Immunoinformatics-Based Multi-Epitope Vaccine Design for Crimean-Congo Hemorrhagic Fever in Cattle

Bhakti Zanzrukiya[1], Abhishek Das[2]
[1]*Post Graduate Student, Department of Biotechnology, Patkar Varde College*
[2]*Assistant Professor, Department of Biotechnology, Patkar Varde College*

***Abstract*— Crimean-Congo Hemorrhagic Fever (CCHF) is a serious viral illness with no licensed vaccine. In this study we employ an in-silico approach to design a potential vaccine against the CCHF virus in cattles. Utilizing immunoinformatics methodologies, we systematically identified critical protein epitopes by evaluating their antigenicity and allergenicity. These candidate antigens were subsequently analyzed for their ability to elicit immune responses, with a particular focus on mapping T-cell and B-cell epitopes, identified using the Immune Epitope Database (IEDB) and assessed with Vaxijen. Molecular docking tools like ClusPro are utilized to analyze the binding interactions between the epitopes and immune receptors like TLRs. This research aims to advance the field of computational vaccinology and add to the groundwork for developing an effective vaccine against CCHF affecting the cattle, which could ultimately help reduce the human transmission.**

***Index Terms*—CCHFV,** **Crimean-Congo hemorrhagic fever, Immunoinformatics, *In silico* vaccine, cattles.**

I. INTRODUCTION

Crimean-Congo Hemorrhagic Fever Virus (CCHFV) is a highly pathogenic virus belonging to the Nairoviridae family and is the causative agent of Crimean-Congo Hemorrhagic Fever (CCHF) [1]. The disease is characterized by severe hemorrhagic manifestations, multi-organ failure, and high fatality rates, making it one of the most dangerous tick-borne viruses known [2]. Hyalomma ticks are the primary vectors of CCHFV, playing a crucial role in its transmission cycle by maintaining the virus in the environment and facilitating its spread among animals and humans [3], [4], [5]. However, ticks are not the sole contributors to viral persistence—livestock, particularly cattle, serve as silent amplifying hosts [3]. Once infected through tick bites, these animals sustain viral replication without showing clinical symptoms, allowing the virus to circulate undetected in endemic regions[6]. Humans, on the other hand, are incidental hosts, acquiring the infection either through tick bites or direct contact with the blood and tissues of infected animals, especially during slaughter and veterinary procedures. This enzootic cycle between ticks and livestock, with occasional spillover into human populations, makes CCHFV a significant One Health challenge [3].

Despite the clear role of livestock in sustaining viral transmission, vaccine development efforts have primarily focused on human protection. Several experimental vaccines, including inactivated and recombinant candidates, are under investigation for human use, but no widely approved vaccine is currently available [7]. More importantly, there is a complete lack of veterinary vaccines, which means that livestock continue to serve as reservoirs, perpetuating the virus and maintaining the risk of human infection. This gap in preventive strategies underscores the need for an effective cattle vaccine that could reduce viral circulation at the animal level, thereby indirectly protecting human populations.

To address this gap, this study focuses on the rational design of a CCHFV vaccine specifically for cattle. Using immunoinformatics-based approaches, we aim to develop a subunit vaccine capable of inducing a protective immune response in bovines [8]. By reducing viral loads in livestock, this approach has the potential to disrupt the tick-to-animal transmission cycle and, consequently, lower the risk of human exposure [9]. Through this study, we seek to lay the foundation for a proactive, livestock-based vaccination strategy that can complement existing efforts to mitigate CCHFV transmission and reduce its public health burden.

II. METHODS

A. Antigenic Peptides Identification

Through a comprehensive literature review, various surface proteins were identified as potential candidates for vaccine development.

B. Protein Sequence Retrieval

To construct a vaccine for Crimean-Congo Hemorrhagic Fever Virus (CCHFV), protein sequences were selected from publically available databases like UniProt (https://www.uniprot.org/), PDB (https://www.rcsb.org/) and NCBI (https://www.ncbi.nlm.nih.gov/). The sequences were check for their transmembrane position using online tool TMHMM (https://services.healthtech.dtu.dk/services/DeepTMHMM-1.0/)[10]. The sequence data is listed in Table 1.

