



# Designing a conserved peptide-based subunit vaccine against SARS-CoV-2 using immunoinformatics approach

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## Abstract

The widespread of coronavirus (COVID-19) is a new global health crisis that poses a threat to the world. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) originated in bats and was discovered first in Wuhan, Hubei province, China in December 2019. Immunoinformatics and bioinformatics tools were employed for the construction of a multi-epitope subunit vaccine to prevent the diseases. The antigenicity, toxicity and allergenicity of all epitopes used in the construction of the vaccine were predicted and then conjugated with adjuvants and linkers. Vaccine Toll-Like Receptors (2, 3, 4, 8 and 9) complex was also evaluated. The vaccine construct was antigenic, non-toxic and non-allergic, which indicates the vaccines ability to induce antibodies in the host, making it an effective vaccine candidate.

**Keywords** SARS-CoV-2 · Vaccine · Immunoinformatics · Adjuvants · Non-allergic · Epitopes

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## Introduction

The Coronavirus (COVID-19) has been established to be caused by severe acute respiratory syndrome virus known as SARS-CoV-2 (Dagur and Dhakar 2020). SARS-CoV-2 is similar to the severe acute respiratory syndrome virus

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(SARS-CoV) by ~80%, also has ~50% similarity to the Middle East respiratory syndrome virus (MERS-CoV) and ~96% closeness to bat coronavirus (RaTG13) (Oladipo et al. 2020a, b). SARS-CoV-2 is a member of betacoronavirus, a positive polar single-stranded RNA genome, like MERS-CoV and SARS-CoV. Previously, in 2002 and 2003, SARS-CoV caused SARS outbreaks in Guangdong Province, China. In 2012 MERS-CoV caused MERS outbreak in the Middle East (Chakraborty et al. 2020a). The genome of the virus is segmented into three parts; ORF1a/b region, which compasses most of the non-structural proteins (nsp) is the first part. The rest parts of the virus genome encode accessory proteins and four essential structural proteins, including spike (S) glycoprotein, small envelope (E) protein, matrix (M) protein, and nucleocapsid (N) protein (Kang et al. 2020).

The SARS-CoV-2 spike glycoprotein is the receptor binding site, a core target for neutralizing antibody, and facilitates virus internalization and membrane fusion (Ou et al. 2020). Regarding epidemiology, human-to-human transmission of the virus through the sneezes, cough, and respiratory droplets has been confirmed, yet the zoonotic nature has not been established (Bhattacharya et al. 2020a).

Emerging and reemerging viruses are often the cause of epidemics, its global spread, and public health challenge more specifically, the novel strains involved in outbreaks of varying intensity such as the SARS epidemic of 2003 and the MERS epidemic of 2012; and the present emergence of respiratory distress associated with SARS-CoV-2 (2019) (Nadeem et al. 2020). Pandemic has dotted man's history and acts as markers pointing to the end of an era and the beginning of a new one. Like the previous pandemic, recent COVID-19 has claimed many lives and halted economies, thereby putting the world in a state of panic. One would think with the development in science, widespread infections like this would have been curbed or eradicated.

Currently, there are no officially approved drugs or vaccines available against SARS-CoV-2 (Chakraborty et al. 2020b). The rapid transmission of SARS-CoV-2 in the human population therefore requires an urgent development for medicines and vaccines to curtail the pandemic. Though the different drug has been tested, one of these drugs is remdesivir (RDV), which shows a broad spectrum of antiviral activity against many viruses like Ebola, Nipah, respiratory syncytial virus (RSV) family and a diverse category of coronaviruses including SARS CoV and MERS CoV (Saha et al. 2020a, b). Vaccines are crucial tools in the prevention and halting of the global spread of SARS-CoV-2 new viral outbreaks. A different approach in vaccine technology which utilizes whole virus, live-attenuation, nucleic acid, virus-like particle, peptide, viral vector, recombinant protein, inactivated virus and subunit has been employed in

vaccine development. However, no specific antiviral drugs or vaccines against the newly emerged SARS-CoV-2 are currently available.

Subunit vaccines for SARS-CoV-2 rely on eliciting an immune response against the S-spike protein to prevent its cleavage with the host ACE 2 (Chen et al. 2020).

Hence this study seeks to explore the SARS-CoV-2 spike glycoprotein for the development of subunit vaccine using reverse vaccinology.

## Methodology

### Selection of SARS-CoV-2 virus sequence for vaccine design

Whole-genome sequences for SARS-CoV-2 available from NCBI and GISAID databanks were retrieved for this study. The first coronavirus isolate from Wuhan submitted to NCBI was also retrieved to annotate and select literature established antigenic gene (surface glycoprotein) (Kumar et al. 2020) from the retrieved whole-genome sequences. The annotated surface glycoprotein was then tested for antigenicity using ANTIGENpro on the scratch protein and VaxiJen (Pandey et al. 2016) of which only sequences meeting the threshold for antigenicity (0.8 on ANTIGENpro and 0.4 on VaxiJen) were used for further analysis. We retrieved a total of 35 sequences available from eight African countries (DR. Congo-16, South Africa-4, Senegal-7, Gambia-2, Egypt-1, Ghana-5, Nigeria-1, Tunisia-3) and the first SARS-CoV-2 isolate (reference sequence) of Wuhan on May 3, 2020. Only the isolates that met the antigenicity criteria were used for this study. Only 3 isolates (DR. Congo-2 and Gambia-1) passed the selection process and were thus subjected to further analysis. The accession number and antigenic status from the servers for each of the sequences are recorded in Supplementary Table 1.

### Prediction of linear B-cell epitopes

BepiPred 2.0 server developed with annotated epitopes from the structure of the antigen-antibody complex of proteins using forest-based algorithms was employed for the prediction of B-cell epitopes. The forest-based algorithm used by the server enables it to make more reliable and accurate predictions when compared with other available tools (Jespersen et al. 2017; Majid and Andleeb 2019). ABCpred prediction server is accessible online and was used for the linear B-cell prediction. The server was used to justify the selection of the B-cell epitopes from the result of BepiPred. The ABCpred server works using Recurrent Neural Network (RNN) which depends on different windows interval length

to achieve high accuracy (Saha and Raghava 2006; Shey et al. 2019).

### Prediction of MHC-II binding epitopes

The IEDB MHC-II tool was used in predicting epitopes (15-mer) for the selected antigenic protein using H2-IAb, H2-IAd and H2-IEd mouse allele. The server was curated to express the MHC-II and peptides binding potential on its inhibitory concentration of score (IC<sub>50</sub>). IC<sub>50</sub> below 5000 nM indicates the lowest binding affinity, IC<sub>50</sub> less than 500 nM depicts midrange affinity while IC<sub>50</sub> values less than 50 nM implies highest binding affinity towards MHC-II. The percentile rank is inversely equivalent to the IC<sub>50</sub> score. The server algorithm was improved significantly by training with a larger dataset, and homologous peptides were avoided to give a real-world estimation of the predicted parameters to the user (Wang et al. 2010; Solanki et al. 2019).

