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A SARS-CoV-2 vaccine candidate: *In-silico* cloning and validation

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ABSTRACT

SARS-CoV-2 is spreading globally at a rapid pace. To contain its spread and prevent further fatalities, the development of a vaccine against SARS-CoV-2 is an urgent prerequisite. Thus, in this article, by utilizing the *in-silico* approach, a vaccine candidate for SARS-CoV-2 has been proposed. Moreover, the effectiveness and safety measures of our proposed epitopic vaccine candidate have been evaluated by *in-silico* tools and servers (AllerTOP and AllergenFP servers). We observed that the vaccine candidate has no allergenicity and successfully combined with Toll-like receptor (TLR) protein to elicit an inflammatory immune response. Stable, functional mobility of the vaccine-TLR protein binding interface was confirmed by the Normal Mode Analysis. The *in-silico* cloning model demonstrated the efficacy of the construct vaccine along with the identified epitopes against SARS-CoV-2. Taken together, our proposed *in-silico* vaccine candidate has potent efficacy against COVID-19 infection, and successive research work might validate its effectiveness in *in vitro* and *in vivo* models.

1. Introduction

The epidemiology of coronavirus infection COVID-19 was first reported in the Wuhan City of China, and later, it has spread rapidly throughout China along with other countries, following a pandemic nature. On January 30, 2020, the World Health Organization (WHO) declared a public health emergency of international concern considering the quick outbreak of the disease [1]. To date, a total of 10,719,946 confirmed cases and 517,337 deaths have been reported by WHO. Infected patients develop symptoms like fever, cough, fatigue, myalgia, dyspnea, decreased leukocyte counts, etc. [2–4]. The established mode of transmission for this disease is through physical contact by any of the following means: cough, sneezes, and respiratory droplets [5–7].

The virus, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), earlier known as Novel Coronavirus (2019-nCoV), is the pathogenic identity for COVID-19 [8–10]. It is an enveloped virus consisting of positive-strand RNA as the genomic component [11]. The virus has a characteristic of crown-like appearance due to the spike glycoprotein on the viral envelope [12]. Spike glycoprotein could be the most suitable target for drug designing, vaccine development, and immunotherapy because of its outer surface localization. All the selected epitopes were either predicted or validated in the human immunological framework model. Such an approach could provide a practical method for designing and developing vaccine candidates against the SARS-CoV-2. Initially, the selection of the desired antigens or considering specific proteins as immunogens is a challenging job. Therefore, the epitope-based vaccine design, *in-silico* cloning and validation, can allow the evaluation of a particular vaccine for its novelty and effectiveness in a given time frame.

Recently, we have proposed an epitope-based *in-silico* peptide

Abbreviations: SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2; NMA, Normal Mode Analysis; TLR, Toll-like receptor; ACC, Auto Cross-Covariance; CAI, Codon Adaptation Index.

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Table 1
List showing the epitopes with encountering MHC-I and MHC-II alleles.

Sl. no	Epitope encountering MHC-I allele	Sl. No.	Epitope encountering MHC-II allele
1	SQCVNLTTR	1	IHVSGTNGT
2	GVYYHKNNK	2	VYYHKNNKS
3	GKQGNFKNL	3	FKNHTSPDV
4	GIYQTSNFR		
5	VSPTKLNDL		
6	KIADYNYKL		
7	KVGGNYNYL		
8	EGFNCYFPL		
9	GPKKSTNLV		
10	SPRRARSVA		
11	LGAENSVAY		
12	FKNHTSPDV		
13	DEDDSEPVL		

vaccine against the SARS-CoV-2 [13]. Herein, we have tried to perform *in-silico* characterizations and validations of these identified epitopes. An understanding of the allergenic nature is a must for the safety and effectiveness of any vaccine. Thus, we analyzed the allergenicity of the constructed vaccine candidate and verified whether these epitopes are feasible for developing a robust vaccine against SARS-CoV-2. Moreover, molecular docking was performed in a web-based docking server, and molecular dynamic simulation was executed to compare and validate the interactions between receptor-ligand complex. Additionally, codon adaptation and *in-silico* cloning were designed for future amplification of targeted vaccine into the expression vector as per requirement. Collectively, this *in-silico* validation has tried to prove the effectiveness of the developed vaccine candidate with identified epitopes to control the SARS-CoV-2 infection.

