In Silico Design of a Multi-Epitope Vaccine Targeting 60 kDa Heat Shock Protein of Hepatitis C Virus

By

Md. Rezoanul Hasan
Student ID: 19346015

A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy.

School of Pharmacy
BRAC University
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Declaration

It is hereby declared that

1. The thesis submitted is my original work while completing degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

Student's Full Name & Signature:

Md. Rezoanul Hasan
Student ID: 19346015

Approval

The thesis titled 'In Silico Design of a Multi-Epitope Vaccine Targeting 60 kDa Heat Shock Protein of Hepatitis C Virus submitted by Md. Rezoanul Hasan (ID: 19346015), Summer 2019, has been accepted as satisfactory in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy.

Supervised

Supervisor:

_____

Mohammad Kawsar Sharif Siam

Senior Lecturer

School of Pharmacy, BRAC University

Approved By

Dean:

_____

A F M Yusuf Haidar, PhD

Acting Dean, School of Pharmacy

Professor, Department of Mathematics and Natural Science

BRAC University

Ethics Statement

All ethical standards were maintained in the completion of this thesis. Conducting the research with integrity and following the ethical principles was given utmost importance.

Abstract

Hepatitis C virus is a type of viral hepatitis which primarily affects the liver and it causes liver swelling, failure and liver cancer. This research paper details the in-silico- design of a multi-epitope vaccine aimed at the 60 kDa heat shock protein, shows suitable antigenic characteristics against Hepatitis C virus. The procedure of in silico design of vaccine used computational technique to search Cytotoxic T Lymphocytes (CTL), Helper T Lymphocytes (HTL), B cell epitope. Each of the epitope from CTL, HTL and B-cell underwent evaluation for their antigenicity, allergenicity and toxicity and find out the suitable candidates. Linkers were used to connect the suitable epitope with the amino acid sequence. By using homology modeling the structural and biochemical stability were confirmed. Molecular docking shows strong binding interactions between the vaccine and Toll-like receptor 4 (TLR4). Immune simulation predict the development of antibodies and the memory cells. All the results suggest that the proposed vaccination has potential for addressing Hepatitis C, pending additional validation for both in vivo and in vitro.

Keywords: Hepatitis C, 60 kDa protein, Multi-epitope vaccine, In silico design, Molecular docking, Immune simulation.

Dedication

I would like to dedicate this work to my beloved parents who always support me in every step of good things in my life. Very much grateful to you for your unwavering support and guidance.

Acknowledgement

I want to express my cordial gratitude to my supervisor Mohammad Kawsar Sharif Siam, Senior Lecturer, School of Pharmacy BRAC University for his tremendous support, motivation and important feedback. I could not pass this phase without his guidance and motivation.

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List of Acronyms

HCV	Hepatitis C Virus
CTL	Cytotoxic T-Lymphocyte
HTL	Helper T-Lymphocyte
GRAVY	Grand Average of Hydropathy
T3DB	The Toxin and Toxin-Target Database
BA	Binding Affinity

Chapter 1

Introduction

Hepatitis C is a liver inflammation caused by the hepatitis C virus. The virus can cause acute and chronic hepatitis, with severity ranging from moderate to serious, lifelong sickness, including liver cirrhosis and cancer. The hepatitis C virus is a bloodborne virus, and the majority of infections develop as a result of blood exposure via hazardous injection practices, unsafe health care, unscreened blood transfusions, injectable drug usage, and sexual activities that include blood contact. An estimated 50 million people worldwide have chronic hepatitis C virus infection, with around 1.0 million new cases emerging each year. Hepatotropic RNA viruses, such as the hepatitis C virus (HCV), generate progressive liver damage that may result in cirrhosis and hepatocellular cancer. Between 64 and 103 million people globally suffer from a chronic infection (Michael P. Manns, 2017).

In nations where HCV is very common, improper injectable drug use and iatrogenic infections and unsterile medical procedures are major risk factors for this blood-borne virus infection. Serum HCV antibody testing, HCV RNA measurement, viral genotype and subtype recognition, and, more recently, assessment of resistance-associated mutations are examples of diagnostic techniques (Michael P Manns, 2017).

According to the World Health Organization, about 242 000 individuals died from hepatitis C in 2022, the majority of which were due to cirrhosis and hepatocellular carcinoma. Direct acting antiviral medications (DAAs) can cure more than 95% of hepatitis C infections, but access to testing and treatment is limited. The monitoring of hepatitis C virus (HCV) genotypes gives real-time insight into the dynamic changes in the worldwide epidemiological picture of HCV infection. Currently, the main cause of HCV transmission in developed as well as developing countries is intravenous drug use. People who inject drugs have a substantially different distribution of HCV genotypes and subtypes than the entire population. In this high-risk population, HCV genotypes that previously showed a limited regional distribution (3a,4) are becoming widespread (Simona Ruta,2015).

Chapter 2

Methodology

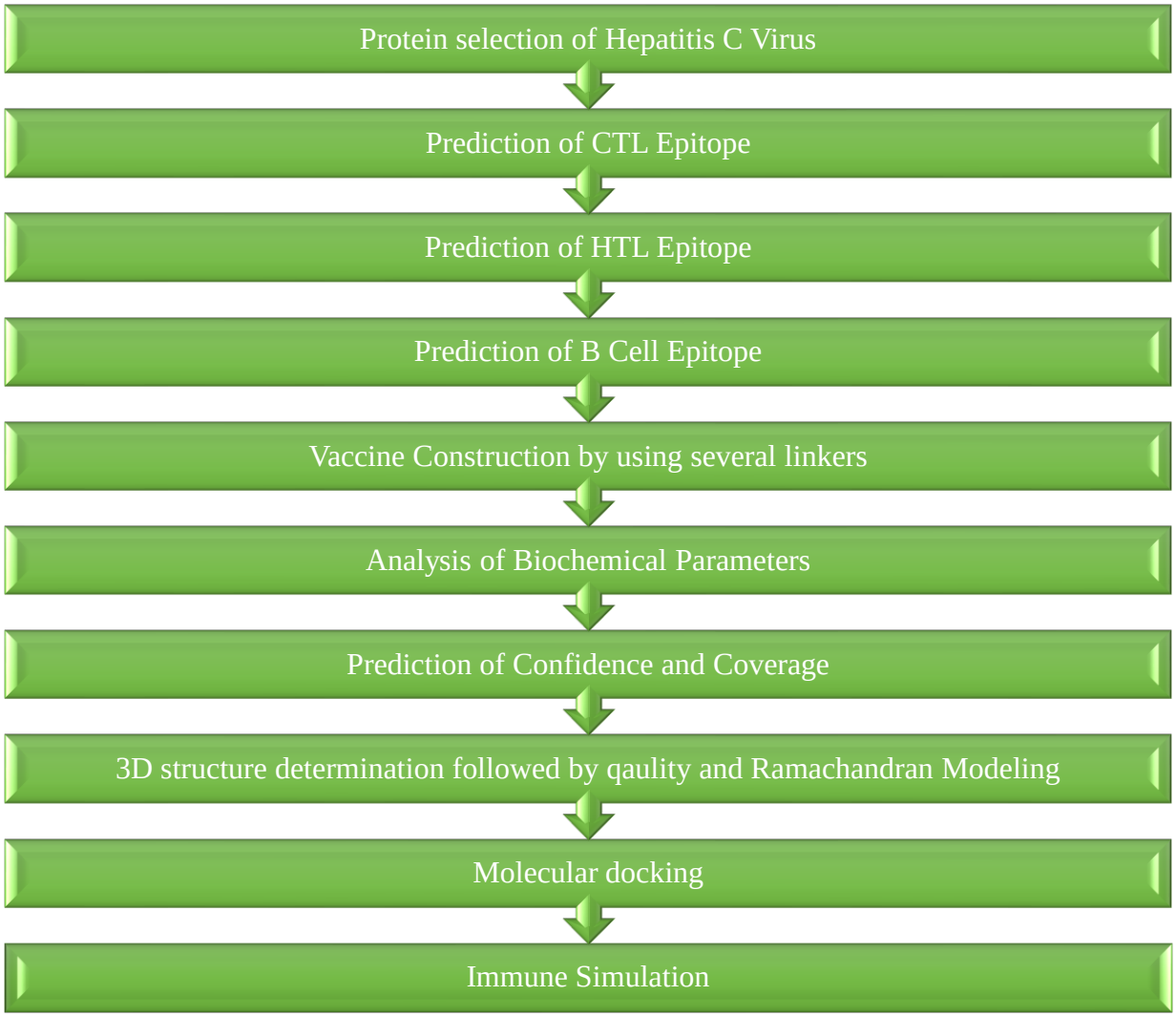


Figure 1 : Schematic overview of In Silico Vaccine Design

2.1 Choosing a suitable Protein sequence

The Uniprot protein database was used to choose a suitable protein sequence. There were 330 proteins in the Uniprot database and this protein was selected according to the antigenicity analysis. The amino acid sequences of the protein were downloaded in FASTA format. The antigenicity was checked by the Vaxigen v.2.0 server. Virus was set in this server as the target organism. To minimize the risk of false positive result 0.5 threshold value was set.