### Prediction of MHC-I binding epitopes

The NetCTL 1.2 server operates through the combination of MHC class I binding peptides, proteasomal C-terminal cleavage and transporter that is linked with antigen processing (TAP) transporter competence. It was used in predicting cytotoxic T lymphocytes for the selected proteins. Outcome projection is based on artificial neural networks, and a weight matrix is created at Default settings (threshold, 0.75), for the evaluation of CTL epitopes 50. NetCTL method of prediction shows higher predictive ability than any other prediction method and has sensitivity 5% more than any server (Larsen et al. 2007; Chauhan et al. 2019). The server predicted a lot of other parameters; therefore, the CTL epitopes and the corresponding scores were extracted and tabulated from the result using regular expression (REGEX) and pandas modules of python programming language.

### Building of multi-epitope vaccine candidate

High scored CTLs, HTLs and B cells epitopes were integrated to build the vaccine candidate. RS09 peptide adjuvant, which is safer and considered an improvement over traditional vaccination methods, was used to enhance the immunogenicity of the potential vaccine (Shanmugam et al. 2012). The adjuvant was connected to the first B cell epitope by EAAAK linker, while B-cells and HTL epitopes were joined together by GPGPG linker and AAY linker was used for attaching the CTLs epitopes from N to C terminal (Chauhan et al. 2019).

### Calculation of the protein antigenicity, allergenicity and toxicity

VaxiJen v2.0 server was used in examining the antigenicity potential of the protein using non-alignment dependent algorithm but focuses on the physiochemical attributes of the selected prototype vaccine (Yasmin et al. 2016; Sanami et al. 2020). ANTIGENPro is a free server which functions with specific microarray data for the evaluation of protein antigenicity index. The accuracy of the server was reported to be 76% based on cross-validation experiments (Magnan et al. 2010; Kalita et al. 2019). AllerTOP v2.0 server (employs machine learning techniques like amino acid E-descriptors, auto and cross variance transformation and the k nearest neighbours, for the allergenic classification with accuracy of 85.3% (Sanami et al. 2020).

### The physiochemical and solubility properties analysis

ProtParam (ExpASy) a free online tool was used in the estimation of the in vivo and in-vitro half-life, amino acid composition, molecular weight, aliphatic score, instability index, theoretical pI (isoelectric point), and grand average of hydropathicity (GRAVY). The solubility of the putative vaccine sequence was evaluated by identifying negligible hydrophobicity or hydrophilicity of an amino acid residue within the protein (Garg et al. 2016; Saadi et al. 2017).

### Analysis of secondary structure of the construct

SOPMA was employed in the evaluation of the vaccine construct. The pathway uses specific BLAST for the characterization and selection of the sequences showing definite similarity to the designed vaccine (Geourjon and Deléage 1995; Chauhan and Singh 2020). The PSIPRED 3.2 was used in validating the secondary structure (Dehghani et al. 2020).

### Assembled vaccine tertiary configuration projection

The 3D structure of the vaccine sequence was projected by I-TASSER (Iterative Treading ASSEMBLY Refinement) server for homology model, I-TASSER operates to design a 3D atomic model by utilizing the multiple threading alignments and iterative structural assemblage (Zhang 2008; Negahdaripour et al. 2018). The generation of automated protein structures and prediction is best achieved with I-TASSER. Phyre2, which uses the latest recognition modulus to construct 3D structures and performs the prediction of ligand binding sites, was also used for the homology modelling of the peptides designed (Majid and Andleeb 2019).

## Refinement and validation of putative vaccine

An online web, Galaxy Refine which employs the CASP10 method for the filtration of the 3D structure was utilized in the refinement of our multi-epitope vaccine candidate (Ko et al. 2012; Chauhan et al. 2019). This server enhanced the structural and global quality of the 3D structure by reconstruction, repacking and simulations of the 3D structure for relaxation. ProSA-web tool accessible and RAMPAGE was employed in the validation of the refined 3D structure (Wiederstein and Sippl 2007; Khatoon et al. 2017). RAMPAGE server probes Ramachandran plot and the principles of PROCHECK for validation via Ramachandran plot. At the same time, excellent scores of the calculated errors in the queried 3D structure were highlighted with Prosa-Web.

## Disulfide engineering

Disulfide by Design 2 server (DbD2) available online was used for the disulfide engineering of the refined protein structure. The input to the server was the advanced tertiary structure. All parameters on the server were set to default, and the best mutants possible were selected based on energy level and Chi3 value (Craig and Dombkowski 2003; Pandey et al. 2018).

## TLR interaction with vaccine

The Toll-like receptor (TLR) proteins identify pathogenic microbial elements and activate the pathway for functioning and regulation of adaptive immune response (Bhattacharya et al. 2020b). ClusPro server is based on PIPER and generates structures using different coefficient sets; the radius of the near-native region, defined in terms of the root mean square deviation (RMSD) from the X-ray structure of the dimer and the number of docked structures that are expected to cluster in the near-native region to classify the dimer biological rather than crystallographic. Human toll-like receptors (2, 3, 4, 8 and 9) was assembled with the vaccine using ClusPro (Kozakov et al. 2017; Solanki et al. 2019).

## Molecular dynamics (MD) simulation

The estimation of direction and motion of molecules of the vaccine-receptor complex with regards to covariance, B-factor, eigenvalues and deformability was done using iMODs online server. The deformability is dependent on the potential of a specific molecule to deform at its residues. The eigenvalue represents how rigid the motion is, and it has a relationship with the energy required to deform the structure of the vaccine-receptor complex as low eigenvalue depicts structures that can be easily deformed (López-Blanco et al. 2014; Sayed et al. 2020).

## Authentication

### Codon optimization and in silico vaccine expression

Reverse translation and augmentation of codon were performed using Jcat for optimum vector (*E. coli*) cellular expression (Grote et al. 2005). The GC content and CAI scores of the sequence to ensure maximum expression was determined. Restriction sites of XhoI and NdeI were selected to the reverse translated sequence. The final vaccine was then cloned into pET-28a (+) plasmid using snap-gene software from Insightful Science (Li et al. 2016).

### Immune model

Agent-based algorithm for the estimation of antigen and foreign particles on immune activity was used by *C-ImmSim online* server to determine the immune actions against the antigen and foreign particles. The interferon, cytokines and antibody level upon vaccine administration, including Th1 and Th2 responses are also estimated by the webserver. Th1 and Th2 responses are also projected by the webserver (Khan et al. 2019).

## Result

### Prediction of linear B-cell epitopes

Twenty linear B-cell epitopes were selected from the generated epitopes based on the length ( $\geq 6$  mers) and the score of the epitopes (0.5). The selected epitopes were used for further analysis. Table 1 below contains all the selected B-cell epitopes.

### Prediction of MHC-II binding epitopes

MHC-II binding epitopes, otherwise known as HTL to be utilized in further analyses, were selected from the list of predicted epitopes based on their MHC binding affinity score and antigenicity. Only Twenty 15 mer epitopes met the selection criteria and were used in vaccine construction. Table 1 below also shows the selected epitopes.

### Prediction of MHC-I binding epitopes

A total of 612 epitopes were predicted for MHC-I binding epitopes out of which 20 was found to be antigenic as well as possess high MHC binding affinity. The selected 9-mer epitopes are presented in Table 1 below.

