DESIGNING A SHORT PEPTIDE VACCINE AGAINST *CLOSTRIDIUM BOTULINUM* TO DEVELOP IMMUNITY AGAINST FOOD POISONING

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Abstract

Clostridium botulinum is considered the most commonly reported bacterium that causes food poisoning. It has a huge impact on the health of people around the world. Given the pressing nature of the situation, it is crucial to expedite the development of a vaccine to effectively counter it. Most experimental work without bioinformatics preliminary work is time-consuming. *Clostridium botulinum* is a type of Gram-positive bacterium that can form spores and is considered a leading cause of food poisoning related to antibiotic use. The prevalence of infections caused by this bacterium has increased significantly over time, resulting in more hospitalizations and deaths. When the spores are ingested, they can germinate in the gastrointestinal tract, causing contamination and resulting in an infection. The bacterium's ability to colonize surfaces is attributed to surface layer proteins and flagellar proteins, while sporophyte proteins facilitate spore invasion. The recurrence of *Clostridium botulinum* infection in hospitalized patients is mainly attributed to two factors. While previous studies have focused on specific proteins, this study aims to analyze the entire proteome to identify more immunogenic proteins. Using immunoinformatics, a chimeric vaccine candidate is formed by selecting proteins to create a multivalent epitope. To ensure the fidelity of our vaccine candidates, we performed stability tests using in silico and molecular dynamics simulations. Additionally, docking studies were performed to confirm the stable interaction of the vaccine with Toll-Like receptors and MHC receptors present in innate immune cells. In-silico codon optimization was also carried out to optimize the vaccine, which was then introduced into a cloning vector. The efficacy of the vaccine was evaluated by examining its expression in both Homo sapiens and E. coli expression systems. The efficacy of vaccine candidates in inducing defensive immune responses was evaluated using an in silico immune simulation system. Our study has successfully generated promising short-peptide vaccine candidates against *Clostridium botulinum* using computational tools, which could potentially save time and resources. These findings provide valuable information for future in vitro and in vivo studies on this topic.

Keywords: *Clostridium botulinum*, immunoinformatic, in silico, chimeric, Toll-Like Receptors, food poisoning.

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INTRODUCTION

Clostridium botulinum is an anaerobic microorganism that is commonly distributed in soil and water and has a widespread presence, characterized by its Gram-positive, rod-shaped, and spore-forming morphology. *Clostridium botulinum* strains produce seven different neurotoxins and one binary toxin. Toxins A, B, E and F are the main pollutants affecting humans **(Ramachandran 2014**). Botulism in birds and mammals is caused by the Type C and D toxins, while *Clostridium botulinum* strains that produce toxins A, B and F are typically found in regions with low rainfall and moderate temperatures. As a spore-forming bacterium, *Clostridium botulinum's* spores are highly resistant to heat and can survive at 100°C for at least six hours, but they can be eliminated in just five minutes at a temperature of 120°C (**Fiock et al., 1963**).

The neurotoxins of *Clostridium botulinum*, known as *Botulinum* neurotoxins (BoNTs), consist of a group of seven proteins with distinct antigenic properties (serotypes A to G). These toxins are known to be some of the most potent protein poisons, with human botulism cases often caused by the ingestion of contaminated food containing serotypes A, B, or E. Other types of botulism, such as wound botulism resulting from contaminated wounds and infant botulism caused by colonization in neonates, are also possible. Due to their high lethality and potential for misuse, the Centers for Disease Control and Prevention (CDCP) classify BoNTs as category-A bio-threats. New methods for producing BoNT vaccines have centred on utilizing the recombinant carboxyl terminus of the toxin-heavy chain (Hc) as an immunogen. The initial stage of the immune response to vaccination involves the collection, processing, and presentation of antigens by dendritic cells (DCs), which are specialized antigen-presenting cells. DCs gather antigens at peripheral sites, transport them to lymphoid tissues, and effectively activate B and T cells **(*David M. White. et.al 2011***).

Food poisoning is a severe illness resulting from the ingestion of a potent neurotoxin produced by the growth of pathogenic microorganisms in food. Three main factors have increased the likelihood that this pathogen is the cause of foodborne illness, and community-acquired *C. botulinum* infection is increasingly recognized. Recent studies recognize this pathogen in animals and foods, and the animal similarities to botulinum isolates and humans **(Dembek et al., 2007)**. Initial diagnosis and management of botulism are critical to reducing the risk of death, as botulism can be a debilitating disease that disrupts the body's nervous system and begins with a combination of potent neurotoxins produced by the bacterium *Clostridium botulinum* **(Czerwiński et al., 2016)**. Gastrointestinal disturbances are often associated with the consumption of *botulinum* toxin (BoNT), which is already present in food, and so this toxin enters the human body through the gastrointestinal tract to produce a toxic substance. Digestive disorders, in the intestine, are called food consumption. poisoning. The outbreak of the BoNT-contaminated food industry could represent a public health crisis. A rapid epidemiological study is crucial to prevent new cases, wherever hazardous food area units are accessible (***Kanaan & Tarek, 2020***).

The Centers for Disease Control and Prevention's decision to discontinue the *botulinum* antigen vaccine has led to a rise in the need for potent botulism vaccines. *Botulinum* toxin (BoNT) is the primary virulence and immunization factor against food poisoning. Elimination of the receptor binding ability enhances the potency of the HCR subunit vaccine. Vaccines that lack receptor binding have the additional property of limiting off-target toxicity **(Amanda Przedpelski et.al.2013).**

MHC class I proteins are important components of the adaptive immune system as they have the fundamental function of presenting peptides on the cell surface for recognition by T cells. The resulting immunogenic peptide-MHC class I (pMHCI) complexes are expressed in nucleated cells and induce cytotoxicity in CD8+ T cells. After a prolonged interaction between a T-cell receptor and particular pMHC complexes, which involves an exhaustive exploration in secondary lymphoid organs, the T-cell undergoes proliferation, resulting in the formation of a specific cellular immune response (**Wieczorek et al. 2017**).

Clostridium botulinum, or botulism, is a neurological disease that has been recognized by humans for centuries. The anaerobic, spore-forming bacterium C. botulinum and closely related species produce botulinum neurotoxin (BoNT), which causes a severe illness considered to be one of the most potent poisons known to humans. Without treatment, severe poisoning can result in respiratory muscle paralysis and death. Although the disease has been known for a long

time, it was not until 1870 when Müller named the newly described disease "poisoning" (**Hill et al 2007**).

Botulinum toxin poses a serious threat to biological weapons due to its extreme potency and lethality. It is simply about manufacturing, shipping and misuse; therefore, prolonged intensive care is required in those affected **(Arnon et al., 2001)**.

Botulinum toxin is an extremely potent poison, as just one gram of the crystallized toxin, if inhaled or ingested, could potentially kill over a thousand people **(Rao et al.,2017)**. The exceptional effectiveness of the toxin is due to its enzyme, a zinc proteinase that cleaves proteins involved in the release of neurotransmitters in the neuromuscular junction. Because of its potential for mass harm, *botulinum* toxin is considered a dangerous biological agent and can be used as a weapon of terrorism or warfare, with aerosolized forms or contaminated water and food as possible delivery methods **(Huang et al., 2022)**.

However, recent developments in research have shown that *botulinum* toxin, despite its lethal potential, also has therapeutic uses. It is now approved as a treatment for various medical conditions, including certain muscle disorders and chronic migraines, among others. This repurposing of the toxin from a deadly agent to a beneficial molecule is an intriguing aspect of *botulinum* toxin analysis in recent years **(Peck et al., 2017)**.

By utilizing the 'Immunological Epitope Database and Analytical Resources' (IEDB), researchers have been able to review data on immunological epitopes from *Clostridium botulinum*, a bacterial pathogen responsible for botulism. The majority of reported epitopes are derived from the class A neurotoxin protein, which is a key component in the pathogenesis of *botulinum* toxin. The use of bioinformatics tools has significantly improved our capacity to handle and process vast quantities of scientific data **(Fadaka et al., 2021)**. By conducting meta-analyses of data, researchers can obtain a comprehensive assessment of current knowledge in a specific area of study. Led by scientists at the La Jolla Institute of Allergy and Immunology and supported by the National Institute of Allergy and Infectious Diseases (NIAID), the Immune Epitope Database (IEDB) aims to present immunological data and analysis tools through a unified interface **(Khorasan et al., 2020)**. The database is populated by published literature and direct submissions of epitope discovery groups, containing a vast collection of B and T cell epitopes, as well as MHC topology and detailed annotations of the immunological context in which each epitope was identified. This information includes the immune/infected species, source organism, epitope antigen, and experimental techniques used **(Smith 2009)**.

The IEDB website offers a range of analysis resources, allowing for further analysis of the compiled and normalized data. The IEDB represents a significant contribution to the field of immunology, providing a valuable resource for researchers and clinicians working to develop vaccines and therapeutic interventions against pathogens such as *Clostridium botulinum* (**Zarebski et al. 2012**).

The In-silico approach has become increasingly important in epitope prediction and validation. In recent studies, researchers have used this approach to search for bacterial proteins and predict potential epitopes using computational methods such as molecular docking and MD simulation. These techniques can provide insights into the interactions between epitopes and antibodies, as well as their potential therapeutic effectiveness. By using these computational tools, researchers can save time and resources by identifying potential epitopes before conducting expensive and time-consuming experimental studies. Overall, In-silico prediction and validation of epitopes is an important tool for advancing our understanding of the immune response and developing new therapeutics **(Joshi et al. 2021)**.

