Non-toxic	Non-allergenic
CRPCLTGFRMAQYEL	Antigen	0.8756	Non-toxic	Non-allergenic
LSYSGRRCIAVKHAI	Antigen	0.618	Non-toxic	Non-allergenic
MAQYELYGPPPPPPA	Antigen	0.423	Non-toxic	Non-allergenic
LERLSYSGRRCIAVK	Antigen	0.7431	Non-toxic	Non-allergenic
RLERLSYSGRRCIAV	Antigen	0.9684	Non-toxic	Non-allergenic
VCRPCLTGFRMAQYE	Antigen	0.5967	Non-toxic	Non-allergenic
VIGPMFAGKSTESCR	Antigen	0.9742	Non-toxic	Non-allergenic
QAVCRPCLTGFRMAQ	Antigen	0.6107	Non-toxic	Non-allergenic
RLSYSGRRCIAVKHA	Antigen	0.9607	Non-toxic	Non-allergenic
GAESYQAVCRPCLTG	Antigen	0.5457	Non-toxic	Non-allergenic
AVCRPCLTGFRMAQY	Antigen	0.5345	Non-toxic	Non-allergenic
RYTEESKVAMHSGAT	Antigen	0.5301	Non-toxic	Non-allergenic
FRMAQYELYGPPPPP	Antigen	0.4894	Non-toxic	Non-allergenic
GFRMAQYELYGPPPP	Antigen	0.589	Non-toxic	Non-allergenic
ESYQAVCRPCLTGFR	Antigen	0.4851	Non-toxic	Non-allergenic
LVIGPMFAGKSTESC	Antigen	1.1855	Non-toxic	Non-allergenic
MRDAPFTVRISQGTD	Antigen	0.9967	Non-toxic	Non-allergenic
RDAPFTVRISQGTDL	Antigen	1.0586	Non-toxic	Non-allergenic
KMRDAPFTVRISQGT	Antigen	1.3035	Non-toxic	Non-allergenic
KCKMRDAPFTVRISQ	Antigen	1.3989	Non-toxic	Non-allergenic
CMKCKMRDAPFTVRI	Antigen	1.7118	Non-toxic	Non-allergenic
AMHSGATYPAISAGY	Antigen	0.5806	Non-toxic	Non-allergenic
FKQVTALVPMADKLD	Antigen	0.5018	Non-toxic	Non-allergenic
FTVRISQGTDLVQVG	Antigen	0.9261	Non-toxic	Non-allergenic
LDKLTAVCMKCKMRD	Antigen	1.5243	Non-toxic	Non-allergenic
MKCKMRDAPFTVRIS	Antigen	1.7305	Non-toxic	Non-allergenic