**Table 1** Selected predicted epitopes for further analysis

Linear B-cell epitopes	MHC-II binding epitopes	MHC-I binding epitopes
NITNLCPFGEVFNATRFASVYAWNKRISNC	VFVYVNVNFPLAVMAS	TSNQVAVLY
SFSTFKCYGVSPTKLNDL	EVFVYVNVNFPLAVMA	QLTPTWRVY
IRGDEVQRQIAPGQTGKIADYNYKLP	FVYVNVNFPLAVMASN	GAEHVNNSY
NNLDSKVGGNYNLY	QEVFVYVNVNFPLAVM	VASQSIIAY
LFRKSNLKPFERDISTEIQAGST	QQEVFVYVNVNFPLAV	KTSVDCTMY
EGFNCFYFPLQS	VYVNVNFPLAVMASNF	STECSNLLL
PTNGVGF	LLEFLIFWSKRTKY	ECSNLLQY
HAPATVCGPKKSTNLV	RFQRYYYRRAKAPT	RSFIEDLLF
SNKKFLPF	EFLIFWSKRTKYIS	VGGNYNYLY
TNTSNQVA	LEFLIFWSKRTKYI	ERDISTEY
NCTEVPVAIHADQLTPTWRVYSTGSNVF	GRFQRYYYRRAKAPT	TSNQVAVLY
VNNSYECDIPIGA	FQRYYYRRAKAPTME	QLTPTWRVY
CASYQTQNSPRRARSVASQ	LIRAAEIRASANLAA	GAEHVNNSY
TMSLGAENSVAYSNN	VTQQLIRAAEIRASA	VASQSIIAY
VEQDKNTQE	TQQLIRAAEIRASAN	KTSVDCTMY
KQIYKTPPIKDFGGFN	QQLIRAAEIRASANL	STECSNLLL
LPDPSKPSKR	QLIRAAEIRASANLA	ECSNLLQY
TLADAGFIKQ	YVTQQLIRAAEIRAS	RSFIEDLLF

### Building of multi-epitope vaccine candidate

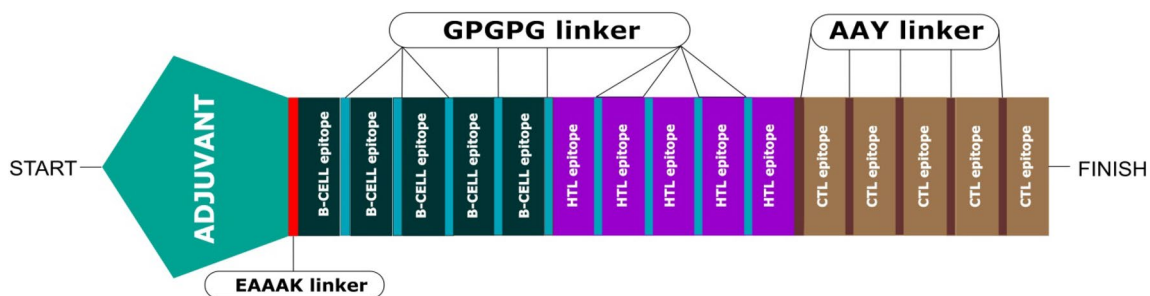
A multi-epitope subunit vaccine candidate was constructed using the selected epitopes predicted above. RS09 adjuvant was attached to the N-terminal of the potential vaccine candidate construct which was linked to the other parts by EAAAK linker. GPGPG and AAY were employed in connecting the different epitopes that make up the vaccine construct. Figure 1 below shows the arrangement of the epitopes, adjuvant and linkers that makes up the vaccine construct.

### Calculation of the protein antigenicity, allergenicity, toxicity and host homology

The vaccine construct was submitted to various servers which predicted the antigenicity, allergenicity and toxicity of the construct. The vaccine construct was found to be antigenic, non-allergenic and non-toxic. BLASTp server was used to analyze the vaccine construct to detect and avoid epitopes homologous with the human protein. The construct was then subjected to further analysis to elucidate its nature and validate the construct.

### The physiochemical analysis

The vaccine construct was predicted by ProtParam to have a molecular weight of 61.32 kDa. The theoretical pI was also calculated by the server to be 6.70, indicating a slightly



**Fig. 1** Schematic representation of the final multi-epitope vaccine candidate. The vaccine candidate contains an adjuvant (green) at the amino-terminal end linked with the multi-epitope sequence through

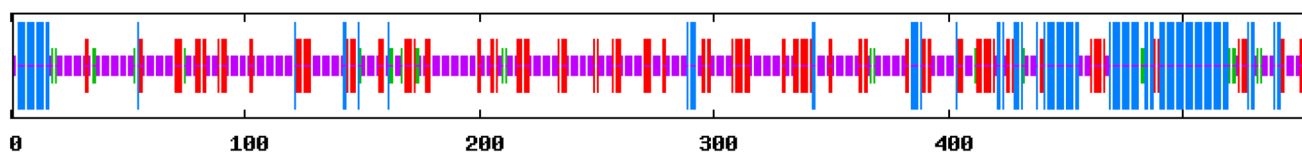
an EAAAK linker (red). B epitopes and HTL epitopes are linked using GPGPG linkers (cyan) while the CTL epitopes are linked with the help of AAY linkers (brown)

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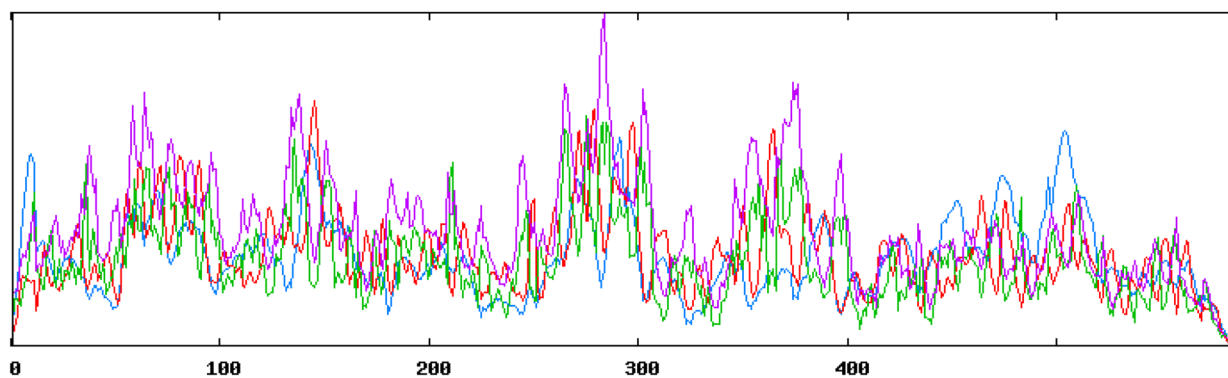
SOPMA:
Alpha helix (Hh): 117 is 28.00%
310 helix (Gg): 0 is 0.00%
Pi helix (Ii): 0 is 0.00%
Betabridge (Bb): 0 is 0.00%
Extended strand (Ee): 145 is 24.79%
Betaturn (Tt): 31 is 5.30%
Bend region (Ss): 0 is 0.00%
Random coil (Cc): 292 is 49.91%
Ambiguous states (?): 0 is 0.00%
Other states : 0 is 0.00%

