

MERS virus spike protein HTL-epitopes selection and multi-epitope vaccine design using computational biology

Amit Joshi, Nahid Akhtar, Neeta Raj Sharma, Vikas Kaushik & Subhomoi Borkotoky

To cite this article: Amit Joshi, Nahid Akhtar, Neeta Raj Sharma, Vikas Kaushik & Subhomoi Borkotoky (2023): MERS virus spike protein HTL-epitopes selection and multi-epitope vaccine design using computational biology, *Journal of Biomolecular Structure and Dynamics*, DOI: [10.1080/07391102.2023.2191137](https://doi.org/10.1080/07391102.2023.2191137)

To link to this article: <https://doi.org/10.1080/07391102.2023.2191137>



[View supplementary material](#)



Published online: 19 Mar 2023.



[Submit your article to this journal](#)



[View related articles](#)



CrossMark

[View Crossmark data](#)



MERS virus spike protein HTL-epitopes selection and multi-epitope vaccine design using computational biology

Amit Joshi^{a,b} , Nahid Akhtar^c , Neeta Raj Sharma^d , Vikas Kaushik^d and Subhomoi Borkotoky^a

^aDepartment of Biotechnology, Invertis University, Bareilly, Uttar Pradesh, India; ^bDepartment of Biochemistry, Kalinga University, Raipur, India; ^cDepartment of Molecular Biology and Genetic Engineering, School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, Punjab, India; ^dDomain of Bioinformatics, School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, Punjab, India

Communicated by Ramaswamy H. Sarma

ABSTRACT

MERS-CoV, a zoonotic virus, poses a serious threat to public health globally. Thus, it is imperative to develop an effective vaccination strategy for protection against MERS-CoV. Immunoinformatics and computational biology tools provide a faster and more cost-effective strategy to design potential vaccine candidates. In this work, the spike proteins from different strains of MERS-CoV were selected to predict HTL-epitopes that show affinity for T-helper MHC-class II HTL allelic determinant (HLA-DRB1:0101). The antigenicity and conservation of these epitopes among the selected spike protein variants in different MERS-CoV strains were analyzed. The analysis identified five epitopes with high antigenicity: QSFYRLNGVGITQQ, DTIKYYSIIPHSIRS, PEPITSNLTKVAPQ, INGRLTTLNAFVAQQ and GDMYVYSAGHATGTT. Then, a multi-epitope vaccine candidate was designed using linkers and adjuvant molecules. Finally, the vaccine construct was subjected to molecular docking with TLR5 (Toll-like receptor-5). The proposed vaccine construct had strong binding energy of -32.3 kcal/mol when interacting with TLR5. Molecular dynamics simulation analysis showed that the complex of the vaccine construct and TLR5 is stable. Analysis using *in silico* immune simulation also showed that the prospective multi-epitope vaccine design had the potential to elicit a response within 70 days, with the immune system producing cytokines and immunoglobulins. Finally, codon adaptation and *in silico* cloning analysis showed that the candidate vaccine could be expressed in the *Escherichia coli* K12 strain. Here we also designed support vaccine construct MEV-2 by using B-cell and CD8+ CTL epitopes to generate the complete immunogenic effect. This study opens new avenues for the extension of research on MERS vaccine development.

ARTICLE HISTORY

Received 4 July 2022
Accepted 3 January 2023

KEYWORDS

MERS-CoV; immunoinformatics; molecular docking; immunosimulation; multi epitope vaccine

Introduction

The Middle East respiratory syndrome (MERS) is caused by MERS-CoV belonging to the beta-coronavirus family. MERS is a respiratory disease that was reported for the first time in 2012 in Saudi Arabia and since then it has been reported in 27 countries in Asia, Africa, Europe and North America (WHO, 2019). So far, 2589 laboratory confirmed cases of MERS have been reported with the case fatality ratio of 34.5% (WHO, 2022). Humans can become infected by coming into direct or indirect contact with infected dromedary camels (Cauchemez et al., 2016). The human-to-human transmission can occur among close household contacts (Alsalamy & Arabi, 2015). Currently, no specific vaccine is available against MERS; however, various vaccine candidates are being developed and are in different stages of clinical trials (Bosaeed et al., 2022; Choi et al., 2020). As MERS has epidemic potential, high case-fatality ratio and lacks specific treatment or vaccine it is important to continue the search for novel and

effective therapies to protect humans from this highly pathogenic virus (Memish et al., 2020).

The MERS-CoV genome has at least 10 open reading frames, generating four structural proteins (Spike, Envelope and Membrane), five accessory proteins (ORF3, ORF4a, ORF4b, ORF5 and ORF8b), N proteins and 16 non-structural proteins, like SARS-CoV (NSP1-NSP16) (Molaei et al., 2021). Different studies have reported that the spike protein of MERS CoV can be an ideal target for vaccine development and various MERS candidate vaccines that are currently being developed are based on the MERS-CoV spike protein (Zhang et al., 2015; Zhou et al., 2018). The interaction between the cellular receptor dipeptidyl peptidase-4 (DPP4) and surface spike protein assists MERS-CoV entrance into host cells, preceded by membrane fusion between the virus and the active site. The MERS-CoV spike protein is a major target for treatments aimed at preventing virus replication and membrane fusion (Al Johani & Hajer, 2016). Because of

CONTACT Subhomoi Borkotoky subhomoy.bk@gmail.com Department of Biotechnology, Invertis University, Bareilly, Uttar Pradesh 243123, India; Vikas Kaushik vikas.1466@lpu.co.in Domain of Bioinformatics, School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, Punjab, India

Supplemental data for this article can be accessed online at <https://doi.org/10.1080/07391102.2023.2191137>.

© 2023 Informa UK Limited, trading as Taylor & Francis Group

its functional characterization and role as the principal antigenic component responsible for triggering host immune responses, spike protein is an important target for vaccine development. For both SARS-CoV and MERS-CoV virions, several inactivated vaccines are available and tested in animal models. UV and formaldehyde have been used to inactivate the MERS-CoV to develop inactivated virus vaccine that could considerably boost antibody levels against MERS-CoV virions (Zhuang et al., 2022). A formaldehyde-based inactivated MERS-CoV vaccine has been reported to induce neutralizing antibodies in rodents (Deng et al., 2018). MERS001, a candidate simian adenovirus-vectored vaccine expressing the full-length spike surface glycoprotein of MERS-CoV has completed its phase 1 clinical trial (Folegatti et al., 2020). In a plethora of studies the immunoinformatics and computational biology tools have aided in the development of vaccine candidates against a wide range of harmful pathogens (Dey et al., 2022a, 2022c; Mahapatra et al., 2022). Strategies like structural and reverse vaccinology have remarkably increased the rate of viral candidate vaccine development (Maria et al., 2017). In the present study, different strains of MERS-CoV were selected and epitopes from their spike protein that shows an affinity for T-helper MHC-class II allelic determinant (HLA-DRB1:0101) were predicted. These epitopes were also checked for antigenicity and conservancy analysis across the selected spike protein variants. Then, a novel multi-epitope vaccine candidate (MEV 1) was designed using linkers and adjuvant molecules. The structure of the final vaccine construct was assessed by various structure validation tools. To create the full immunogenic impact, we additionally built the support vaccine construct MEV-2 employing B-cell and CD8+ CTL epitopes. CD8+ CTL epitopes are peptide sequences that the adaptive immune system's T-cytotoxic cells are able to identify. These epitopes, which are often formed from proteins or antigens, aid in the recognition and destruction of foreign or aberrant cells by T lymphocytes. Protein sequences known as B-cell epitopes are identified by B-cells, which are a component of the adaptive immune system. These epitopes, which come from proteins or antigens, aid the B-cells in identifying and eliminating foreign or aberrant cells. Epitopes on B-cells are frequently linear and continuous. Finally, the vaccine constructs were subjected to molecular docking and molecular dynamics analysis with TLR5. The computational immune simulation analysis of the vaccine candidate was also performed. This study aims to aid in the development of a vaccine candidate against the MERS-CoV virion to investigate the therapeutic role of epitopes in stimulating the immune system.

Methodology

Strain selection

Available strains of MERS virions were selected from the NCBI-GenBank database based on available literature to access spike protein sequences for each of the strains (Table 1). All spike protein sequences retrieved from the 5 different strains of MERS-CoV (Table 1) were subjected to multiple sequence alignment using the Clustal-Omega server

(Sievers & Higgins, 2014). In addition, the AllergenFP program (Dimitrov et al., 2014) was utilized to assess the allergenicity of spike proteins for the MERS strains under consideration (Table 1).

HTL epitopes screening

Studies have demonstrated that CD4+ T-cell epitopes (HTL epitopes) are less variable than B-cell and CD8+ T-cell epitopes, and the availability of data supporting the multiple MHC Class II HLA variations makes HTLs preferable for our study (Sanchez-Trincado et al., 2017). HTL epitopes were analyzed for MERS wild type, Saudi Arabian strain by deploying NetMHC II Pan 4.0 server (Reynisson et al., 2020) against HLA DRB1:0101, later population coverage analysis was also done by using IEDB server (<http://tools.iedb.org/population/>) against all major MHC ClassII HLA determinants (HLA-DRB1*03:01, HLA-DRB1*11:01, HLA-DRB1*13:01 and HLA-DRB1*15:01) as our study is primarily focused on HTL epitopes determination. For the determination of the antigenicity of the epitopes Vaxjen2.0 server was used which predicts if the bacterial, fungal, viral and tumour peptides are antigenic or not by evaluating various physiochemical properties of the proteins using an auto-cross-covariance method (Doytchinova & Flower, 2007). In the VaxiJen 2.0 server, the epitopes' amino acid sequences in plain format were used as input, and "Virus" was selected as the target organism. The threshold score for virus peptides to be antigenic is 0.4. Further, we conducted conservancy analysis for all the selected epitopes in five strains by deploying the IEDB server. ToxinPred which applies an SVM-based approach to predict peptide toxicity was employed to predict the epitopes' toxicity (Gupta et al., 2013). The reason for using the ToxinPred server for toxicity analysis of the epitopes was that it can predict the toxicity of peptides with 94.5% accuracy (Gupta et al., 2013). Furthermore, the potential of the selected epitopes to induce interleukin-2, interleukin-4 and interferon- γ were also predicted using IL2Pred (Lathwal et al., 2021), IL4Pred (Dhanda et al., 2013a) and IFNepitope webserver (Dhanda et al., 2013b), respectively. In IL2Pred, IL4Pred and IFNepitope epitopes sequences were used as input in FASTA format and the default parameters were used. Finally, the allergenicity of the epitopes was predicted by AllerCatPro version 2.0 (Nguyen et al., 2022a). AllerCatPro was used to predict the allergenicity of the selected epitopes because it has higher accuracy (84.7%) in comparison to other allergenicity prediction tools such as AlgPred 2.0 (accuracy of 52.3%) and AllerTOPv2 (accuracy of 75.0%; Nguyen et al., 2022a).

Multiepitope vaccine construct

Strong binding and antigenic epitopes were used for designing the final vaccine construct. *Salmonella Dublin* flagellin protein and RS09 were used as adjuvants and PADRE sequence was used to improve stability against enzymatic degradation and provide universal immune stimulation in a heterogeneous population as it can bind to 15 of the 16 most common human HLA-DR types with high affinity

Table 1. List of Spike protein Accession numbers for different MERS Virus strains (retrieved from NCBI-GenBank database) analysed for allergenicity (AllergenFP TSI: Tanimoto similarity index).

MERS strains	Accession number	AllergenFP TSI Score	Allergenicity Inference
South Korean Strain	QKF93418.1	0.84	Non-Allergen
Abu Dhabi, Dubai Strain	ASU90340.1	0.84	Non-Allergen
Oman-Thailand Common Strain	ALDS1904.1	0.84	Non-Allergen
Saudi Arabian Strain	AHI48572.1	0.84	Non-Allergen
Wild Type, Saudi Arabian Strain	YP_009047204.1	0.84	Non-Allergen

(Ghaffari-Nazari et al., 2015; Robinson et al., 2017; Skwarczynski & Toth, 2016). Finally, GPGPG linker peptides were used to connect epitopes, adjuvants and PADRE with each other (Akhtar et al., 2021; Jain et al., 2021).