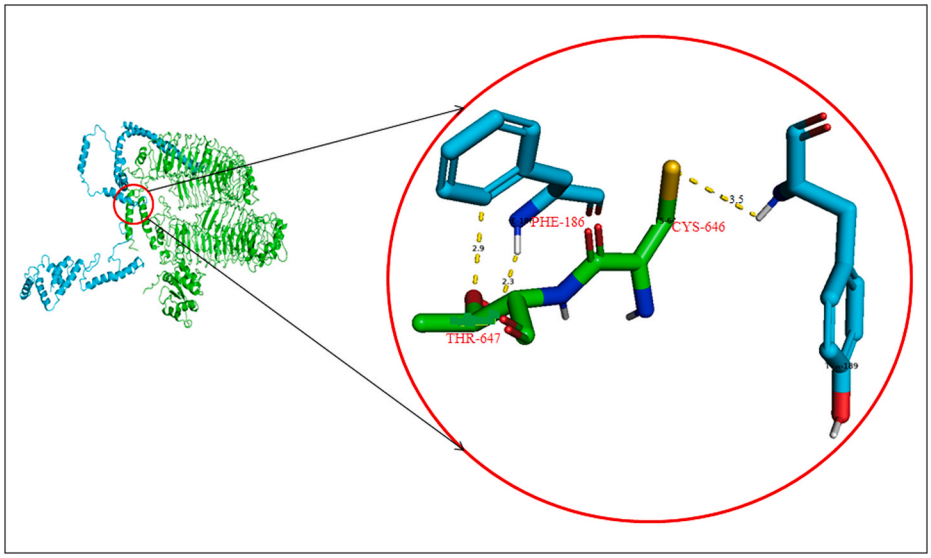

Fig. 1. Molecular docking shows non-covalent interactions between TLR protein and construct vaccine candidate.

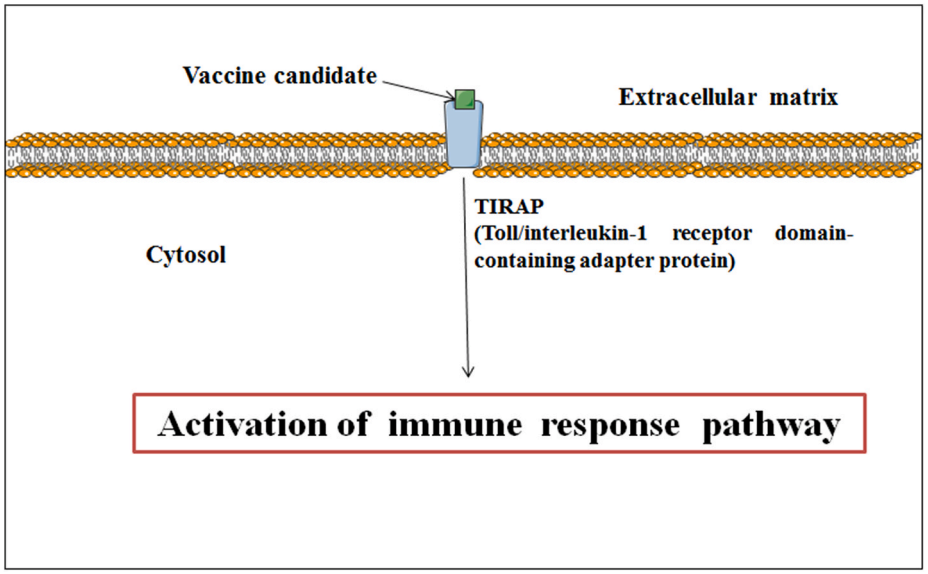

Fig. 2. Schematic diagram of TLR induced immune response pathway.