In this study, our objective was to identify potential epitopes within the membrane-binding proteins of *Clostridium botulinum* that could stimulate an immune response in the host by interacting with MHC class I proteins. To achieve this goal, we employed bioinformatics tools to predict epitopes that could interact with the MHC class I HLA allele and generate a short peptide vaccine for protection against *C. botulinum* induced food poisoning. By using a computational approach, we aimed to provide a comprehensive understanding of the immune response elicited by potential epitopes of *C. botulinum* and to identify novel targets for the development of effective vaccines.

METHODOLOGY

The objective of our study was to anticipate probable epitopes within the membrane-binding

proteins of *Clostridium botulinum* that have the potential to bind with MHC class I proteins and generate an immune response against this noxious bacterium **(Simpson 2004)**. To achieve this goal, we employed a series of bioinformatics tools and procedures, including: (1) Selection of target membrane-binding proteins, (2) Screening of potential epitopes within the selected proteins, (3) Retrieval of the structural information of the MHC class I HLA allele, (4) Prediction of the structure of the epitopes, (5) Molecular docking to evaluate the binding affinity of the epitopes to the MHC class I HLA allele, and (6) Molecular dynamics simulation to further analyze the stability of the epitope-MHC complex. By utilizing a comprehensive approach, we were able to successfully identify potential epitopes in the membrane-binding proteins of *C. botulinum* that have the ability to interact with MHC class I proteins and potentially elicit an immune response against the bacterium. These epitopes can be further explored to evaluate their immunogenicity and their potential use in the development of a short peptide vaccine for food poisoning caused by *C. botulinum*.

The proteome of *Clostridium botulinum* was retrieved, and its structure was analyzed.

The structural proteins of *Clostridium botulinum* were selected from the NCBI (*National Centre for Biotechnology Information Database*) (https://www.ncbi.nlm.nih.gov/) PDB database on their allergenicity. The AllerTop 2.0 is a web-based tool that offers improved precision in predicting the likelihood of protein allergenicity when compared to other computational approaches **(Dimitrov et al., 2014)**. Additionally, it includes novel attributes that assist evaluators in making well-informed judgments. VaxiJen Ver2.0 for the antigenicity of a protein sequence **(Zaharieva et al., 2017)**. The ExPASy-ProtParam tool was used to examine the physicochemical properties of selected proteins. The tools described here can calculate various physical and chemical properties of user-entered protein sequences or proteins stored in Swiss-Prot or TrEMBL. The tool can compute various physical and chemical parameters for a protein sequence or a protein stored in Swiss-Prot or TrEMBL, including the molecular weight of amino acids and atomic composition, extinction coefficient, estimated half-life, instability index, aliphatic index, and grand average of hydropathicity (GRAVY). Additionally, the protein's secondary structure was analyzed by means of the PSIPRED online tool, available at http://bioinf.cs.ucl.ac.uk/psipred/.

Screening of epitopes of Clostridium botulinum protein

B-cell epitopes were predicted using the Immune Epitope Database (IEDB) bioinformatics tool, which can be found at http://tools.iedb.org/main/. IEDB uses a sophisticated algorithm based on the protein structure of antigen-antibody complexes and exclusively relies on epitope data from crystalline structures. The tool is highly accurate, efficient, and of excellent quality, and it only employs epitope data derived from crystalline structures. The NIAID sponsors IEDB, and it is freely available.

The Immune Epitope Database (IEDB) organizes experimental data into several divisions, including T-cell epitopes, animal infections, autoimmunity, allergy, non-human primates, and transplantation. In addition, the tool is used for both epitope analysis and prediction. Based on the Type I immunogenicity service offered by IEDB, HLA-1-binding peptides were predicted as per **(Chen et al. 2020)**. Furthermore, AllerTop 2.0 is a web server that provides higher accuracy in predicting protein allergenicity than other computational methods, along with additional features that aid reviewers in making well-informed decisions. The antigenicity of protein sequences was evaluated using VaxiJen Ver2.0.

MHC I HLA allele structure retrieval

Retrieve the MHC I HLA by predicting T-Cell epitopes. In addition, allele allergenicity was analyzed using the AllerTop2.0 web server and antigenicity using the VexiJen2.0 web server tool. Analyzing population coverage using IEDB, population coverage cuts more than 60% of this value for better vaccines **(Trolle et al., 2015)**.

Prediction of the structure of the epitope and validation

In order to assess the protein's key features such as digestibility, mutagenicity, toxicity, allergenicity, and hydrochemical and physicochemical properties, a number of bioinformatics tools were utilized including Vaxijen 2.0, Protein Digestion Server **(Hebditch et al., 2017)**, AllerTOP Server v2.0, ExPAsy Portparam, and ToxinPred Server **(Chen et al. 2020)**. Validate vaccines using PROCHECK from the Ramachandran chart **(Laskowski et al., 1996)** and analyze immunogenicity using the vaccine's c-ImmSim server. Screening vaccines using the GalaxyRefine web server to improve energy. The GalaxyRefine

online server (https://galaxy.seoklab.org/cgi-bin/submit.cgi?type=REFINE) was utilized to validate and refine the 3D structure of the vaccine construct. This server is recognized for its high accuracy in screening and validation. Side-chain recovery and reconstruction were performed as part of the refinement process **(Park et al., 2012)**.

Evaluation of peptide binding (Molecular Docking)

To investigate the binding affinity between selected epitopes and MHC HLA allelic determinants, we conducted a molecular docking analysis using the ClusPro 2.0 server (https://cluspro.bu.edu/) **(Kozakov D, et al., 2017)**. This computational tool is widely recognized for its high accuracy in predicting protein-protein interactions and provides insights into the structural basis of protein interactions **(Kozakov D, et al., 2020)**. After retrieving the results from the Cluspro2.0 tool, all results were analyzed by deploying the Auto dock vina offline software utility. Then, complete relaxation of the structure was performed using a molecular dynamics simulation procedure **(Floresta G, et al., 2020)**.

Molecular dynamic simulation (Optimization)

MD simulations for anchor assemblies were performed using iMods online software **(Aliaga JI, et al., 2014)**. We utilized the COPASI software package for modeling and simulation to support the entire process. This software allows for input of specifications based on model response and provides a simulation of change dynamics over time. Our strategy for identifying the model was incremental, meaning that we developed the model gradually in stages. As we progressed, we incorporated additional biological evidence and assumptions, which allowed us to construct a more comprehensive and accurate model. This approach enabled us to streamline the process of model identification and produce a more robust and reliable model. This facilitated a more nuanced and comprehensive representation of the underlying biological mechanisms, thereby enhancing the accuracy and predictive power of the final model (**Mura I et al. 2016**). To enable the efficient expression of the mosaic proteins within our chosen expression system, we utilized the EMBOSS 6.0.1 backtranseq program (https://www.ebi.ac.uk/Tools/st/emboss_backtranseq/) to reverse-translate their amino acid sequences into corresponding DNA sequences. This approach ensured that the nucleotide sequences were compatible with our expression system, allowing us to successfully express the mosaic proteins. The EMBOSS analysis package provides automated processing of data in different formats and facilitates the retrieval of sequence data from Web28. Additionally, we used the Java Codon Adaptation tool (JCat), an online web-based server (http://www.jcat.de/), to perform codon optimization **(Hiller K et al., 2022).**

RESULTS

The proteome of Clostridium botulinum was retrieved, and its structure was analyzed.

Membrane proteins of *clostridium botulinum* were selected from the NCBI database i.e., 3AH4_B and these proteins, were structure analysis performed and then tested for allergenicity and antigenicity by using several tools. The selected protein ID is 3AH4_B with 288 amino acids. The physicochemical properties of the chosen protein sequence were analyzed through ExPAsy Portparam to determine its structural characteristics. The analysis revealed that the protein has a molecular weight of 34052.04 and a theoretical pI of 8.29. The chemical formula of selected proteins is $C_{1519}H_{2322}N_{412}O_{467}S_7$ and the total number of atoms is 4727. Regarding the analysis of the protein's structure, we assessed the estimated half-life of the membrane protein in different settings. Specifically, we found that the protein had an estimated half-life of 2.8 hours in Mammalian reticulocytes (In vitro), 3 minutes in Yeast (In vivo), and 2 minutes in Escherichia coli (In vivo). Additionally, we analyzed the protein's Instability Index, which was found to be 32.23, indicating that the protein is stable. Furthermore, we also analyzed the protein's Aliphatic Index, which was found to be 86.94. The chosen membrane protein from Clostridium botulinum has a Grand Average of Hydropathicity (GRAVY) of 0.607. The selected *Clostridium botulinum* membrane protein is an antigenic (VaxiJen 2.0) score of 0.5630 (At Threshold=0.4) and Non-allergenic (AllerTOP 2.0).

Screening of epitopes of *Clostridium botulinum* protein

The selected proteins were subjected to B-cell epitope (http://tools.iedb.org/main/bcell/) choices against MHC Class I HLA allelic determinant was 17 epitopes, all epitopes pick value represented in graph 1. The total number of B-cell 4 epitopes was selected based on antigenicity scores (VaxiJen 2.0). Then one B-cell epitope (QTNANDLRW) was finalized based on allergenic (AllerTOP 2.0), see Table 1.