PFTVRISQGTDLVQV	Antigen	0.709	Non-toxic	Non-allergenic
KLDKLTAVCMKCKMR	Antigen	1.2252	Non-toxic	Non-allergenic
VAMHSGATYPAISAG	Antigen	0.5088	Non-toxic	Non-allergenic
DAPFTVRISQGTDLV	Antigen	0.7576	Non-toxic	Non-allergenic
TGFRMAQYELYGPPP	Antigen	0.5767	Non-toxic	Non-allergenic
ELVIGPMFAGKSTES	Antigen	1.0699	Non-toxic	Non-allergenic
ISQGTDLVQVGGAES	Antigen	0.668	Non-toxic	Non-allergenic
MAMLELVIGPMFAGK	Antigen	1.0084	Non-toxic	Non-allergenic
QGTDLVQVGGAESYQ	Antigen	0.7967	Non-toxic	Non-allergenic
SQGTDLVQVGGAESY	Antigen	0.6723	Non-toxic	Non-allergenic
TYPAISAGYLYEVMQ	Antigen	0.4497	Non-toxic	Non-allergenic
LTGFRMAQYELYGPP	Antigen	0.4583	Non-toxic	Non-allergenic
APFTVRISQGTDLVQ	Antigen	0.6734	Non-toxic	Non-allergenic
DLVQVGGAESYQAVC	Antigen	0.7897	Non-toxic	Non-allergenic
LVQVGGAESYQAVCR	Antigen	0.4910	Non-toxic	Non-allergenic
CLTGFRMAQYELYGP	Antigen	0.5975	Non-toxic	Non-allergenic
AMLELVIGPMFAGKS	Antigen	0.9833	Non-toxic	Non-allergenic
LELVIGPMFAGKSTE	Antigen	1.2806	Non-toxic	Non-allergenic
MHSGATYPAISAGYL	Antigen	0.5898	Non-toxic	Non-allergenic
QLLTAGKYVIVAALD	Antigen	0.4303	Non-toxic	Non-allergenic
RPCLTGFRMAQYELY	Antigen	0.8958	Non-toxic	Non-allergenic
GTDLVQVGGAESYQA	Antigen	0.634	Non-toxic	Non-allergenic
PCLTGFRMAQYELYG	Antigen	0.8955	Non-toxic	Non-allergenic
AISAGYLYEVMQRLE	Antigen	0.4379	Non-toxic	Non-allergenic
MLELVIGPMFAGKST	Antigen	1.0939	Non-toxic	Non-allergenic
PFKQVTALVPMADKL	Antigen	0.4035	Non-toxic	Non-allergenic
VPMADKLDKLTAVCM	Antigen	0.8357	Non-toxic	Non-allergenic
IVSAAPPRSCNIYPV	Antigen	0.7191	Non-toxic	Non-allergenic
PIVSAAPPRSCNIYP	Antigen	0.6174	Non-toxic	Non-allergenic
TDLVQVGGAESYQAV	Antigen	0.6181	Non-toxic	Non-allergenic
DKLDKLTAVCMKCKM	Antigen	1.0224	Non-toxic	Non-allergenic
HSGATYPAISAGYLY	Antigen	0.4478	Non-toxic	Non-allergenic
MADKLDKLTAVCMKC	Antigen	0.9534	Non-toxic	Non-allergenic
ADKLDKLTAVCMKCK	Antigen	1.1234	Non-toxic	Non-allergenic
PMADKLDKLTAVCMK	Antigen	0.8666	Non-toxic	Non-allergenic
ATYPAISAGYLYEVM	Antigen	0.4983	Non-toxic	Non-allergenic
GATYPAISAGYLYEV	Antigen	0.4719	Non-toxic	Non-allergenic
VQLLTAGKYVIVAAL	Antigen	0.4353	Non-toxic	Non-allergenic
APIVSAAPPRSCNIY	Antigen	0.4020	Non-toxic	Non-allergenic
GAPIVSAAPPRSCNI	Antigen	0.5186	Non-toxic	Non-allergenic
GPPPPPPAHNLLGAP	Non-Antigen	0.2413	Non-toxic	Non-allergenic
VAVDEGQFFPDLYEG	Non-Antigen	0.2232	Non-toxic	Non-allergenic
AVAVDEGQFFPDLYE	Non-Antigen	0.2093	Non-toxic	Non-allergenic
DAVAVDEGQFFPDLY	Non-Antigen	0.2999	Non-toxic	Non-allergenic
YGPPPPPPAHNLLGA	Non-Antigen	0.3828	Non-toxic	Non-allergenic