2.2 Selecting CTL Epitopes

Potential subunit vaccination candidates for a variety of diseases are cytotoxic T lymphocyte (CTL) epitopes. A large number of T cell epitope prediction methods now in use are indirect methods which predict MHC class I binders rather than CTL epitopes (Manoj Bhasin,2004). Considering their ability to recognize virus-infected cells and respond either directly—by lysing the infected cell—or indirectly—by secreting cytokines that prevent viral replication and/or create extra nonspecific inflammatory cells to the liver—CTL may be a key player in the pathophysiology of chronic HCV infection (J Immunol,1998).

2.3 Selecting HTL Epitopes

The induction of humoral and cellular immune responses is greatly influenced by helper T lymphocyte (HTL) responses. HTL epitopes will likely become an essential part of immunotherapeutic and preventive vaccinations. This resulted in the development of Pan DR helper T cell epitopes (PADRE), which are designed to bind a large number of common HLA-DR molecules with high affinity and function as potent immunogens (J Alexander, 1998).

A variety of preventative vaccinations are inefficient, need multiple booster shots, and/or only protect a small percentage of individuals. In addition, novel immunotherapeutic vaccines that trigger potent cellular immune responses are important in the case of malignant or virally infected cells. Consequently, a new generation of vaccines that are highly successful may be possible if HTL activity is optimized by the use of synthetic epitopes like PADRE or pathogen-derived, deeply cross-reactive epitopes (J Fikes, 1998).

2.4 Selecting B Cell Epitope

The humoral and cell-mediated immune responses rely mainly on B-cell and T-cell recognition of epitopes (Reche P.A., 2017). A wide range of epitopes, also known as B-cell epitopes, can be detected by the humoral branch (B cells): those found on the exposed parts of bacteria or viral particles, and also those found on soluble proteins, glycoproteins, polysaccharides, or lipopolysaccharides provides by pathogenic organisms that invade. Activated B cells underwent class shifting, affinity maturation, and plasma cell differentiation upon exposure to T-dependent antigens. A significant amount of antibodies relevant to the epitope that their immunoglobulin receptor detects are generated by plasma cells (Powell, 2001).

2.5 Constructed Vaccine by using linkers

By using particular linkers to link multiple epitopes, a vaccine has been developed in silico. This study utilized three linkers regardless of the four considered in traditional vaccination designs. The EAAAK linker was used to bind the main protein sequence to CTL epitopes as an adjuvant. HTL epitopes were linked together by the GPGPG linker. The KK linker was used to connect B Cell epitopes (Garg, 2016).

2.6 Antigenicity assessment of the constructed vaccine

The bioinformatic tool for the In silico identification and prediction of antigens that are protective from different sources is the VaxiJen 2.0 website. As far as we know, it is the sole technique for predicting and identifying protective antigens of bacterial, fungal, parasitic, viral, and cancerous origin In silico (Nikolet Doneva, 2024). VaxiJen 2.0 employs a partial minimum squares algorithm (PLS) for antigen prediction and an alignment-independent method based on auto-and cross-covariance (ACC) translation of protein sequences into homogeneous equal-length vectors.

2.7 Biochemical Analysis of the Constructed Vaccine using ProtParam

The ProtParam server was used to evaluate the vaccine's physicochemical characteristics (Garg, 2016). Numerous Physico-chemical characteristics that can be derived from a protein sequence are calculated by ProtParam. Important information was provided by this tool, such as the grand average of hydropathicity (GRAVY), index of instability, molecular weight, total amount of amino acids, and additional physicochemical characteristics. When evaluating the vaccine's stability and possible effectiveness, these factors are crucial (Garg, 2016).

2.8 Allergenicity Prediction of the Constructed Vaccine

The sequence was uploaded in plain text format to an Allergen Online server, which analyzed the vaccine's allergenicity in order to ensure its safety. Following criteria outlined by the Codex Alimentarius Commission (2003), Allergen Online offers an informative peer-reviewed tool for determining the main possible risks of allergy for GMOs and novel foods (Rechard E Goodman, 2016).

2.9 Toxicity Prediction of the Constructed Vaccine

The T3DB database was used for the toxicity testing, which helped identify whether the vaccine design had any harmful qualities or not. Significant bioinformatics resource, the T3DB gathers detailed data regarding common or ubiquitous toxins and their toxin-targets (Emilia Lim, 2010).

2.10 3D Structural modeling of the constructed Vaccine

The phyre2 server was applied to estimate the vaccine's three-dimensional structure. In simple format, the vaccination sequence was supplied, and Phyre2 produced primary and tertiary structures (Mark N Wass, 2015). While assessing the model's structural reliability, coverage and

confidence were crucial considerations. After being emailed the resultant PDB file, PyMOL software was used to display it for structural analysis (Lawrence A Kelley, 2015).

2.11 Evaluation and assessment of Ramachandran Plots

The Swiss Model Expasy structure assessment tool was used to generate a Ramachandran plot in order to confirm the modeled vaccine's structural integrity (Kiefer, 2009). As an increasing number of experimentally determined models of protein 3D structures have become readily accessible, especially at high and ultra-high resolution, their exact structure has been continuously analyzed and improved (RA LASKOWSKI, 2013). The graphic showed the polypeptide chain's phi and psi angles in the PDB file that was obtained using Phyre2. This analysis ensured that the protein structure was stable and folded correctly.

2.12 Assessment of Z score for 3D-Model

Z-scores are employed to represent how a particular physical or anatomical measurement differs from a population mean that is related to its size or age. Z-scores may help with clinical evaluation and decision-making since they may be applied to blood pressure, height, weight, and echocardiogram readings (Alexander E Curtis, 2016). The Z-score of the constructed vaccination model was ascertained using the ProSA-web server. This method examined the energy distribution across the model and assessed the overall quality of the 3D structure (Wiederstein & Sippl, 2007). When the PDB file is uploaded, the output comprises a knowledge-based energy against sequence position graph, a Z-score, and a Z-score versus residue count graph. These assessments were essential in establishing the vaccine's structural trustworthiness.

2.13 Assessment of Molecular Docking

In computerized assistance drug design and structural molecular biology, molecular docking is an essential technique. Identifying the main binding mode or modes of a ligand with a protein of known three-dimensional structure is the aim of ligand—protein docking (Garrett M. Morris, 2008). The binding interactions between the desired vaccination and an appropriate human toll-like receptor (TLR) were assessed using molecular docking. The Cluspro server was used for molecular docking. The source of the TLRs PDB file was as the ligand molecule, the vaccine's PDB file was published to the RCSB protein Data Bank. The complex with the highest binding energy score was chosen for additional examination out of the 10 docked complexes produced by ClusPro (Kozakov, 2017).

2.14 Simulation and immune Response of the constructed vaccine

The C-ImmSim 10.1 server, which mimics immunological reactions, was used to assess the vaccine's immunogenic potential. Three injections were simulated at time increments of 1, 84, and 168 using the vaccine sequence that was submitted in FASTA format with eight hours being represented by each time step (Catiglione, 2011). This simulation produced graphical depictions of immunoglobulin synthesis, lymphocyte responses, and antigen levels, offering information on humoral and cellular immune responses after vaccination.

Chapter 3

Result

3.1 Protein selection based on amino acid sequence and antigenicity

Protein Name	60 kDa heat shock protein
Gene name	HSPD1
Amino Acid	573
Organism Name	Homo Sapiens (Human)
Status	UniprotKB reviewed (Swiss-prot)

Amino Acid Sequence

MLRLPTVFRQMRPVSRVLAPHLTRAYAKDVKFGADARALMLQGVDLLADAVAVTMG
PKGRTVIIEQSWGSPKVTKDGVTVAKSIDLKDKYKNIGAKLVQDVANNTNEEAGDGTT
TATVLARSIAKEGFEKISKGANPVEIRRGVMLAVDAVIAELKKQSKPVTTPEEIAQVATIS
ANGDKEIGNIISDAMKKVGRKGVITVKDGKTLNDELEIIEGMKFDRGYISPYFINTSKGQ
KCEFQDAYVLLSEKKISSIQSIVPALEIANAHRKPLVIIAEDVDGEALSTLVLNRLKVGLQ
VVAVKAPGFGDNRKNQLKDMAIATGGAVFGEEGLTLNLEDVQPHDLGKVGEVIVTKD
DAMLLKGKGDKAQIEKRIQEIIEQLDVTTSEYEKEKLNERLAKLSDGVAVLKVGGTSDV
EVNEKKDRVTDALNATRAAVEEGIVLGGGCALLRCIPALDSLTPANEDQKIGIEIIKRTLK
IPAMTIAKNAGVEGSLIVEKIMQSSSEVGYDAMAGDFVNMVEKGIIDPTKVVRTALLDA
AGVASLLTTAEVVVTEIPKEEKDPGMGAMGGMGGGMGGGMF

Model selected: virus

Threshold for this model: 0.5

Your Sequence:

MLRLPTVFRQMRPVSRVLAPHLTRAYAKDVK
FGADARALMLQGVDLLADAVAVTMGPKGRTV
IIEQSWGSPKVTKDGVTVAKSIDLKDKYKNI
GAKLVQDVANNTNEEAGDGTTTATVLARSIA
KEGFEKISKGANPVEIRRGVMLAVDAVIAEL
KKQSKPVTTPEEIAQVATISANGDKEIGNII
SDAMKKVGRKGVITVKDGKTLNDELEIIEGM
KFDRGYISPYFINTSKGQKCEFQDAYVLLSE
KKISSIQSIVPALEIANAHRKPLVIIAEDVD
GEALSTLVLNRLKVGLQVVAVKAPGFGDNRK
NQLKDMAIATGGAVFGEEGLTLNLEDVQPHD
LGKVGEVIVTKDDAMLLKGKGDKAQIEKRIQ
EIIEQLDVTTSEYEKEKLNERLAKLSDGVAV
LKVGGTSDVEVNEKKDRVTDALNATRAAVEE
GIVLGGGCALLRCIPALDSLTPANEDQKIGI
EIIKRTLKIPAMTIAKNAGVEGSLIVEKIMQ
SSSEVGYDAMAGDFVNMVEKGIIDPTKVVRT
ALLDAAGVASLLTTAEVVVTEIPKEEKDPGM
GAMGGMGGGMGGGMF

Overall Prediction for the Protective Antigen = **0.5709** (Probable **ANTIGEN**).