MHC I HLA allele structure retrieval

To assist in T-cell epitope prediction, the IEDB offers a variety of resources, one of which is MHC I HLA allele predictions that can be accessed directly through their website at http://tools.iedb.org/main/tcell/. Utilizing artificial neural network (ANN) technology, these tools are capable of predicting potential T-cell epitopes that can bind with MHC I HLA. The selection process involves choosing T-cell epitopes with an IC50 value of less than 100 and also evaluating their allergenicity and antigenicity. This evaluation is performed for all selected epitopes within the IC50 range, see Table 2. In addition to predicting T-cell epitopes, we also evaluated the population coverage of MHC I HLA alleles using the population coverage analysis tools available on the IEDB website (http://tools.iedb.org/population/). This tool analyzes a text file containing all selected epitopes to determine the coverage of different MHC I HLA alleles in the population. They give graphical and table value results, for *Clostridium botulinum* selected epitopes population coverage is 73.62%, see Table 3 and also analyses the graphical value, in Graph 2.

The epitope's vaccine structure was predicted and then validated

The prediction of B-cell and T-cell epitopes for MHC class I, start to construction of vaccine structure using selected epitopes. The construction of a vaccine with specific linkers such as EAAAK, AYY and GPGPG, respectively N to C terminal of vaccine structure. The prediction of the vaccine structure of selected epitopes analyzes the antigenic value, which is 0.9067 and allergenic expression, which is non-allergen. We also analyze the toxicity using the ToxinPred tool, which results in the vaccine being non-toxic and also analyze the solubility (SoLpro tool) of the vaccine as 0.9202, which refers to the in Escherichia coli solubility values greater than 0.5, which vaccine better for human users. The analysis of physiochemical properties of the vaccine, like half-life in mammalian 01 hours and GRAVY score 0.321. The 3D structure of the vaccine was visualized using trRosetta (https://yanglab.nankai.edu.cn/trRosetta/) and then further revised using 3D Pro, a protein editor that allows for modifications and adjustments to the protein structure, shown in figure 1. The vaccine 3D structure refinement by GalaxyRefine webserver, see Figure 2. For validation of the structure, we analyzed the Ramachandran plot (PROCHECK webserver) for selected epitopes, in Figure 3.

Evaluation of peptide binding (Molecular Docking)

We conducted a molecular docking analysis using the Cluspro2.0 tool for the selected epitopes and MHC class I HLA allelic determinants. The results indicated a significant interaction between the HLA receptor and epitope QTNANDLRN, with an Atomic Contact Energy value of -576.5., see Figure 4. In order to increase the precision and dependability of our results, we took into account multiple factors, including data obtained from small-angle X-ray scattering (SAXS), constructing homo-multimers, implementing attraction or repulsion forces, applying pairwise distance restraints, removing unstructured protein regions, and identifying heparin-binding sites. Notably, we identified the interaction site as ARG 13 (auth A), which played a crucial role in the binding process, see Figure 5. After obtaining results from the Cluspro2.0 tool, the results were further examined by deploying Auto Dock vina offline software utility. To refine the structure further, a molecular dynamics simulation procedure was employed.

Molecular dynamic simulation (Optimization)

We employed the iMods online software (https://imods.iqfr.csic.es/) to simulate the docked complexes, and ran molecular dynamics (MD) simulations. To aid in the modeling process, we also used the COPASI modeling and simulation software package. To simplify the model definition process, we adopted an incremental approach by utilizing the model's reaction-based specifications as input, which generates simulated time courses displaying variable dynamics. By gradually incorporating additional biological evidence and assumptions with each iteration, we were able to build a more comprehensive and accurate model. By adopting a systematic and gradual approach, we were able to increase the complexity of the model while preserving its accuracy and relevance. This involved constructing progressively more intricate versions of the model, with each new iteration incorporating additional biological evidence and modeling assumptions, see Graph 3.

The process of codon optimization and in silico cloning of the vaccine

In order to facilitate efficient expression of the vaccine in Homo sapiens, we employed the Java Codon Adaptation Tool, an online web server, to optimize codon usage. We then used the

EMBOSS software analysis package to process and retrieve the data in different formats. As a result, the cDNA sequence was optimized, resulting in a GC content of 57.037%. as shown in Graph 5. We used SnapGene software provided by Insightful Science, available at snapgene.com, to clone the optimized multidrug resistance vaccine DNA sequence into the pET-29a (+) vector, see Fig. 5.

Immune simulation.

To assess the immunogenicity and immunoreactivity of the genetically engineered peptide, we carried out an in-silico simulation of the immune response using the C-IMMSIM server, which can be accessed at https://kraken.iac.rm.cnr.it/C-IMMSIM/.

The simulation was carried out over a period of around 15 months, with a total of 1400 time steps completed, and each individual time step set at approximately 8 hours. The vaccination protocol was implemented following the recommended interval of 4 weeks between the first and second doses. The administration of three peptide injections was scheduled on day 1, day 30, and day 60, respectively. The efficacy of the vaccination protocol was evaluated through a bacterial challenge conducted on day 240, which corresponds to 8 months or 6 months post-vaccination. The simulation utilized an agent-based model with a site-specific scoring matrix (SSSM) derived from machine learning methods to predict immune interactions, see Figure 6.

DISCUSSION

Our research was centered on the identification of immunogenic epitopes from bacterial membrane proteins of *Clostridium botulinum*, which is a pathogenic bacterium responsible for causing food poisoning in humans. Our findings revealed that these epitopes are associated with MHC class I HLA allelic determinants and demonstrated high levels of antigenicity. Our study focused on identifying immunogenic epitopes from bacterial membrane proteins of Clostridium botulinum, a pathogenic bacterium that causes food poisoning in humans. We found that these epitopes were associated with MHC class I HLA allelic determinants and exhibited high levels of antigenicity. The high variability of HLA allelic determinants suggests that they could potentially associate with a range of bacterial, fungal, and viral pathogens, thus facilitating the development of vaccines for various botulinum-related pathogens in the future. Furthermore, we observed that the identified epitope was structurally stable and capable of internalizing in subcellular organelles. In a similar study, choline-binding protein epitopes from Clostridium botulinum were also found to exhibit antigenic properties **(Munia et al. 2021)**,

Therefore, we sought to expand this in silico investigation to include bacterial membrane proteins. Recent studies have shown that it activates thromboxane receptors, leading to cardiac and vascular dysfunction in rats. As mice age, those with weak interleukin (IL)-17B knockouts exhibit cardiac and vascular dysfunction. In patients with Crohn's disease, treatment with recombinant interleukin-17B-producing bacteria has been found to be effective, highlighting the importance of IL-17B in regulating an overactive immune response in the human body. Direct investigations in rats have indicated that this cytokine functions as a crucial immunomodulator in the gastrointestinal or intestinal tract. Thus, this approach aided in determining that the selected epitope did not elicit an excessive immune response in this study **(Sikka et al. 2013)**. Interleukin 4 has been shown to mediate several pro-inflammatory functions in food poisoning, including the rearrangement of IgG and IgM phenotypes, expression of VCAM-1, promotion of eosinophil migration across the endothelial layer, secretion of mucus and Tc2 cytotoxic agents leading to the release of cytokines. IL-4 also plays a crucial role in clearing bacteria from the lungs and reducing the risk of death from food born disease. In this study, we focused on perfecting the QTNANDLRN epitope from the bacterial membrane domain of *Clostridium botulinum*, which can interact with the MHC class I HLA allele determinant. This could have implications for the development of vaccines against botulinum-related pathogens in the future.

After conducting MD simulation analysis, we observed the anchor complexes to be stable with RMSD values of approximately 1 angstrom. Furthermore, we successfully achieved in silico cloning of the codon-optimized epitope vaccine into the pET29a (+) expression vector, yielding significant outcomes.

CONCLUSION

This study demonstrates new methods for predicting immunogenic epitopes against Clostridium botulinum for the treatment of food poisoning. The sequence of the peptide vaccine

can be designed using a variety of techniques, including bioinformatics analysis. The peptide should include amino acid sequences from the toxin produced by *Clostridium botulinum*, such as the Hc and LC regions of the *botulinum* toxin. Additionally, the vaccine should include sequences from the surface proteins of *Clostridium botulinum*, such as the capsular polysaccharide and flagellin. The peptide should also include sequences from other foodborne pathogens, such as Escherichia coli to protect against multiple foodborne illnesses. Finally, peptide vaccines must be designed to be potent and stable enough to induce an effective immune response. Here we have identified the epitaph QTNANDLRN. This epithelium is stable and can readily penetrate the subcellular compartments to obtain an engineered vaccine that exhibits both antigenic and immunogenic properties without causing allergic reactions. The robust immune response was confirmed using immunosimulation studies. In addition, detailed molecular dynamics and assembly simulation studies have shown that the vaccine is stable. In future studies, the identified epitope can be further tested in wet lab experiments by incorporating it into post-synthetic adjuvants and linkers. Utilizing the identified immunogenic epitopes from bacterial membrane proteins associated with MHC class I HLA allelic determinants that showed antigenicity, we conducted molecular dynamics simulations, confirming the stability of the anchor complexes with RMSD values of about 1 angstrom. Additionally, the in silico cloning of the codon-optimized epitope vaccine into the pET29a (+) expression vector was successful, indicating its potential effectiveness in developing a vaccine against *Clostridium botulinum* and related pathogens.