Structural modelling

The secondary structure of the MERS-CoV vaccine constructs (MEV-1 and MEV-2) was predicted by the PSIPRED server (Buchan & Jones, 2019). This web server helps in the prediction of beta-sheets, alpha helices, and coils in proteins by using feed-forward neural networks (Buchan & Jones, 2019). The tertiary structure of the candidate multi-epitope vaccine construct was modelled using the I-TASSER server. The model was further submitted to CABS-flex 2.0 server (Kuriata et al., 2018) for optimization. Ramachandran plot analysis (<https://zlab.umassmed.edu/bu/rama/index.pl>), ProSA-web server (Wiederstein & Sippl, 2007) and ERRAT plot (<https://saves.mbi.ucla.edu/>) were used for the validation of the structure. The full-length structure of TLR5 (UniProt ID: O60602) was obtained from the AlphaFold server (Varadi et al., 2022).

Molecular docking

For protein-protein docking, the rigid-body protein-protein docking program: ZDOCK server (Pierce et al., 2014) was used. ZDOCK scores employ the Fast Fourier Transform algorithm and a combination of shape complementarity, electrostatics, and statistical potential terms to enable an efficient global docking search on a 3D grid. The top ten models were downloaded and their binding energies were compared using the PBDePISA server (Krissinel & Henrick, 2007). The docked conformations were visualized using UCSF Chimera (Pettersen et al., 2004).

Immune simulation

To determine the immunogenicity and immune response profile of the CanineCV MEV, the C-IMMSIM webserver available at <https://kraken.iac.rm.cnr.it/C-IMMSIM/> was used (Rapin et al., 2010). The C-IMMSIM webserver applies position-specific scoring matrices obtained from the machine learning approach to predict the immune response. It has been recommended that the minimum interval between the first and second dose of the vaccine should be for weeks and in some cases, the minimum interval can be 8 weeks, 3 months or 6 months (Castiglione et al., 2012; Kar et al., 2020; Robinson et al., 2017). Hence, in this study immune response to the vaccination was predicted using three

injections at four weeks interval (Abraham Peele et al., 2021; Safavi et al., 2020). In this analysis, the default parameters were used except for the time step of injection. The time steps of 1, 84 (equivalent to 4 weeks) and 168 (equivalent to 8 weeks) were used.

Physiochemical characterization of multi-epitope vaccine construct

The ProtParam online server was used to predict the vaccine construct's physicochemical characteristics (Gasteiger et al., 2005). This webserver aids in assessing different physicochemical attributes of query protein like isoelectric point, number of amino acids, stability, amount of positively and negatively charged amino acids, molecular weight, half-life, extinction coefficient and aliphatic index. The amino acid sequence of the vaccine construct in the form of one letter code was used as input. The final vaccine construct's solubility was assessed using the SOLpro online program (Magnan et al., 2009). The vaccine construct's amino acid sequence in plain format without a header was used as input and default parameters of the SOLpro webserver were used. The vaccine construct's allergenic potential was determined by AllerCatPro 2.0 server (Nguyen et al., 2022a). The vaccine construct's amino acid sequence in FASTA format was used as input and default parameters were used in the AllerCatPro 2.0 server.

In silico cloning and codon use adaption parameters

The JCcat website was used to optimize the codons of the candidate MERS vaccine construct for expression in *E. coli* K12 strain to ensure effective cloning and expression of the vaccine construct (Grote et al., 2005). Rho-independent transcription terminators, bacterial ribosomal binding sites and cleavage sites of various restriction enzymes were eliminated during the codon optimization of the candidate MERS vaccine construct. Finally, *in silico* cloning was performed using the SnapGene restriction cloning module. During *in silico* cloning, the codon-optimized vaccine construct's sequence was inserted between the pET28a(+) vector's Xhol (158) and EcoRI (192) restriction sites.

Molecular dynamics simulation

Molecular dynamics (MD) is a widely used tool for determining the dynamic interactions of biomolecules with their binding partners (Borkotoky et al., 2021). MD simulation was performed to better understand the binding dynamics and stability of the vaccine construct-TLR5 complex. The MD

simulations were carried out with GROMACS v.2018 (Van Der Spoel et al., 2005) and with the Gromos54a7 force field (Huang et al., 2011). The systems were solvated with SPC water molecules in a cubic box of dimension $1.2\text{ nm} \times 1.2\text{ nm} \times 1.2\text{ nm}$. The solvated systems were neutralized by adding 5 NA^+ ions followed by steepest descent minimization for 50,000 steps with a tolerance of $1000\text{ kJ mol}^{-1}\text{ nm}^{-1}$. Equilibrations of 500 ps NVT and 1 ns NPT were performed at 300 K and 1 bar using the V-rescale thermostat and Berendsen barostat (Kneller et al., 2020). Leap-frog integration with Nose-Hoover and Parrinello-Rahman couplings (Martonak et al., 2003) was used for the final production run of 50 ns. Using the built-in GROMACS modules, a thorough analysis of the final trajectory was performed.

Support vaccine construct MEV-2 designing from B-cell and T-cytotoxic cell epitopes

B-Cell epitopes were identified for spike protein by deploying ABCPred tool (Saha & Raghava, 2006), and CD8+ MHC Class I T cytotoxic cell epitopes were identified by using NetMHCpan-4.0 (Reynisson et al., 2020). Like MEV-1, *Salmonella dublin* flagellin protein and RS09 were used as adjuvants and PADRE sequence was used to improve stability against enzymatic degradation and provide universal immune stimulation in a heterogeneous population.

Results

Spike protein sequence analysis for selected strains

Retrieved FASTA sequences of spike proteins for all the strains of MERS-CoV were analyzed for the comparative assessment using Clustal-Omega and it was found that all 4 selected strains showed more than 99% similarity with wild type (see Supplementary Figure 1, Supplementary Table 1, Supplementary Figure 2 for multiple alignment and phylogenetic tree).

HTL-epitopes screening from spike protein

Altogether 1339 HTL-epitopes from the wild-type Spike protein were predicted of which 27 were weak binders to HLA-DRB allelic determinant while eight epitopes were found to be strong binders. Strong binding (SB) epitopes were checked for antigenicity and it was noted that 5 epitopes: QSIFYRLNGVGITQQ, DTIKYYSIIPHSIRS, PEPITSLNKTYVAPQ,

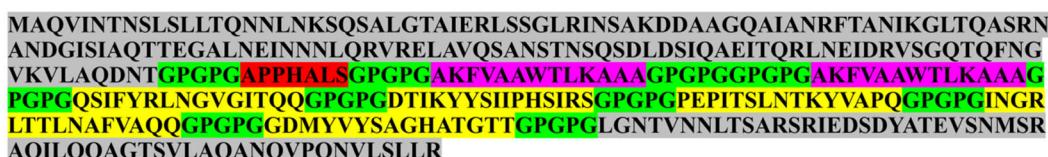
INGRLTTLNAFVAQQ and GDMYVYSAGHATGTT have antigenicity greater than 0.4 threshold value (see Table 2). Toxicity analysis for selected epitopes was conducted by the ToxinPred tool (Gupta et al., 2013), and all the considered epitopes were found to be non-toxic in nature on the basis of their SVM scores (see Table 3). Furthermore, all the selected epitopes were predicted as non-allergic by the AllerCatPro webserver (Table 3). Four out of five epitopes selected for the MERS-CoV vaccine construct design were predicted to have the ability to induce interleukin-4 production (Table 3). Similarly, three out of five epitopes selected for the MERS-CoV vaccine construct design were predicted to have the ability to induce interleukin-4 production (Table 3).

Multi-epitope vaccine construct

Salmonella dublin flagellin protein and RS09 peptide were used as adjuvants. RS09 is a toll-like receptor 4 agonist, while flagellin protein is a toll-like receptor 5 agonist. Toll-like receptor agonists help to activate both innate and adaptive immunity. Hence, these adjuvants were employed in the MERS-CoV vaccine construct design. The PADRE sequence was added to the vaccine to improve its metabolic stability as it contains D-alanine, L-cyclo-hexylalanine and amino-caproic acid residues that can significantly increase the stability against enzymatic degradation (Skwarczynski & Toth, 2016). The schematic representation of the designed MERS-CoV candidate multi-epitope vaccine is provided in Figure 1.

Modeling and docking of the vaccine construct (MEV-1)

The secondary structure prediction of the MEV-1 using PSIPRED shows the presence of coil, helix and strand (Supplementary Figure 3). The tertiary model of the vaccine construct predicted by I-TASSER webserver with the best C-score ($C\text{-score} = -1.07$) was selected for further analysis (Figure 2A). Typically, the C-score value ranges from -5 to 2 and higher C-score values imply the model of higher significance (Yang et al., 2015). Among the total 357 residues, 91.84% are in highly preferred observations, 6.38% residues are in preferred observations, 1.77% residues are in questionable observations of the Ramachandran plot, and the Z-Score obtained from the ProSA-web server was -4.95 (Figure 2B, C). The ERRAT plot score for the vaccine construct model was 50.14 (Figure 2D). In the case of the TLR5 model (Figure 2E), out of 784 residues 98.59% are in highly preferred observations, 1.28% residues are in preferred observations,



Legend:

Linker HTL Epitopes RS09 Adjuvant PADRE Sequence Flagellin protein

Figure 1. Schematic representation of multi-epitope vaccine design.

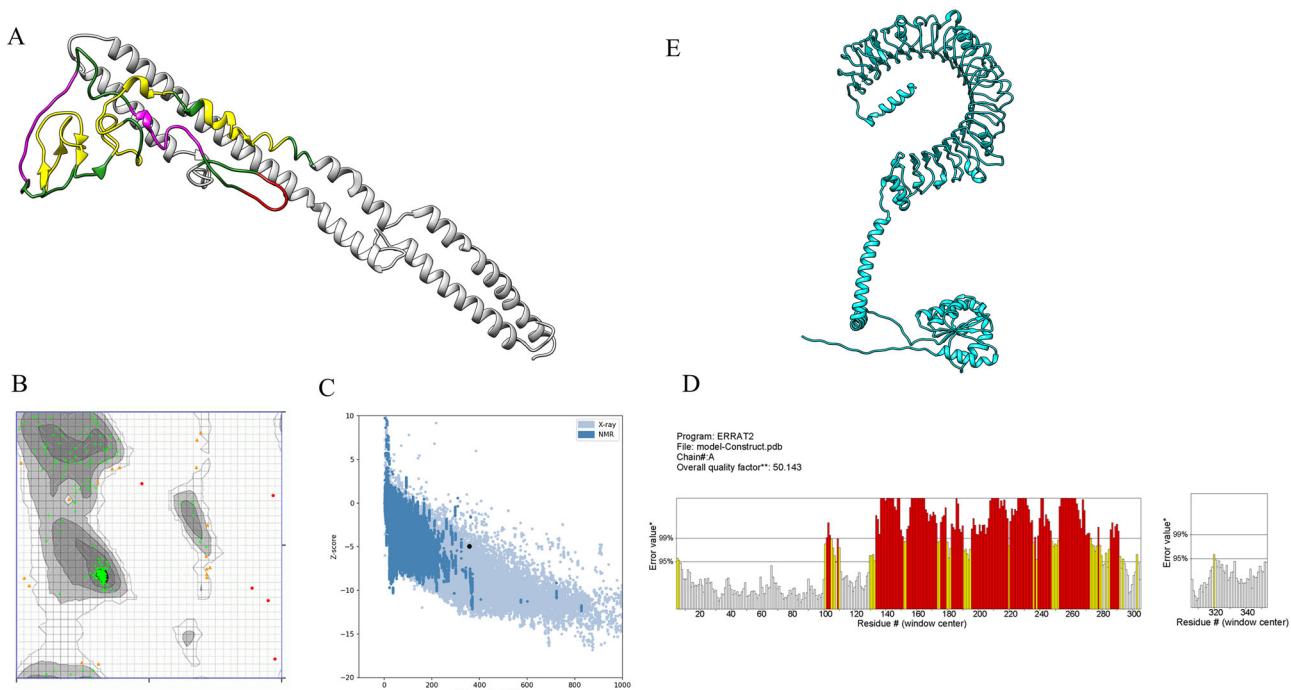


Figure 2. (A) Three-dimensional model of the multi-epitope vaccine, MEV-1 (Linker: green, HTL epitopes: yellow, RS09 adjuvant: red, PADRE sequence: magenta, *Salmonella dublin* flagellin protein: light grey), (B) Ramachandran Plot, (C) ProSA model quality, (D) ERRAT plot of the vaccine construct, (E) full-length model of TLR5.