AVDEGQFFPDLYEGV	Non-Antigen	0.2069	Non-toxic	Non-allergenic
LYGPPPPPPAHNLLG	Non-Antigen	0.0247	Non-toxic	Non-allergenic
IVAALDGDFMQQPFK	Non-Antigen	0.0262	Non-toxic	Non-allergenic
LYEVMQRLEEYDAVA	Non-Antigen	0.3935	Non-toxic	Non-allergenic
QVGGAESYQAVCRPC	Non-Antigen	0.3764	Non-toxic	Non-allergenic
VAALDGDFMQQPFKQ	Non-Antigen	-0.0327	Non-toxic	Non-allergenic
DEGQFFPDLYEGVVQ	Non-Antigen	-0.0014	Non-toxic	Non-allergenic
VDEGQFFPDLYEGVV	Non-Antigen	0.1600	Non-toxic	Non-allergenic
ELYGPPPPPPAHNLL	Non-Antigen	-0.0327	Non-toxic	Non-allergenic
QRYTEESKVAMHSGA	Non-Antigen	0.3591	Non-toxic	Non-allergenic
YELYGPPPPPPAHNL	Non-Antigen	0.2009	Non-toxic	Non-allergenic
QYELYGPPPPPPAHN	Non-Antigen	0.3453	Non-toxic	Non-allergenic
SGRRCIAVKHAIDQR	Non-Antigen	0.3179	Non-toxic	Non-allergenic
EGQFFPDLYEGVVQL	Non-Antigen	0.1802	Non-toxic	Non-allergenic
VGGAESYQAVCRPCL	Non-Antigen	0.3554	Non-toxic	Non-allergenic
VIVAALDGDFMQQPF	Non-Antigen	0.0468	Non-toxic	Non-allergenic
GGAESYQAVCRPCLT	Non-Antigen	0.3096	Non-toxic	Non-allergenic
PPPPPPAHNLLGAPI	Non-Antigen	0.3115	Non-toxic	Non-allergenic
TALVPMADKLDKLTA	Non-Antigen	0.2711	Non-toxic	Non-allergenic
AALDGDFMQQPFKQV	Non-Antigen	-0.0125	Non-toxic	Non-allergenic
AESYQAVCRPCLTGF	Non-Antigen	0.2011	Non-toxic	Non-allergenic
VCMKCKMRDAPFTVR	Non-Antigen	2.039	Non-toxic	Non-allergenic
QVTALVPMADKLDKL	Non-Antigen	0.3452	Non-toxic	Non-allergenic
VTALVPMADKLDKLT	Non-Antigen	0.2269	Non-toxic	Non-allergenic
KQVTALVPMADKLDK	Non-Antigen	0.2671	Non-toxic	Non-allergenic
GQFFPDLYEGVVQLL	Non-Antigen	-0.1455	Non-toxic	Non-allergenic
YLYEVMQRLEEYDAV	Non-Antigen	0.3504	Non-toxic	Non-allergenic
GYLYEVMQRLEEYDA	Non-Antigen	0.1978	Non-toxic	Non-allergenic
TEESKVAMHSGATYP	Non-Antigen	0.3684	Non-toxic	Non-allergenic
YTEESKVAMHSGATY	Non-Antigen	0.3612	Non-toxic	Non-allergenic
SKVAMHSGATYPAIS	Non-Antigen	0.3921	Non-toxic	Non-allergenic
YPAISAGYLYEVMQR	Non-Antigen	0.3921	Non-toxic	Non-allergenic
ALDGDFMQQPFKQVT	Non-Antigen	0.0405	Non-toxic	Non-allergenic
KVAMHSGATYPAISA	Non-Antigen	0.3989	Non-toxic	Non-allergenic
LDGDFMQQPFKQVTA	Non-Antigen	-0.0465	Non-toxic	Non-allergenic
QFFPDLYEGVVQLLT	Non-Antigen	-0.2004	Non-toxic	Non-allergenic
YVIVAALDGDFMQQP	Non-Antigen	0.0736	Non-toxic	Non-allergenic
DGDFMQQPFKQVTAL	Non-Antigen	0.1525	Non-toxic	Non-allergenic
EESKVAMHSGATYPA	Non-Antigen	0.2666	Non-toxic	Non-allergenic
ESKVAMHSGATYPAI	Non-Antigen	0.3488	Non-toxic	Non-allergenic
AGYLYEVMQRLEEYD	Non-Antigen	0.3059	Non-toxic	Non-allergenic
FFPDLYEGVVQLLTA	Non-Antigen	-0.1031	Non-toxic	Non-allergenic
FPDLYEGVVQLLTAG	Non-Antigen	0.171	Non-toxic	Non-allergenic
ISAGYLYEVMQRLEE	Non-Antigen	0.3097	Non-toxic	Non-allergenic
PAISAGYLYEVMQRL	Non-Antigen	0.2095	Non-toxic	Non-allergenic