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Table's

B cell epitope's

No.	Start	End	Peptide	Length	Antigenic	Allergic
1	5	13	QTNANDLRN	9	1.2424	NON-ALLERGEN
2	73	79	LTWNAPL	7	1.6285	ALLERGEN
3	125	136	TLMVSTQTSSSN	12	0.7921	ALLERGEN
4	261	267	NSQIANG	7	1.3148	ALLERGEN

Table 1: *Epitope prediction from clostridium botulinum interacting MHC Class I HLA, on the bases of antigenicity (Threshold VaxiJen score > 0.5) and allergenicity (AllerTOP), the selected B-cell epitope in green (QTNANDLRN).*

MHC I HLA epitopes

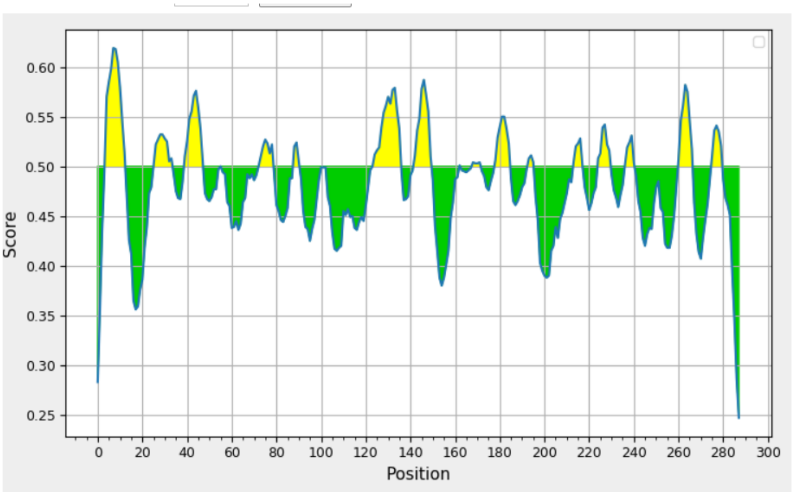
Allele	Seq. num.	start	end	length	Allergic	Antigenic	peptide	ic50	rank
HLA-A*02:06	4	20	28	9	NON-ALLERGEN	1.1594	IQYWNINYL	12.65	0.13
HLA-A*02:03							IQYWNINYL	13.78	0.23
HLA-A*02:01							IQYWNINYL	66.52	0.6
HLA-B*15:01	1	58	66			1.1342	KQAYKIKVM	70.34	0.34
HLA-B*58:01	3	30	38			0.8669	LNSQIIVLW	70.27	0.33
HLA-A*02:06	2	48	56			0.6514	LQYNIDDTL	48.41	0.49
HLA-B*40:01	3	53	61			1.0913	NETKSAYTL	16.06	0.04
HLA-B*58:01	3	32	40			1.4191	SQIIVLWQW	50.19	0.27
HLA-B*44:03							SQIIVLWQW	63.67	0.08
HLA-A*02:03	4	19	28	10		1.0341	LIQYWNINYL	57.35	0.83
HLA-A*02:06							LIQYWNINYL	95.68	0.83
HLA-B*15:01	2	48	57			0.6448	LQYNIDDTLM	47.06	0.26
HLA-A*02:06	2	3	12			0.9285	LTWNAPLSSV	47.14	0.48
HLA-A*68:01	1	3	12			1.3969	MSQTNANDLR	25.83	0.23
HLA-B*58:01	3	31	40			1.3266	NSQIIVLWQW	12.93	0.06
HLA-B*57:01							NSQIIVLWQW	33.36	0.12
HLA-B*58:01	4	14	23			0.584	QSTDSLIQYW	17.47	0.1
HLA-A*23:01	2	23	32			0.9296	QYWYLLQNYI	88.3	0.26
HLA-B*15:01	4	18	27			0.9663	SLIQYWNINY	84.56	0.39
HLA-B*15:01	3	32	41			1.7088	SQIIVLWQWF	97.59	0.44
HLA-A*33:01	2	25	34			0.7977	WYLLQNYISR	49.68	0.2
HLA-B*15:01	2	41	50			1.1488	YMNPNLVLQY	13.3	0.07
HLA-A*01:01							YMNPNLVLQY	68.48	0.13

Table 2*: Shows the T-cell binding MHC I HLA epitopes prediction on the bases of allergen and antigenic value.*

population/area	Class I		
	coverage[a]	average_hit[b]	pc90[c]
World	73.32%	1.53	0.37
Average	**73.32**	**1.53**	**0.37**
Standard deviation	**0.0**	**0.0**	**0.0**

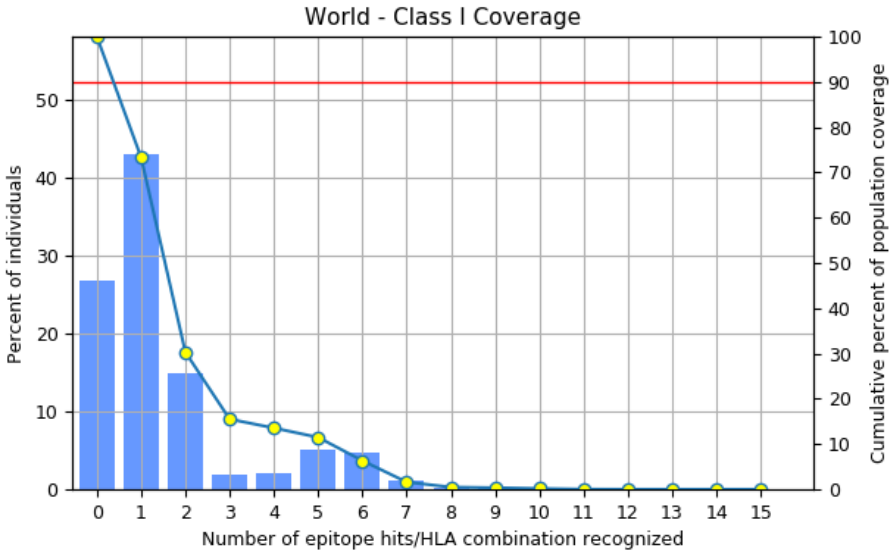
Table 3: *MHC I alleles cover worldwide effective value in table.*

Graph & Figure



Average: 0.481 **Minimum:** 0.247 **Maximum:** 0.619

Graph 1: *shows the pick of B-cells epitopes at a threshold value of 0.5.*



Graph 2: *shows the population coverage of MHC I alleles.*

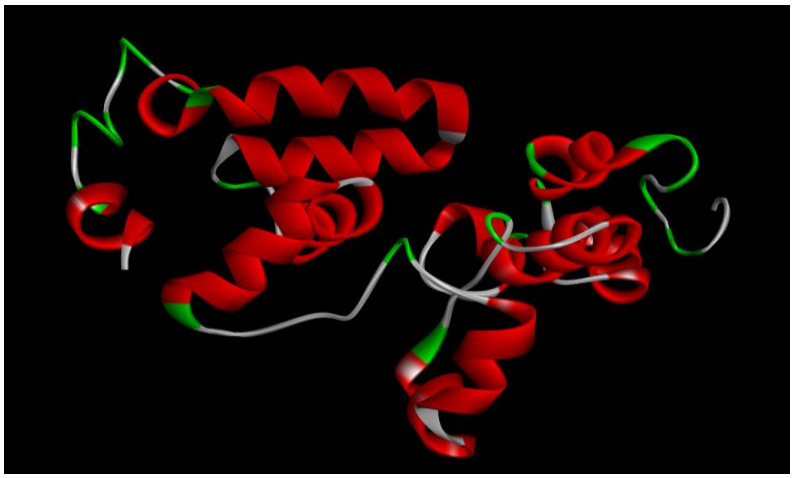
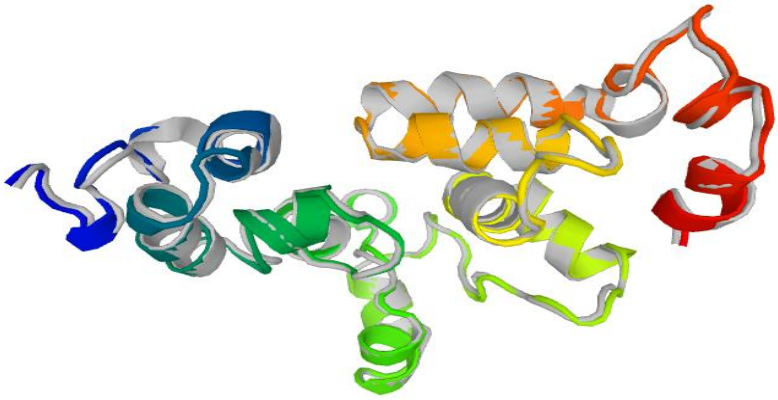