Figure 2 : Antigenicity of the amino acid sequence

3.2 Identification and valuation of the CTL Epitopes

Using a composite score, 0.05 tap transit efficiency, an A1 supertype, and a threshold of 0.75, the NetCTL1.2 software made it easier to identify CTL epitopes (M. V. Larsen, 2007).

```
NetCTL-1.2 predictions using MHC supertype A1. Threshold 0.750000

 377 ID FASTA pep QLDVTTSEY aff   0.6937 aff_rescale   2.9451 cle 0.9775 tap   2.6140 COMB   3.2225 <-E
 409 ID FASTA pep TSDVEVNEK aff   0.2800 aff_rescale   1.1888 cle 0.6654 tap   0.2780 COMB   1.3025 <-E
 397 ID FASTA pep LSDGVAVLK aff   0.2173 aff_rescale   0.9228 cle 0.7729 tap   0.4340 COMB   1.0604 <-E
 421 ID FASTA pep VTDALNATR aff   0.1844 aff_rescale   0.7830 cle 0.7724 tap   1.4810 COMB   0.9729 <-E
  33 ID FASTA pep GADARALML aff   0.1629 aff_rescale   0.6917 cle 0.9123 tap   0.5470 COMB   0.8559 <-E
  82 ID FASTA pep KSIDLKDKY aff   0.1442 aff_rescale   0.6122 cle 0.4282 tap   3.2950 COMB   0.8412 <-E
 495 ID FASTA pep MQSSSEVGY aff   0.1344 aff_rescale   0.5707 cle 0.7462 tap   3.0520 COMB   0.8353 <-E
  18 ID FASTA pep LAPHLTRAY aff   0.1284 aff_rescale   0.5450 cle 0.9191 tap   3.0130 COMB   0.8336 <-E
 239 ID FASTA pep FQDAYVLLS aff   0.2156 aff_rescale   0.9153 cle 0.0313 tap  -2.6080 COMB   0.7896 <-E
 147 ID FASTA pep AVDAVIAEL aff   0.1184 aff_rescale   0.5026 cle 0.9779 tap   1.1090 COMB   0.7048
  47 ID FASTA pep LADAVAVTM aff   0.1245 aff_rescale   0.5285 cle 0.9242 tap   0.2330 COMB   0.6788
 235 ID FASTA pep QKCEFQDAY aff   0.1053 aff_rescale   0.4472 cle 0.4616 tap   2.9660 COMB   0.6647
 351 ID FASTA pep TKDDAMLLK aff   0.1224 aff_rescale   0.5197 cle 0.7407 tap   0.3640 COMB   0.6490
 506 ID FASTA pep MAGDFVNMV aff   0.1063 aff_rescale   0.4515 cle 0.9514 tap   0.4050 COMB   0.6144
 498 ID FASTA pep SSEVGYDAM aff   0.1267 aff_rescale   0.5381 cle 0.3389 tap   0.3080 COMB   0.6043
 317 ID FASTA pep AIATGGAVF aff   0.0770 aff_rescale   0.3270 cle 0.8655 tap   2.8600 COMB   0.5998
```

Figure 3 : NetCTL forecasts of CTL epitopes

In order to anticipate MHC-I binding alleles, the discovered CTL epitopes were examined using NetMHCpan 4.1. This artificial neural network-based method evaluated 9-mer peptides for every HLA gene. The threshold for weak binders was set at 2, and the threshold for strong binding peptides was 0.5. Peptides were chosen for additional research because of their strong binding affinity (C. W. K., 2001). Antigenicity was assessed using VaxiJen v.2.0, allergenicity with AllerTop v.2.0, and toxicity with ToxinPred to guarantee their efficacy and safety. The vaccine was produced with one common CTL epitope.

Table 1 : Identifying CTL Epitopes with Antigenicity, Allergenicity and Toxicity

CTL Epitope	Antigenicity	Allergenicity	Toxicity
QLD TOTSY	Antigen	Allergen	Non Toxin
KSIDLKDKY	Antigen	Antigen	Non Toxin
MASS SEVGI	Antigen	Non Allergen	Non Toxin

3.3 Prediction and valuation of the HTL epitopes

By promoting the release of cytokines such as IFN-gamma, IL-4, and IL-10, which affect the immune system following vaccination, HTL epitopes play a significant part in immunological responses. The IFNepitope server was used to examine the induction of IFNγ, which activates MHC molecules. The IL-4 pred server was used to assess IL-4, which promotes B cell growth, while IL-10, crucially the IL-10 pred server was used to evaluate immune modulation. The antigenicity, allergenicity, and toxicity of epitopes that met all three requirements were further investigated (Larsen et al., 2007). For additional research, only antigenic, non-allergic, and non-toxic epitopes were chosen. One HTL epitope was used in the preparation of the vaccine.
HTL epitopes are an important part of immunotherapeutic and preventative vaccinations (J Alexander, 1998).

Table 2 : Identifying HTL epitopes with Antigenicity, Allergenicity, Toxicity, IL-4, IL-10, IFN-Gamma

HTL Epitope	Antigenicity	Allergenicity	Toxicity	IFN	IL4	IL10
KDGVTVAKSIDLKDK	Antigen	Allergen	Non Toxin	Negative	Non Inducer	Non Inducer
DLKDKYKNIGAKLVQ	Antigen	Allergen	Non Toxin	Negative	Non Inducer	Non Inducer
LKDKYKNIGAKLVQD	Antigen	Non Allergen	Non Toxin	Negative	Non Inducer	Non Inducer
KDKYKNIGAKLVQDV	Antigen	Non Allergen	Non Toxin	Negative	Non Inducer	Non Inducer
DKYKNIGAKLVQDVA	Non Antigen	Non Allergen	Non Toxin	Positive	Non Inducer	Non Inducer
FQDAYVLLSEKKISS	Antigen	Non Allergen	Non Toxin	Negative	Non Inducer	Inducer

QDAYVLLSEKKISSI	Antigen	Non Allergen	Non Toxin	Negative	Non Inducer	Inducer
SEVGYDAMAGDFVNM	Antigen	Allergen	Non Toxin	Negative	Non Inducer	Non Inducer
EVGYDAMAGDFVNMV	Antigen	Allergen	Non Toxin	Negative	Non Inducer	Non Inducer
TKDGVTVAKSIDLKD	Antigen	Allergen	Non Toxin	Positive	Non Inducer	Non Inducer
DGVTVAKSIDLKDKY	Antigen	Non Allergen	Non Toxin	Negative	Inducer	Non
EFQDAYVLLSEKKIS	Antigen	Non Allergen	Non Toxin	Negative	Non Inducer	Inducer
GVMLAVDAVIAELKK	Antigen	Non Allergen	Non Toxin	Negative	Non Inducer	Inducer
IGNIISDAMKKVGRK	Non Antigen	Non Allergen	Non Toxin	Negative	Non Inducer	Non Inducer

TAEVVVTEIPKEEKD	Non Antigen	Non Allergen	Non Toxin	Positive	Non Inducer	Non Inducer
VTKDGVTVAKSIDLK	Antigen	Allergen	Non Toxin	Negative	Inducer	Non Inducer
IDLKDKYKNIGAKLV	Antigen	Allergen	Non Toxin	Negative	Inducer	Non Inducer
IAKEGFEKISKGANP	Non Antigen	Non Allergen	Non Toxin	Negative	Non Inducer	Inducer
AKEGFEKISKGANPV	Non Antigen	Non Allergen	Non Toxin	Negative	Non Inducer	Non Inducer
KEGFEKISKGANPVE	Non Antigen	Non Allergen	Non Toxin	Negative	Non Inducer	Inducer
EGFEKISKGANPVEI	Antigen	Non Allergen	Non Toxin	Negative	Non Inducer	Non Inducer

VFRQMRPVSRVLAPH	Non Antigen	Non Allergen	Non Toxin	Negative	Non Inducer	Inducer
FRQMRPVSRVLAPHL	Non Antigen	Non Allergen	Non Toxin	Negative	Non Inducer	Non Inducer
RQMRPVSRVLAPHLT	Antigen	Allergen	Non Toxin	Negative	Non Inducer	Non Inducer
LAVDAVIAELKKQSK	Antigen	Allergen	Non Toxin	Negative	Non Inducer	Non Inducer
AVDAVIAELKKQSKP	Antigen	Non Allergen	Non Toxin	positive	Inducer	Inducer
VDAVIAELKKQSKPV	Antigen	Non Allergen	Non Toxin	Negative	Inducer	Inducer
DAVIAELKKQSKPVT	Antigen	Non Allergen	Non Toxin	positive	Inducer	Inducer
DAYVLLSEKKISSIQ	Antigen	Non Allergen	Non Toxin	Negative	Non Inducer	Inducer