Table 2. Screened HTL-Epitopes from spike protein of MERS CoV.

Epitope from MERS virus spike protein	Binding affinity score	Affinity (nM)	Binding level	VaxiJen Score (antigenicity)	Conservancy in Selected strains	Inference
QSIFYRLNGVGITQQ	0.864	4.35	SB	0.4984	100% (5/5)	Selected
YDAYQNLVGYYSDDG	0.717	21.24	SB	-0.2796	100% (5/5)	Not-selected
DTIKYYSIIPHSIRS	0.845	5.31	SB	0.4097	100% (5/5)	Selected
RGVFQNCTAVGVRQQ	0.748	15.19	SB	0.1415	100% (5/5)	Not-selected
GGGWLVASGSTVAMT	0.795	9.14	SB	0.2852	100% (5/5)	Not-selected
PEPITSLNKTYVAPQ	0.661	38.92	SB	0.4794	100% (5/5)	Selected
INGRLTTLNAFVAQQ	0.777	11.05	SB	0.7138	100% (5/5)	Selected
GDMVVYSAAGHATGTT	0.695	27.01	SB	1.085	100% (5/5)	Selected

Table 3. Toxicity, allergenicity, IL-2, IL-4 and interferon- γ inducing potential analysis of selected epitopes.

Peptide ID	Peptide sequence	Toxicity	Allergenicity	Interleukin-2 inducing potential	Interleukin-4 inducing potential	Activation potential of interferon- γ
Seq1	QSIFYRLNGVGITQQ	Non-toxin	Non-allergen	IL-2 non-inducer	Non IL4 inducer	No
Seq2	DTIKYYSIIPHSIRS	Non-toxin	Non-allergen	IL-2 inducer	IL4 inducer	No
Seq3	PEPITSLNKTYVAPQ	Non-toxin	Non-allergen	IL-2 inducer	IL4 inducer	No
Seq4	INGRLTTLNAFVAQQ	Non-toxin	Non-allergen	IL-2 inducer	IL4 inducer	No
Seq5	GDMVVYSAAGHATGTT	Non-toxin	Non-allergen	IL-2 non-inducer	IL4 inducer	No

0.13% residues are in questionable observations of the Ramachandran plot and the Z-Score obtained from the ProSA-web server was -7.76 . The ERRAT plot score for the TLR5 model was 81.48 (Supplementary Figure 4). These results suggest that the overall qualities of the models are good.

Post optimization and validation of the vaccine construct's tertiary structure, the vaccine construct was docked with the TLR5 structure. The top ten models obtained from the Z-Dock server were further evaluated for their binding energy using the PDBePISA server (Krissinel & Henrick, 2007). Based on the binding energy complex 8 having -32.3 kcal/mol (Supplementary Table 2) was selected for further evaluation.

The construct formed a total of six H-bonds (Table 4, Figure 3). All the H-bonds are formed in the epitope region of the construct namely Arg235, Ser236, Arg265, Thr293 and Tyr287.

Immune-simulation of candidate multi-epitope vaccine (MEV-1)

The immune simulation shows that the first injection of the vaccine increased the levels of immunoglobulin activity (i.e., IgG1 + IgG2, IgM and IgG + IgM antibodies). Moreover, the second and third injections significantly increased the antibody titer in comparison to the first injection (Figure 4A).

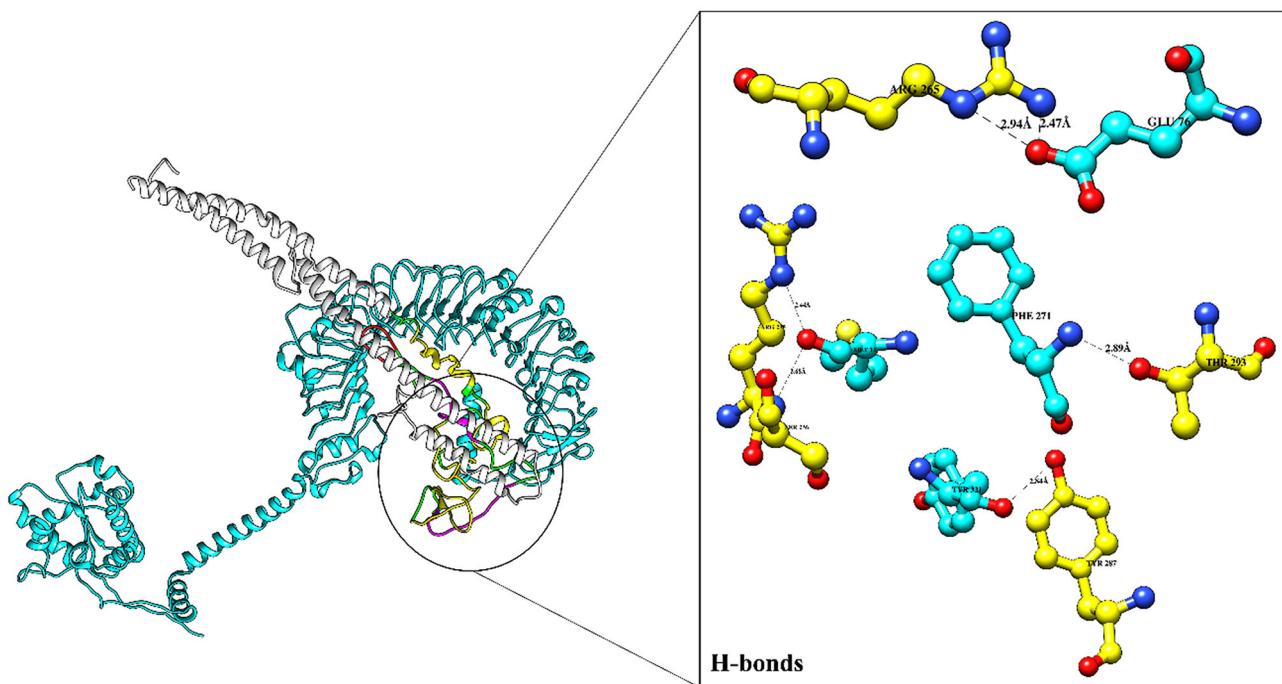


Figure 3. The docked complex of TLR5 (cyan) with the MEV-1. The H-bonds are shown in the inset. The color scheme of the vaccine construct is identical to Figure 2.

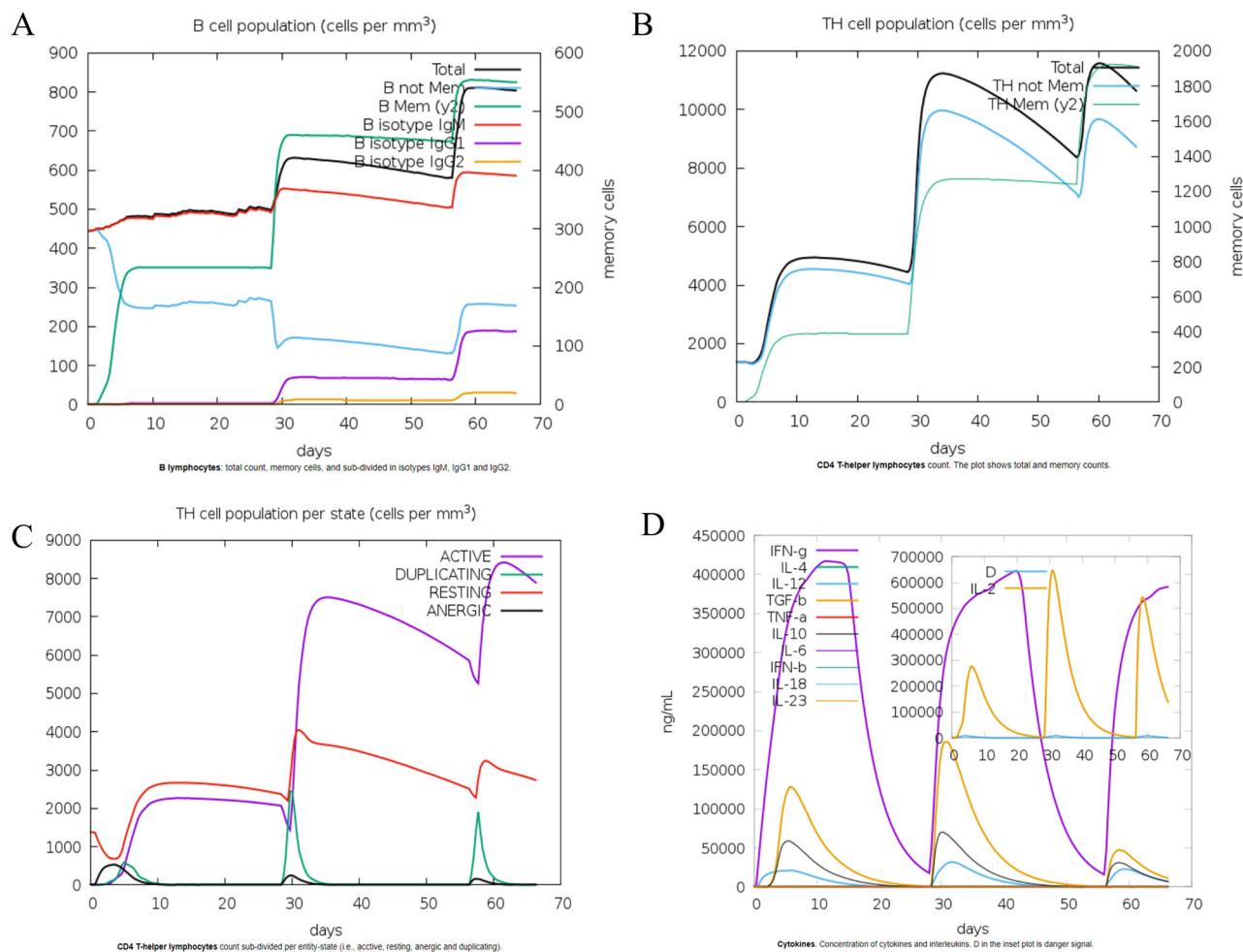


Figure 4. Immune simulation results: (A) Levels of immunoglobulins after injecting multi-epitope vaccine construct, (B) CD4 + T-helper lymphocytes population after injecting multi-epitope vaccine construct, (C) CD4 + T-helper lymphocytes levels based on active, resting, anergic and duplicating entry states and (D) Cytokines levels after injecting multi-epitope vaccine construct.

Table 4. The details of H-bond interactions of the TLR5 and the MEV-1.

Sl. No.	TLR 5			Distance (Å)	MEV-1		
	Residue name	Residue number	Atom name		Residue name	Residue number	Atom name
1	MET	14	O	2.44	ARG	235	NE
2	MET	14	O	2.68	SER	236	N
3	GLU	76	OE1	2.94	ARG	265	NE
4	GLU	76	OE1	2.47	ARG	265	NH2
5	PHE	271	N	2.89	THR	293	OG1
6	TYR	321	O	2.84	TYR	287	OH

Table 5. Physiochemical analysis of multi-epitope vaccine, MEV-1.

Physiochemical properties	Values/Results
Allergenicity	Non-allergen
No. of Amino Acids	357 residues
Molecular weight	36529.62 Dalton
Total number of negatively charged residue	20
Total number of positively charged residue	25
Formula	C ₁₅₈₀ H ₂₅₄₃ N ₄₇₅ O ₅₁₅ S ₃
Total number of amino acids	5116
Solubility	0.537 (Soluble)
Estimated half-life	30 h (in mammalian reticulocytes) >20 h (in yeast cells) >10 h (in <i>E. coli</i>) 32.06 (Stable)
Instability Index	79.10
Aliphatic Index	
Grand average of hydropathicity (GRAVY Score)	-0.342 (below 0 values indicate hydrophilic globular nature while above 0 values indicate hydrophobic membranous nature)

The third injection also increased the titer of the IgG2 antibody. The analysis also predicted that the immunization could increase the total B-cell population after every injection of the vaccine candidate (Figure 4A). The same figure also shows the increase in IgM, an antibody that is generated after the initial exposure to antigen. Additionally, the expression of memory cells is also increased after each vaccination suggesting activation of robust secondary immune response (Figure 4A). However, there is no significant increase in the B not memory cells (Figure 4A). The T-helper cell population also increased after each vaccination (Figure 4B). Furthermore, there is an increase of active T helper cell population after the vaccinations (Figure 4C). The resting and duplicating T helper cell population increased after the first two injections, however; after the third injection their population slightly decreased (Figure 4C). Thus, increase in memory B cells and resting T cells after vaccination imply the activation of adaptive immune response on which the vaccination is based. The vaccination also stimulated the interferon- γ , TGF-beta, interleukin-10, and interleukin-12 production (Figure 4D). There was an increase in the number of interferon- γ , TGF-beta, interleukin-12 and interleukin-10 concentration after the first two vaccinations; however, their concentration decreased after the third vaccination in comparison to the first two (Figure 4D). Overall, the C-IMMSIM simulations predicted that the MERS-MEV-1 could activate the immune response.