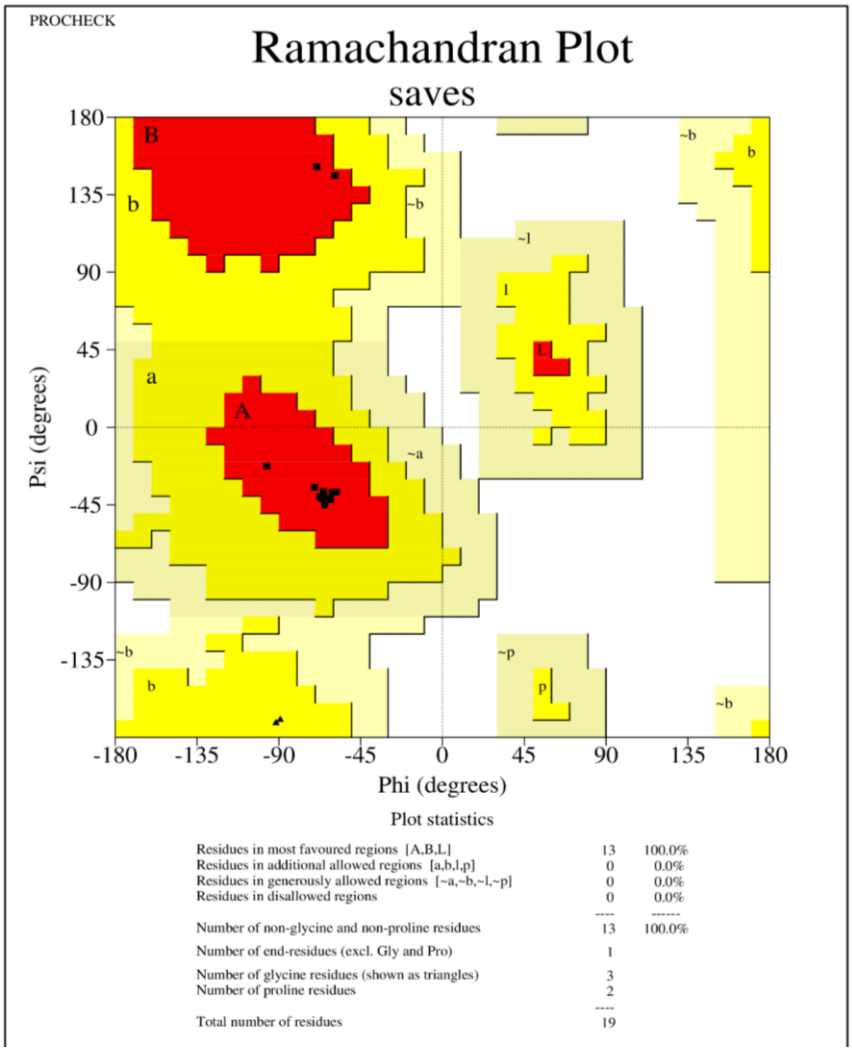
Figure 1*: 3D structure of vaccine by trRosetta webserver.*



Model	GDT-HA	RMSD	MolProbity	Clash score	Poor rotamers	Rama favored
Initial	1.0000	0.000	4.200	309.7	12.0	93.3
MODEL 1	0.8583	0.609	2.753	60.1	1.8	95.5
MODEL 2	0.8722	0.601	2.946	65.2	4.7	97.2
MODEL 3	0.8694	0.613	2.815	61.7	2.4	96.1
MODEL 4	0.8722	0.595	2.814	58.9	1.8	94.4
MODEL 5	0.8625	0.618	2.840	62.4	3.0	96.6

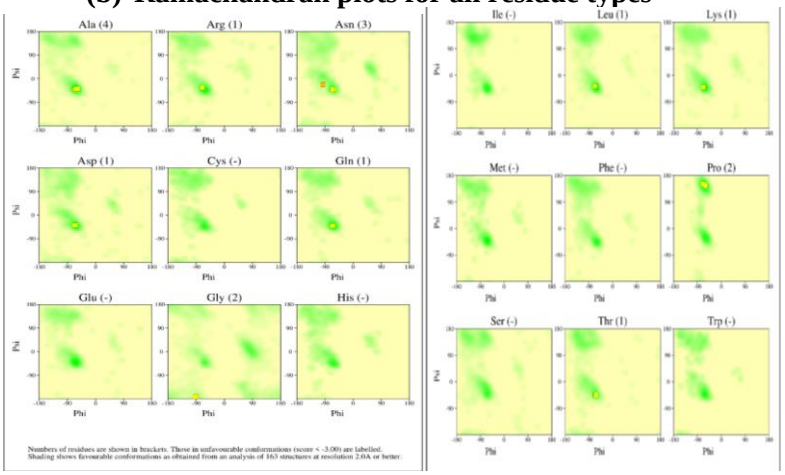
Figure 2: *show the vaccine 3D refined structure by GalaxyRefine webserver.*

(a) Ramachandran plot for epitope: QTNANDLRN

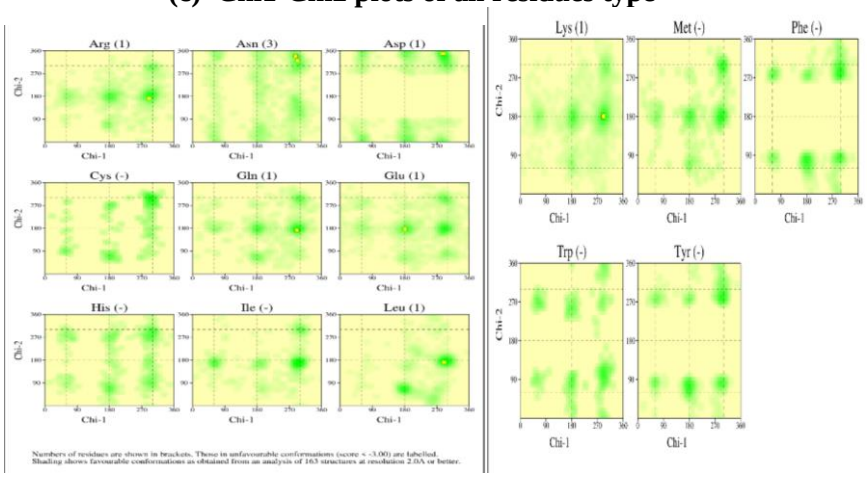


Here, 100% of residues are in the most favourable region.

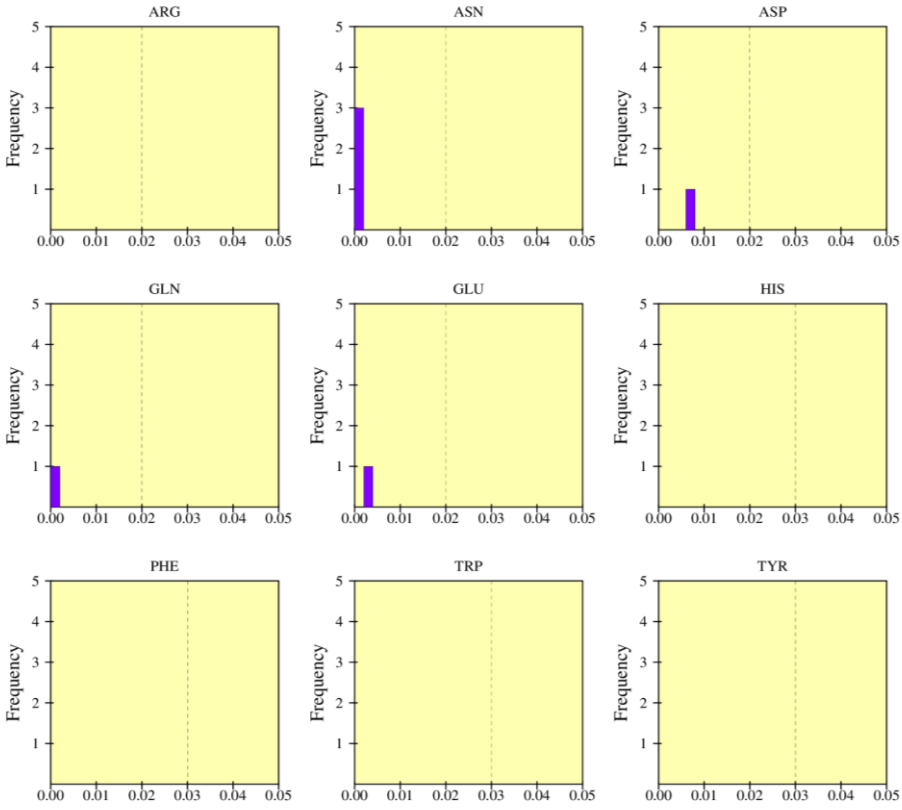
(b) Ramachandran plots for all residue types