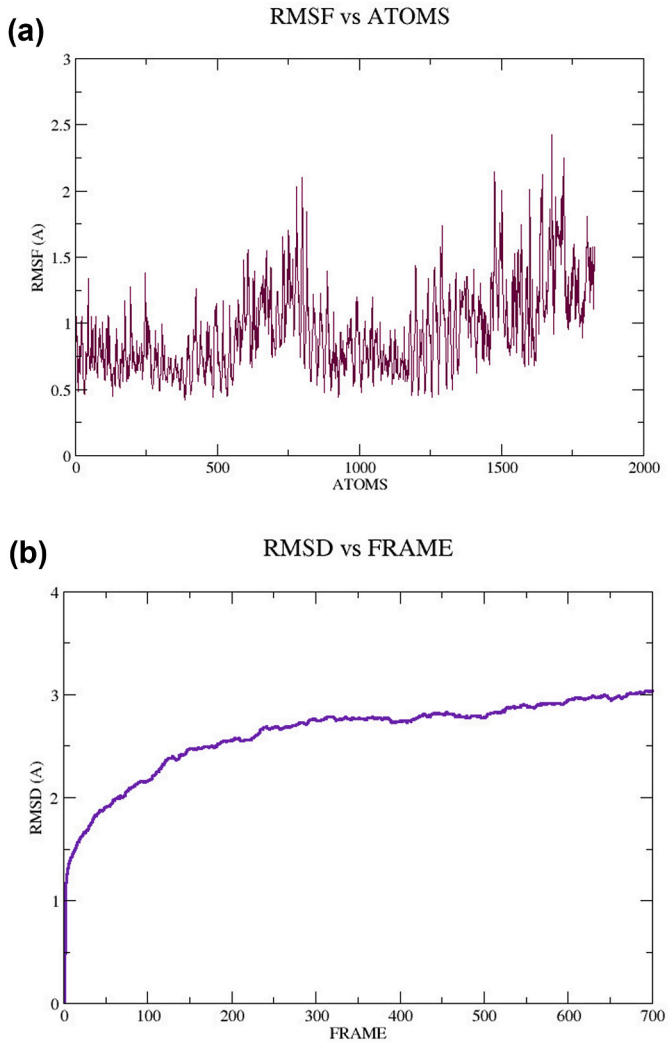

Fig. 3. Molecular Dynamics (MD) simulation of TLR protein and vaccine candidate complex (time against distance) **a**) Root Mean Square Fluctuation (RMSF) plot, **b**) Root mean square deviations (RMSD) plot.

2. Materials and methods

2.1. Retrieval of spike protein multi epitopes and vaccine designing

The multi-epitopes of the SARS-CoV-2 spike protein were selected and retrieved from our previously published work [13]. These epitopes were used to construct a vaccine, and a similar methodology has been employed here for analyzing the effectiveness of the proposed vaccine candidate.

2.2. Allergenicity analysis of vaccine component

The novel vaccine should be non-allergenic for its compelling performance. Here, two servers AllerTOP and AllergenFP, were used for allergenicity assessment of our vaccine candidate [14,15]. Both the servers rely on the principle of Auto Cross-Covariance (ACC) and allergenic evaluation methods. These two methods utilize the physicochemical properties of amino acid residues.

2.3. Molecular docking, molecular dynamics (MD) simulation, and normal mode analysis mobility analysis

Molecular docking analysis was performed to study the stable protein-protein interaction and related sub-cellular functions. For molecular docking, the ClusPro 2.0: protein-protein docking server was used [16]. In the ClusPro server, the rigid body docking phase uses the PIPER docking program, which relies on the Fast Fourier Transform (FFT) correlation approach. PIPER represents the interaction energy between two proteins using an expression of form *E*;

$$E = w_1E_{rep}+w_2E_{attr}+w_3E_{elec}+w_4E_{DARS}$$

Where,

E_{rep} and E_{attr} denote the attractive and repulsive contributions to the van der Waals interaction energy, and E_{elec} is electrostatic energy.

E_{DARS} is a pairwise structure-based potential; it primarily represents desolvation contributions, i.e., free energy change by removal of the water molecules from the interface.

The coefficients w_1, w_2, w_3, and w_4 define the weights of the corresponding residues.

For the analysis and display of molecular assemblies of the TLR4/5 proteins and vaccine component complex, the visual molecular dynamics (VMD 1.8.3.) program was utilized [17]. The root mean square fluctuation (RMSF) is calculated for Cα atoms, and root mean square deviations (RMSD) of total protein is selected against the backbone Cα atom of the protein complex. Applying the present force field, we completed a 10 ps (pico-second) unrestrained MD simulation of the acid-unfolded state of TLR4/5 proteins and vaccine candidate complex. Primarily the magnitude within the state-of-the-art simulation can define the structural and dynamical features of the studied protein [18].

