



# An innovative Approach to in Silico Vaccine Design Against Dengue Virus Type 2

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## Abstract

Dengue virus type 2 (DENV-2) is a significant public health concern, causing dengue fever, dengue hemorrhagic fever, and dengue shock syndrome, which can be life-threatening. Due to the lack of effective antiviral treatments and limitations of current vaccines, there is a need for innovative vaccine development. This study focused on creating a vaccine candidate against DENV-2 using bioinformatics tools for the design process.

A multi-faceted in silico approach was employed to design a novel vaccine against DENV-2, concentrating on identifying immunogenic epitopes essential for strong immune responses. Epitopes were selected based on criteria such as non-toxicity, non-allergenicity, and high immunogenicity. These predicted epitopes were combined with adjuvants, linkers, and a his-tag to create the vaccine construct, followed by a thorough evaluation of its effectiveness.

After the investigations, a non-toxic, non-allergenic protein with an antigenic score of 0.7776 was selected as a candidate for vaccine design. Four epitopes for B cells and 19 epitopes for T cells were predicted, and the vaccine candidate was formulated by combining these epitopes. The vaccine structure was predicted to be non-toxic, non-allergenic, and had a favorable immunogenicity score of 0.7044. Furthermore, the designed vaccine successfully passed all virtual assessments.

Based on the results, this multi-epitope peptide can be used as a promising vaccine candidate that warrants further development. This study makes a significant contribution to the overarching aim of controlling and preventing dengue fever, and provides an innovative vaccine formulation strategy that could lessen the global impact of this disease.

**Key words:** Dengue virus type 2; Vaccine design; Multiepitope vaccine; Immunoinformatics

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## Introduction

Dengue virus type 2 (DENV-2) is one of the four serotypes of the dengue virus, a flavivirus transmitted by mosquitoes that represents a considerable public health risk in tropical and subtropical areas around the globe (Sukupolvi-Petty, Austin et al. 2010). This virus primarily spreads through the bites of infected *Aedes* mosquitoes, particularly *Aedes aegypti* and *Aedes albopictus*, which are frequent in these climates (Zandi, Teoh et al. 2011). Recent outbreaks of DENV-2 have been reported in various regions, including Southeast Asia, the Americas, and parts of Africa, leading to significant morbidity and mortality. For instance, the 2023 outbreak in Brazil resulted in thousands of reported cases, overwhelming healthcare systems and highlighting the urgent need for effective public health interventions (Sarker, Roknuzzaman et al. 2024). DENV-2 is linked to significant illness and mortality worldwide. The clinical spectrum of DENV-2 infection varies widely, ranging from asymptomatic cases to severe disease presentations. Mild infections may present with symptoms like fever, headache, and joint pain, often similar to other viral infections (Sirisena, Mahilkar et al. 2021). However, severe cases can lead to increased vascular permeability, resulting in plasma leakage, hemorrhage, and organ dysfunction. The risk of severe disease is heightened in individuals with a history of previous dengue infections, a phenomenon known as antibody-dependent enhancement (ADE). This complexity in disease presentation underscores the urgent need for effective prevention and treatment strategies (Guzman and Harris 2015, Guzman, Gubler et al. 2016).

Traditional vaccine development approaches have faced hurdles, including safety concerns and the complexity of the virus's serotype diversity. Current strategies for vaccinating against DENV-2 include live-attenuated vaccines, inactivated virus vaccines, and subunit vaccines. However, their effectiveness can differ based on serotype and previous dengue exposure, highlighting the necessity for enhanced vaccine candidates that offer broad and long-lasting protection against all four dengue serotypes (Sridhar,