(c) Chi1-Chi2 plots of all residues type



(d) RMS distances from planarity



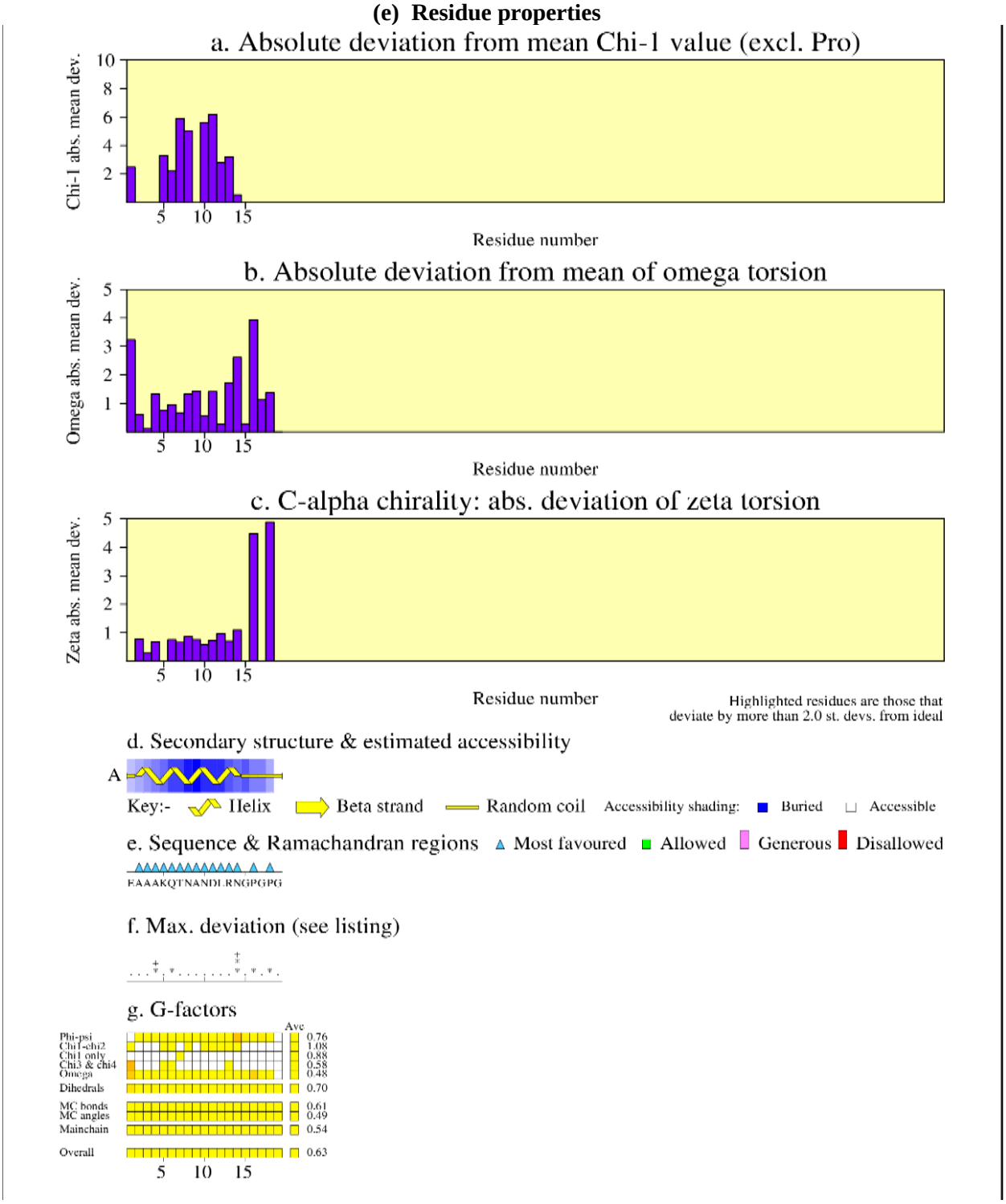
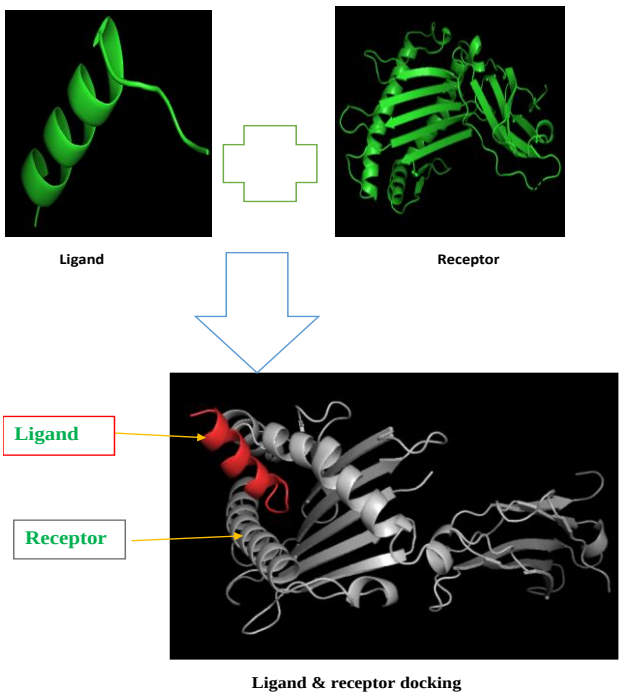
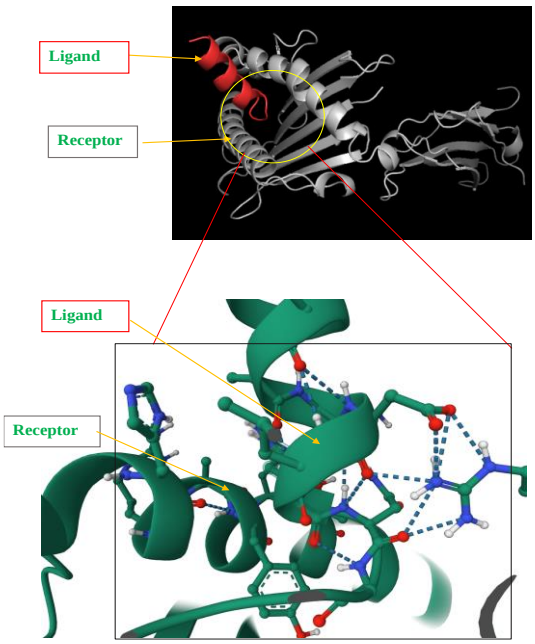


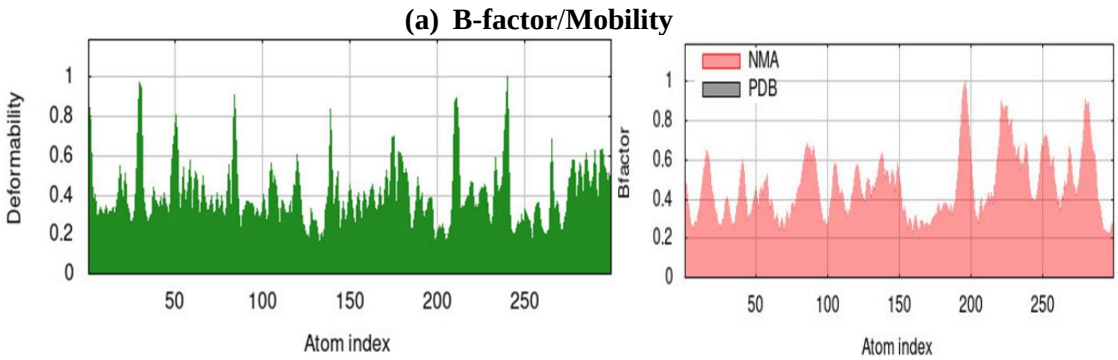
Figure 3: *show all validation plots value along with Ramachandran plots (a-e) for epitope: QTNANDLRN.*



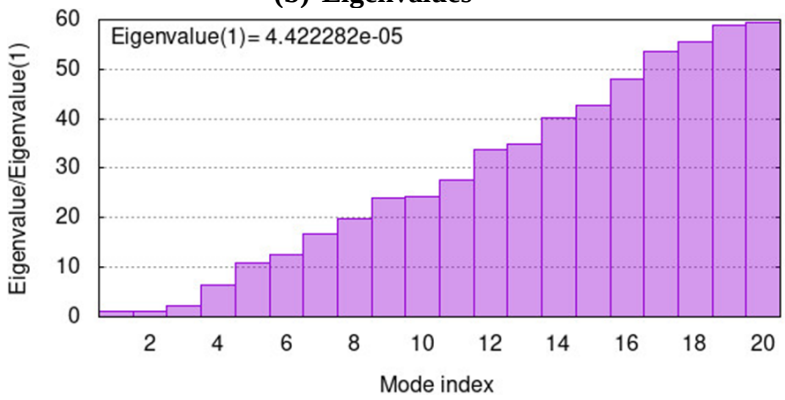
Fig; 4 *docked complexes'; epitope: QTNANDLRN and receptor HLA.*



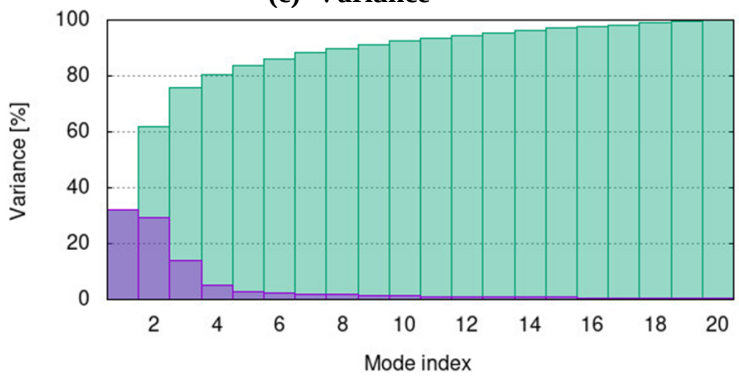
Fig; 5 *docked complexes show bond interaction between epitope: QTNANDLRN at ARG13(auth A) and receptor HLA.*