SAGYLYEVMQRLEEY	Non-Antigen	0.0643	Non-toxic	Non-allergenic
GDFMQQPFKQVTALV	Non-Antigen	0.1384	Non-toxic	Non-allergenic
PDLYEGVVQLLTAGK	Non-Antigen	0.2483	Non-toxic	Non-allergenic
QPFKQVTALVPMADK	Non-Antigen	0.362	Non-toxic	Non-allergenic
KYVIVAALDGDFMQQ	Non-Antigen	0.1526	Non-toxic	Non-allergenic
VVQLLTAGKYVIVAA	Non-Antigen	0.3916	Non-toxic	Non-allergenic
DLYEGVVQLLTAGKY	Non-Antigen	0.2589	Non-toxic	Non-allergenic
QQPFKQVTALVPMAD	Non-Antigen	0.2115	Non-toxic	Non-allergenic
MQQPFKQVTALVPMA	Non-Antigen	0.2181	Non-toxic	Non-allergenic
FMQQPFKQVTALVPM	Non-Antigen	0.1707	Non-toxic	Non-allergenic
PPPPPAHNLLGAPIV	Non-Antigen	0.3356	Non-toxic	Non-allergenic
DFMQQPFKQVTALVP	Non-Antigen	0.1737	Non-toxic	Non-allergenic
GVVQLLTAGKYVIVA	Non-Antigen	0.3715	Non-toxic	Non-allergenic
HNLLGAPIVSAAPPR	Non-Antigen	0.2955	Non-toxic	Non-allergenic
SGATYPAISAGYLYE	Non-Antigen	0.3809	Non-toxic	Non-allergenic
LYEGVVQLLTAGKYV	Non-Antigen	0.0905	Non-toxic	Non-allergenic
PPPPAHNLLGAPIVS	Non-Antigen	0.281	Non-toxic	Non-allergenic
AGKYVIVAALDGDFM	Non-Antigen	0.1143	Non-toxic	Non-allergenic
GKYVIVAALDGDFMQ	Non-Antigen	0.0503	Non-toxic	Non-allergenic
LLTAGKYVIVAALDG	Non-Antigen	0.2081	Non-toxic	Non-allergenic
LTAGKYVIVAALDGD	Non-Antigen	0.1766	Non-toxic	Non-allergenic
TAGKYVIVAALDGDF	Non-Antigen	0.2568	Non-toxic	Non-allergenic
LGAPIVSAAPPRSCN	Non-Antigen	0.3156	Non-toxic	Non-allergenic
LLGAPIVSAAPPRSC	Non-Antigen	0.1699	Non-toxic	Non-allergenic
NLLGAPIVSAAPPRS	Non-Antigen	0.2431	Non-toxic	Non-allergenic
YEGVVQLLTAGKYVI	Non-Antigen	0.0623	Non-toxic	Non-allergenic
EGVVQLLTAGKYVIV	Non-Antigen	0.0839	Non-toxic	Non-allergenic
PPPAHNLLGAPIVSA	Non-Antigen	0.2496	Non-toxic	Non-allergenic
AHNLLGAPIVSAAPP	Non-Antigen	0.2355	Non-toxic	Non-allergenic
PAHNLLGAPIVSAAP	Non-Antigen	0.1644	Non-toxic	Non-allergenic
PPAHNLLGAPIVSAA	Non-Antigen	0.1774	Non-toxic	Non-allergenic

Table S3: LBL epitopes for the vaccine construction.