Luedtke et al. 2018, Deng, Yang et al. 2020). Research on DENV-2 has significantly progressed in recent decades, focusing on its molecular biology, pathogenesis, and interactions with the host immune system (Fadaka, Sibuyi et al. 2021). Innovations in genomic sequencing and bioinformatics have facilitated the identification of viral proteins and potential epitopes that can be targeted for vaccine development. *In silico* approaches have gained attention in recent years, allowing researchers to model viral structures, predict immunogenic epitopes, and design novel vaccines efficiently. These computational tools can accelerate the identification of promising vaccine candidates while minimizing the risks associated with traditional experimental methods (Fahimi, Sadeghizadeh et al. 2016, Ali, Pandey et al. 2017). In this study, a multiepitope peptide vaccine candidate targeting dengue virus type 2 was designed as a candidate vaccine. Subsequently, the vaccine's efficacy was tested and validated using various computational tools.

## Materials and Method

### Genome Retrieval and identification of open reading frames

The complete genome sequence of dengue virus type 2 was obtained from the NCBI database (NCBI Reference Sequence: NC\_001474.2, GenBank: U87411.1). Furthermore, the ORF finder server served as a source for all the available open reading frames (ORFs) of DENV-2 (Rombel, Sykes et al. 2002).

### Prediction of ORF localization

Protein localization prediction was carried out using the Virus-mPLOC server, seeking for membrane proteins. These proteins may be identified by the host's immune system as foreign substances, making them potential candidates for antigens (Xiao, Wu et al. 2011).

### Finding Signal peptide and transmembrane helices

Signal peptide sequences were identified by the SignalP server. Signal peptides act as markers for cellular transport and are subsequently removed during post-translational modification. Therefore, it is advisable to identify and eliminate them (Petersen, Brunak et al. 2011). The



transmembrane regions of many integral membrane proteins consist of a bundle of hydrophobic  $\alpha$ -helices. This structure may arise from a two-stage folding process, where preformed transmembrane helices with independent stability pack together without undergoing topological rearrangement. So this structure remains inaccessible to the immune system and should be removed. TMHMM Server v. 2. was used to analyze the transmembrane helices (Käll, Krogh et al. 2007).

### **Immunogenicity, homology and allergenicity assessment**

The VaxiJen Server with default parameters was used to predict the immunogenicity of ORF. Immunogenicity refers to the capacity of a substance containing antigens to trigger the body to mount an immune response against it (Zaharieva, Dimitrov et al. 2017). An allergen epitope is a molecule segment that can specifically bind to immunoglobulin, leading to allergic reactions. These epitopes were removed using the AllercatPro servers and non-allergenic epitopes were utilized for further analysis (Nasar, Nasar et al. 2022).

### **Epitope Prediction**

The IEDB server with default parameters was used to analytically predict B cell and T cell epitopes. All HLA alleles were considered and analyzed. Each epitope underwent evaluation for toxicity, allergenicity, and immunogenicity by Toxinpred, Allercatpro and IEDB server, respectively (Gupta, Kapoor et al. 2013, Vita, Overton et al. 2015). Immunogenic, non-toxic, and non-allergenic epitope were chosen for further analysis.

### **Vaccine construction**

The process of constructing the vaccine needs the assembly of appropriate his-tag, adjuvant, and linker components. Subsequently, characteristics of the vaccine, such as toxicity, allergenicity, and antigenicity were assessed using the ToxinPred, AllercatPro 2.0 and VaxiJen servers, respectively. The flexible linker used for connecting the epitopes were AAY and GGGGS. His-tag was used to improve peptide expression in *Escherichia coli* and the ribosomal protein L7/L12 was

utilized as adjuvant. The IL-12 sequence was obtained from the NCBI protein database with accession ID AAB37425.2.

### **Physicochemical and Structural characteristics**

The chemical and physical properties assessment includes half-life, instability index, molecular weight, GRAVY, aliphatic index, and theoretical isoelectric point (PI). All items are crucial for developing a vaccine construct. The physicochemical properties of the vaccine formulation were analyzed using the ProtParam server (Garg, Avashthi et al. 2016). An abundance of alpha helices in a protein sequence can impede vaccine development, as it decreases effectiveness by rendering the vaccine less immunogenic. Prabi server was utilized to analyze the secondary structure of the vaccine sequence such as measuring the percentage of alpha helices (Roohparvar Basmenj, Izadkhah et al. 2023). To determine the tertiary structure of the potential vaccine, the Phyre server was used (Kelley and Sternberg 2009). Furthermore, Ramachandran plot was analyzed by the MolProbity server (Davis, Leaver-Fay et al. 2007).