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(a)



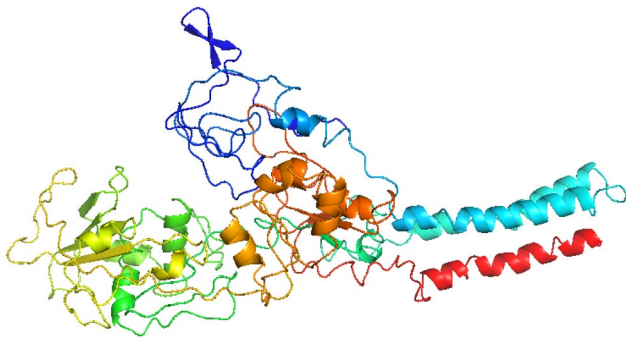
(b)



(c)

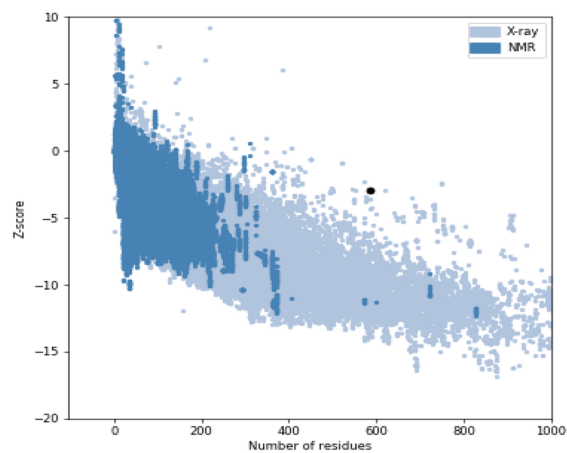
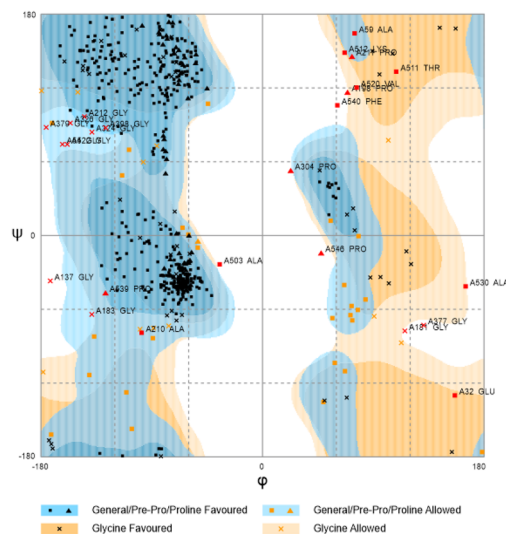
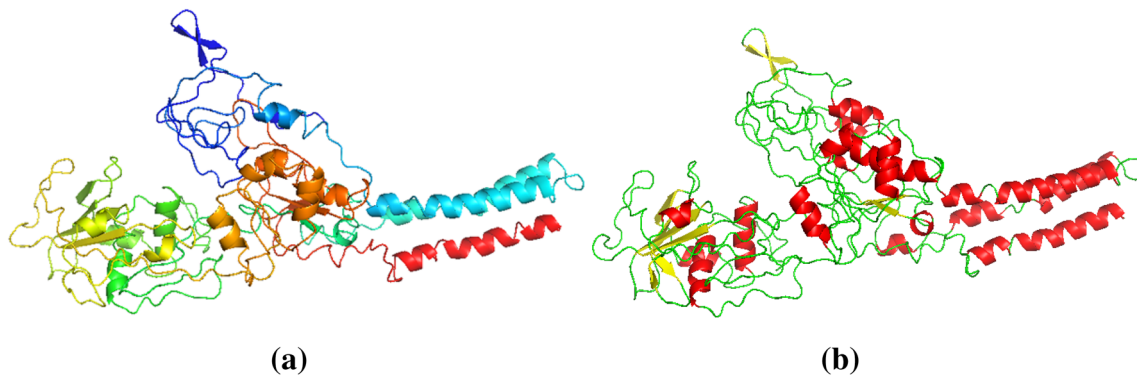
**Fig. 2** The secondary structure of the vaccine construct: **a** secondary structure result showing the percentage of Alpha Helix, Extended Strand, Beta Turn and Random Coil **(b, c)** OMPL prediction of the

secondary structure represented by different colours. Blue is alpha-helix, and green is Beta strands, red is extended strand and yellow random coil



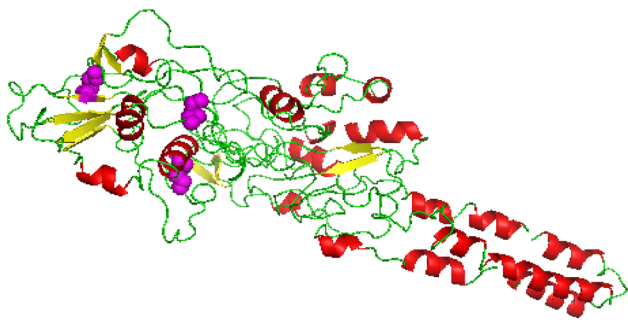
**Fig. 3** The assembled tertiary configuration of vaccine construct

acidic vaccine construct. The total number of negative residues on the vaccine construct was predicted to be 33 while that of positive residue is 31. The molecular formula of the construct is  $C_{2740}H_{4107}O_{838}S_{15}$ , and the total number of atoms present in the construct is 8441. The estimated half-life is 4.4 h, > 20 h and > 10 h in mammalian reticulocytes (in vitro), yeast (in vivo) and *Escherichia coli* (in vivo) respectively. The construct is found to be stable as inferred from its instability index 26.76. Aliphatic index of 65.78 and Grand average of hydropathicity (GRAVY) of  $-0.261$  were also observed.



**Fig. 4** Refinement and validation of putative vaccine: **a** the tertiary structure of the vaccine construct before refinement **b** the refined form of the putative vaccine **c** the Ramachandran map of the refined

structure showing 89.2% of its residue in the favoured region, 6.5% in the allowed area and 4.3% in outlier regions **d** the Z score graph generated from ProSA-web with a Z score of  $-2.93$



**Fig. 5** Disulfide engineering showing disulfide bond as predicted by disulfide by design 2

### Analysis of secondary structure and solubility properties of the construct

SOPMA generated the secondary structure of the vaccine construct, which is composed of 20% Alpha Helix, 24.79% Extended Strand, 5.3% Beta Turn and 49.91% Random Coil (Fig. 2). PSIPRED was used to validate the result generated for the secondary structure. I-TASSER was utilized in solvent accessibility analysis which predicted 14% of the residue as buried, 77% of the residue as medium exposed and 9% as highly exposed.