Physicochemical characterization of multi-epitope vaccine construct

Multi-epitope vaccine construct was checked for physicochemical characters using *in silico* tools like AllerCatPro 2.0, SOLpro (Magnan et al., 2009) and ProtParam. Different

physicochemical parameters of the vaccine construct are listed and represented in Table 5.

In silico cloning and codon use adaption parameters

The candidate MERS vaccine construct's Codon Adaptation Index was 0.993 after the JCcat website codon adaptation, indicating a high degree of sequence expression. The codon-adapted candidate MERS vaccine sequence has 56.11% GC content. The candidate vaccine construct was inserted between Xhol (158) and EcoRI (192) restriction sites of the pET28a (+) vector. The size of the cloned product was 6406 bp. The final cloned vaccine construct is demonstrated in Figure 5 and the inserted vaccine construct is displayed in orange color.

MD simulation of the docked complex

To measure the conformational stability of the MEV1-TLR5 complex after 50 ns of simulations, the RMSD (Root Mean Square Deviation) profile for backbone residues, the Rg (Radius of Gyration) and H-bond (Hydrogen Bond) graphs were generated. From the RMSD graph (Figure 6A), it was observed that the complex is stable after 15 ns. A similar trend was observed in the Rg plot (Figure 6B), where a gradual decrease of Rg value was observed after 15 ns denoting the compactness and stability of the complex. The final snapshot (Figure 6C) from the simulated trajectory was also extracted at 50 ns using 'trjconv' utility. The structure was found to be compact as compared to the docked complex (Figure 3). The number of hydrogen bond interactions of the complex (Figure 6D) reached a maximum of 27 and remained at 15 on average from 20 ns. These results suggest that the MEV1-TLR5 complex is stable.

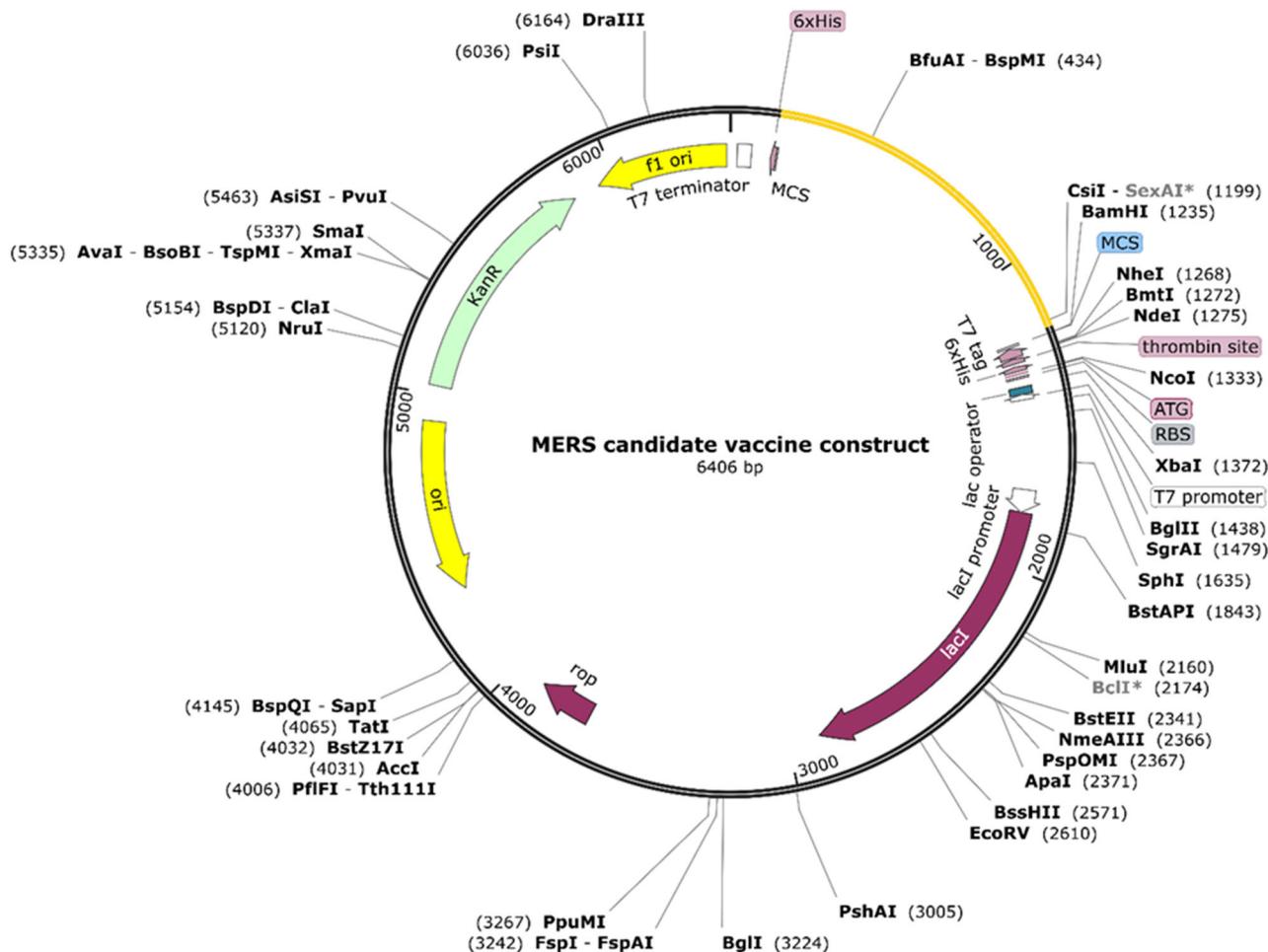


Figure 5. *In silico* cloning of the candidate MERS vaccine in pET28a (+) vector.

Population coverage analysis

The selected epitopes were also checked for population coverage analysis by deploying IEDB server, as the study primarily focused on HTL epitopes so we used HLA-DRB1*01:01, HLA-DRB1*03:01, HLA-DRB1*11:01, HLA-DRB1*13:01 and HLA-DRB1*15:01 alleles. It was found that selected epitopes have 57.59% population coverage worldwide (Table 6 and supplementary Figure 5).

Multi-epitope vaccine (MEV-2) design with B-cell epitopes and T-cytotoxic cell epitopes

For B-cell epitopes (14-mer) determination ABCpred tool (Saha & Raghava, 2006) was used, and the maximum threshold value of 0.9 was set, and we obtained 4 continuous B-cell epitopes out of 1395 epitopes. For T-cytotoxic cell epitopes (9-mer) determination, NetMHCpan 4.0 webserver (Reynisson et al., 2020) was used. Based on scores and ranks total 7 epitopes were selected from 1344 epitopes that were interacting with allelic determinants HLA-A01:01, HLA-A03:01 and HLA-B15:01. 3 epitopes strongly bind with HLA-A01:01 while 4 epitopes strongly bind with HLA-B15:01. No epitope passes the percent rank threshold <0.03 for HLA-A03:01.

After that we conducted antigenicity, allergenicity, toxicity, IL-2, IL-4 induction *in silico* analysis for the epitopes, we finally filtered three B-cell epitopes that can lead to humoral

immunity production (see Table 7); and three T-cytotoxic cell epitopes that interacts with MHC Class-I HLA determinants (see Table 8).

MEV-2 was designed with B-cell epitopes and T-cytotoxic cell epitopes along with flagellin protein and RS09 adjuvants (schematic representation provided in Figure 7). Calculated physiochemical properties for MEV-2 provided in Table 9.

Modeling and docking of the MEV-2 with TLR5

The tertiary model of the vaccine construct predicted by iTASSER webserver with the best C-score (C-score = -0.12) was selected for further analysis (Figure 8A). Among the total 276 residues, 90.58% are in highly preferred observations, 6.52% residues are in preferred observations, 2.89% residues are in questionable observations of the Ramachandran plot and the Z-Score obtained from the ProSA-web server was -4.13 (Figure 8B, C). The ERRAT plot score for the vaccine construct model was 50.14 (Figure 8D). These results suggest that the overall qualities of the models are good.

Post optimization and validation of the vaccine construct's tertiary structure, the vaccine construct was docked with the TLR5 structure. The top ten models obtained from the Z-Dock server were further evaluated for their binding energy using the PDBePISA server (Krissinel & Henrick, 2007). Based on the binding energy complex having -24.0 kcal/mol was

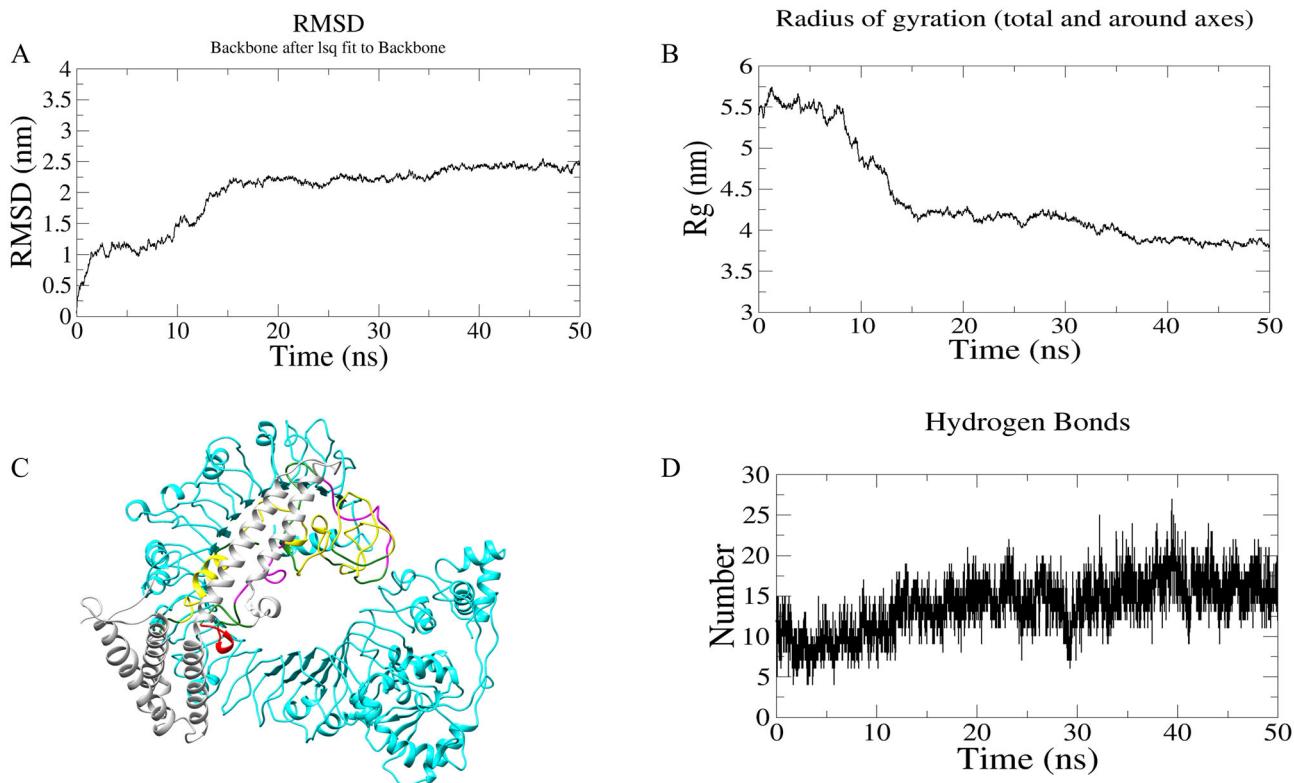


Figure 6. The MD simulation results: (A) RMSD of the protein backbone, (B) Rg evolution, (C) Final snapshot (at 50 ns) of the simulated trajectory and (D) H-bonds between the vaccine construct and TLR5. The color scheme of the vaccine construct is identical to Figure 2.