### **Molecular docking**

Molecular docking experiments were conducted to examine the interactions between the designed vaccine (ligand) and the MHCI (PDB ID: 5YXU) and MHCII (PDB ID: 4MD4) as receptors. The Hdock server was utilized to explore these interactions(Yan, Tao et al. 2020).

## **Result**

### **Finding antigen**

Dengue virus type 2 has a single chromosome with a length of 10,723 Mb. Using the ORF finder, 73 ORFs were predicted. Seven of those ORF predicted to be membrane proteins through analyzing with the Virus-mPLoc server. Analyzing the signal peptides using the signal p server, no signal peptides were detected. Four ORFs were found to have intramembrane domains, by utilizing the TMHMM Server, which is an exclusion criterion due to inaccessibility of the immune system to these proteins. The threshold of the Vaxijen server for immunogenicity of ORFs is 0.4. Through analysis of this server, two non-an-





tigen ORFs were deleted. Following the allergenicity assessment using the Allercatpro server, one protein demonstrated potential for further investigation and advancement in the next phase of research. As a result, ORF 70 was selected for the next step. The ORF sequence and analysis are detailed in table 1.

### **Selection of epitopes**

Utilizing the IEDB server, a total of four epitopes binding to B cells, 10 epitopes binding to MHCI, and 9 epitopes binding to MHCII were identified. The allergenicity, immunogenicity, and toxicity of each epitope were evaluated using the Allercat pro, Vaxijen, and ToxinPred servers, respectively, and the results are summarized in Table 2,3 and 4.

### **Vaccine construction**

His-tag, adjuvant, and linker components were incorporated to finalize the vaccine's sequence. The toxicity assessment of the vaccine's sequence conducted by the ToxinPred server did not identify any 10-mer toxin peptide sequences. Analysis via the Allercat pro server indicated that the vaccine sequence is non-allergenic. Additionally, using the Vaxijen server, the antigenicity of the vaccine yielded a favorable antigenicity score. The final sequence is presented in Table 5.

### **Physical, chemical and structural characteristics**

Physicochemical measurements offer valuable information regarding the protein's size, stability, and hydrophilicity, providing insights into its potential, as detailed in Table 6. All results were obtained from the ProtParam server(SobhZahedi and YektaKooshali 2024). Secondary structure of the candidate vaccine predicted by the Prabi server (figure1 and 2). The Phyre2 Server was used to predict the third structure (figure 3). Using the MolProbity server, the three-dimensional structure of the vaccine construct was evaluated. Analyzing the result, it was noted that the 96.2% amino acids reside within the favored region of the Ramachandran chart, signifying a correct conformational alignment. Also, 100.0% of the amino acids were within the allowed region, indicating admissible deviations from ideal conformations (figure 4).

### **Molecular docking**

The HDock server was utilized to study the molecular docking interactions. Score of -198.98 for binding 5YXU to MHCI and -185.44 for binding 4MD4 to MHCII indicates a suitable connection. Therefore, this vaccine can create a good connection with its receptors in physiological conditions of the human body. Results were showed in figure 5.