C. Antigenic Sites Determination

To identify antigenic regions within the selected proteins, the online tool Antigenic Peptide Prediction server (https://imed.med.ucm.es/Tools/antigenic.pl) was used.

D. Antigenicity and Allergenicity Analysis

To ensure the safety and immunogenicity of the vaccine construct, predicted antigenic peptides from the selected proteins were analyzed for both antigenicity and allergenicity. Antigenicity prediction was performed using VaxiJen v2.0 (https://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html), which evaluates the immunogenic potential of peptides based on an alignment-independent approach [11]. Allergenicity was assessed using AllerTOP v2.0 (https://www.ddg-pharmfac.net/allertop_test/), a tool that classifies peptides as allergens or non-allergens based on physicochemical properties[12]

Peptides with a high antigenicity score (above the threshold of 0.45, as recommended by VaxiJen) were considered immunogenic. Only peptides classified as non-allergens by AllerTOP were included in the final vaccine construct to minimize adverse allergic reactions.

E. B-Cell and T-Cell Epitope Prediction

The identification of potential B-cell and T-cell epitopes is crucial for designing a multi-epitope vaccine. Predicted epitopes were analyzed for MHC binding potential using BepiPred (https://services.healthtech.dtu.dk/services/BepiPred-2.0/) [13] and the Immune Epitope Database and Analysis Resource (IEDB) (http://tools.iedb.org/main/tcell/).

Epitopes were selected based on high antigenicity, binding affinity to prevalent BoLA alleles and HLA alleles, and non-allergenicity.

F. Adjuvant Selection

Based on literature review, 3 adjuvants were selected for the analysis, i.e. human β-defensin, L7/L12 ribosomal protein and 50S ribosome. These adjuvants were studied for enhancing immune responses by stimulating the innate immune system [14], [15], [16]. V1, V2 and V3 constructs were designed with 3 adjuvants respectively added at the N-terminus of the vaccine construct to optimize immune activation.

G. Vaccine Construct

The design of a multi-epitope vaccine incorporates various epitopes linked by specific peptide linkers to maintain structural integrity and functional activity. Adjuvant is included at the N-terminus of the construct and is separated from downstream antigenic sequences using the EAAAK linker, which provides flexibility and prevents interference.

The GPGPG linker separates the B-cell epitope from MHC-II (Helper T-Cell) epitopes and also links multiple MHC-II epitopes, preserving their conformational integrity for effective presentation to helper T-cells.

The AAY linker is used to connect MHC-II epitopes to MHC-I (Cytotoxic T-Cell) epitopes and to link multiple MHC-I epitopes. This ensures proper interaction with T-cell receptors and facilitates efficient binding to MHC molecules, enabling robust cellular immune responses.

H. Modelling of Vaccine Construct

The 3D structure of the designed vaccine construct was modeled using SWISS-MODEL (https://swissmodel.expasy.org/), a web-based automated protein structure homology-modelling server [17]. The primary sequence of the vaccine

construct, which includes the adjuvant, epitopes, and linkers, was submitted in FASTA format.

Quality Assessment:

1. The structure was visualized using Chimera (https://www.cgl.ucsf.edu/chimera/), a molecular visualization tool, to inspect the folding and conformation of the vaccine construct.
2. The model underwent quality checks using the SAVES server prediction (https://saves.mbi.ucla.edu/) tools. An ERRAT score above 95% indicating that the overall quality of the construct is satisfactory.
3. The Z-score of the structure was assessed using ProSa (Protein Structure Analysis tool) (https://prosa.services.came.sbg.ac.at/prosa.php). A Z-score between -10 to 10 is considered acceptable and indicates that the structure is in a stable and reasonable conformation.[18], [19]

I. Physiological Properties

The ProtParam tool (https://web.expasy.org/protparam/) was used to analyze the vaccine construct's physiological properties. The analysis provided key parameters, including the molecular weight, theoretical isoelectric point (pI), instability index and chemical formula [20].