Table 6. Population coverage analysis of selected epitopes (+: restricted, -: not restricted).

Epitope	Coverage	HLA allele						Total HLA hits	
		(Genotypic frequency (%))							
		HLA-DRB1*01:01	HLA-DRB1*03:01	HLA-DRB1*11:01	HLA-DRB1*13:01	HLA-DRB1*15:01			
Epitope #1: QSIFYRLNGVGITQQ	57.59%	+	+	+	+	+	+	5	
Epitope #2: DTIKYYSIIPHSIRS	57.59%	+	+	+	+	+	+	5	
Epitope #3: PEPITSNLNTKYVAPQ	57.59%	+	+	+	+	+	+	5	
Epitope #4: INGRLLTNAFVAQQ	57.59%	+	+	+	+	+	+	5	
Epitope #5: GDMYVYSGHATGTT	57.59%	+	+	+	+	+	+	5	
Epitope set	57.59%	5	5	5	5	5	5	25	

Table 7. B-cell epitopes prediction by using wild type strain.

S.No.	B-Cell Epitopes	Start position	Score (>0.9)	Vaxijen Score (Threshold >0.4)	Toxicity	Allergenicity	IL-2 Inducing Potential	IL-4 Inducing Potential	IFN-gamma activation potential	Inference
1	NITITYQGLFPYQG	121	0.94	1.3203	Non-Toxin	Non-Allergen	Inducer	Inducer	No	Selected
2	WPRPIDVSKADGII	99	0.92	0.4075	Non-Toxin	Non-Allergen	Inducer	Inducer	No	Selected
3	TTTNEAFQKVQDAV	1068	0.91	0.2709	Non-Toxin	Non-Allergen	Inducer	Non-Inducer	No	Not-Selected
4	NMFQFATLPVYDTI	332	0.9	0.4129	Non-Toxin	Non-Allergen	Inducer	Inducer	No	Selected

selected for further evaluation (Supplementary Table 3). The construct formed a total of 19 H-bonds (Table 10, Figure 9).

MD simulation of the MEV-2-TLR5 complex

To measure the conformational stability of the MEV-2-TLR5 complex after 50 ns of simulations, the RMSD profile for backbone residues, the Rg and H-bond graphs were generated. The RMSD graph showed a steady pattern of RMS deviation after 30 ns (Figure 9A). A stable trend was observed in the Rg plot (Figure 9B) without any strong deviations. The final

snapshot (Figure 9C) from the simulated trajectory was also extracted at 50 ns. Both the Rg plot and the snapshot shows that although the complex is stable, it does not adopt more compact conformation like MEV-1. The number of hydrogen bond interactions of the complex (Figure 9D) reached a maximum of 19 and remained at 09 on average. These results suggest that the MEV2-TLR5 complex is stable.

Discussion

MERS-CoV is a unique human coronavirus that presents a serious threat to public health on a global scale, necessitating the

Table 8. T Cytotoxic-cell epitopes (CD8+ CTLs) prediction.

S.No.	T-Cytotoxic Cell Epitopes interacts with HLA-A*01:01	Rank (Threshold <0.03)	Vaxijen Score (Threshold >0.4)	Toxicity	Allergenicity	IL-2 inducing potential	IL-4 inducing potential	IFN-gamma activation potential	Inference
1	QVDQLNSSY	0.0206	0.3844	Non-Toxin	Allergen	Inducer	Inducer	No	Not-Selected
2	ATDCSDGNY	0.0224	0.7838	Non-Toxin	Non-Allergen	Inducer	Inducer	No	Selected
3	FSSRYVDLY	0.0259	1.0488	Non-Toxin	Allergen	Inducer	Non-Inducer	No	Not-Selected
S.No.	T-Cytotoxic Cell Epitopes interacts with HLA-B*15:01	Rank (Threshold <0.03)	Vaxijen Score (Threshold >0.4)	Toxicity	Allergenicity	IL-2 inducing potential	IL-4 inducing potential	IFN-gamma activation potential	Inference
1	SQFNYKQSF	0.0076	1.1913	Non-Toxin	Non-Allergen	Inducer	Inducer	No	Selected
2	IIYPQGRTY	0.0084	0.1547	Non-Toxin	Allergen	Non-Inducer	Inducer	No	Not-Selected
3	GQGTHIVSF	0.0292	0.4695	Non-Toxin	Non-Allergen	Non-Inducer	Non-Inducer	No	Selected
4	VVKALNESY	0.0296	0.4901	Non-Toxin	Allergen	Inducer	Inducer	No	Not-Selected

MAQVINTNSLTLTQNNLNKSQSALGTIAERLSSGLRINSAKDDAAGQAIANRFTANIKGLTQASRNANDGISIAQTTEGALNEIN
 NNLQRVRELAVQSANSTNSQSDLDSIQAETQRLNEIDRVSGQTQFNGVKVLAQDNT GPGPG APPHALS GPGPG AKFVAWTL
 KAAA GPGPG AKFVAWTLKAAA GPGPG NITITYQGLFPYQG GPGPG WPRPIDVSKADGH GPGPG NMFFQFATLPVYDTI GPGP
 ATDCSDGNY GPGPG SQFNYKQSF GPGPG GQGTHIVSF GPGPG LGNTVNNTSARSRIEDSDYATEVSNMSRAQILQQAGTSVL
 AQANQVPQNVLSLLR

Legends:

Linker: B-Cell Epitopes: CTL Epitopes: CD8+ MHC I: RS09 adjuvant:
 PADRE sequence: Flagellin protein:

Figure 7. MEV2 (multi epitope vaccine 2) schematic design with B-cell epitopes and T-cytotoxic cell epitopes.**Table 9.** Physiochemical properties for MEV-2.

Physicochemical properties	Values/results
Allergenicity	Non-allergen
No. of Amino Acids	351 residues
Molecular weight	36039.98 Dalton
Total number of negatively charged residue	22
Total number of positively charged residue	23
Formula	C ₁₅₆₆ H ₂₄₈₄ N ₄₆₂ O ₅₀₈ S ₄ 5024
Total number of amino acids	0.545 (Soluble)
Solubility	30 h (in mammalian reticulocytes) >20 h (in yeast cells) >10 h (in <i>E. coli</i>) 33.69 (Stable)
Estimated half-life	75.7
Instability Index	-0.342 (below 0 values indicate hydrophilic globular nature while above 0 values indicate hydrophobic membranous nature)
Aliphatic Index	
Grand average of hydropathicity (GRAVY Score)	

immediate development of an advanced vaccination. According to estimates, 35% of MERS-CoV patients have died unexpectedly. Since 2012, MERS has spread to 27 nations, with the Kingdom of Saudi Arabia reporting almost 80% of all human cases. People who contracted the disease in the Middle East and then went to other nations account for the cases discovered outside of the region (Alenazi & Arabi, 2022). The binding of the MERS-CoV spike protein to the DPP-4 receptor initiates the internalization of genetic material into the host cells, which later initiates the replication of the virus particles within the host cells (Widagdo et al., 2019). In several recent studies, immunoinformatics techniques have been used in predicting epitopes-based vaccine candidates against pathogens like Dengue (Krishnan et al., 2021), SARS-CoV2 (Joshi et al., 2020), Canine Circovirus (Jain et al., 2021) and *Candida tropicalis*. Previously various computational studies have been performed to design novel vaccine candidates

against MERS (Mahmud et al., 2021; Nguyen et al., 2022b; Srivastava et al., 2018; Tahir Ul Qamar et al., 2019). Nguyen and colleagues (Nguyen et al., 2022b) targeted the surface glycoprotein of MERS-CoV and identified seven epitopes (NITITYQGLFPY, YSNITITYQGLF, YIDLKEGNYTY, SYIDLKEGNYT, TQINTTLLDLTY, PPLMDVNMEAAY and PTNFSFGVTQEY) that could bind with HLA_A0101 allele. Srivastava et al. (2018) targeted 13 different MERS-CoV proteins to identify CTL and HTL epitopes which were then linked using EAAAK and GGGGS linkers to human β-defensin-2 and human β-defensin-3 adjuvants to design two vaccine candidates namely CTL-MEV (500 amino acids) and HTL MEV (657 amino acids; Srivastava et al., 2018). Here we used GPGPG linker not only to connect various epitopes and adjuvants but also to maintain the proper length of the multi epitope vaccine (MEV); as it should ranges from 350 to 600 amino acids (Nosrati et al., 2019; Rouzbahani et al., 2022; Yang et al., 2021). Also, many

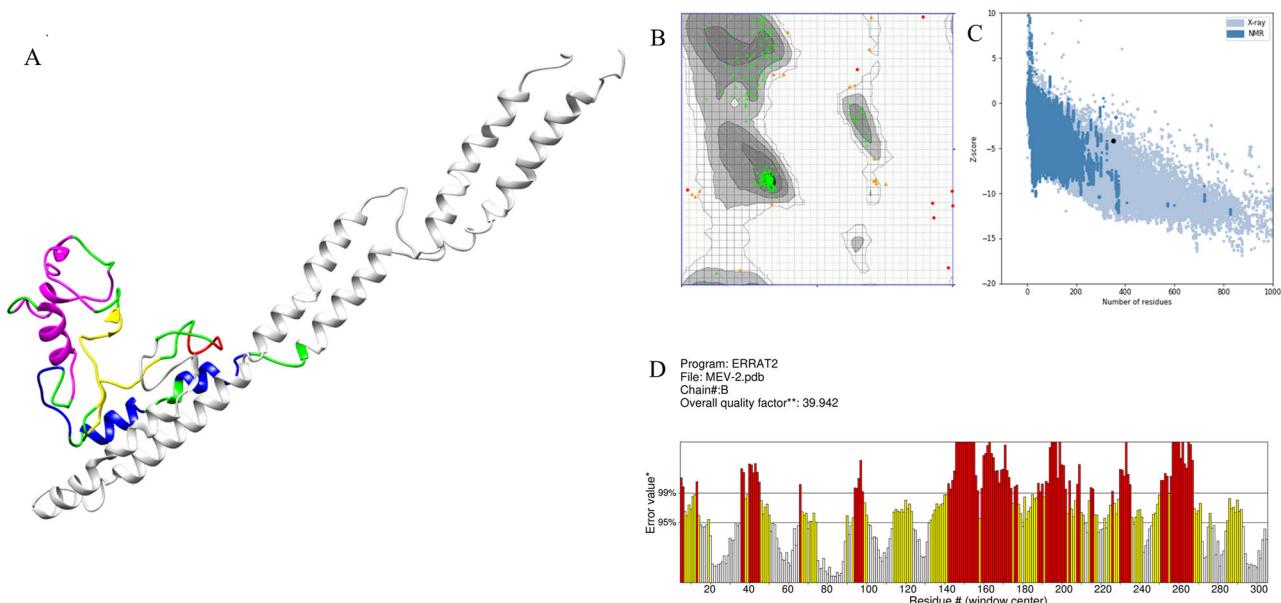


Figure 8. (A) Three-dimensional model of the multi-epitope vaccine (Linker: green, B-cell epitopes: magenta, CTL Epitopes: blue, RS09 adjuvant: red, PADRE: yellow, *Salmonella Dublin* flagellin protein: light grey), (B) Ramachandran Plot, (C) ProSA model quality and (D) ERRAT plot of the vaccine construct.

Table 10. The details of H-bond interactions of the TLR5 and MEV-2.