Normal mode analysis mobility allowed us to investigate the large-scale mobility and the stability of macromolecules. The iMODS server performed the internal coordinates analysis based on the protein-protein structural complex [19]. The server calculates a specific combined motion of large macro-molecule along with the NMA of dihedral coordinates of Cα atoms. Additionally, iMODS estimates B-factor (a disorder of an atom in a protein), structural deformability, and computes the eigenvalue.

2.4. In-silico cloning and physicochemical property assessments of the peptide-based vaccine contrast

Due to the dissimilarity between the codons of human and *E. coli*, codon adaptation tools were used. It is necessary to adapt the codon usage within the prokaryotic organism to boost the expression rate in the respective host system. For the cloning of the vaccine component, *E. coli* strain K12 was selected as a host. The Java Codon Adaptation Tool (JCat) was employed for codon optimization of our vaccine component [20]. Here, we selected the pET28a (+) expression vector for cloning, and its nucleotides sequences were collected from the 'Addgene' vector database [21]. The WebDSVver 2.0 (http://www.molbiotools.com/WebDSV/) was used for pursuing the *in-silico* cloning of peptide-based vaccine component against SARS-CoV-2. Solubility and physicochemical property assessments of the primary sequence of vaccine candidates are essential for determining the state, stability, and accessibility of a vaccine. The solubility of the construct vaccine was predicted against the average solubility of *E. coli* protein in the Protein-Sol webserver [22]. The ProtParam server was accessed for further analysis of various physicochemical properties of the designed vaccine candidate [23].

3. Result

3.1. Retrieval of spike protein multi-epitopes and vaccine designing

We collected 13 MHC-I (SQCVNLTTR, GVYYHKNNK, GKQGNFKNL, GIYQTSNFR, VSPTKLNDL, KIADYNYKL, KVGGNYNYL, EGFNCYFPL, GPKKSTNLV, SPRRARSVA, LGAENSVAY, FKNHTSPDV, and DEDDSEPVL) and 3 MHC-II (IHVSGTNGT, VYYHKNNKS, and FKNHTSPDV) 9mer epitopes. These epitopes were used to construct a peptide-based