Cluster	Members	Representative	Weighted Score
0	38	Center	-1065.1
0	38	Lowest Energy	-1105.2
1	36	Center	-1004.9
1	36	Lowest Energy	-1064.6
2	35	Center	-891.9
2	35	Lowest Energy	-1057.9
3	31	Center	-944.4
3	31	Lowest Energy	-1059.3
4	25	Center	-996.9
4	25	Lowest Energy	-1021.6
5	23	Center	-912.1

5	23	Lowest Energy	-1064.7
6	23	Center	-973.2
6	23	Lowest Energy	-1093.2
7	23	Center	-895.6
7	23	Lowest Energy	-1128.9
8	22	Center	-952.7
8	22	Lowest Energy	-981.8
9	21	Center	-1010.4
9	21	Lowest Energy	-1041.4
10	21	Center	-1059.7
10	21	Lowest Energy	-1112.8
11	20	Center	-1215.8
11	20	Lowest Energy	-1215.8
12	20	Center	-907.4
12	20	Lowest Energy	-1019.5
13	19	Center	-896.8
13	19	Lowest Energy	-995
14	18	Center	-914.4
14	18	Lowest Energy	-1051.7
15	18	Center	-951.1
15	18	Lowest Energy	-951.1
16	16	Center	-1005.1
16	16	Lowest Energy	-1005.1
17	15	Center	-923.1
17	15	Lowest Energy	-1016.3
18	15	Center	-889
18	15	Lowest Energy	-978.5
19	14	Center	-904.3
19	14	Lowest Energy	-977.4
20	14	Center	-905.2
20	14	Lowest Energy	-969.1
21	13	Center	-930
21	13	Lowest Energy	-1016.8
22	13	Center	-901.6
22	13	Lowest Energy	-1015.8
23	13	Center	-891.3
23	13	Lowest Energy	-942.6
24	13	Center	-902.9
24	13	Lowest Energy	-940
25	13	Center	-1100.9
25	13	Lowest Energy	-1100.9
26	13	Center	-927
26	13	Lowest Energy	-1036.8
27	13	Center	-905.8
27	13	Lowest Energy	-1002.2

28	13	Center	-905.1
28	13	Lowest Energy	-1037.6
29	12	Center	-921.7
29	12	Lowest Energy	-1032.8

Table S4: The list of energy scores from the TLR9 complex.

Receptor interface residue(s):
ASN 15A 3.632
SER 16A 2.309
LYS 17A 2.949
GLY 18A 3.237
ARG 32A 3.787
GLU 34A 4.297
GLU 36A 1.617
SER 37A 1.450
ASN 38A 3.770
SER 60A 4.695
ARG 61A 2.171
ASN 62A 4.881
HIS 85A 2.880
ASP 107A 4.646
SER 109A 4.261
HIS 110A 2.851
ASP 131A 2.646
TYR 134A 2.698
ALA 163A 4.023
HIS 164A 3.107
ASP 184A 3.284
SER 186A 3.177
ASP 214A 4.242
SER 216A 4.788
GLN 217A 3.024
SER 239A 4.858
ARG 241A 3.318
ASP 242A 2.952
ASP 263A 3.943
SER 290A 4.882
ASN 291A 4.810
SER 292A 3.856
SER 313A 3.911
HIS 314A 1.796
ASN 315A 4.049
ILE 316A 4.846
ASP 338A 4.274