### Assembled vaccine tertiary configuration projection

Ten threading templates were used to generate five tertiary structure model whose C-score ranges from  $-3.58$  to  $-1.05$ . The C-score value whose standard ranges between  $-5$  and  $2$  indicates more stability as it tends towards the positive numbers. Therefore, the model with the highest value of C-score (Model 1 with  $-1.05$  C-score) was selected (Fig. 3). The model chosen has an estimated TM-score of  $0.58 \pm 0.14$ , which shows that the topology of the structure is accurate because the TM-score is greater than  $0.5$ . The server also predicted RMSD value of  $10.1 \pm 4.6$  Å. Figure 3 shows the assembled tertiary structure of the vaccine construct.

### Refinement and validation of putative vaccine

In the refinement of the generated tertiary structure of the vaccine construct, the tool Galaxy web was employed which developed models from which model 5 was preferentially chosen based on its Clash score of 22.1, Poor rotamers which of 0.9, GDT-HA of 0.9397, Ramachandran favoured of 86.8%, RMSD of 0.444 and MolProbity of 2.461, this is shown in Fig. 4b as compared to the unrefined structure in Fig. 4a. The refined constructed tertiary structure of the

putative vaccine was validated by different servers. Rampage was used for the Ramachandran map plot which validated the refined structure has 89.2% of its residue in the select region, 6.5% in the allowed area and 4.3% in outlier regions (Fig. 4c). ProSA-web was utilized to further validate the refined structure with resultant Z score of  $-2.93$  (Fig. 4d). ERRAT with a quality score of 74.4 and other data predicted above helped in authenticating the selected model.

### Disulfide engineering

In a bid to stabilize the model of the refined vaccine candidate, disulfide engineering was performed on the refined construct using Disulfide by design v2.0. 66 pairs of residues were predicted usable for stability enhancement for the refined construct. After evaluation of all possible pairs of residues, only five pairs of residues were selected based on energy level less than or equal to 2.0 and Chi 3 value between  $-87$  and  $+97$  degree as shown in Fig. 5. Five mutants were generated, which includes LEU16-ARG27, SER123-GLU155, ALA218-TYR231, GLY267-TYR274 and ASN474-LEU515.

### TLR-vaccine interaction

The interaction of the vaccine construct with multiple TLRs (TLR2, TLR4, TLR8, TLR9) was analyzed combining each TLR (receptor) and vaccine construct (ligand). The ClusPro server used for the analysis output 30 models for each receptor-ligand interaction of which the best model was selected based on the binding energy weight for the balanced coefficient. The selected models are shown in Fig. 6 below.

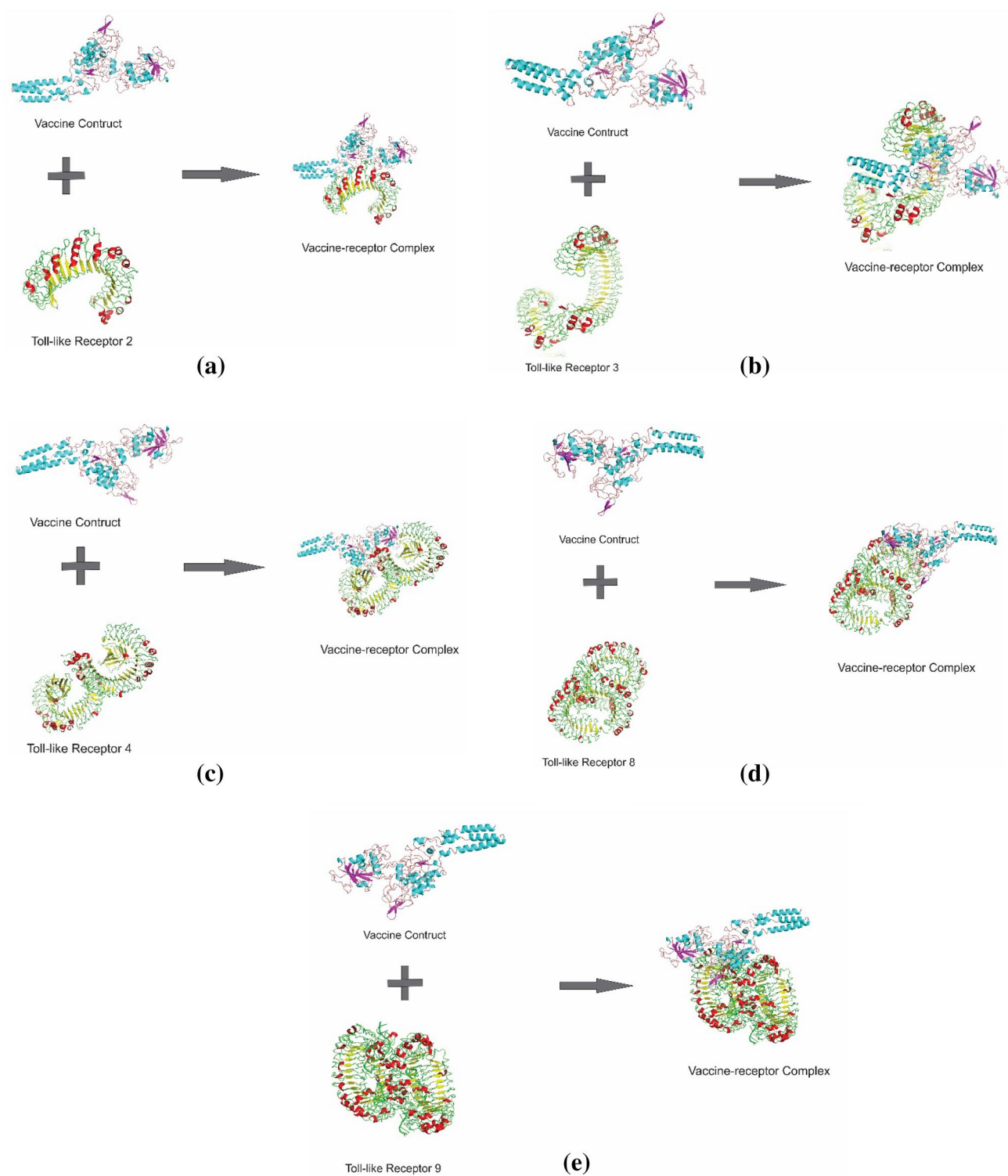
### Molecular dynamics simulation

The vaccine-TLR complex was examined for its molecular dynamics to establish residual stability and function. The spin direction of the vaccine and receptor protein was predicted by the server as indicated in Fig. 7. The server also analyzed the vaccine-TLR deformability region and Fig. 8 shows the result. B-factor, Eigenvalue, Variance, residual index and the elastic network model was also predicted (Figs. 9, 10, 11, 12, 13) by the iMODs server which was used for the molecular dynamics simulation.