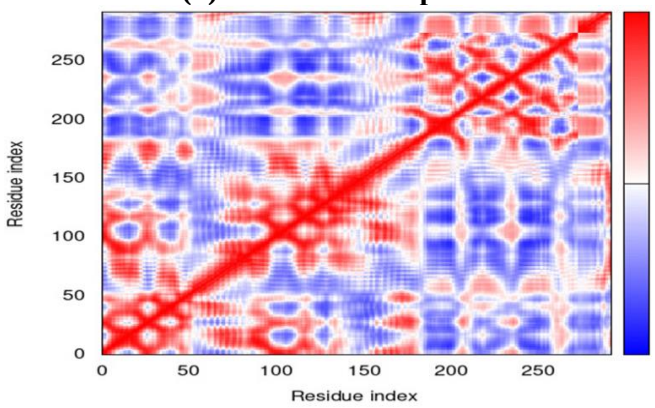
(b) Eigenvalues



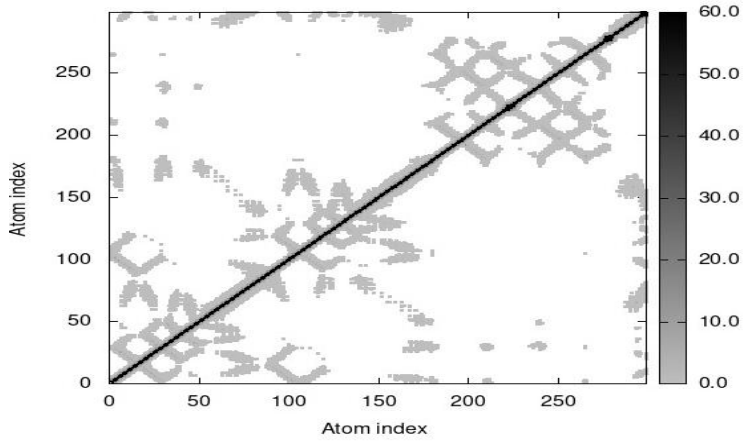
(c) Variance



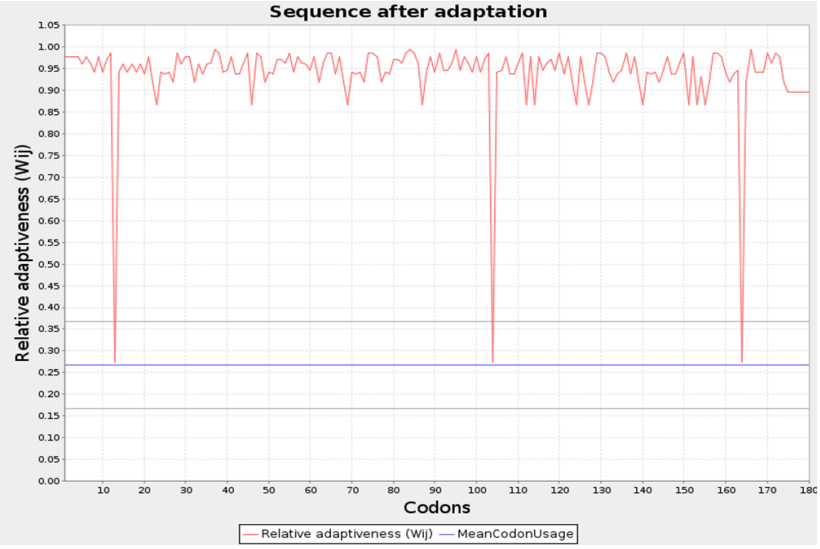
(d) Covariance map



(e) Elastic network



Graph 4: *In above graphs (a-e) show the Molecular simulation curve value of designed vaccine.*



***Graph 5**: Show the Codon Adaptation value for the designed vaccine sequence.*

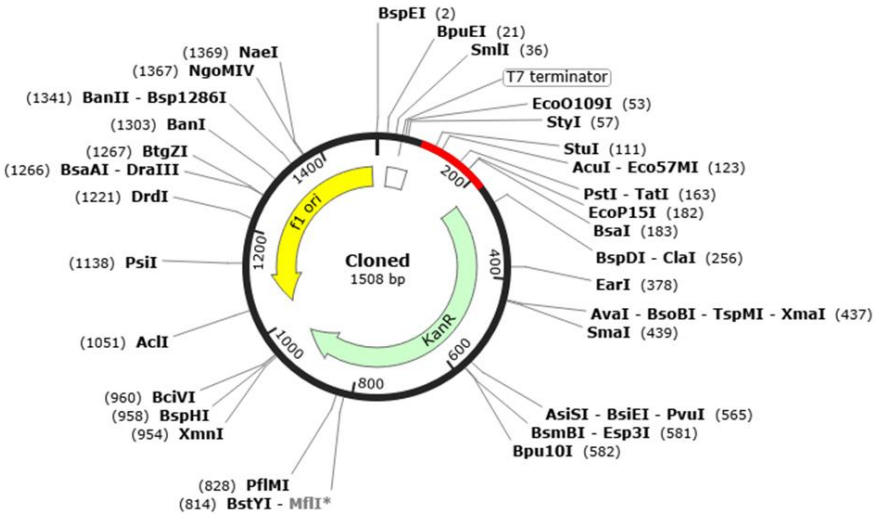
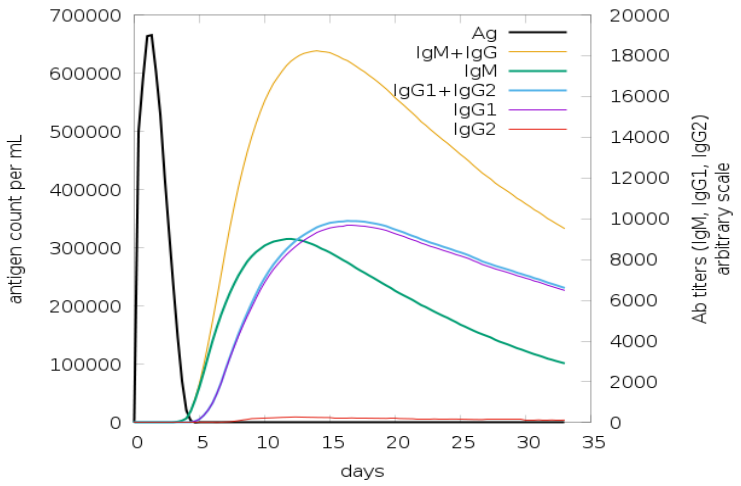
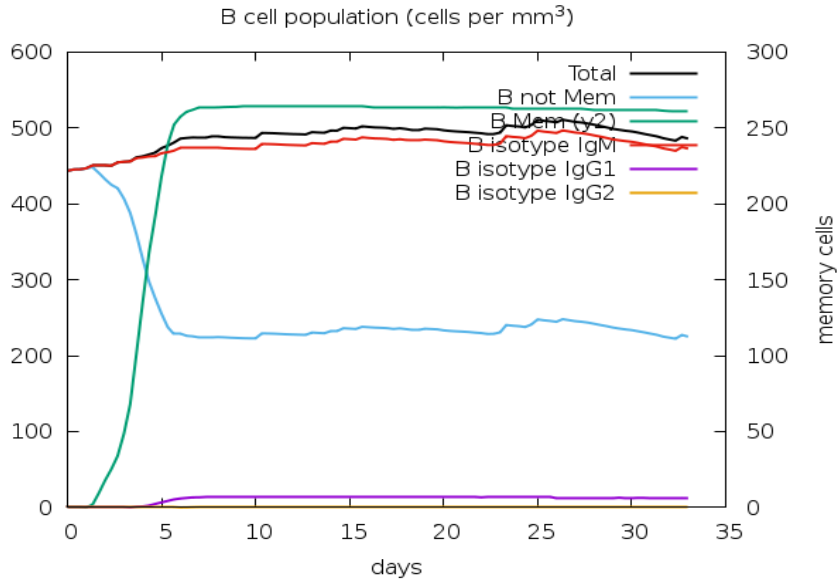


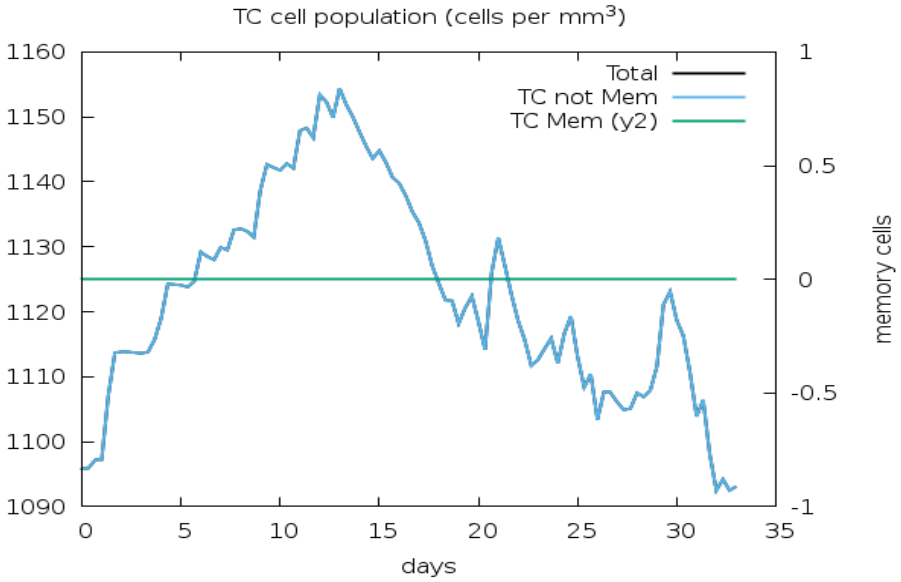
***Figure 6:** In silico restriction cloning. The black colour part represents the pET29a (+) expression vector in which the codon-optimized multi-epitope vaccine is inserted (red colour part).*