VKFGADARALMLQGV	Antigen	Non Allergen	Non Toxin	Negative	Inducer	Non Inducer
GAKLVQDVANNTNEE	Non Antigen	Non Allergen	Non Toxin	Negative	Inducer	Non Inducer
GRTVIIEQSWGSPKV	Non Antigen	Non Allergen	Non Toxin	Negative	Non Inducer	Inducer
TTPEEIAQVATISAN	Antigen	Non Allergen	Non Toxin	Positive	Non Inducer	Non Inducer
DRGYISPYFINTSKG	Antigen	Non Allergen	Non Toxin	Negative	Inducer	Inducer
RGYISPYFINTSKGQ	Antigen	Allergen	Non Toxin	Negative	Non Inducer	Non Inducer
EIAQVATISANGDKE	Non Antigen	Non Allergen	Non Toxin	Negative	Inducer	Non Inducer
IAQVATISANGDKEI	Non Antigen	Non Allergen	Non Toxin	Negative	Non Inducer	Non Inducer

AYVLLSEKKISSIQS	Antigen	Non Allergen	Non Toxin	Negative	Non Inducer	Inducer
TLNDELEIIEGMKFD	Non Antigen	Non Allergen	Non Toxin	Negative	Non Inducer	Non Inducer
LNDELEIIEGMKFDR	Non Antigen	Non Allergen	Non Toxin	Negative	Non Inducer	Non Inducer
NDELEIIEGMKFDRG	Non Antigen	Non Allergen	Non Toxin	Negative	Non Inducer	Non Inducer
DELEIIEGMKFDRGY	Non Antigen	Non Allergen	Non Toxin	Negative	Inducer	Non Inducer
KVGLQVVAVKAPGFG	Antigen	Non Allergen	Non Toxin	Negative	Inducer	Non Inducer
VGLQVVAVKAPGFGD	Antigen	Allergen	Non Toxin	Negative	Non Inducer	Inducer
GLQVVAVKAPGFGDN	Antigen	Non Allergen	Non Toxin	Negative	Inducer	Non Inducer
LQVVAVKAPGFGDNR	Antigen	Allergen	Non Toxin	Negative	Non Inducer	Non Inducer
VSRVLAPHLTRAYAK	Non Antigen	Non Allergen	Non Toxin	Negative	Inducer	Non Inducer
SRVLAPHLTRAYAKD	Non Antigen	Non Allergen	Non Toxin	Negative	Non Inducer	Inducer
TSDVEVNEKKDRVTD	Antigen	Non Allergen	Non Toxin	Negative	Non Inducer	Non Inducer
DQKIGIEIIKRTLKI	Antigen	Non Allergen	Non Toxin	Negative	Non Inducer	Non Inducer
AGDFVNMVEKGIIDP	Antigen	Allergen	Non Toxin	Negative	Non Inducer	Non Inducer
GDFVNMVEKGIIDPT	Antigen	Non Allergen	Non Toxin	Positive	Inducer	Non Inducer

DFVNMVEKGIIDPTK	Antigen	Non Allergen	Non Toxin	Negative	Non Inducer	Non Inducer
LVQDVANNTNEEAGD	Non Antigen	Non Allergen	Non Toxin	Negative	Inducer	Non Inducer
VQDVANNTNEEAGDG	Non Antigen	Non Allergen	Non Toxin	Negative	Non Inducer	Inducer
GYISPYFINTSKGQK	Antigen	Non Allergen	Non Toxin	Negative	Inducer	Inducer
YISPYFINTSKGQKC	Antigen	Non Allergen	Non Toxin	Negative	Inducer	Inducer
ISPYFINTSKGQKCE	Antigen	Non Allergen	Non Toxin	Negative	Non Inducer	Non Inducer
SPYFINTSKGQKCEF	Antigen	Non Allergen	Non Toxin	Negative	Non Inducer	Non Inducer
TKVVRTALLDAAGVA	Non Antigen	Non Allergen	Non Toxin	Negative	Non Inducer	Non Inducer

KVVRTALLDAAGVAS	Non Antigen	Non Allergen	Non Toxin	Positive	Non Inducer	Non Inducer
SIVPALEIANAHRKP	Antigen	Non Allergen	Non Toxin	Negative	Non Inducer	Non Inducer
IVPALEIANAHRKPL	Antigen	Non Allergen	Non Toxin	Negative	Inducer	Inducer
PALEIANAHRKPLVI	Antigen	Non Allergen	Non Toxin	Positive	Inducer	Inducer

3.4 Prediction and assessment of the B cell Epitopes

When creating peptide-based vaccines or producing antibodies, it is preferable to predict B-cell epitopes, particularly when purified protein is hard to come by and vaccination must be done using synthetic peptides produced from proteins (Reimer U., 2009). The IEDB Resource Analysis server was employed to forecast B cell epitopes using the "Bepipred Linear Prediction 2.0" technique. After being submitted for processing, the protein sequence produced a tabular dataset and a graphical representation that prioritized antigenic areas. The selection of antigenic areas with significant scores was ensured by applying a threshold value of 0.5 to find pertinent epitopes.

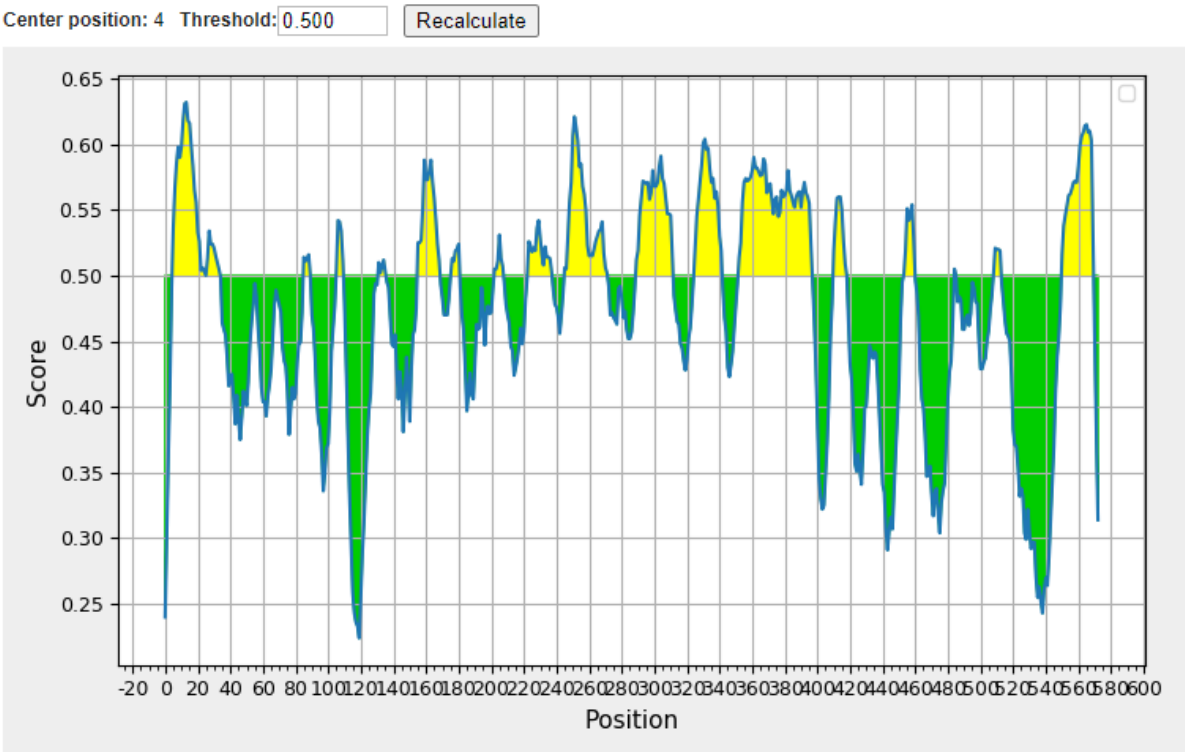


Figure 4 : B-cell epitope score versus location graph

The identical criteria employed for HTL epitopes were used to evaluate the antigenicity, allergenicity, and toxicity of the chosen epitopes. Four B Cell epitopes were found that fulfill the requirements but only one B CELL epitope was used in the preparation of the vaccine.