### Codon adaptation and in silico cloning

The COVID-19 vaccine candidate was expressed in *E. coli* using JCAT and SnapGene server. Adapting the vaccine into *E. coli* k12 strain predicted the GC content of 55.67%,

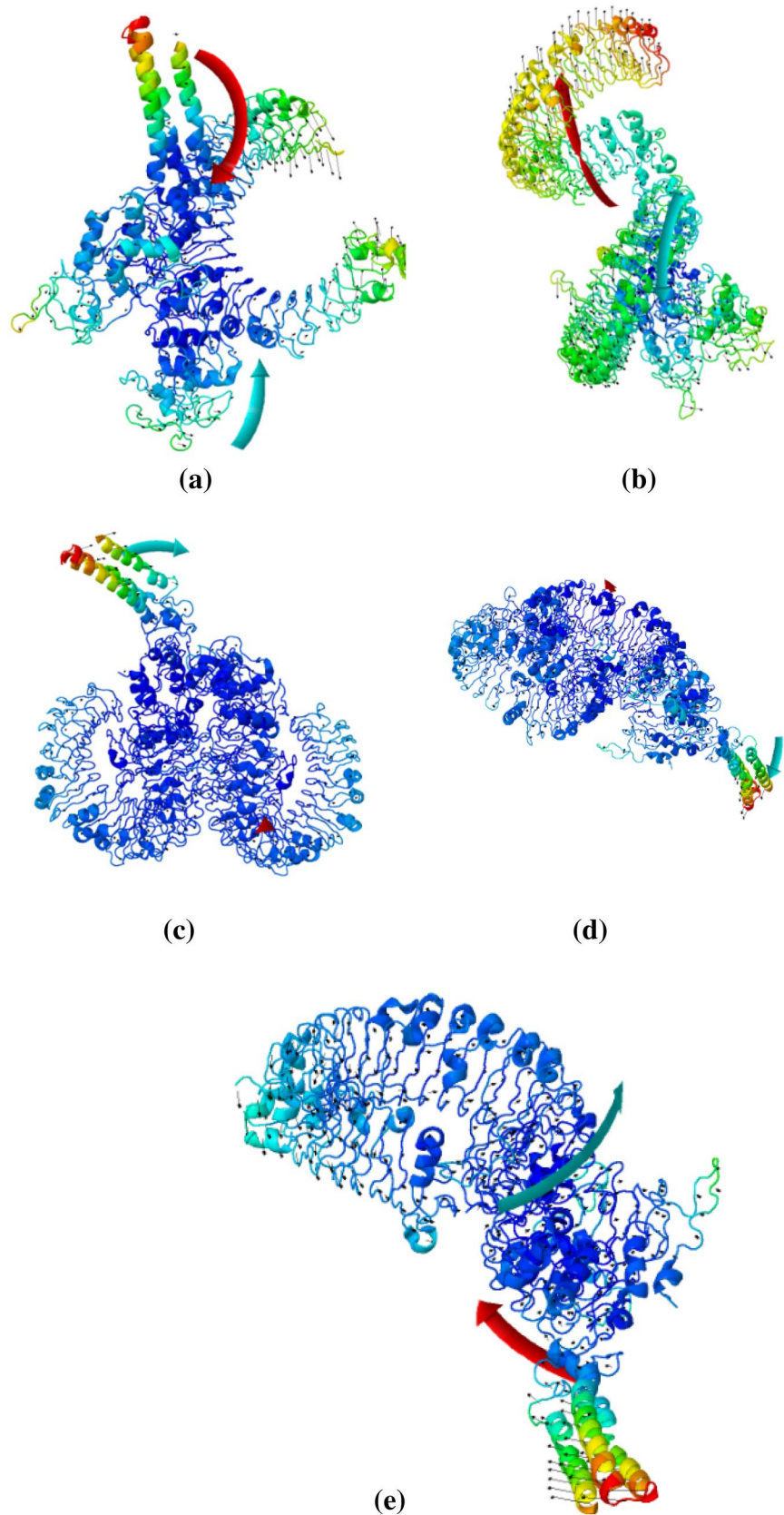


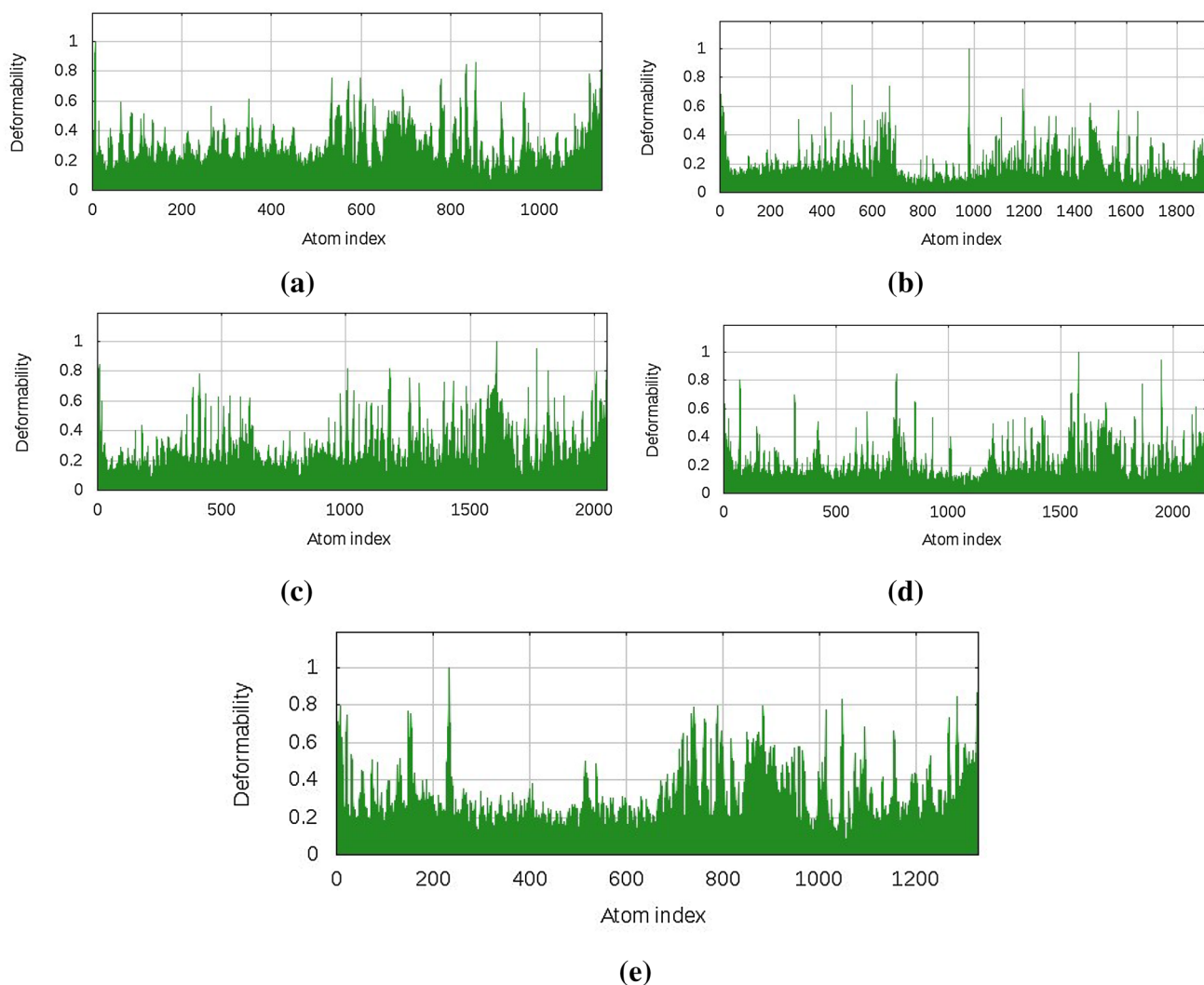


**Fig. 6** Molecular docking of vaccine with TLR showing: **a** docked complexes for vaccine-TLR2 complex with vaccine coloured pink and TLR2 coloured red. **b** Docked complexes for vaccine-TLR3 complex with vaccine coloured pink and TLR3 coloured red. **c** Docked complexes for vaccine-TLR4 complex with vaccine coloured pink and