Sl. No.	TLR 5			MEV-2			
	Residue name	Residue number	Atom name	Distance (Å)	Residue name	Residue number	Atom Name
1	GLY	685	N	2.43	GLY	156	O
2	THR	686	N	3.52	GLY	156	O
3	THR	686	OG1	3.89	GLY	158	O
4	LYS	680	N	3.86	GLY	192	O
5	GLN	675	NE2	2.54	GLY	213	O
6	GLN	675	NE2	2.74	TRP	216	O
7	TYR	774	OH	2.58	LYS	273	O
8	GLN	745	NE2	2.59	PHE	276	O
9	TYR	774	OH	3.08	PHE	276	O
10	ASN	750	N	2.19	THR	285	OG1
11	GLN	675	O	3.64	TRP	216	N
12	GLN	675	OE1	2.52	PRO	214	N
13	ASP	681	OD1	3.09	GLY	192	N
14	GLN	684	O	3.56	GLY	158	N
15	THR	686	OG1	2.48	GLY	158	N
16	GLN	745	O	3.43	GLY	282	N
17	GLN	745	O	3.74	THR	285	OG1
18	ASP	746	O	2.87	THR	285	OG1
19	ASP	746	OD1	2.84	THR	285	N

review literatures indicate the same criteria of selection of linkers in viral proteome dependent epitopes. In current study, we tried to explore available data sets for spike protein of the MERS-CoV to reveal most antigenic and conserved epitopes by deploying latest immunoinformatics tools and servers. For the design of a multi-epitope vaccine, physicochemical factors are always required since one must constantly consider the construct's size, half-life, stability, and pI values. Therefore, for our Multi epitope vaccine construct we used ProtParam tool (Adam, 2021; Pitaloka et al., 2022). Earlier Mahmud et al. (2021) had also targeted the MERS-CoV spike protein to design a vaccine candidate. The epitope QSIFYRLNGVGITQQ was found to be common between this study and the study by Mahmud et al., 2021(Mahmud et al., 2021). The study used TLR2 which is usually found in a dimeric state with TLR1 or TLR6 for molecular docking. In our study, during molecular docking TLR5 interacts with adjuvants like flagellin and initiates immunization via

CD4+ and CD8+ T-cells activation was used (Keshavarz-Fathi & Rezaei, 2019). The simple carrier epitope AKFVAATLKA, also known as the pan HLA DR-binding epitope (PADRE), has been suggested for use in the creation of synthetic and recombinant vaccines. As a Toll-like receptor agonist adjuvant, it aids in developing a multi-epitope construct to a proper size. It has also been seen in several studies that when PADRE adjuvant was used with HTLs and CTLs, it not only supports the structure but also reignites IFN-gamma production, which in contrast activates macrophages and initiates antiviral and antibacterial immunity (Ghaffari-Nazari et al., 2015). In many recent studies, it was found that not only viral proteins but also bacterial proteins were successfully used for T-cell epitopes depending on vaccine construction (Dey et al., 2021; 2022b). Here in the study, we primarily used CD4+ HTL epitopes. Moreover, CD4+ T cell epitopes are less variant in comparison to B-cells and CD8+ CTL epitopes, also the availability of data

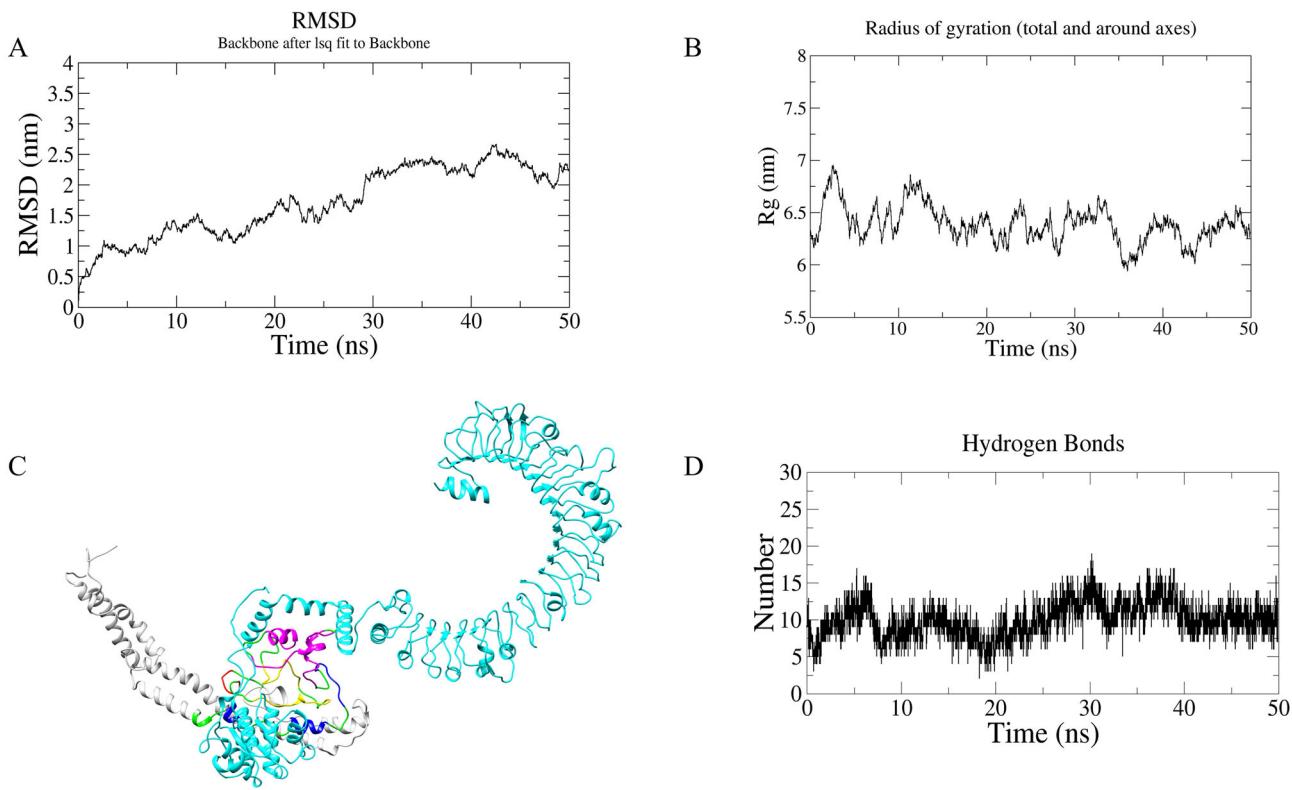


Figure 9. The MD simulation results: (A) RMSD of the protein backbone, (B) R_g evolution, (C) Final snapshot (at 50 ns) of the simulated trajectory and (D) H-bonds between the vaccine construct and TLR5.

supports the multiple MHC Class II HLA variants makes this choice preferable (Bibi et al., 2021; Knowlden et al., 2019; Sanchez-Trincado et al., 2017). Population coverage analysis reveals that these five selected epitopes cover all the major MHC Class II HLA determinants across the world-wide population with 57.59% coverage. The difference in the methodology used and the selection criterion of the epitopes among the earlier studies which targeted MERS-CoV spike protein and this study could be the reason for the prediction of unique epitopes for the vaccine design. Later the epitopes that fulfilled the selection criterion in this study were linked with adjuvants and linkers to design candidate multi-epitope vaccine construct. The vaccine construct was docked with TLR5 (Toll like receptor 5) successfully with binding energy of -32.3 kcal/mol that indicates possible internalization. Similarly, Mahmud et al. (2021) had also performed molecular docking studies and predicted that the candidate multi-epitope vaccine designed by them could interact with TLR2 and TLR4 with the binding energy of -22.13 and -21.16 kcal/mol, respectively. Allergenicity can be a significant challenge during vaccine development (Mahmud et al., 2021). It was predicted that this candidate multi-epitope vaccine is non-allergic. The predicted vaccine was found to be hydrophilic and globular which confirms its solubility to induce physiological interactions with exposed HLA determinants. According to a recent work (Alhabbab et al., 2022) MERS-CoV virion might produce potent long-lasting virus-specific affinity and neutralizing immunoglobulin and T and B cell responses up to 7 years after exposure. So, in this study we also look up for immune simulation analysis and noted that our predicted multi-epitope-based vaccine construct produces cytokines as well as immunoglobulins within 70-day

framework. Further, the MD simulation revealed that the complex between the vaccine construct and TLR5 is stable. The findings in this work could offer better avenues for extension of research on MERS vaccine development. MEV2 was designed to involve continuous B-cell epitopes and CTL epitopes. It acts as supporting vaccine to MEV-1 (constructed by using HTL epitopes). The comparative assessment was made with recently published studies on MERS epitopes and is presented in Table 11. Both MEV1 and MEV2 were collectively referred as double shot vaccine construct, as both vaccines were predicted to support the complete immunogenic effect on patients.

Conclusion

MERS-CoV poses a danger to public health globally and requires the immediate development of an advanced vaccination strategy. We constructed a multi-epitope vaccine with five screened epitopes QSIFYRLNGVGITQQ, DTIKYYSIIPHSIRS, PEPITSLNKYVAPQ, INGRLLTLNAFVAQQ and GDMYVYSAGHATGTT from spike protein of the same virus. The vaccine construct (MEV-1) was docked with TLR5 (Toll-like receptor 5) successfully with the binding energy of -32.3 kcal/mol and *in silico* immune simulations showed the immune response elicitation potential of the candidate MERS vaccine. Future perspectives of this research include synthesis of a multi-epitope vaccine construct and testing its efficacy and safety on animal models. Here we also designed support vaccine construct MEV-2 by using B-cell and CD8+ CTL epitopes to generate the complete immunogenic effect.

Table 11. Comparative assessment report on recent MERS epitopes-based vaccines.

Target protein	Number of amino acids	Adjuvants	Final epitopes	Reference
Spike protein for predicting HTL epitopes	MEV1 (HTL epitopes): 357 MEV2 (CTL epitopes & B Cell epitopes): 351	RS09 and <i>Salmonella dublin</i> flagella protein	HTL Epitopes: QSIFYRLNGVGITQQ, DTIKYSHPSHSIRS, PEPITSLENTKVAPQ, INGRITTLNAFVAQQ, GDMVYVSAAGHATGTT	This study
Surface glycoprotein for predicting HTL, CTL and B cell epitopes	Only epitopes were predicted in this study. Vaccine design was not performed. Hence, this data is unavailable. 500	cholera toxin subunit B	Only epitopes were predicted in this study. Vaccine design was not performed. Hence, this data is unavailable.	Nguyen et al. (2022b)
S glycoprotein, E protein, N protein, M protein, ORF3, ORF4a, ORF4b, ORF5, ORF1a, a papain-like protease PL(pro), ORF1a, 3CLpro, ORF1ab, PL(pro); ORF1ab, and ORF8b for predicting CTL epitopes	489	cholera toxin subunit B	Only epitopes were predicted in this study. Vaccine design was not performed. Hence, this data is unavailable.	Mahmud et al. (2021)
Spike protein to predict B cell epitope, CTL and HTL epitopes	Only epitopes were predicted in this study. Vaccine design was not performed. Hence, this data is unavailable.	Only epitopes were predicted in this study. Vaccine design was not performed. Hence, this data is unavailable.	Only epitopes were predicted in this study. Vaccine design was not performed. Hence, this data is unavailable.	Tahir Ull Qamar et al. (2019)

This study opens new avenues for the extension of research on MERS vaccine development.

Acknowledgment

All authors are thankful to the School of Bioengineering and Biosciences, Lovely Professional University, and Invertis University for providing sound computational facilities for the conduction of research work.

Disclosure statement

There are no relevant financial or non-financial competing interests to report.

Funding

The author(s) reported there is no funding associated with the work featured in this article.

ORCID

Amit Joshi  <http://orcid.org/0000-0001-8287-8375>
 Nahid Akhtar  <http://orcid.org/0000-0002-8286-3736>
 Neeta Raj Sharma  <http://orcid.org/0000-0001-8638-4217>
 Vikas Kaushik  <http://orcid.org/0000-0003-2349-1220>
 Subhomoi Borkotoky  <http://orcid.org/0000-0001-6752-4296>

Data availability statement

The results from the NetMHCpan calculations are available at <https://github.com/subhomoi/NetMHCpan-R>.

Author's contribution

AJ, SB, and NA conducted the research work. VK, NRS designed and guided the research work. All authors equally contributed to manuscript writing and verification.