Table 3 : Identifying B Cell epitopes with Antigenicity, Allergenicity and Toxicity

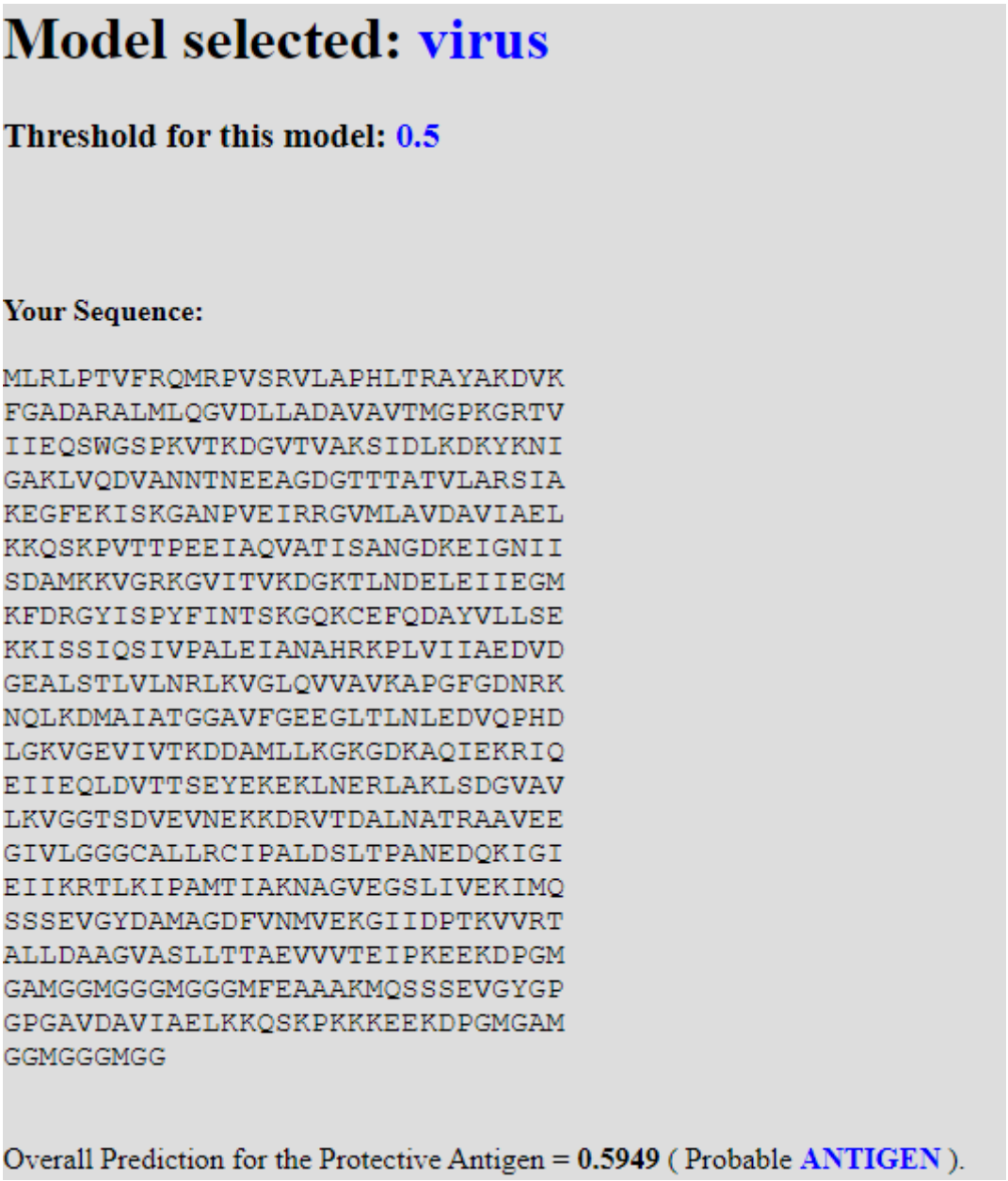
B cell Epitope	Length	Antigenicity	Allergenicity	Toxicity
TVFRQMRPVSRVLAPHLTRA	20	Non Antigen	Allergen	Non
KKQSKPVTTPEEIA	14	Antigen	Allergen	Non
ISPYFINTSKGQKCE	15	Antigen	Non Allergen	Non Toxin
LSEKKISSIQSIVPALEIANAHRKPLV	27	Antigen	Non Allergen	Non Toxin
RLKVGLQVVAVKAPGFGDNRKNQ	23	Antigen	Non Allergen	Non Toxin
KEEKDPGMGAMGGMGGGMGG	20	Antigen	Non Allergen	Non Toxin

3.5 Construction of vaccine by adding the selected epitopes the with amino acid sequence using linkers

Databases and in silico tools have distinct but complementary functions in vaccine design. In silico technologies offer computational techniques for forecasting and designing novel vaccines and vaccine components (Yongqun He , 2013).An in-silico method was used in this research to construct a multi-epitope vaccine, in which distinct epitopes were joined by particular linkers. The current research only used three linkers, in contrast to conventional vaccine designs. The primary protein sequence, which functions, was fused to CTL epitopes using the EAAAK linker as an adjuvant. The GP GPG linker was utilized to connect HTL epitopes, whereas the KK linker was used to connect B cell epitopes.One CTL epitope, one HTL epitope, and one B CELL epitope were used to construct the vaccine.

MLRLPTVFRQMRPVSRVLAPHLTRAYAKDVKFGADARALMLQGVDLLADAVAVTMG
PKGRTVIIEQSWGSPKVTKDGVTVAKSIDLKDKYKNIGAKLVQDVANNTNEEAGDGTT
TATVLARSIAKEGFEKISKGANPVEIRRGVMLAVDAVIAELKKQSKPVTTPEEIAQVATIS
ANGDKEIGNIISDAMKKVGRKGVITVKDGKTLNDELEIIEGMKFDRGYISPYFINTSKGQ
KCEFQDAYVLLSEKKISSIQSIVPALEIANAHRKPLVIIAEDVDGEALSTLVLNRLKVGLQ
VVAVKAPGFGDNRKNQLKDMAIATGGAVFGEEGLTLNLEDVQPHDLGKVGEVIVTKD
DAMLLKGKGDKAQIEKRIQEIIEQLDVTTSEYEKEKLNERLAKLSDGVAVLKVGGTSDV
EVNEKKDRVTDALNATRAAVEEGIVLGGGCALLRCIPALDSLTPANEDQKIGIEIIKRTLK
IPAMTIAKNAGVEGSLIVEKIMQSSSEVGYDAMAGDFVNMVEKGIIDPTKVVRTALLDA
AGVASLLTTAEVVVTEIPKEEKDPGMGAMGGMGGGMGGGMFEAAAKMQSSSEVGYG
PGPGAVDAVIAELKKQSKPKKKEEKDPGMGAMGGMGGGMGG

3.6 Antigenicity of the constructed vaccine



Model selected: virus

Threshold for this model: 0.5

Your Sequence:

MLRLPTVFRQMRPVSRVLAPHLTRAYAKDVK
FGADARALMLQGVDLLADAVAVTMGPKGRTV
IIEQSWGSPKVTKDGVTVAKSIDLKDKYKNI
GAKLVQDVANNTNEEAGDGTTTATVLARSIA
KEGFEKISKGANPVEIRRGVMLAVDAVIAEL
KKQSKPVTTPEEIAQVATISANGDKEIGNII
SDAMKKVGRKGVITVKDGKTLNDELEIIEGM
KFDRGYISPYFINTSKGQKCEFQDAYVLLSE
KKISSIQSIVPALEIANAHRKPLVIIAEDVD
GEALSTLVLNRLKVGLQVVAVKAPGFGDNRK
NQLKDMAIATGGAVFGEEGLTLNLEDVQPHD
LGKVGEVIVTKDDAMLLKGKGDKAQIEKRIQ
EIIEQLDVTTSEYEKEKLNERLAKLSDGVAV
LKVGGTSDVEVNEKKDRVTDALNATRAAVEE
GIVLGGGCALLRCIPALDSLTPANEDQKIGI
EIIKRTLKIPAMTIAKNAGVEGSLIVEKIMQ
SSSEVGYDAMAGDFVNMVEKGIIDPTKVVRT
ALLDAAGVASLLTTAEVVVTEIPKEEKDPGM
GAMGGMGGGMGGGMFEAAAKMQSSSEVGYGP
GPGAVDAVIAELKKQSKPKKKEEKDPGMGAM
GGMGGGMGG

Overall Prediction for the Protective Antigen = **0.5949** (Probable **ANTIGEN**).

Figure 5 : Antigenicity of the amino acid sequence for constructed vaccine

The antigenicity of the constructed vaccine was counted 0.5949 (Probable Antigen).

The antigenicity of the constructed vaccine is greater than the antigenicity of the initial amino acid sequence.

3.7 Stability Test and Biochemical Analysis of this Constructed vaccine

ProtParam provided useful information on a number of properties and was used to analyze the vaccine's physicochemical characteristics. As part of this analysis, the grand average of hydropathicity, instability index, molecular weight, and total amino acid count were calculated (GRAVY), among other essential qualities. When evaluating the vaccine's stability and possible efficacy, these elements are crucial. The therapeutic pI was 5.86, the instability index 31.62 which was below 40, and the GRAVY value was negative (-0.127). This implies that the vaccine possesses hydrophila and stability (Garg, 2016).