TLR4 coloured red. **d** Docked complexes for vaccine-TLR8 complex with vaccine coloured pink and TLR8 coloured red. **e** Docked complexes for vaccine-TLR9 complex with vaccine coloured pink and TLR9 coloured red

**Fig. 7** Molecular dynamics simulation spin prediction result: **a** spin prediction of the vaccine–TLR2 interaction. **b** Spin prediction of the vaccine–TLR3 interaction. **c** Spin prediction of the vaccine–TLR4 interaction. **d** Spin prediction of the vaccine–TLR8 interaction. **e** Spin prediction of the vaccine–TLR9 interaction





**Fig. 8** Molecular dynamics simulation deformability B-factor result: **a** deformability B-factor region of the vaccine–TLR2 interaction. **b** Deformability B-factor region of the vaccine–TLR3 interaction. **c**

Deformability B-factor region of the vaccine–TLR4 interaction. **d** Deformability B-factor region of the vaccine–TLR8 interaction. **e** Deformability B-factor region of the vaccine–TLR9 interaction

Codon Adaptation Index (CAI) of 0.93; and back-translated the protein sequence to an *E. coli* codon compatible nucleotide. The back-translated nucleotide was adapted into the *E. coli* expression system using restriction enzyme XhoI (158) and XbaI (335) as a cloning site (Fig. 14).

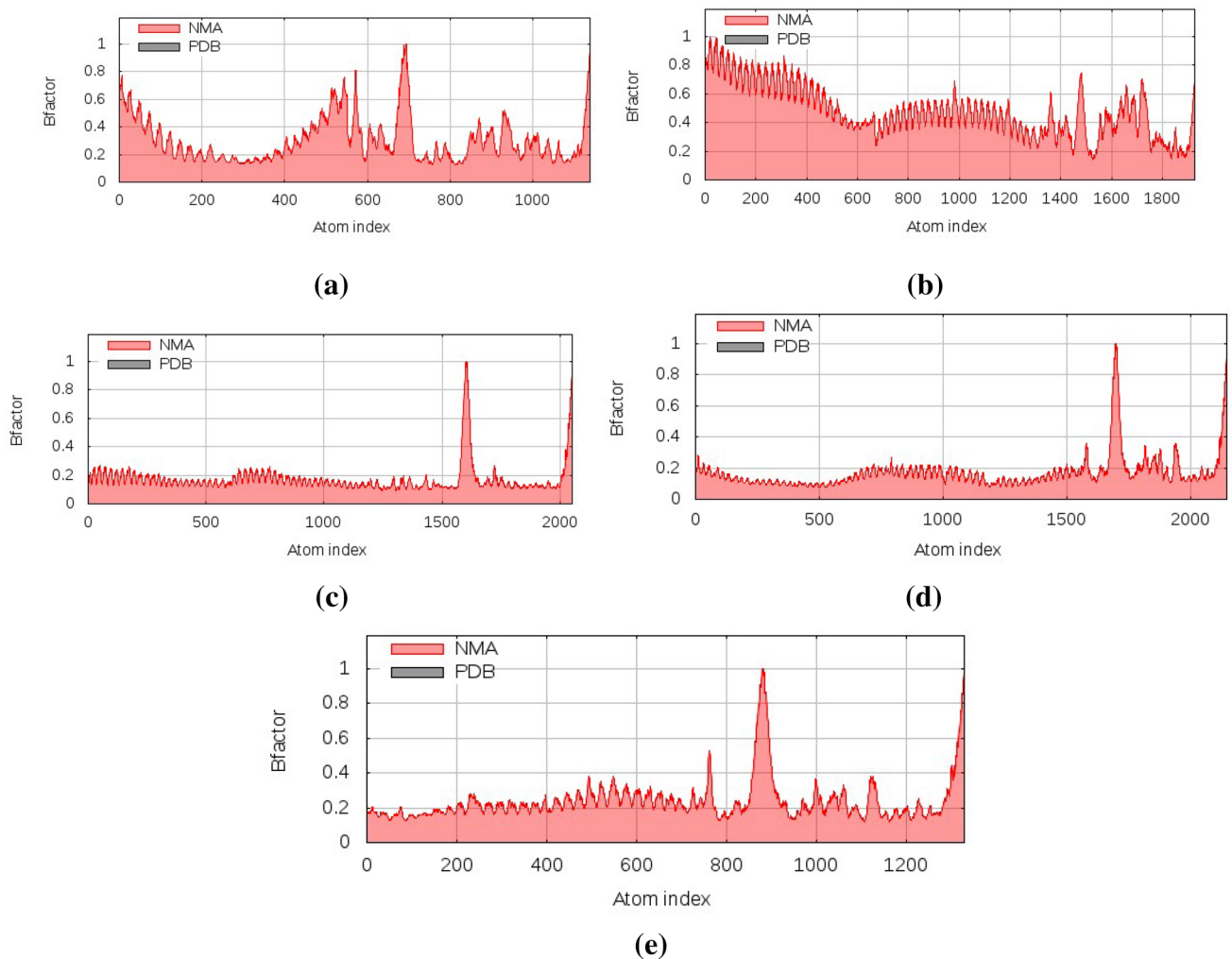
### Immune simulation

The C-ImmSim server revealed the system’s successful immune response and the increased half-life of the vaccine candidate. The specific immunoglobulin and interleukin concentrate was shown (Fig. 15). Activity of the CD4, T-helper lymphocytes count as well as B lymphocytes count was uncovered.

### Discussion

The existence of man has been in close association with microorganisms like bacteria, parasites, prions and viruses; however, the anthropogenic ways of men has led to the emergence, extinction while some of this organisms in a bid to survive has evolved into other forms. Such is the case of SARs CoV-2, which is the etiologic organism of the coronavirus disease 2019 (COVID-19). The havoc wrecked by COVID-19 has destabilized man, all hands are on deck, and the quest to find a lasting solution is the ultimate.