References

- Kuriata, A., Gierut, A. M., Oleniecki, T., Cierny, M. P., Kolinski, A., Kurncinski, M., & Kmiecik, S. (2018). CABS-flex 2.0: A web server for fast simulations of flexibility of protein structures. *Nucleic Acids Research*, 46(W1), W338–W343. <https://doi.org/10.1093/nar/gky356>
- Abraham Peele, K., Srihansa, T., Krupanidhi, S., Ayyagari, V. S., & Venkateswarulu, T. C. (2021). Design of multi-epitope vaccine candidate against SARS-CoV-2: a in-silico study. *Journal of Biomolecular Structure and Dynamics*, 39(10), 3793–3801. <https://doi.org/10.1080/07391102.2020.1770127>
- Adam, K. M. (2021). Immunoinformatics approach for multi-epitope vaccine design against structural proteins and ORF1a polyprotein of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). *Tropical Diseases, Travel Medicine and Vaccines*, 7(1), 22. <https://doi.org/10.1186/s40794-021-00147-1>
- Akhtar, N., Joshi, A., Singh, J., & Kaushik, V. (2021). Design of a novel and potent multivalent epitope based human cytomegalovirus peptide vaccine: An immunoinformatics approach. *Journal of Molecular Liquids*, 335, 116586. <https://doi.org/10.1016/j.molliq.2021.116586>
- Al Johani, S., & Hajer, A. H. (2016). MERS-CoV diagnosis: An update. *Journal of Infection and Public Health*, 9(3), 216–219. <https://doi.org/10.1016/j.jiph.2016.04.005>
- Alenazi, T. H., & Arabi, Y. M. (2022). Severe Middle East respiratory syndrome (MERS) pneumonia. *Encyclopedia of Respiratory Medicine*, 362–372. <https://doi.org/10.1186/s13099-022-00495-z>
- Alhabbab, R. Y., Algaissi, A., Mahmoud, A. B., Alkayyal, A. A., Al-Amri, S., Alfaleh, M. A., Basabrain, M., Alsubki, R. A., Almarshad, I. S., Alhudaithi, A. M., Gafari, O. A. A., Alshamlan, Y. A., Aldossari, H. M., Alsafi, M. M., Bukhari, A., Bajhmom, W., Memish, Z. A., Alsalem, W., & Hashem, A. M. (2022). MERS-CoV infection elicits long-lasting specific antibody, T and B cell immune responses in recovered individuals. *Clinical Infectious Diseases*, 76(3), e308–e318. <https://doi.org/10.1093/cid/ciac456>
- Alsalamy, S., & Arabi, Y. M. (2015). Infection with Middle East respiratory syndrome coronavirus. *Canadian Journal of Respiratory Therapy*, 51(4), 102. <https://www.ncbi.nlm.nih.gov/pubmed/26566382>
- Bibi, S., Ullah, I., Zhu, B., Adnan, M., Liaqat, R., Kong, W.-B., & Niu, S. (2021). In silico analysis of epitope-based vaccine candidate against tuberculosis using reverse vaccinology. *Scientific Reports*, 11(1), 1249. <https://doi.org/10.1038/s41598-020-8089-6>
- Borkotoky, S., Banerjee, M., Modi, G. P., & Dubey, V. K. (2021). Identification of high affinity and low molecular alternatives of boceprevir against SARS-CoV-2 main protease: A virtual screening approach. *Chemical Physics Letters*, 770, 138446. <https://doi.org/10.1016/j.cplett.2021.138446>
- Bosaeed, M., Balkhy, H. H., Almaziad, S., Aljami, H. A., Alhatmi, H., Alanazi, H., Alahmadi, M., Jawhary, A., Alenazi, M. W., Almasoud, A., Alanazi, R., Bittaye, M., Aboagye, J., Albaalharith, N., Batawi, S., Folegatti, P., Ramos Lopez, F., Ewer, K., Almoaike, K., ... Khalaf Alharbi, N. (2022). Safety and immunogenicity of ChAdOx1 MERS vaccine candidate in healthy Middle Eastern adults (MERS002): An open-label, non-randomised, dose-escalation, phase 1b trial. *Lancet Microbe*, 3(1), e11–e20. [https://doi.org/10.1016/S2666-5247\(21\)00193-2](https://doi.org/10.1016/S2666-5247(21)00193-2)
- Buchan, D. W. A., & Jones, D. T. (2019). The PSIPRED Protein Analysis Workbench: 20 years on. *Nucleic Acids Research*, 47(W1), W402–W407. <https://doi.org/10.1093/nar/gkz297>
- Castiglione, F., Mantile, F., De Berardinis, P., & Prisco, A. (2012). How the interval between prime and boost injection affects the immune response in a computational model of the immune system. *Computational and Mathematical Methods in Medicine*, 2012, 842329. <https://doi.org/10.1155/2012/842329>
- Cauchemez, S., Nouvellet, P., Cori, A., Jombart, T., Garske, T., Clapham, H., Moore, S., Mills, H. L., Salje, H., Collins, C., Rodriguez-Barraquer, I., Riley, S., Truelove, S., Algarni, H., Alhakeem, R., AlHarbi, K., Turkistani, A., Aguas, R. J., Cummings, D. A., ... Ferguson, N. M. (2016). Unraveling the drivers of MERS-CoV transmission. *Proceedings of the National Academy of Sciences of United States of America*, 113(32), 9081–9086. <https://doi.org/10.1073/pnas.1519235113>
- Choi, J. A., Goo, J., Yang, E., Jung, D. I., Lee, S., Rho, S., Jeong, Y., Park, Y. S., Park, H., Moon, Y. H., Park, U., Seo, S. H., Lee, H., Lee, J. M., Cho, N. H., Song, M., & Kim, J. O. (2020). Cross-protection against MERS-CoV by prime-boost vaccination using viral spike DNA and protein. *Journal of Virology*, 94(24), e01176-20. <https://doi.org/10.1128/JVI.01176-20>
- Deng, Y., Lan, J., Bao, L., Huang, B., Ye, F., Chen, Y., Yao, Y., Wang, W., Qin, C., & Tan, W. (2018). Enhanced protection in mice induced by immunization with inactivated whole viruses compare to spike protein of middle east respiratory syndrome coronavirus. *Emerging Microbes & Infections*, 7(1), 1–10. <https://doi.org/10.1038/s41426-018-0056-7>
- Dey, J., Mahapatra, S. R., Lata, S., Patro, S., Misra, N., & Suar, M. (2022a). Exploring Klebsiella pneumoniae capsule polysaccharide proteins to design multiepitope subunit vaccine to fight against pneumonia. *Expert Review of Vaccines*, 21(4), 569–587. <https://doi.org/10.1080/14760584.2022.2021882>
- Dey, J., Mahapatra, S. R., Patnaik, S., Lata, S., Kushwaha, G. S., Panda, R. K., Misra, N., & Suar, M. (2022b). Molecular characterization and designing of a novel multiepitope vaccine construct against *Pseudomonas aeruginosa*. *International Journal of Peptide Research and Therapeutics*, 28(2), 49. <https://doi.org/10.1007/s10989-021-10356-z>
- Dey, J., Mahapatra, S. R., Raj, T. K., Kaur, T., Jain, P., Tiwari, A., Patro, S., Misra, N., & Suar, M. (2022c). Designing a novel multi-epitope vaccine to evoke a robust immune response against pathogenic multidrug-resistant *Enterococcus faecium* bacterium. *Gut Pathogens*, 14(1), 21. <https://doi.org/10.1186/s13099-022-00495-z>