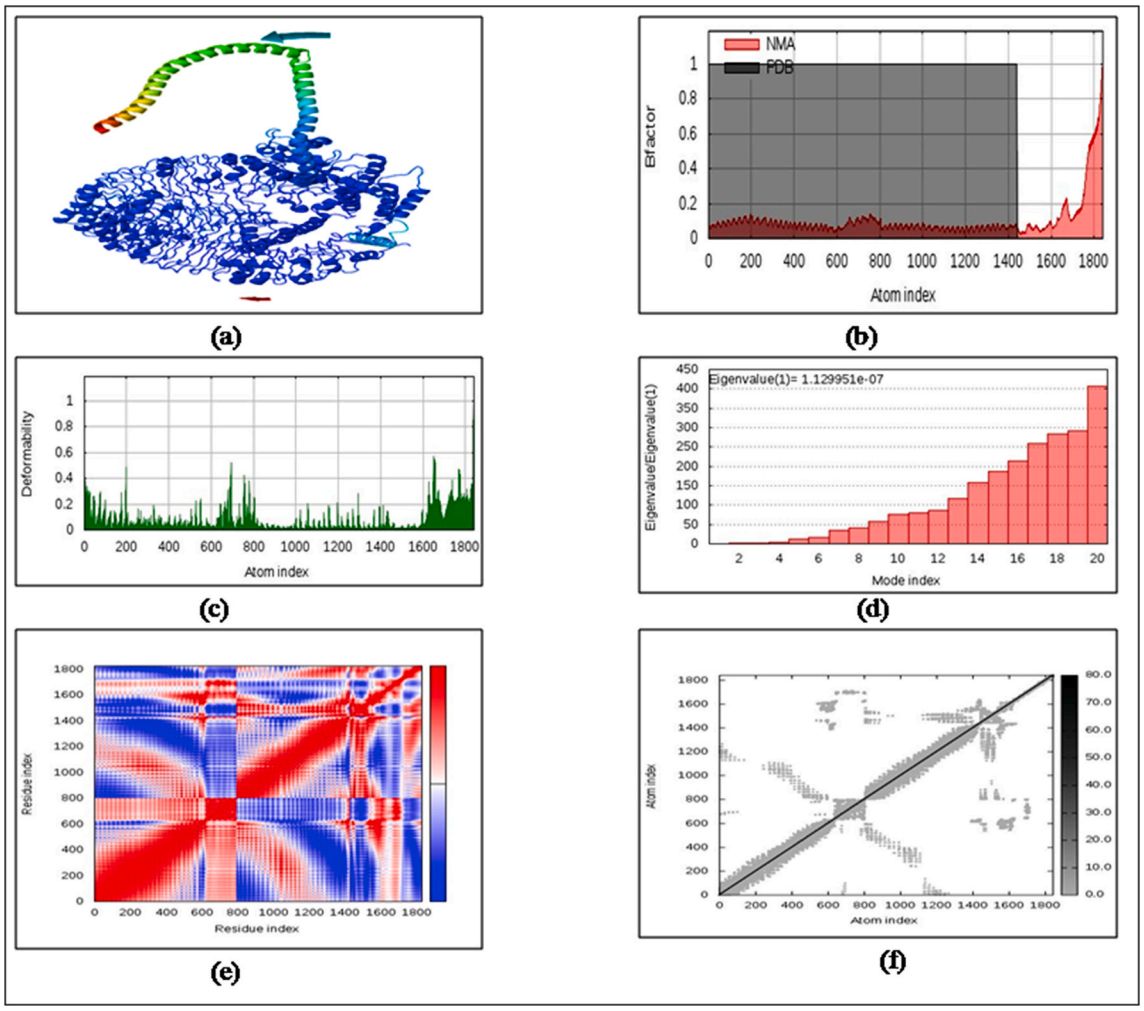

Fig. 4. Outputs of NMA study a) NMA mobility of protein domains b) B-factor plot of PDB and NMA c) deformability plot of atomic fluctuation d) eigenvalue plot e) covariance matrix plot f) elastic network plot.

vaccine against SARS-CoV-2 (Table 1).

3.2. Allergenicity analysis of vaccine component

Both AllerTOP and AllergenFP servers confirmed the non-allergenic nature of the proposed vaccine component. Therefore, the vaccine might not show any harmful allergic reaction after administration.

3.3. Molecular docking, molecular dynamics simulation, and normal mode analysis mobility analysis

The outputs of molecular docking in ClusPro 2.0 showed high negative energy (−1362.3 kcal/mol) for the docking between TLR4/5 proteins and the vaccine component. The cysteine 646 of TLR4/TLR5 formed a non-covalent bond (3.5 Å) with tyrosine 189 of the vaccine construct, whereas, threonine 647 of TLR4/TLR5 formed two non-covalent bonds (2.9 Å and 2.3 Å) with phenylalanine 186 (Fig. 1). The probable immune cascade mechanism of the vaccine candidate in the TIRAP receptor protein has been depicted in Fig. 2 [24].

The molecular dynamic simulation of TLR4/5 proteins and the vaccine candidate was performed by the Visual Molecular Dynamics (VMD 1.8.3.) program, and it produced 700 frames (100 frame = 5000 TS = 10 ps) and RMSD plot (Fig. 3a and b). RMSF was calculated for Cα atoms, and in RMSD, total protein was selected against the backbone of Cα. The plot displayed that the protein complex was in a steady-state.

As per the normal mode analysis mobility, upon binding, the vaccine component and the receptor proteins (TLR4/TLR5) were significantly directed to each other (Fig. 4a) [25]. The deformability plot showed a little bit of fluctuation (Fig. 4c), whereas; the normal mode analysis B-factor was highly minimized from its PDB B-factor (Fig. 4b). The eigenvalue showed an inverse relationship with the variance of the protein-protein docking complex, and the estimated eigenvalue was $1.129951e^{-07}$ (Fig. 4d). In the present work, the covariance matrix is illustrated through the graphical representation via white, red and blue color variations indicating the correlated, uncorrelated, and anti-correlated pairs of amino acid residues, respectively (Fig. 4e). Springs of atomic contact are plotted as grey dots in the elastic network model, where the stiffness of interaction was proportional to the gradient of the grey color (Fig. 4f).