J. Solubility Prediction

The solubility of the vaccine construct was predicted using the Protein Sol tool (https://protein-sol.manchester.ac.uk/). The tool assesses the construct's potential solubility in water, which is an important factor for ensuring that the vaccine can be effectively delivered and administered. A favorable solubility profile ensures that the vaccine is amenable to formulation. [21]

K. Secondary Structure Prediction

The secondary structure of the vaccine construct was predicted using SopMA (https://npsa.lyon.inserm.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_sopma.html). SopMA is a reliable tool for predicting the secondary structure of proteins based on their amino acid sequences. The prediction provided insights into the proportion of α-helices, β-strands, and random coils, which are important for understanding the protein's folding and stability. This information is crucial for assessing how the vaccine construct might interact with immune cells. [22]

L. Selection of Receptors

Different TLRs as well as Bovine RP-105 were selected as the receptors for docking studies due to their critical role in viral recognition. TLR3 is predicted to induce IFN response[23]. TLR4 is utilized by virus for host entry[24]. TLR8 receptor, along with TLR9, is known to participate in the detection of viral pathogens, activating immune responses [24], [25]. Bovine cell surface receptor RP-105 MD has 30% similarity to bovine TLR-4 receptor[25], [26], [27]

M. Energy Minimization

After modeling the vaccine construct using SWISS-MODEL, the structure was refined in Chimera by removing non-stranded segments. The vaccine and receptors were then subjected to energy minimization using Swiss PDB Viewer (https://spdbv.unil.ch/) to optimize their geometry[28].

N. Molecular Docking

Docking of the V1, V2 and V3 constructs and TLRs and bovine receptors was performed using ClusPro (http://cluspro.bu.edu/), a protein-protein docking software that predicts the most likely binding modes between two interacting molecules. ClusPro works by generating multiple docking poses through rigid-body docking, followed by energy minimization to refine the protein-protein interactions. The software ranks the generated models based on their energy scores, and the model with the lowest energy is selected as the most stable and biologically relevant interaction. [29], [30], [31], [32], [33].

O. Codon Optimization

To enhance the expression of the V1 vaccine construct, codon optimization was performed using the NovoPro Codon Optimization Tool (https://www.novoprolabs.com/tools/codon-optimization). This tool optimizes the DNA sequence by selecting codons that are more efficiently translated in the target expression system. The optimized sequence ensures higher protein yield and reduces the likelihood of misfolding or degradation [34]. The codon-optimized version of the vaccine construct was generated for expression in *Escherichia coli*.

P. Immune Simulation

Immune simulation of the vaccine construct was performed using C-IMMSIM (http://www.c-imm-sim.org/), a computational tool that simulates the immune response to a given antigen [35], [36], [37]. The V1 vaccine construct was input into the model to simulate interactions with antigen-presenting cells, T-cells, and B-cells.

Q. Vector Builder

The vaccine construct was cloned into a Bacterial Protein Expression Vector using VectorBuilder (https://en.vectorbuilder.com/). The expression vector chosen was based on the pET system, which is commonly used for high-level protein expression in *Escherichia coli K12*. To facilitate protein purification, a 6x His-tag was added at the C-terminus of the vaccine construct. This His-tag allows for efficient purification of the expressed protein using nickel affinity chromatography [38].

III. RESULTS AND DISCUSSIONS

A. Protein Sequence Retrieval

From the retrieved sequences, the sequences depicting highest antigenic score as well as were non-allergen and positioned as outer membrane were selected. These sequences included regions of non-structural proteins, envelopment protein and glycoprotein chains as mentioned in Table 1.

B. Antigenic Sites Determination

Antigenic Peptide Prediction server employs predictive algorithms to calculate antigenicity based on the physicochemical properties of amino acid sequences. To create a multiepitope vaccine that covers a broad range of antigenic sites or epitopes, an epitope was selected from each protein.

C. Antigenicity and Allergenicity Analysis

Table 2 summarizes the analysis results for each peptide with their antigenic score.