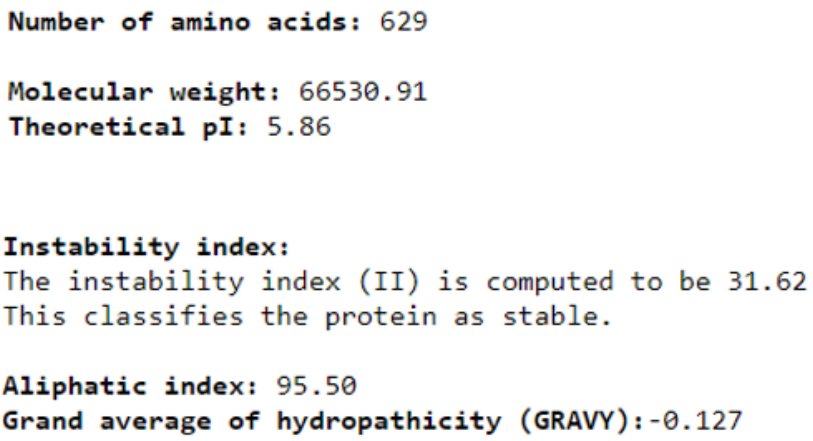
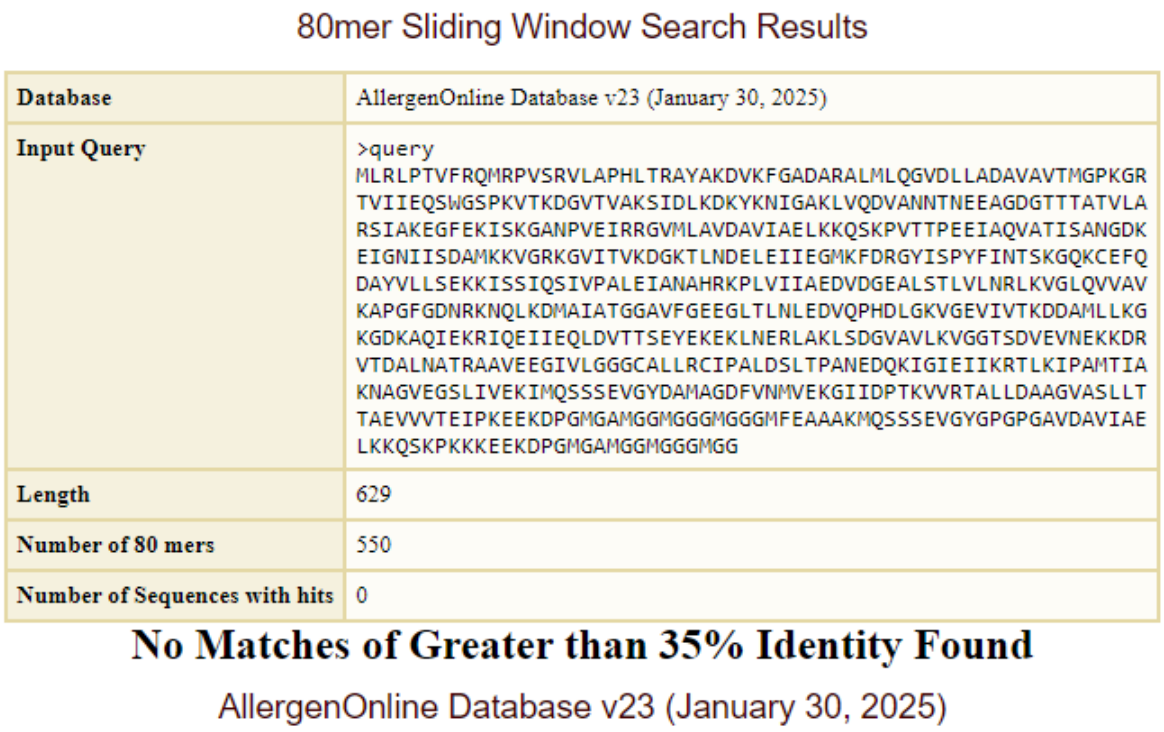


Figure 6 : Biochemical prediction of constructed vaccine

3.8 Prediction of the Allergenicity and Toxicity of this constructed Vaccine

The sequence was uploaded in plain text format to the Allergen Online service, which evaluated the vaccine's possible allergenicity in order to assess its safety (Goodman, 2016). Additionally, toxicity evaluation was carried out using the T3DB database, which made it possible to identify any harmful characteristics of the vaccine's architecture (Ebisawa, 2016).



80mer Sliding Window Search Results

Database	AllergenOnline Database v23 (January 30, 2025)
Input Query	>query MLRLPTVFRQMRPVSRVLAPHLTRAYAKDVKFGADARALMLQGVDLLADAVAVTMGPKGR TVIIEQSWGSPKVTKDGVTVAKSIDLKDKYKNIGAKLVQDVANNTNEEAGDGTTTATVLA RSIAKEGFEKISKGANPVEIRRGVMLAVDAVIAELKKQSKPVTTPEEIAQVATISANGDK EIGNIISDAMKKVGRKGVITVKDGKTLNDELEIIEGMKFDRGYISPYFINTSKGQKCEFQ DAYVLLSEKKISSIQSIVPALEIANAHRKPLVIIAEDVDGEALSTLVLNRLKVGLQVVAV KAPGFGDNRKNQLKDMAIATGGAVFGEEGLTLNLEDVQPHDLGKVGEVIVTKDDAMLLKG KGDKAQIEKRIQEIIEQLDVTTSEYEKEKLNERLAKLSDGVAVLKVGGTSDVEVNEKKDR VTDALNATRAAVEEGIVLGGGCALLRCIPALDSLTPANEDQKIGIEIIKRTLKIPAMTIA KNAGVEGSLIVEKIMQSSSEVGYDAMAGDFVNMVEKGIIDPTKVVRTALLDAAGVASLLT TAEVVVTEIPKEEKDPGMGAMGGMGGGMGGGMFEAAAKMQSSSEVGYGPGPGAVDAVIAE LKKQSKPKKKEEKDPGMGAMGGMGGGMGG
Length	629
Number of 80 mers	550
Number of Sequences with hits	0

No Matches of Greater than 35% Identity Found

AllergenOnline Database v23 (January 30, 2025)

Figure 7 : Allergen online resource for predicting allergenicity

Your search returned no results

Figure 8 : T3DB online resource for predicting toxicity

3.9 Prediction confidence and coverage of the constructed vaccine (3D Structural Modelling)

Protein structure, function, and mutations can be predicted and analyzed using the Phyre2 set of online tools. The three-dimensional structure of the vaccine was predicted using the Phyre2 server. Phyre2 was able to create primary and tertiary structures because the sequence was given in plain text format. After being provided by email, the generated PDB file was examined using PyMOL software for structural visualization (Christopher M Yates, 2015). There was 84% coverage and 100% confidence. The accuracy and efficacy of vaccines are enhanced by high confidence and adequate coverage.

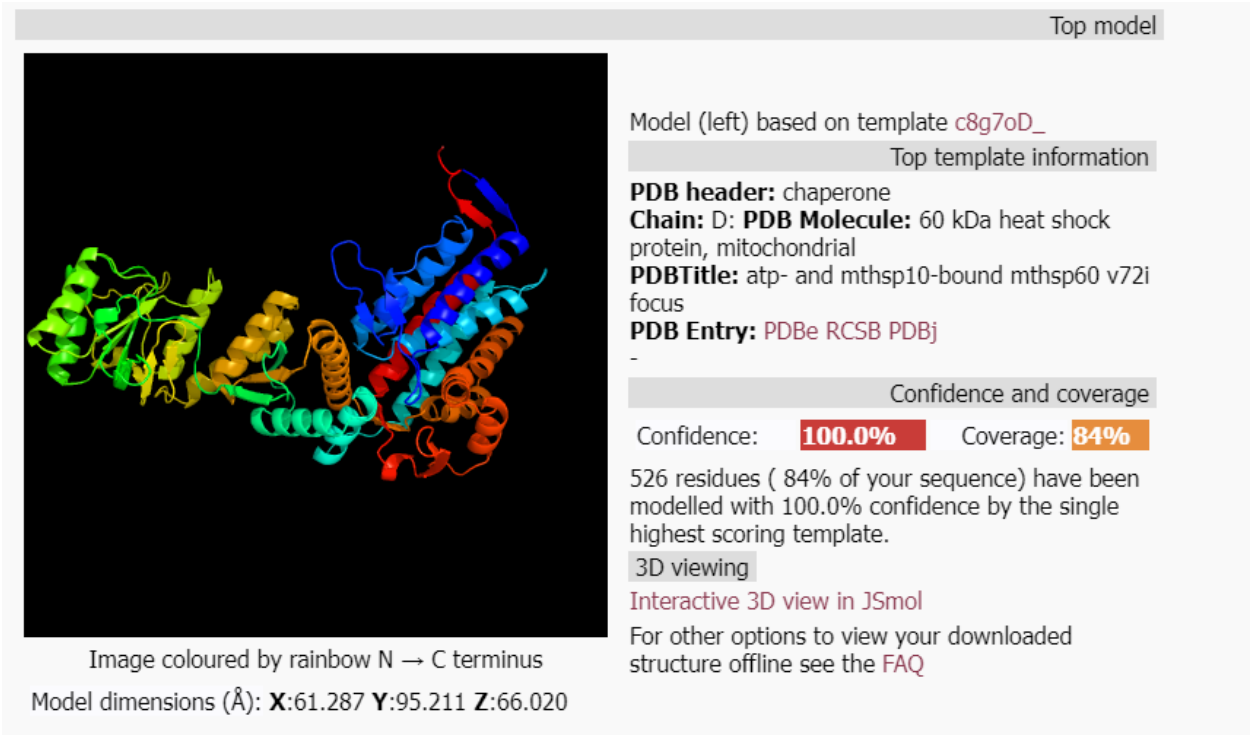


Figure 9 : Phyre2's score for homology modeling

3.10 Analysis of the Ramachandran Plots

A Ramachandran plot produced by the Swiss Model Expasy structure assessment tool was used to assess the modeled vaccine's structural integrity. After uploading the PDB file from Phyre2, the phi and psi angles inside the polypeptide could be seen chain. Verifying the proper folding and stability of the protein structure required this evaluation (Kiefer, 2009). The Ramachandran outlier is 0.00%, and the Ramachandran Favored region is more than 92% (98.09%)