THR 339A 3.385
TRP 360A 4.142
HIS 362A 4.206
THR 363A 2.730
GLY 389A 3.480
SER 390A 4.284
LYS 392A 1.976
CYS 393A 4.375
SER 394A 2.912
GLY 395A 2.468
SER 396A 3.165
GLY 397A 3.011
LYS 398A 3.317
Ligand interface residue(s):
CYS 33A 3.049
ASP 77A 4.407
ARG 110A 3.317
ASP 111A 3.011
ALA 112A 3.606
TYR 114A 2.468
CYS 143A 3.480
MET 144A 1.976
CYS 146A 4.206
LYS 147A 3.283
PRO 149A 3.629
GLY 150A 3.411
PRO 151A 2.730
LYS 153A 2.785
CYS 154A 3.728
ARG 157A 3.856
VAL 194A 4.733
LYS 197A 4.142
ARG 201A 1.796
PRO 257A 4.483
PRO 258A 3.318
PRO 259A 4.161
HIS 261A 3.177
LYS 262A 2.952
ARG 264A 2.646
MET 265A 2.698
GLN 267A 2.171
TYR 268A 2.851

LEU 270A 4.522
TYR 271A 3.096
ARG 285A 2.309
TYR 288A 1.450
SER 289A 3.626
ARG 291A 2.819
ARG 292A 1.617
ALA 295A 3.787
Receptor-ligand interface residue pair(s):
15A - 289A 4.633
15A - 292A 3.632
16A - 285A 2.309
16A - 288A 3.107
16A - 289A 3.626
17A - 285A 2.949
18A - 285A 3.237
32A - 295A 3.787
34A - 292A 4.964
34A - 295A 4.297
36A - 288A 4.440
36A - 292A 1.617
36A - 295A 4.172
37A - 285A 4.700
37A - 288A 1.450
37A - 289A 4.743
37A - 291A 3.947
37A - 292A 2.664
38A - 288A 3.770
60A - 288A 4.728
60A - 292A 4.695
61A - 267A 2.171
61A - 270A 4.522
61A - 271A 3.096
61A - 288A 2.866
61A - 291A 2.819
61A - 292A 2.421
62A - 288A 4.881
85A - 267A 2.880
85A - 271A 3.680
107A - 264A 4.646
109A - 264A 4.261
110A - 264A 4.450

110A - 268A 2.851
110A - 271A 4.384
131A - 264A 2.646
134A - 261A 4.839
134A - 264A 4.569
134A - 265A 2.698
134A - 268A 3.234
163A - 261A 4.023
164A - 261A 3.840
164A - 265A 3.107
184A - 261A 3.284
186A - 261A 3.177
214A - 261A 4.242
216A - 261A 4.788
217A - 262A 3.316
217A - 265A 3.024
239A - 258A 4.858
241A - 257A 4.483
241A - 258A 3.318
241A - 259A 4.161
242A - 262A 2.952
263A - 258A 3.943
290A - 153A 4.882
291A - 157A 4.810
292A - 157A 3.856
313A - 201A 3.911
314A - 149A 3.629
314A - 150A 3.669
314A - 153A 2.785
314A - 201A 1.796
315A - 153A 4.049
316A - 157A 4.846
338A - 201A 4.274
339A - 150A 3.411
339A - 151A 4.435
339A - 153A 3.385
339A - 154A 3.728
339A - 201A 4.305
360A - 197A 4.142
362A - 146A 4.206
362A - 147A 4.488
363A - 146A 4.358
363A - 147A 3.283

363A - 150A 3.863
363A - 151A 2.730
389A - 143A 3.480
389A - 194A 4.733
390A - 143A 4.284
392A - 112A 4.954
392A - 144A 1.976
393A - 114A 4.375
394A - 114A 2.912
395A - 33A 3.049
395A - 77A 4.407
395A - 114A 2.468
396A - 33A 4.473
396A - 110A 3.935
396A - 111A 3.807
396A - 112A 4.907
396A - 114A 3.165
397A - 111A 3.011
397A - 112A 3.606
397A - 114A 3.363
397A - 144A 4.929
398A - 110A 3.317
398A - 111A 3.472

Table S5: The list of receptor and ligand interface residues.