D. B-cell Epitope Prediction, MHC-I and MHC-II Epitopes Prediction

B-cell epitope for each epitope depicting highest antigenic score and non-allergenicity were selected. The B-cell epitopes antigenic score was between 1.5 to 0.5.

MHC Class I BoLA allele BoLA-1:00902, BoLA-1:01901, BoLA-1:02101, BoLA-1:00901, and BoLA-1:02001 were identified for vaccine construct.

MHC Class II HLA alleles include HLA-DRB1*13:02, HLA-DRB1*12:01, and HLA-DQA1*05:01/DQB1*02:01 were identified. Table 3,4 and 5 displays data for B-cell epitopes, MHC-I and MHC-II epitopes respectively.

E. Vaccine Construct

The vaccine construct (V1, V2 and V3) were made by strategically placing the obtained sequences along with the adjuvant and linkers.

F. Modelling & Quality Assessment of Vaccine Construct

The modelling software SWISS-MODEL selected suitable templates based on sequence similarity, and a three-dimensional structural model was generated as shown in Fig. 1. The ERRAT score and Z-score of the constructs is mentioned in Table 7.

G. Physiological Properties

Table 8 presents the physiochemical properties of the three vaccine constructs (V1, V2, and V3). These properties, including molecular weight, theoretical isoelectric point (pI), instability index, aliphatic index, and grand average of hydropathicity (GRAVY), are essential for evaluating the stability and solubility of the designed vaccine candidates.

Amino Acid Composition & Molecular Weight:

The three constructs vary in size, with V1 (334 AA, 36.57 kDa) being the smallest, followed by V2 (390 AA, 41.44 kDa), and V3 (542 AA, 59.00 kDa). Larger molecular weight may impact expression efficiency and structural complexity, making V1 the most feasible for synthesis.

Theoretical pI (Isoelectric Point):

The theoretical pI values suggest the constructs' behavior in different pH environments. V1 (pI 8.46) and V3 (pI 9.87) are basic in nature, while V2 (pI 5.97) is slightly acidic, indicating a potential difference in solubility and interaction with host immune components.

Instability Index:

The instability index provides insight into the potential in vivo stability of the constructs. All three constructs have values below 40, indicating that they are stable proteins suitable for further studies. Among them, V1 exhibits the highest stability, making it a promising candidate for vaccine development.

Aliphatic Index:

The aliphatic index, which indicates the relative volume occupied by aliphatic side chains (Ala, Val, Ile, Leu), reflects protein thermostability. V2 has the highest aliphatic index, suggesting greater thermal stability. Higher aliphatic index values are desirable for vaccine constructs as they contribute to structural integrity under physiological conditions.

GRAVY (Grand Average of Hydropathicity):

The GRAVY values for all constructs are negative suggesting that they are hydrophilic and likely to be soluble in aqueous environments, a critical property for proper expression and immunogenicity. V3 has the most negative GRAVY score, indicating higher solubility but potentially lower membrane affinity.

G. Solubility Prediction

The solubility of the designed vaccine constructs was predicted using the Protein-Sol software (as seen in Fig. 2). The solubility analysis indicates that V3 is the most soluble, followed by V2, with V1 showing the least solubility. While V2 has an advantage due to its pI being closer to physiological pH, V3's overall higher solubility could make it easier to express and purify. On the other hand, V1 may require solubility-enhancing strategies (e.g., fusion tags, expression system modifications) to ensure efficient production. The data for solubility prediction is mentioned in Table 9.

H. Secondary Structure Prediction

The secondary structure of the designed epitope was predicted using the SOPMA tool, following the consensus prediction method outlined by Geourjon and Deléage (1995). The analysis revealed that the V2 appears to be the most stable construct due to its highest alpha helix content and lowest random coil proportion, indicating a well-folded and stable protein structure. V1 maintains a balanced distribution of secondary structures, with moderate alpha helix content and a reasonable amount of random coil, making it a structurally viable option for vaccine development as depicted in Fig 3. On the other hand, V3 exhibits greater flexibility, with lower alpha helix content and a higher random coil percentage, which could affect its stability but might be beneficial for antigenic exposure.