### **Discussion**

The emergence and re-emergence of dengue virus (DENV), particularly dengue virus type 2, pose significant public health challenges globally. The nuances of DENV-2 immunopathology, characterized by a spectrum of clinical manifestations from mild illness to severe dengue with hemorrhagic manifestations, necessitate a comprehensive understanding of its biology and immune responses for effective vaccine development(Yenamandra, Koo et al. 2021). The immune response to DENV-2 is complex, involving both humoral and cellular immunity. Neutralizing antibodies are crucial for controlling viral replication; however, the presence of non-neutralizing antibodies from prior infections can worsen the disease through mechanisms such as antibody-dependent enhancement (ADE) (Roy and Bhattacharjee 2021). This dual interaction complicates vaccine development, highlighting the need for vaccines that can produce a robust and balanced immune response effective against all dengue serotypes. An effective vaccine for this virus has not yet been developed (Prompetchara, Ketloy et al. 2020). In silico methodologies, leveraging genomic data and computational algorithms, allow for a thorough analysis of genetic diversity, facilitating the identification of conserved epitopes across genotypes that can be targeted for vaccine development (Martinelli 2022).

After predicting (ORFs), we identified a potential ORF suitable for vaccine development against DENV-2. Through comprehensive computational evaluations, our study revealed that this specific protein does not contain any toxic regions. Additionally, the protein demonstrated a high antigenicity score, indicating its potential to generate an immune response while posing



minimal risk to the host. By using various bio-informatics tools, we selected epitopes that are non-toxic, non-allergenic, exhibit a favorable antigenic score, and are accessible to the immune system. These selected epitopes were subsequently integrated into a vaccine design by linking them with appropriate linkers, an adjuvant, and a His-tag. The toxicity evaluation indicated that the vaccine construct does not include any 10-mer toxin peptide sequences, allowing for its administration without any toxic risks to the recipient. Additionally, the antigenicity analysis yielded a promising score of 0.7044, suggesting that the vaccine construct has the potential to effectively stimulate an immune response, which is vital for its role as a preventive measure against the targeted virus. Furthermore, the assessment for allergenicity showed that the vaccine construct is non-allergenic, implying that it is likely to be well-tolerated by individuals with various sensitivities or allergies. This is encouraging, as it reduces the likelihood of inducing adverse allergic reactions upon administration. The physical and chemical properties of the vaccine design provide important insights into its characteristics. The vaccine construct is anticipated to maintain stability under typical physiological conditions, supported by a stability index of 35.51. An aliphatic index of 90.38 indicates that it is a hydrophobic protein. The analysis of the Ramachandran plot revealed that a significant proportion (96.2%) of the amino acids in the vaccine construct fall within the favored region, suggesting an appropriate conformational structure. Additionally, the docking analysis examined how the vaccine interacts with the MHCI and MHCII receptors. The leading cluster models exhibited strong interactions, as evidenced by the weighted scores and the considerable number of interacting amino acids. These findings suggest that the vaccine construct can effectively bind to the target receptors, potentially facilitating the initiation of an immune response.

These results highlight the significance of this protein as a promising candidate for further research, enhancing our understanding of the vaccine design's potential and its suitability for development. To validate the findings, subsequent

research may include animal model studies, in vitro and in vivo experiments, and clinical trials to confirm the vaccine construct's long-term effects, efficacy, and safety. These steps are crucial for advancing the vaccine toward eventual clinical application and ensuring its readiness for human use.

## Conclusion

The use of in silico techniques in vaccine development offers a promising path toward creating a safe and effective vaccines for DENV-2. Focusing on genetic variability, mapping immunogenic epitopes, and conducting safety evaluations establish a solid framework for progressing vaccine candidates through the preclinical phases. Collaborative efforts that merge computational predictions with experimental validation will be crucial in turning these designs into practical vaccine options for preventing dengue. By leveraging computational tools to predict and refine vaccine candidates, researchers can tackle the challenges presented by DENV-2's genetic variability and immune evasion mechanisms. Although obstacles persist, combining in silico methods with conventional vaccine development practices has the potential to greatly improve the effectiveness and safety of dengue vaccines, thereby enhancing public health outcomes in regions where dengue is prevalent.

## CONFLICTS OF INTEREST:

The authors have declared that no competing interests exist.

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## Data Availability:

All relevant data are within the paper and the supporting files.

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