3.4. In-silico cloning and physicochemical property assessments of peptide-based vaccine construct

In-silico cloning is a rapid method to assess the possibility of developing a potent multi-epitopic vaccine in a given time frame. Here, the JCat server calculated the codon adaptation index (CAI) and GC content of the optimized nucleotides sequence of constructed SARS-CoV-2 peptide-based vaccine. The value was noted as 1.0 and 46.61654, respectively. These are expected values for possible efficient expression of the construct vaccine into the host. Finally, the WebDSV tool was applied for inserting the adapted codon sequences of the peptide-based vaccine candidate within the pET28a(+) vector for expression (Fig. 5). The

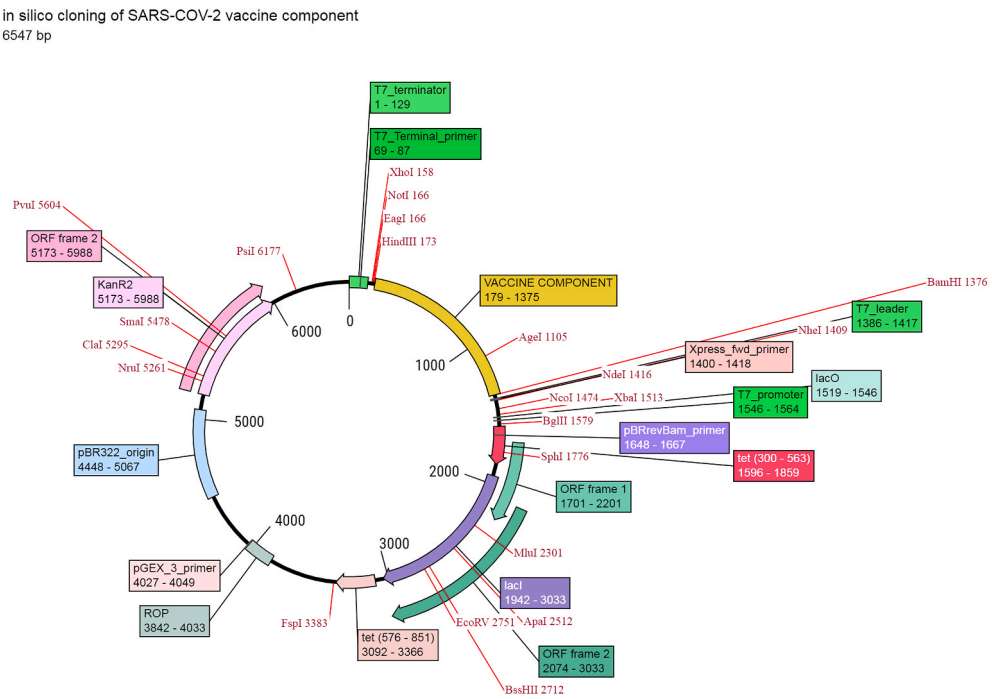

Fig. 5. Schematic representation of our *in-silico* cloning of vaccine candidate within pET28a(+) expression vector.

Table 2
Different physicochemical properties of our vaccine candidate.

Sl. No.	Physicochemical property	Analytical values
1	Solubility	0.698 (Soluble)
2	Number of amino acids	399
3	Molecular weight	40198.87 Da
4	Theoretical Isoelectric point (pI)	8.94
5	Total number of atoms	5667
6	Formula	$C_{1740}H_{2838}N_{510}O_{577}S_2$
7	Estimated half-life	1 h (mammalian reticulocytes, *in vitro*) >20 h (yeast, *in vivo*) >10 h (*Escherichia coli*, *in vivo*)
8	Instability index	16.64 (Stable)
9	Aliphatic index	61.03
10	Grand average of hydropathicity (GRAVY)	−0.552

physiochemical properties and details of solubility of the peptide-based-epitope vaccine candidate are listed in Table 2. Our vaccine candidate showed an instability index of 16.64 (<40, Stable) and a solubility score of 0.698. The estimated half-life was determined as 1 h (mammalian reticulocytes, *in vitro*) and >10 h (*Escherichia coli*, *in vivo*).