- Dey, J., Mahapatra, S. R., Singh, P., Patro, S., Kushwaha, G. S., Misra, N., & Suar, M. (2021). B and T cell epitope-based peptides predicted from clumping factor protein of *Staphylococcus aureus* as vaccine targets. *Microbial Pathogenesis*, 160, 105171. <https://doi.org/10.1016/j.micpath.2021.105171>
- Dhanda, S. K., Gupta, S., Vir, P., & Raghava, G. P. S. (2013a). Prediction of IL4 inducing peptides. *Clinical & Developmental Immunology*, 2013, 263952. <https://doi.org/10.1155/2013/263952>
- Dhanda, S. K., Vir, P., & Raghava, G. P. S. (2013b). Designing of interferon-gamma inducing MHC class-II binders. *Biology Direct*, 8(1), 30. <https://doi.org/10.1186/1745-6150-8-30>
- Dimitrov, I., Naneva, L., Doytchinova, I., & Bangov, I. (2014). AllergenFP: Allergenicity prediction by descriptor fingerprints. *Bioinformatics*, 30(6), 846–851. <https://doi.org/10.1093/bioinformatics/btt619>
- Doytchinova, I. A., & Flower, D. R. (2007). VaxiJen: A server for prediction of protective antigens, tumour antigens and subunit vaccines. *BMC Bioinformatics*, 8(1), 4. <https://doi.org/10.1186/1471-2105-8-4>
- Folegatti, P. M., Bittaye, M., Flaxman, A., Lopez, F. R., Bellamy, D., Kupke, A., Mair, C., Makinson, R., Sheridan, J., Rohde, C., Halwe, S., Jeong, Y., Park, Y.-S., Kim, J.-O., Song, M., Boyd, A., Tran, N., Silman, D., Poult, I., ... Gilbert, S. (2020). Safety and immunogenicity of a candidate Middle East respiratory syndrome coronavirus viral-vectored vaccine: A dose-escalation, open-label, non-randomised, uncontrolled, phase 1 trial. *The Lancet Infectious Diseases*, 20(7), 816–826. [https://doi.org/10.1016/S1473-3099\(20\)30160-2](https://doi.org/10.1016/S1473-3099(20)30160-2)
- Gasteiger, E., Hoogland, C., Gattiker, A., Duvaud, S. e., Wilkins, M. R., Appel, R. D., & Bairoch, A. (2005). Protein identification and analysis tools on the ExPASy server. In J. M. Walker (Ed.), *The proteomics protocols handbook* (pp. 571–607). Humana Press. <https://doi.org/10.1385/1-59259-890-0:571>
- Ghaffari-Nazari, H., Tavakkol-Afshari, J., Jaafari, M. R., Tahaghoghi-Hajghorbani, S., Masoumi, E., & Jalali, S. A. (2015). Improving multi-epitope long peptide vaccine potency by using a strategy that enhances CD4+ T help in BALB/c mice. *PLoS One*, 10(11), e0142563. <https://doi.org/10.1371/journal.pone.0142563>
- Grote, A., Hiller, K., Scheer, M., Munch, R., Nortemann, B., Hempel, D. C., & Jahn, D. (2005). JCat: a novel tool to adapt codon usage of a target gene to its potential expression host. *Nucleic Acids Research*, 33(Web Server), W526–W531. <https://doi.org/10.1093/nar/gki376>
- Gupta, S., Kapoor, P., Chaudhary, K., Gautam, A., Kumar, R., Open Source Drug Discovery, C., & Raghava, G. P. (2013). In silico approach for predicting toxicity of peptides and proteins. *PLoS One*, 8(9), e73957. <https://doi.org/10.1371/journal.pone.0073957>
- Huang, W., Lin, Z., & van Gunsteren, W. F. (2011). Validation of the GROMOS 54A7 force field with respect to beta-peptide folding. *Journal of Chemical Theory and Computation*, 7(5), 1237–1243. <https://doi.org/10.1021/ct100747y>
- Jain, P., Joshi, A., Akhtar, N., Krishnan, S., & Kaushik, V. (2021). An immunoinformatics study: Designing multivalent T-cell epitope vaccine against canine circovirus. *Journal of Genetic Engineering and Biotechnology*, 19(1), 121. <https://doi.org/10.1186/s43141-021-00220-4>
- Joshi, A., Joshi, B. C., Mannan, M. A., & Kaushik, V. (2020). Epitope based vaccine prediction for SARS-CoV-2 by deploying immuno-informatics approach. *Informatics in Medicine Unlocked*, 19, 100338. <https://doi.org/10.1016/j.imu.2020.100338>
- Kar, T., Narsaria, U., Basak, S., Deb, D., Castiglione, F., Mueller, D. M., & Srivastava, A. P. (2020). A candidate multi-epitope vaccine against SARS-CoV-2. *Scientific Reports*, 10(1), 10895. <https://doi.org/10.1038/s41598-020-67749-1>
- Keshavarz-Fathi, M., & Rezaei, N. (2019). Chapter 3 - vaccines, adjuvants, and delivery systems. In N. Rezaei & M. Keshavarz-Fathi (Eds.), *Vaccines for cancer immunotherapy* (pp. 45–59). Academic Press. <https://doi.org/10.1016/B978-0-12-814039-0.00003-5>
- Kneller, D. W., Phillips, G., O'Neill, H. M., Jedrzejczak, R., Stols, L., Langan, P., Joachimiak, A., Coates, L., & Kovalevsky, A. (2020). Structural plasticity of SARS-CoV-2 3CL M(pro) active site cavity revealed by room temperature X-ray crystallography. *Nature Communications*, 11(1), 3202. <https://doi.org/10.1038/s41467-020-16954-7>
- Knowlden, Z. A. G., Richards, K. A., Moritzky, S. A., & Sant, A. J. (2019). Peptide epitope hot spots of CD4 T cell recognition within influenza hemagglutinin during the primary response to infection. *Pathogens*, 8(4), 220. <https://www.mdpi.com/2076-0817/8/4/220> <https://doi.org/10.3390/pathogens8040220>
- Krishnan, G. S., Joshi, A., Akhtar, N., & Kaushik, V. (2021). Immunoinformatics designed T cell multi epitope dengue peptide vaccine derived from non structural proteome. *Microbial Pathogenesis*, 150, 104728. <https://doi.org/10.1016/j.micpath.2020.104728>
- Krissinel, E., & Henrick, K. (2007). Inference of macromolecular assemblies from crystalline state. *Journal of Molecular Biology*, 372(3), 774–797. <https://doi.org/10.1016/j.jmb.2007.05.022>
- Lathwal, A., Kumar, R., Kaur, D., & Raghava, G. P. S. (2021). In silico model for predicting IL-2 inducing peptides in human. *bioRxiv*. <https://doi.org/10.1101/2021.06.20.449146>
- Magnan, C. N., Randall, A., & Baldi, P. (2009). SOLpro: Accurate sequence-based prediction of protein solubility. *Bioinformatics*, 25(17), 2200–2207. <https://doi.org/10.1093/bioinformatics/btp386>
- Mahapatra, S. R., Dey, J., Raj, T. K., Kumar, V., Ghosh, M., Verma, K. K., Kaur, T., Kesawat, M. S., Misra, N., & Suar, M. (2022). The potential of plant-derived secondary metabolites as novel drug candidate against Klebsiella pneumoniae: Molecular docking and simulation investigation. *South African Journal of Botany*, 149, 789–797. <https://doi.org/10.1016/j.sajb.2022.04.043>
- Mahmud, S., Rafi, M. O., Paul, G. K., Promi, M. M., Shimu, M. S. S., Biswas, S., Emran, T. B., Dhama, K., Alyami, S. A., Moni, M. A., & Saleh, M. A. (2021). Designing a multi-epitope vaccine candidate to combat MERS-CoV by employing an immunoinformatics approach. *Scientific Reports*, 11(1), 15431. <https://doi.org/10.1038/s41598-021-92176-1>
- María, R., Arturo, C., Alicia, J. A., Paulina, M., & Gerardo, A. O. (2017). The impact of bioinformatics on vaccine design and development. *Vaccines*, 2, 3–6. <https://doi.org/10.5772/intechopen.69273>
- Martonak, R., Laio, A., & Parrinello, M. (2003). Predicting crystal structures: The Parrinello-Rahman method revisited. *Physical Review Letters*, 90(7), 075503. <https://doi.org/10.1103/PhysRevLett.90.075503>
- Memish, Z. A., Perlman, S., Van Kerkhove, M. D., & Zumla, A. (2020). Middle East respiratory syndrome. *The Lancet*, 395(10229), 1063–1077. [https://doi.org/10.1016/S0140-6736\(19\)33221-0](https://doi.org/10.1016/S0140-6736(19)33221-0)
- Molaei, S., Dadkhah, M., Asgharizadeh, V., Karami, C., & Safarzadeh, E. (2021). The immune response and immune evasion characteristics in SARS-CoV, MERS-CoV, and SARS-CoV-2: Vaccine design strategies. *International Immunopharmacology*, 92, 107051. <https://doi.org/10.1016/j.intimp.2020.107051>
- Nguyen, M. N., Krutz, N. L., Limviphuad, V., Lopata, A. L., Gerberick, G. F., & Maurer-Stroh, S. (2022a). AllerCatPro 2.0: A web server for predicting protein allergenicity potential. *Nucleic Acids Research*, 50(W1), W36–W43. <https://doi.org/10.1093/nar/gkac446>
- Nguyen, T. L., Lee, Y., & Kim, H. (2022b). Immunogenic epitope-based vaccine prediction from surface glycoprotein of MERS-CoV by deploying immunoinformatics approach. *International Journal of Peptide Research and Therapeutics*, 28(3), 77. <https://doi.org/10.1007/s10989-022-10382-5>
- Nosrati, M., Behbahani, M., & Mohabatkar, H. (2019). Towards the first multi-epitope recombinant vaccine against Crimean-Congo hemorrhagic fever virus: A computer-aided vaccine design approach. *Journal of Biomedical Informatics*, 93, 103160. <https://doi.org/10.1016/j.jbi.2019.103160>
- Pettersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblatt, D. M., Meng, E. C., & Ferrin, T. E. (2004). UCSF Chimera—a visualization system for exploratory research and analysis. *Journal of Computational Chemistry*, 25(13), 1605–1612. <https://doi.org/10.1002/jcc.20084>
- Pierce, B. G., Wiehe, K., Hwang, H., Kim, B. H., Vreven, T., & Weng, Z. (2014). ZDOCK server: interactive docking prediction of protein-protein complexes and symmetric trimers. *Bioinformatics*, 30(12), 1771–1773. <https://doi.org/10.1093/bioinformatics/btu097>
- Pitaloka, D. A. E., Izzati, A., Amirah, S. R., & Syakuran, L. A. (2022). Multi epitope-based vaccine design for protection against Mycobacterium tuberculosis and SARS-CoV-2 coinfection. *Advances and Applications in Bioinformatics and Chemistry*, 15, 43–57. <https://doi.org/10.2147/AABC.S366431>
- Rapin, N., Lund, O., Bernaschi, M., & Castiglione, F. (2010). Computational immunology meets bioinformatics: The use of prediction tools for

- molecular binding in the simulation of the immune system. *PLoS One*, 5(4), e9862. <https://doi.org/10.1371/journal.pone.0009862>
- Reynisson, B., Alvarez, B., Paul, S., Peters, B., & Nielsen, M. (2020). NetMHCpan-4.1 and NetMHClipan-4.0: improved predictions of MHC antigen presentation by concurrent motif deconvolution and integration of MS MHC eluted ligand data. *Nucleic Acids Research*, 48(W1), W449–W454. <https://doi.org/10.1093/nar/gkaa379>
- Robinson, C. L., Romero, J. R., Kempe, A., & Pellegrini, C. (2017). Advisory Committee on Immunization Practices recommended immunization schedule for children and adolescents aged 18 years or younger—United States, 2017. *MMWR. Morbidity and Mortality Weekly Report*, 66(5), 134–135. <https://doi.org/10.15585/mmwr.mm6605e1>
- Rouzbahani, A. K., Kheirandish, F., & Hosseini, S. Z. (2022). Design of a multi-epitope-based peptide vaccine against the S and N proteins of SARS-CoV-2 using immunoinformatics approach. *Egyptian Journal of Medical Human Genetics*, 23(1), 16. <https://doi.org/10.1186/s43042-022-00224-w>
- Safavi, A., Kefayat, A., Mahdevar, E., Abiri, A., & Ghahremani, F. (2020). Exploring the out of sight antigens of SARS-CoV-2 to design a candidate multi-epitope vaccine by utilizing immunoinformatics approaches. *Vaccine*, 38(48), 7612–7628. <https://doi.org/10.1016/j.vaccine.2020.10.016>
- Saha, S., & Raghava, G. P. (2006). Prediction of continuous B-cell epitopes in an antigen using recurrent neural network. *Proteins: Structure, Function, and Bioinformatics*, 65(1), 40–48. <https://doi.org/10.1002/prot.21078>
- Sanchez-Trincado, J. L., Gomez-Perez, M., & Reche, P. A. (2017). Fundamentals and methods for T- and B-Cell epitope prediction. *Journal of Immunology Research*, 2017, 2680160. <https://doi.org/10.1155/2017/2680160>
- Sievers, F., & Higgins, D. G. (2014). Clustal omega. *Current Protocols in Bioinformatics*, 48(1), 3 13 11–16. <https://doi.org/10.1002/0471250953.bi0313s48>
- Skwarczynski, M., & Toth, I. (2016). Peptide-based synthetic vaccines. *Chemical Science*, 7(2), 842–854. <https://doi.org/10.1039/C5SC03892H>
- Srivastava, S., Kamthania, M., Singh, S., Saxena, A. K., & Sharma, N. (2018). Structural basis of development of multi-epitope vaccine against Middle East respiratory syndrome using in silico approach. *Infection and Drug Resistance*, 11, 2377–2391. <https://doi.org/10.2147/IDR.S175114>
- Tahir Ul Qamar, M., Saleem, S., Ashfaq, U. A., Bari, A., Anwar, F., & Alqahtani, S. (2019). Epitope-based peptide vaccine design and target site depiction against Middle East Respiratory Syndrome Coronavirus: an immune-informatics study. *Journal of Translational Medicine*, 17(1), 362. <https://doi.org/10.1186/s12967-019-2116-8>
- Van Der Spoel, D., Lindahl, E., Hess, B., Groenhof, G., Mark, A. E., & Berendsen, H. J. (2005). GROMACS: Fast, flexible, and free. *Journal of Computational Chemistry*, 26(16), 1701–1718. <https://doi.org/10.1002/jcc.20291>
- Varadi, M., Anyango, S., Deshpande, M., Nair, S., Natassia, C., Yordanova, G., Yuan, D., Stroe, O., Wood, G., Laydon, A., Zidek, A., Green, T., Tunyasuvunakool, K., Petersen, S., Jumper, J., Clancy, E., Green, R., Vora, A., Lutfi, M., ... Velankar, S. (2022). AlphaFold Protein Structure Database: Massively expanding the structural coverage of protein-sequence space with high-accuracy models. *Nucleic Acids Research*, 50(D1), D439–D444. <https://doi.org/10.1093/nar/gkab1061>
- WHO. (2019). Middle East respiratory syndrome coronavirus (MERS-CoV). [https://www.who.int/news-room/fact-sheets/detail/middle-east-respiratory-syndrome-coronavirus-\(mers-cov\)](https://www.who.int/news-room/fact-sheets/detail/middle-east-respiratory-syndrome-coronavirus-(mers-cov))
- WHO. (2022). MERS outbreaks. World Health Organization - Regional Office for the Eastern Mediterranean. <http://www.emro.who.int/health-topics/mers-cov/mers-outbreaks.html>
- Widagdo, W., Sookawasdi Na Ayudhya, S., Hundie, G. B., & Haagmans, B. L. (2019). Host determinants of MERS-CoV transmission and pathogenesis. *Viruses*, 11(3), 280. <https://doi.org/10.3390/v11030280>
- Wiederstein, M., & Sippl, M. J. (2007). ProSA-web: Interactive web service for the recognition of errors in three-dimensional structures of proteins. *Nucleic Acids Research*, 35(Web Server), W407–W410. <https://doi.org/10.1093/nar/gkm290>
- Yang, Z., Bogdan, P., & Nazarian, S. (2021). An in silico deep learning approach to multi-epitope vaccine design: A SARS-CoV-2 case study. *Scientific Reports*, 11(1), 3238. <https://doi.org/10.1038/s41598-021-81749-9>
- Yang, J., Yan, R., Roy, A., Xu, D., Poisson, J., & Zhang, Y. (2015). The iTASSER Suite: Protein structure and function prediction. *Nature Methods*, 12(1), 7–8. <https://doi.org/10.1038/nmeth.3213>
- Zhang, N., Tang, J., Lu, L., Jiang, S., & Du, L. (2015). Receptor-binding domain-based subunit vaccines against MERS-CoV. *Virus Research*, 202, 151–159. <https://doi.org/10.1016/j.virusres.2014.11.013>
- Zhou, Y., Jiang, S., & Du, L. (2018). Prospects for a MERS-CoV spike vaccine. *Expert Review of Vaccines*, 17(8), 677–686. <https://doi.org/10.1080/14760584.2018.1506702>
- Zhuang, Z., Liu, D., Sun, J., Li, F., & Zhao, J. (2022). Immune responses to human respiratory coronaviruses infection in mouse models. *Current Opinion in Virology*, 52, 102–111. <https://doi.org/10.1016/j.coviro.2021.11.015>