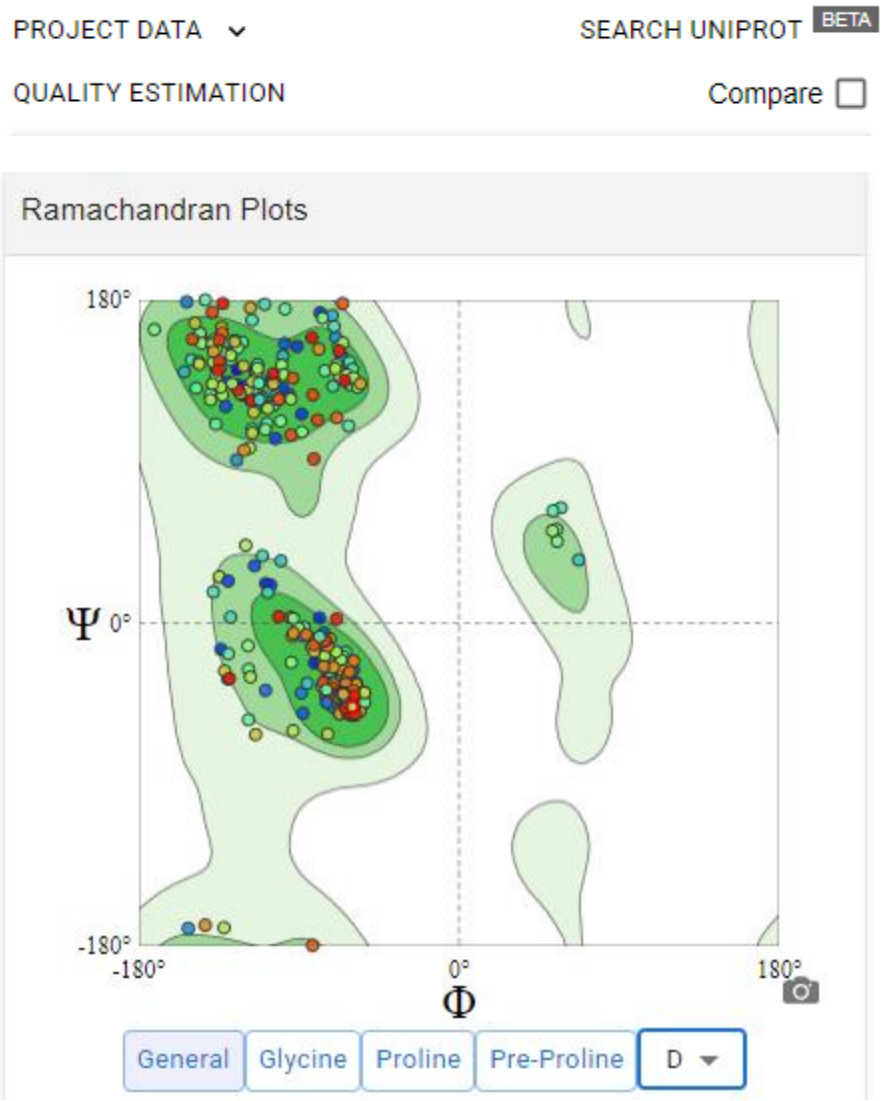


Figure 10 : Evaluation report of Ramchandran plot

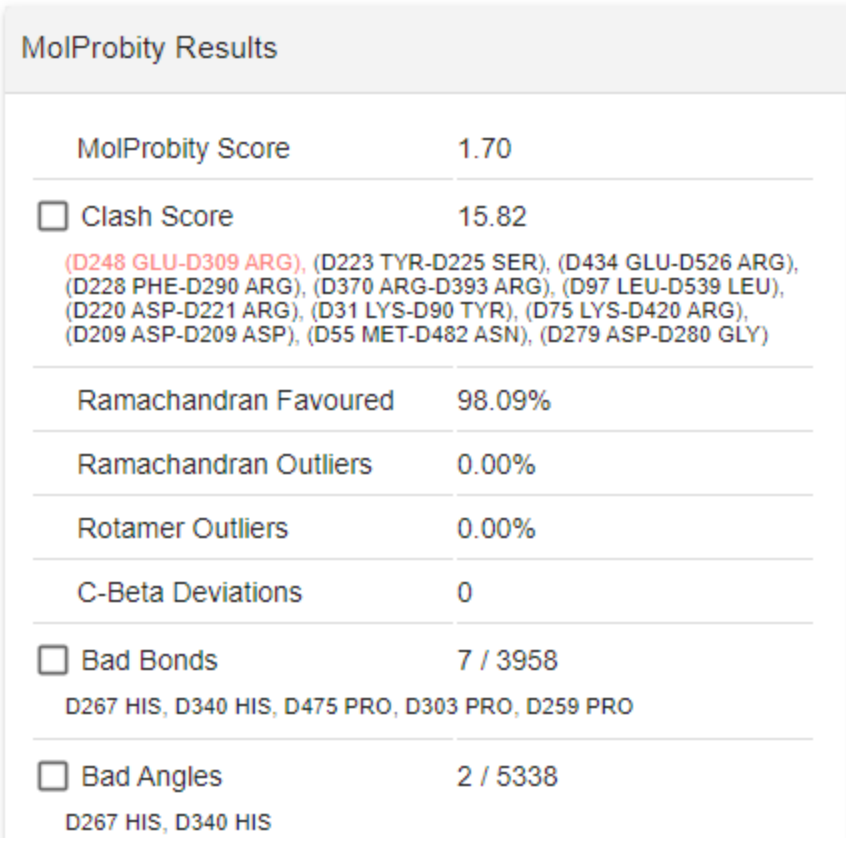


Figure 11 : Molprovity report from Swiss-Model Expasy

3.11 Prediction and analysis Z score for 3D -Model

The constructed vaccination model's Z-score was evaluated using the ProSA-web server. Following the PDB file upload, the server returned findings that included the Z-score, Z-score versus residue count in one graph, and energy distribution versus sequence location in another (Wiederstein & Sippl, 2007). In order to confirm the vaccine's structural reliability, these assessments were crucial. The Z-score was 11.2. This score is slightly higher than the ideal range of score.

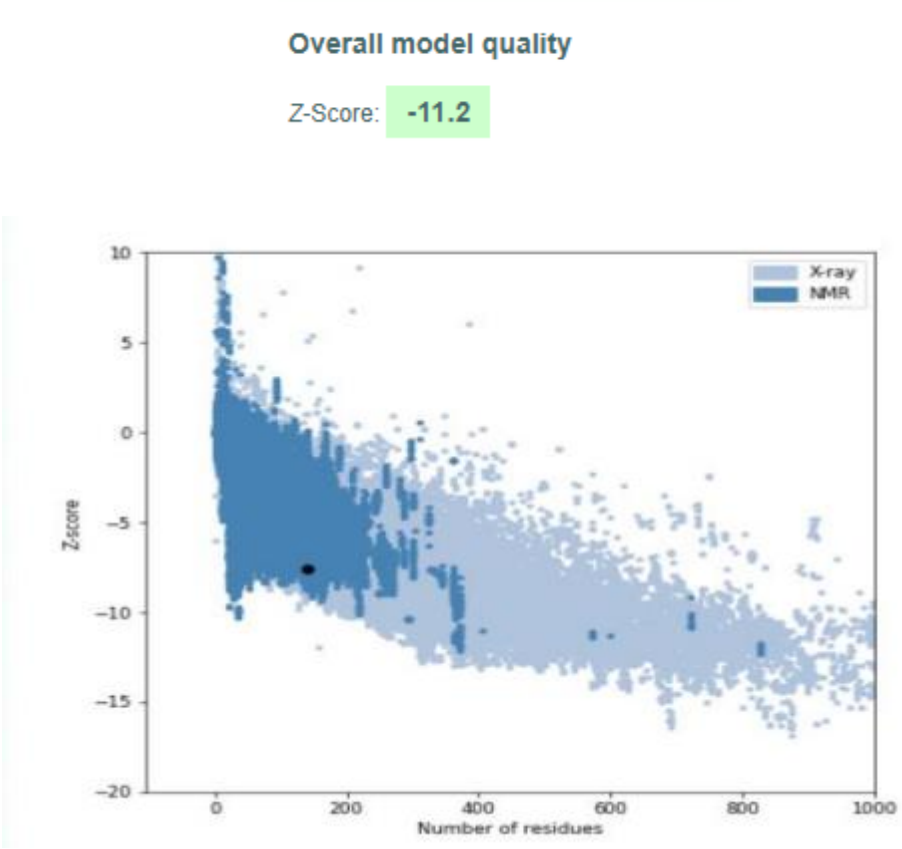


Figure 12 : Overall model quality of the costructed vaccine

3.12 Molecular Docking of the constructed Vaccine

Computer-assisted drug design and structural molecular biology both heavily rely on molecular docking. Predicting the dominant binding mode or modes of a ligand with a protein of known three-dimensional structure is the aim of ligand-protein docking (Garrett M Morris,2008). In order to assess the binding interactions between the constructed molecules and human TLR-4, the ClusPro server was used for molecular docking. The vaccine's PDB file was submitted as the ligand, and the TLR's PDB file was obtained from the RCSB Protein Data Bank. The initial docked complex was the most stable of the ten docked complexes that were produced. The highest score that was punished was -749.7 and lowest -533.2. With a high cluster density and low energy, the optimal binding cluster was chosen.