I. Molecular Docking

Molecular docking of the three vaccine constructs (V1, V2, and V3) was performed with various Toll-like receptors (TLRs) and Bovine RP105. The docking scores reflect the binding affinity of each vaccine construct to the receptors, which plays a critical role in determining the immune response activation and overall effectiveness of the vaccine candidates. The more negative the docking score, the stronger the binding affinity between the receptor and the vaccine construct.

Based on the molecular docking results in Table 11, V1 appears to be the most promising candidate, exhibiting the strongest binding to TLR3, TLR4, TLR8, TLR9, and Bovine RP105. This suggests that V1 is likely to elicit the most robust immune response through activation of these receptors. Fig 4 shows the docking of V1 with receptor RP-105. V3 shows moderate binding, and V2 has the weakest binding affinity, which may limit its potential as a strong immunogen.

J. Codon Optimization

The V1 vaccine construct was optimized for expression in E. coli as shown in Fig. 5, resulting in a Codon Adaptation Index (CAI) of 0.81, indicating strong alignment with E. coli's codon usage. This optimization enhances translation efficiency and supports higher protein expression within the bacterial host.

Additionally, the GC content of the optimized sequence was 54.19%, which is ideal for E. coli. This level of GC content promotes mRNA stability and reduces the formation of secondary structures, ensuring better translation and protein folding. The optimized sequence, provided in the supplementary data sheet, demonstrates the suitability of the construct for high-yield expression in E. coli, offering a promising approach for large-scale vaccine production.

K. Immune Simulation

Immune Cell Counts:

Fig. 6 and 7 depict the dynamics of various immune cell populations over time, including active, internalized antigen-presenting, MHC II-presenting, mitotic, anergic, and resting cells. The data reveal a distinct trend where the number of active immune cells steadily increases, indicating an effective immune response triggered by the vaccine. In contrast, the resting immune cells remain relatively constant, suggesting that only a subset of cells is actively engaged in the immune response. Notably, the activation of antigen-presenting cells (APCs) and the presentation of MHC II molecules highlight the efficient processing and presentation of the antigen, which is crucial for initiating a strong adaptive immune response. Anergic cells, which represent immune tolerance, were observed in small quantities, suggesting that the vaccine may not induce immune tolerance or anergy at levels that would compromise its efficacy.

Virus, Immunoglobulins & Immune Complexes:

Fig. 8 illustrates the interaction between the virus, immunoglobulins (Ig), and immune complexes. The high concentration of immunoglobulins, particularly IgG, indicates a robust humoral immune response, which is critical for neutralizing the virus. The presence of immune complexes, formed when antibodies bind to viral antigens, suggests efficient antibody-virus interactions, leading to a reduced viral load and contributing to virus clearance. Comparing these results with other vaccine candidates or existing vaccines could provide insight into the relative efficacy of the vaccine construct in inducing a strong antibody response and protecting against viral infections.

Cytokine & Interleukin Concentrations:

Fig. 9 displays the concentration levels of key immune signaling molecules, including IL-2 and danger signals. Elevated IL-2 levels indicate T-cell activation and proliferation, which are essential for mounting an effective cellular immune response. The presence of danger signals suggests strong immune activation, although excessive levels may point to potential risks of excessive inflammation, which can be detrimental. An optimal cytokine response is expected to correlate with effective vaccine-induced immunity, balancing the need for robust immune activation while minimizing harmful inflammation.

Epitope Predictions (Parker_B Scale & Peptide Lists):

The Parker B-cell scale and peptide lists predict potential B-cell and T-cell epitopes. The strongest epitopes, based on their high binding affinity to MHC I and MHC II molecules, were identified and are expected to be the key determinants of immunogenicity. These epitopes were compared with known immunodominant epitopes of CCHF, highlighting their relevance for inducing a strong immune response. The implications of these predictions suggest that the vaccine may offer broad population coverage, potentially inducing cross-reactive immunity across different HLA alleles.