4. Discussion

The SARS-CoV-2 has become a global concern and is rapidly emerging as a threat to human civilization. COVID-19, as a pandemic, has already caused many deaths around the globe and still is affecting the human population at a rapid rate. Hence, the new therapeutic approach by targeting TLRs (TLR4/TLR5) modulation may serve as a better choice for vaccine or adjuvant development against the infection of SARS-CoV-2 [26].

Kaur et al. developed a multi-epitope peptide-based chimeric vaccine against the *Taenia solium* by *in-silico* approaches [26]. The structural vaccinological analysis and computational validation of the vaccine candidate were also performed against the Mayaro virus [27]. In considerate to the human pathogenic viruses (West Nile virus and Ebola virus) specific peptide-based vaccine has been developed by employing immuno-pharmacoinformatic techniques [28,29].

The principal focus of our current study was the *in-silico* evaluation of potent vaccine candidates from identified common epitopes. Primarily, we have chosen 16 common B-cell and T-cell epitopes from our previous published work [13]. Afterward, an analysis of the allergenic property was carried out on the designed vaccine candidate. Safe efficacy is a prerequisite for a vaccine, for successful human administration [30]. From the analysis in the AllerTOP and AllergenFP servers, it was found that the constructed vaccine candidate is non-allergenic and safe for future *in vivo* and *in vitro* work. Further, molecular docking of vaccine candidates with TLR proteins was performed in the ClusPro 2.0 server to compare and validate the accuracy, stable protein-protein binding, and molecular interactions [31,32]. It was observed that the molecular docking was significant with a high negative energy value (lowest energy value −1362.3 kcal/mol) of the top ordered protein-protein docking complex [33,34]. The molecular dynamics simulation based refinement of the TLR4/TLR5 and vaccine candidate protein complex established the fact that the conformational model was in a steady state. The residues in the protein complex accelerated conformation transitions among the local energy state in 700 frames (5000 TS = 10 ps), time against distance trajectory. The mobility of the protein complex formed by molecular docking was justified through the normal mode analysis mobility study [35]. Outputs of the normal mode analysis study using iMODS revealed that both TLR4/TLR5 and vaccine candidates have stable interaction movement towards each other upon molecular binding.

Additionally, the complex was not easily deformable as there were significantly lower peaks in the deformability plot. Furthermore, codon adaptation within the prokaryotic system was analyzed to obtain a better adaptation and high expression profile in a eukaryotic expression system. When the construct vaccine candidate was cloned into the pET28 (+) expression vector with the help of WebDSVver 2.0, higher expression of the cloned codons was projected.

The pI value predicted in the ProtParam tool indicates the essential (cation rich) character of the vaccine candidate. The prediction also helps to compute the stability of the vaccine into the host body. An

aliphatic index of 61.03 predicted that the vaccine is thermostable while the instability index lower than 40 (16.60) indicated higher stability of the vaccine structure after expression.

5. Conclusion

Our *in-silico* work reveals that the proposed vaccine candidate is non-allergenic, and has efficient as well as stable molecular interaction with the TLR proteins of humans. Results also indicate a high potential and effectiveness of the selected epitopes against SARS-CoV-2. The codon adaptation and *in-silico* cloning established the impending amplification ability of the vaccine construct within the expression vector as per the conditional requirement. Furthermore, our *in-silico* analysis might be encouraging to the researchers who are trying to develop efficient therapy against COVID-19. However, constructed vaccine candidates will require successive laboratory validation in *in vitro* and *in vivo* models.

Ethics approval and consent to participate

Not Applicable.

Availability of data and material

All data generated or analyzed during this study are included in this published article.

Consent for publication

Not applicable.

Author contributions

MB and ARS designed the model of the computational framework, *in-silico* analysis, and wrote the manuscript. PP, PG, and GS carried out the implementation and validations., BCP and RPS helped with the analysis editing the manuscript. SSL and CC conceived the study and were in charge of overall direction and planning.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.imu.2020.100394.

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