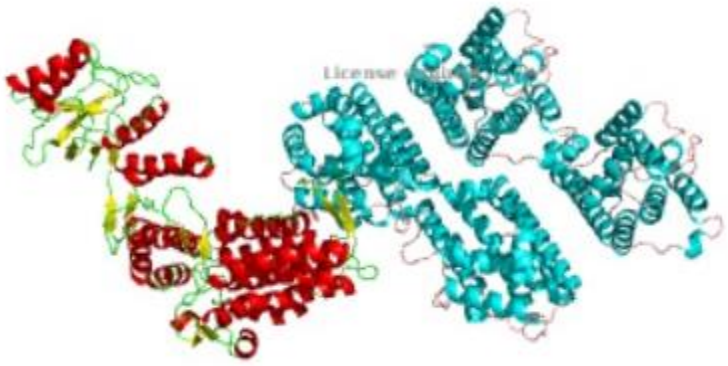


Figure 13 : Molecular docking with TLR-4 receptor

Cluster	Members	Representative	Weighted Score
0	49	Center	-554.5
		Lowest Energy	-613.9

Figure 14 : Suitable docked complex from ClusPro

3.13 Immune Simulation of the constructed Vaccine

In order to maintain a sufficient amount of vaccination in the body and produce the intended prolonged immunological response, longer doses—such as booster doses—are usually needed. Monitoring the levels of specific antibodies or immunoglobulins in connection with the vaccination schedule and timing is necessary to assess the immune response induced in the body. The vaccine's immune response simulation was examined using c-Immsim. After immunization,

the graph shows an increase in antigen levels. It also shows that antibody concentrations increased noticeably by day with IgG and IgM levels peaking at 65 days. This implies that the vaccination successfully boosted an immune answer. Moreover, the production of memory cells and antibodies by lymphocytes was noted (Rapin,2011). These memory cells are essential for immunological memory retention, which allows for a quicker reaction in the event that the human Hepatitis C Virus re-enters the body.

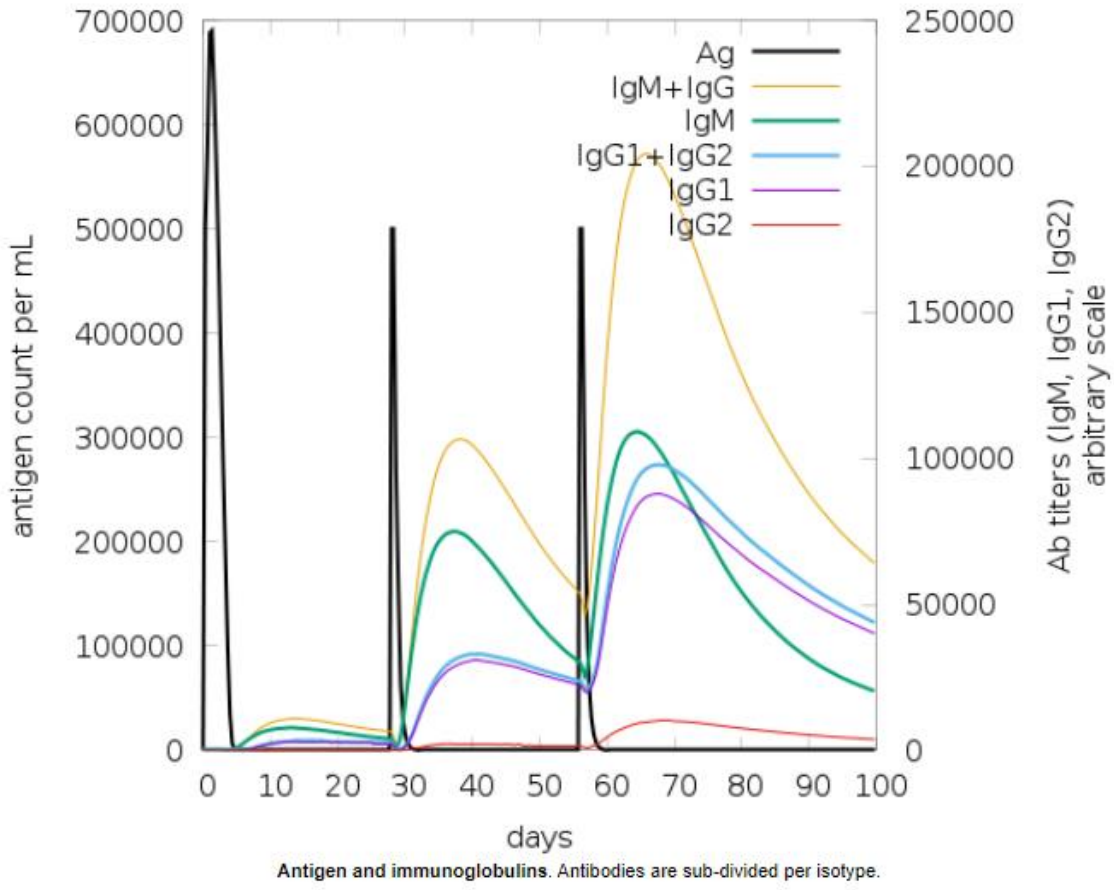


Figure 15 : Immune simulation of the constructed vaccine is displayed visually using C-Immsim

Chapter 4

Discussion

Hepatitis C virus (HCV) is a viral pandemic that is five times more common than human immunodeficiency virus type 1 infection, with an estimated 170 million people infected globally. Blood-screening procedures have reduced the risk of transfusion-associated hepatitis to a very low level in developed nations; however, injection drug use and, to a lesser extent, other forms of percutaneous or mucous membrane exposure continue to be the primary causes of new cases. The majority of HCV-infected individuals develop chronic illness, and viral infection has emerged as the primary reason for liver transplantation. The biggest number of HCV infections has been documented in Egypt, however prevalence varies globally. It is believed that the use of parenteral antischistosomal therapy in Egypt has contributed to the prevalence of HCV antibodies in different locations, which range from 6 to 28 percent. In the United States, 1.8% of people have HCV antibody positive status. An estimated 2.7 million people in the US are actively infected with HCV, since according to currently available diagnostics, 3 out of 4 seropositive individuals also have viremia. Using the VaxiJen 2.0 server, a main protein was chosen based on antigenicity to create a new vaccine against the Human Papillomavirus (HPV). Using a variety of computational methods, the protein's sequence was examined to find four Bcell epitopes, one helper T lymphocyte (HTL) epitope, and one cytotoxic T lymphocyte (CTL) epitope. These epitopes underwent a thorough screening process based on a number of parameters. The ProtParam tool was used to assess the stability of the vaccine design, validating its stability. The Grand Average of Hydropathy (GRAVY) score was negative, indicating better solubility and absorption, and the amino acid composition fell within the intended range. The 3D structural model with 84% coverage at 100% confidence was produced using Phyre2, and this was considered suitable. Using Allergen Online and T3DB, safety assessments were conducted to verify that the vaccine was non-toxic and non-allergenic, two essential characteristics of the perfect vaccination to guarantee it does not cause adverse responses upon management. ProsaWeb was used to assess the 3D model's structural quality, and the Z-score showed that the model had an appropriate energy distribution. Swiss-Model Expasy was also used to do a Ramachandran plot analysis, which showed that the

structural parameters fell within a reasonable range. The human Toll-like receptor 4 (TLR4) was chosen as the appropriate receptor for the Hepatitis C virus for molecular docking. Lastly, C-IMMSIM was used to perform immune response simulations, with positive outcomes. This study finds that the constructed vaccine has potential as a novel preventive measure against the Hepatitis C virus after taking into account all evaluations.

Chapter 5

Conclusion

HCV infection will likely continue to affect health around the world for some time to come. It is necessary to distinguish HCV infection from other causes of viral hepatitis due to the high rates of progression to chronic infection and the absence of effective preventative measures. Even with recent advancements, research into more potent treatments needs to continue to be a top priority. The creation of a successful vaccination is the best chance for a global response to the HCV infection epidemic.

This study's main goal was to use an in-silico method to construct a multi-epitope vaccination. A significant drawback, though, is that this study only examines the vaccine's computer modeling without evaluating its efficacy or safety. A vaccination must be taken into consideration for practical use, it needs to pass stringent in-vitro and in-vivo testing to verify its efficacy and safety. Prior to moving on with clinical trials and commercialization, these experimental validations are crucial.

To raise awareness and understanding of viral hepatitis, WHO hosts World Hepatitis Day campaigns every year. In order to highlight the significance of the liver for a healthy life and the necessity of increasing viral hepatitis prevention, testing, and treatment in order to prevent liver illnesses and meet the 2030 hepatitis eradication objective, WHO has chosen the theme "One life, one liver" for World Hepatitis Day 2023.

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