Correlating immune cell activation, antibody responses, and cytokine signaling underscores the vaccine's potential to induce a balanced and effective immune response. The observed strong humoral and cellular responses, combined with optimal cytokine activation, suggest that the vaccine may be highly effective. However, potential limitations such as insufficient memory cell formation or the risk of excessive inflammation need to be considered and addressed in future studies. When compared with existing vaccine candidates or in vivo data, the current vaccine construct demonstrates promising results but requires further validation to ensure its long-term efficacy and safety.

L. Vector Builder

A vector clone containing the vaccine construct as shown in Fig. 10 can be synthesized and used for clinical trials.

ACKNOWLEDGMENT

We express our heartfelt gratitude to Patkar Varde College for providing the resources and infrastructure essential for the successful completion of this research.

REFERENCES

[1] V. M. Deyde, M. L. Khristova, P. E. Rollin, T. G. Ksiazek, and S. T. Nichol, “Crimean-Congo Hemorrhagic Fever Virus Genomics and Global Diversity,” J Virol, vol. 80, no. 17, pp. 8834–

8842, Sep. 2006, doi: 10.1128/JVI.00752-06/SUPPL_FILE/DEYDE_TABLE_S1.DOC.

[2] N. Shahhosseini et al., "Crimean-Congo Hemorrhagic Fever Virus in Asia, Africa and Europe," Microorganisms 2021, Vol. 9, Page 1907, vol. 9, no. 9, p. 1907, Sep. 2021, doi: 10.3390/MICROORGANISMS9091907.

[3] J. I. Thierry Ebogo Belobo et al., "Worldwide epidemiology of Crimean-Congo Hemorrhagic Fever Virus in humans, ticks and other animal species, a systematic review and meta-analysis," 2021, doi: 10.1371/journal.pntd.0009299.

[4] O. E. Igah et al., "Sokoto Journal of Veterinary Sciences Detection of Crimean-Congo haemorrhagic fever virus circulating in ticks and cattle in Plateau and Kaduna States, Nigeria," /Sokoto Journal of Veterinary Sciences, vol. 22, no. 4, pp. 283–290, 2024, doi: 10.4314/sokjvs.v22i4.7.

[5] A. M. Ibrahim, I. A. Adam, B. T. Osman, and I. E. Aradaib, "Epidemiological survey of Crimean Congo hemorrhagic fever virus in cattle in East Darfur State, Sudan," Ticks Tick Borne Dis, vol. 6, no. 4, pp. 439–444, Jun. 2015, doi: 10.1016/J.TTBDIS.2015.03.002.

[6] D. Oluwayelu, A. Adebiyi, and O. Tomori, "Endemic and emerging arboviral diseases of livestock in Nigeria: a review," Parasites & Vectors 2018 11:1, vol. 11, no. 1, pp. 1–12, Jun. 2018, doi: 10.1186/S13071-018-2911-8.

[7] B. Ahata and G. B. Akçapınar, "CCHFV vaccine development, current challenges, limitations, and future directions," Front Immunol, vol. 14, p. 1238882, 2023, doi: 10.3389/FIMMU.2023.1238882.

[8] A. Z. Siddiki et al., "Construction of a multi-epitope in silico vaccine against Anaplasma Marginale using immunoinformatics approach," Biocatal Agric Biotechnol, vol. 50, p. 102706, Jul. 2023, doi: 10.1016/J.BCAB.2023.102706.

[9] S. A. Atim et al., "Risk factors for Crimean-Congo Haemorrhagic Fever (CCHF) virus exposure in farming communities in Uganda," J Infect, vol. 85, no. 6, p. 693, Dec. 2022, doi: 10.1016/J.JINF.2022.09.007.

[10] J. Hallgren et al., "DeepTMHMM predicts alpha and beta transmembrane proteins using deep neural networks," bioRxiv, p. 2022.04.08.487609, Apr. 2022, doi: 10.1101/2022.04.08.487609.

[11] I. A. Doytchinova and D. R. Flower, "VaxiJen: A server for prediction of protective antigens, tumour antigens and subunit vaccines," BMC Bioinformatics, vol. 8, no. 1, pp. 1–7, Jan. 2007, doi: 10.1186/1471-2105-8-4/TABLES/2.