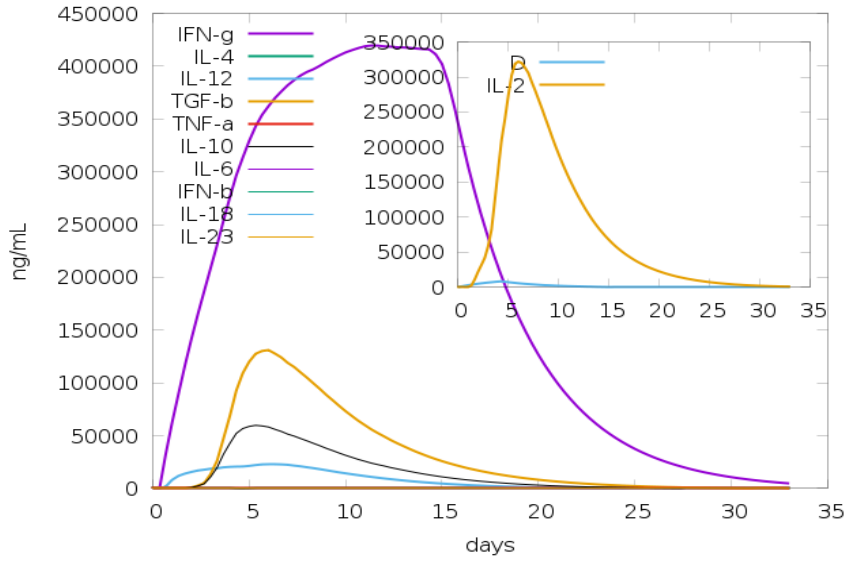
***(a)-Antigen and immunoglobulins**. Antibodies are sub-divided per isotype.*



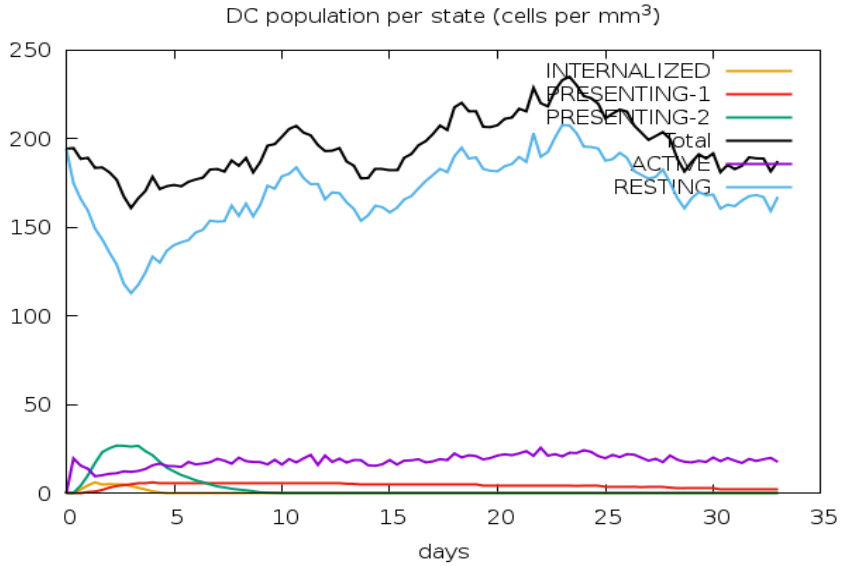
***(b)-B-lymphocytes**: total count, memory cells, and sub-divided in isotypes IgM, IgG1 and IgG2.*



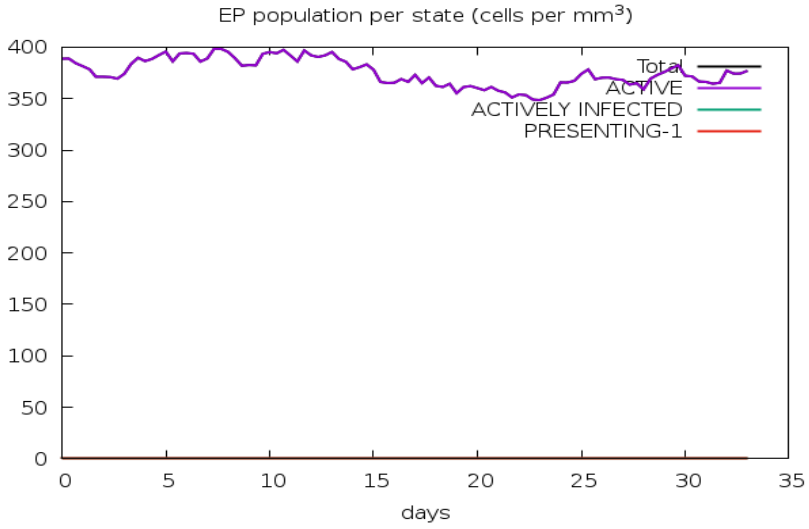
***(c)- CD8 T-cytotoxic lymphocytes** count. Total and memory shown.*



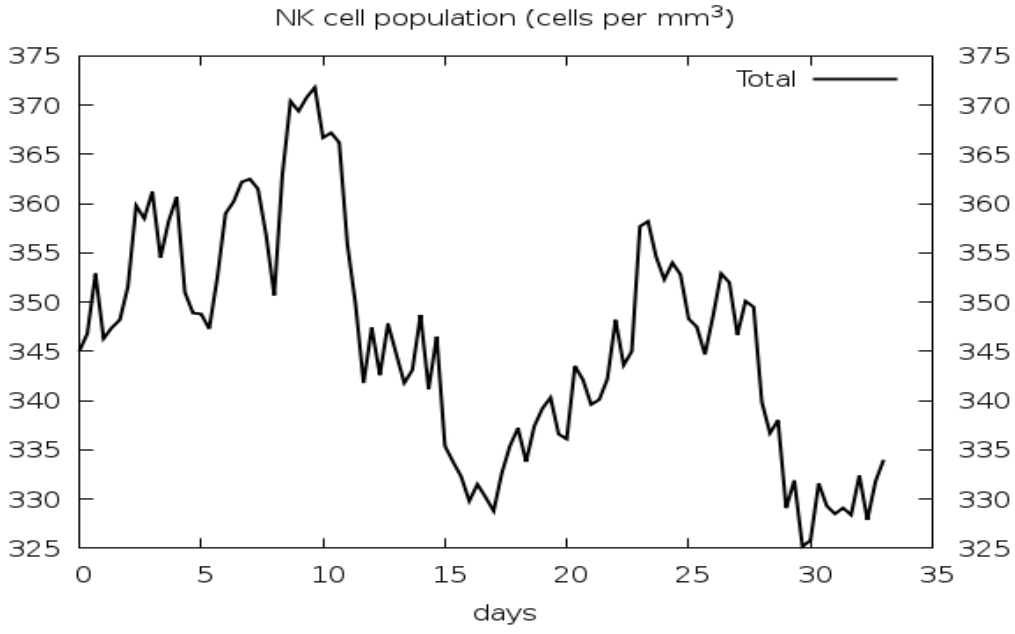
***(d)-Cytokines**. Concentration of cytokines and interleukins. D in the inset plot is danger signal.*



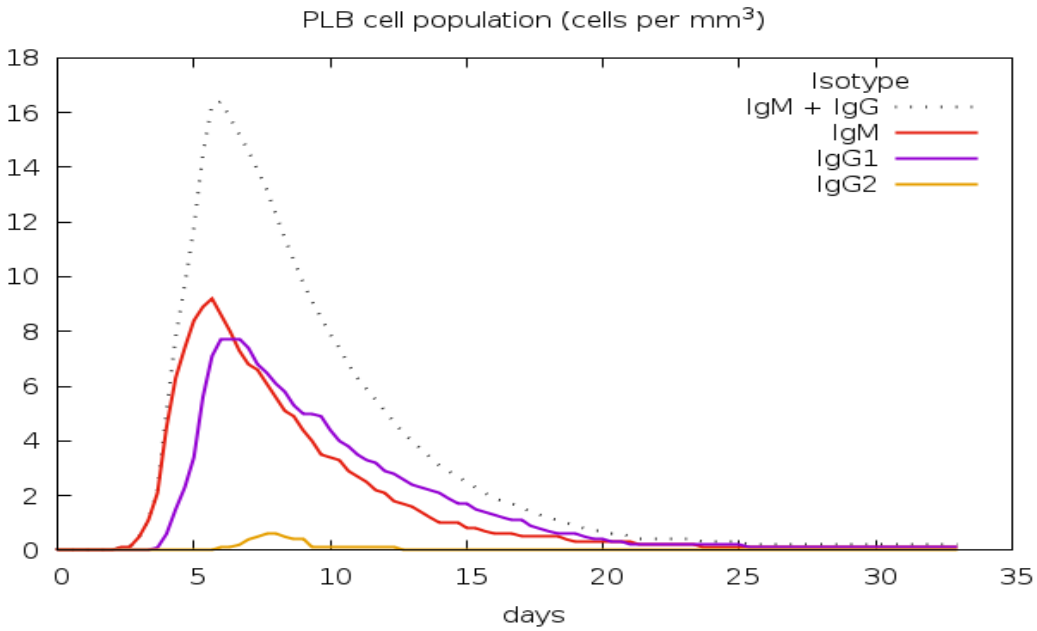
***(e)-Dendritic cells**. DC can present antigenic peptides on MHC class-I molecules. The curves show the total number broken down to active, resting, internalized and presenting the Ag.*



***(f)-Epithelial cells**. Total count broken down to active, bacterial or virus infected and presenting on class-I MHC molecule*



***(g)-Natural Killer cells**. Show the positive impact on Ag.*



***(h)**-Plasma B lymphocytes count sub-divided per isotype (IgM, IgG1 and IgG2).*

***Graph 6**: **Immune simulation.** Above all graphs (a-h) show the immunity development within the body in less than 5 days and neutralize the Ag by generating large amounts of Antibody.*