[12] I. Dimitrov, D. R. Flower, and I. Doytchinova, "AllerTOP - a server for in silico prediction of allergens," BMC Bioinformatics, vol. 14, no. Suppl 6, p. S4, Apr. 2013, doi: 10.1186/1471-2105-14-S6-S4.

[13] J. N. Clifford, M. H. Høie, S. Deleuran, B. Peters, M. Nielsen, and P. Marcatili, "BepiPred-3.0: Improved B-cell epitope prediction using protein language models," Protein Sci, vol. 31, no. 12, p. e4497, Dec. 2022, doi: 10.1002/PRO.4497.

[14] M. A. Imran, M. R. Islam, A. Saha, S. Ferdousee, M. A. Mishu, and A. Ghosh, "Development of Multi-epitope Based Subunit Vaccine Against Crimean-Congo Hemorrhagic Fever Virus Using Reverse Vaccinology Approach.," Int J Pept Res Ther, vol. 28, no. 4, p. 124, Jul. 2022, doi: 10.1007/s10989-022-10430-0.

[15] A. A. Omoniyi et al., "In silico design and analyses of a multi-epitope vaccine against Crimean-Congo hemorrhagic fever virus through reverse vaccinology and immunoinformatics approaches," Sci Rep, vol. 12, no. 1, Dec. 2022, doi: 10.1038/S41598-022-12651-1.

[16] S. Z. Shah et al., "An Immunoinformatics Approach to Design a Potent Multi-Epitope Vaccine against Asia-1 Genotype of Crimean-Congo Haemorrhagic Fever Virus Using the Structural Glycoproteins as a Target," Vaccines (Basel), vol. 11, no. 1, Jan. 2022, doi: 10.3390/VACCINES11010061.

[17] A. Waterhouse et al., "SWISS-MODEL: homology modelling of protein structures and complexes," Nucleic Acids Res, vol. 46, no. W1, pp. W296–W303, Jul. 2018, doi: 10.1093/NAR/GKY427.

[18] M. Wiederstein and M. J. Sippl, "ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins," Nucleic Acids Res, vol. 35, no. suppl_2, pp. W407–W410, Jul. 2007, doi: 10.1093/NAR/GKM290.

[19] M. J. Sippl, "Recognition of errors in three-dimensional structures of proteins," Proteins, vol. 17, no. 4, pp. 355–362, 1993, doi: 10.1002/PROT.340170404.

[20] E. Gasteiger et al., "Protein Identification and Analysis Tools on the ExPASy Server," The Proteomics Protocols Handbook, pp. 571–607, 2005, doi: 10.1385/1-59259-890-0:571.

[21] M. Hebditch, M. A. Carballo-Amador, S. Charonis, R. Curtis, and J. Warwicker, "Protein–Sol: a web tool for predicting protein solubility from sequence," Bioinformatics, vol. 33, no. 19, p. 3098, Oct. 2017, doi: 10.1093/BIOINFORMATICS/BTX345.

[22] C. Combet, C. Blanchet, C. Geourjon, and G. Deléage, "NPS@: network protein sequence analysis," Trends Biochem Sci, vol. 25, no. 3, pp. 147–150, Mar. 2000, doi: 10.1016/S0968-0004(99)01540-6.

[23] R. Alam et al., "In silico formulation of a next-generation multiepitope vaccine for use as a prophylactic candidate against Crimean-Congo hemorrhagic fever," BMC Medicine 2023 21:1, vol. 21, no. 1, pp. 1–19, Feb. 2023, doi: 10.1186/S12916-023-02750-9.

[24] R. Alam et al., "In silico formulation of a next-generation multiepitope vaccine for use as a prophylactic candidate against Crimean-Congo hemorrhagic fever," BMC Medicine 2023 21:1, vol. 21, no. 1, pp. 1–19, Feb. 2023, doi: 10.1186/S12916-023-02750-9.

[25] A. Engin et al., "Toll-like receptor 8 and 9 polymorphisms in Crimean-Congo hemorrhagic fever," Microbes Infect, vol. 12, no. 12–13, pp. 1071–1078, Nov. 2010, doi: 10.1016/J.MICINF.2010.07.012.

[26] R. Alam et al., "In silico formulation of a next-generation multiepitope vaccine for use as a prophylactic candidate against Crimean-Congo hemorrhagic fever," BMC Medicine 2023 21:1, vol. 21, no. 1, pp. 1–19, Feb. 2023, doi: 10.1186/S12916-023-02750-9.

[27] S. Il Yoon, M. Hong, and I. A. Wilson, "An unusual dimeric structure and assembly for TLR4 regulator RP105–MD-1," Nature Structural & Molecular Biology 2011 18:9, vol. 18, no. 9, pp. 1028–1035, Aug. 2011, doi: 10.1038/nsmb.2106.

[28] M. U. Johansson, V. Zoete, O. Michielin, and N. Guex, "Defining and searching for structural motifs using DeepView/Swiss-PdbViewer," BMC Bioinformatics, vol. 13, no. 1, pp. 1–11, Jul. 2012, doi: 10.1186/1471-2105-13-173/FIGURES/4.

[29] S. Vajda et al., "New additions to the ClusPro server motivated by CAPRI," Proteins: Structure, Function and Bioinformatics, vol. 85, no. 3, pp. 435–444, Mar. 2017, doi: 10.1002/prot.25219.

[30] D. Kozakov et al., "The ClusPro web server for protein-protein docking," Nat Protoc, vol. 12, no. 2, pp. 255–278, Feb. 2017, doi: 10.1038/NPROT.2016.169.

[31] G. Jones et al., "Elucidation of protein function using computational docking and hotspot analysis by ClusPro and FTMap," Acta Crystallogr D Struct Biol, vol. 78, no. Pt 6, p. 690, Jun. 2022, doi: 10.1107/S2059798322002741.

[32] I. T. Desta, K. A. Porter, B. Xia, D. Kozakov, and S. Vajda, "Performance and Its Limits in Rigid Body Protein-Protein Docking," Structure, vol. 28, no. 9, pp. 1071-1081.e3, Sep. 2020, doi: 10.1016/J.STR.2020.06.006.

[33] D. Kozakov et al., "How good is automated protein docking?," Proteins, vol. 81, no. 12, pp. 2159–2166, Dec. 2013, doi: 10.1002/PROT.24403.

[34] S. Zaib et al., "Designing multi-epitope monkeypox virus-specific vaccine using immunoinformatics approach," J Infect Public Health, vol. 16, no. 1, pp. 107–116, Jan. 2023, doi: 10.1016/J.JIPH.2022.11.033.

[35] N. Rapin, O. Lund, M. Bernaschi, and F. Castiglione, "Computational Immunology Meets Bioinformatics: The Use of Prediction Tools for Molecular Binding in the Simulation of the Immune System," PLoS One, vol. 5, no. 4, p. e9862, 2010, doi: 10.1371/JOURNAL.PONE.0009862.

[36] P. Stolfi et al., "In-silico evaluation of adenoviral COVID-19 vaccination protocols: Assessment of immunological memory up to 6 months after the third dose," Front Immunol, vol. 13, Oct. 2022, doi: 10.3389/FIMMU.2022.998262.

[37] C. Ragone et al., "Identification and validation of viral antigens sharing sequence and structural homology with tumor-associated antigens (TAAs).," J Immunother Cancer, vol. 9, no. 5, p. e002694, May 2021, doi: 10.1136/JITC-2021-002694.

[38] V. P. Kutyshenko, G. V. Mikoulinskaia, S. V. Chernyshov, A. Y. Yegorov, D. A. Prokhorov, and V. N. Uversky, “Effect of C-terminal His-tag and purification routine on the activity and structure of the metalloenzyme, l-alanyl-d-glutamate peptidase of the bacteriophage T5,” Int J Biol Macromol, vol. 124, pp. 810–818, Mar. 2019, doi: 10.1016/J.IJBIOMAC.2018.11.219.