Overtime vaccine has proven to be the rescue from infectious disease, more importantly, for prophylactic, prevention is preferred to cure, and the portion of the world population at risk of COVID-19 infection is greater than the currently infected. The various mechanism is used in vaccine



**Fig. 9** Molecular dynamics simulation mobility B-factor result: **a** mobility B-factor of the vaccine–TLR2 interaction. **b** Mobility B-factor of the vaccine–TLR3 interaction. **c** Mobility B-factor of the vac-

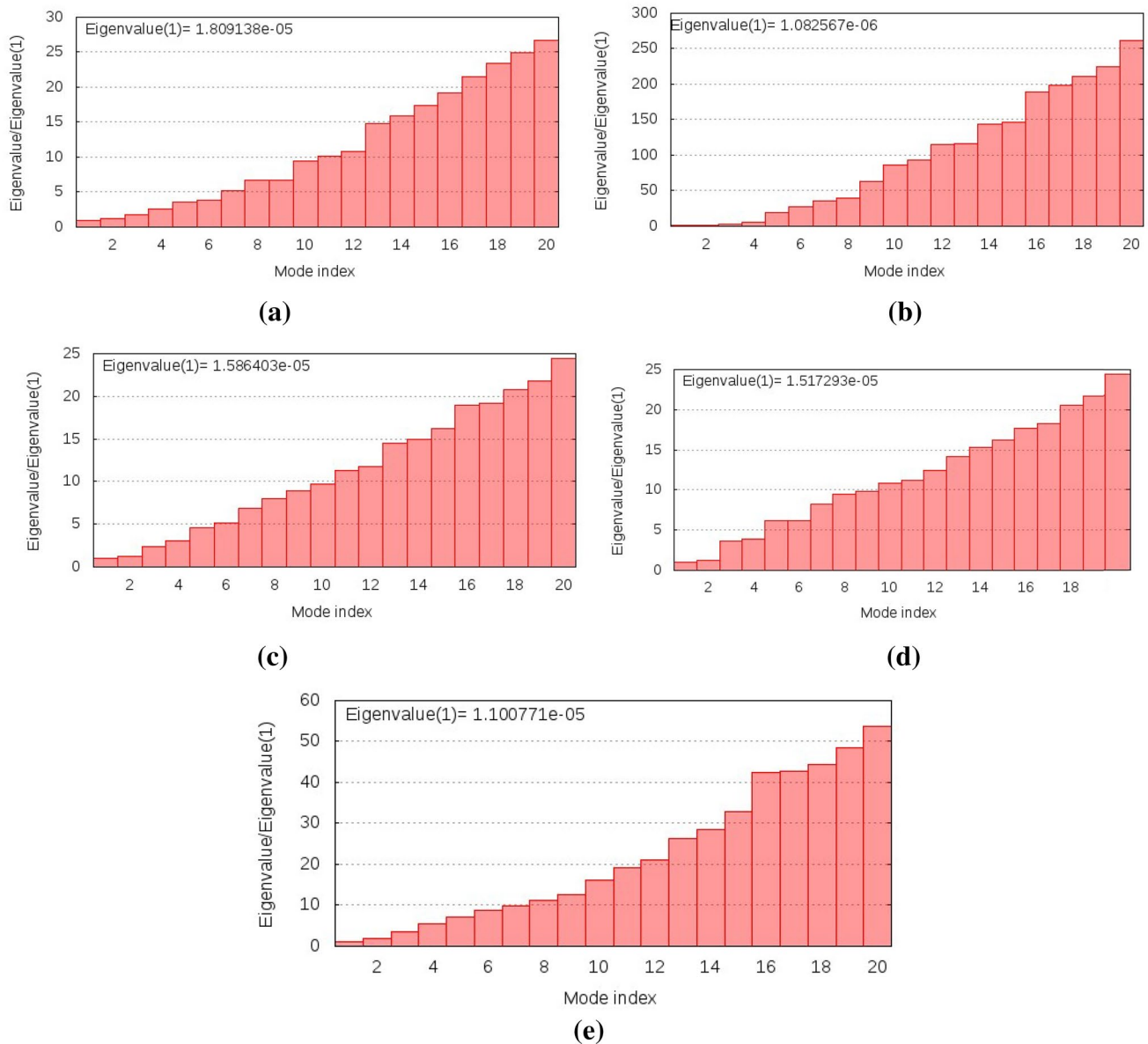
cine–TLR4 interaction. **d** Mobility B-factor of the vaccine–TLR8 interaction. **e** Mobility B-factor of the vaccine–TLR9 interaction

machinery such as live attenuation, conjugated, DNA, mRNA and Subunit (Wadhwa et al. 2020). We explored the surface glycoprotein of COVID 19 to predict the immune capable epitopes for vaccine design. The surface glycoprotein is the antibody stimulating segment of SARs CoV-2 though conserved yet with slight variation. Antibodies are defence army that ward-off foreign agents including pathogenic viruses (Forthal 2014), lymphocytes which is a domain of leucocytes recognizes the presence of the antigen, then stimulates corresponding antibody against it due to its lock-key specificity. The antigens are destroyed by antibodies and are finally engulfed by macrophages.

Nucleotide sequences were recovered from the GISAID database (accession information in Supplementary Table 1) and the B-cell, HTL and CTL epitopes were projected (Table 1). Stimulation and generation of T and B cell-mediated long-term immune response are essential for the

curbing of a deadly virus such as SAR-CoV-2 (Oladipo et al. 2020a, b). B-cells are responsible for antibody maintenance and facilitate response to future exposure to an antigen (Tobón et al. 2013). B-cells determine humoral and adaptive immunity; it functions by presenting the antigens through the MHC class II which is recognized by CD4<sup>+</sup> to produce T-cell immune response during infection thereby conferring early protection (MS 2020). B cells are significant in ordering the specific immunogenic response following an attack on the system. Similarly, T cells decide cellular immune responses; they direct effector roles such as pathogen clearance and autoimmune reactions (Bacchetta et al. 2016). HTL mediates fast and efficient antibodies to infections of non-tissue (Chaplin 2010).

The concept of multiple epitope vaccine tends to identify and build B and T cells that are typically immune activation, thereby provoke effective and specific responses. The



**Fig. 10** Molecular dynamics simulation Eigenvalue result: **a** Eigenvalue of the vaccine–TLR2 interaction. **b** Eigenvalue of the vaccine–TLR3 interaction. **c** Eigenvalue of the vaccine–TLR4 interaction. **d**

Eigenvalue of the vaccine–TLR8 interaction. **e** Eigenvalue of the vaccine–TLR9 interaction

chemical stability, easy generation and non-infectious capacity of epitopes have proved it to be reliable vaccine candidates (Enayatkhani et al. 2020). B and T cell epitopes that met the selection criteria were conjugated with respective linkers for inter, and intra-epitope build up not excluding an adjuvant which is necessary to accelerate antigen-specific immunity (Fig. 1) (Groot et al. 2008). Immune adjuvants trigger monocytes and dendritic cells to express its surface molecule, produce chemokines and cytokines, which then attracts the monocytes, natural killer cells and granulocytes all in the bid to eliminate the pathogen. Hence the dendritic cells are driven towards the lymph node to activate the T

and B cells (Kar et al. 2020). Vaccine development requires an adjuvant which is necessary for augmenting the efficacy of the vaccine (Shey et al. 2019). It has been found that the severity and mortality of the disease is closely associated with high levels of cytokine release in the critical patients which ultimately results in CRS (cytokine release syndrome) or more popularly known as cytokine storm (Saha et al. 2020a, b).

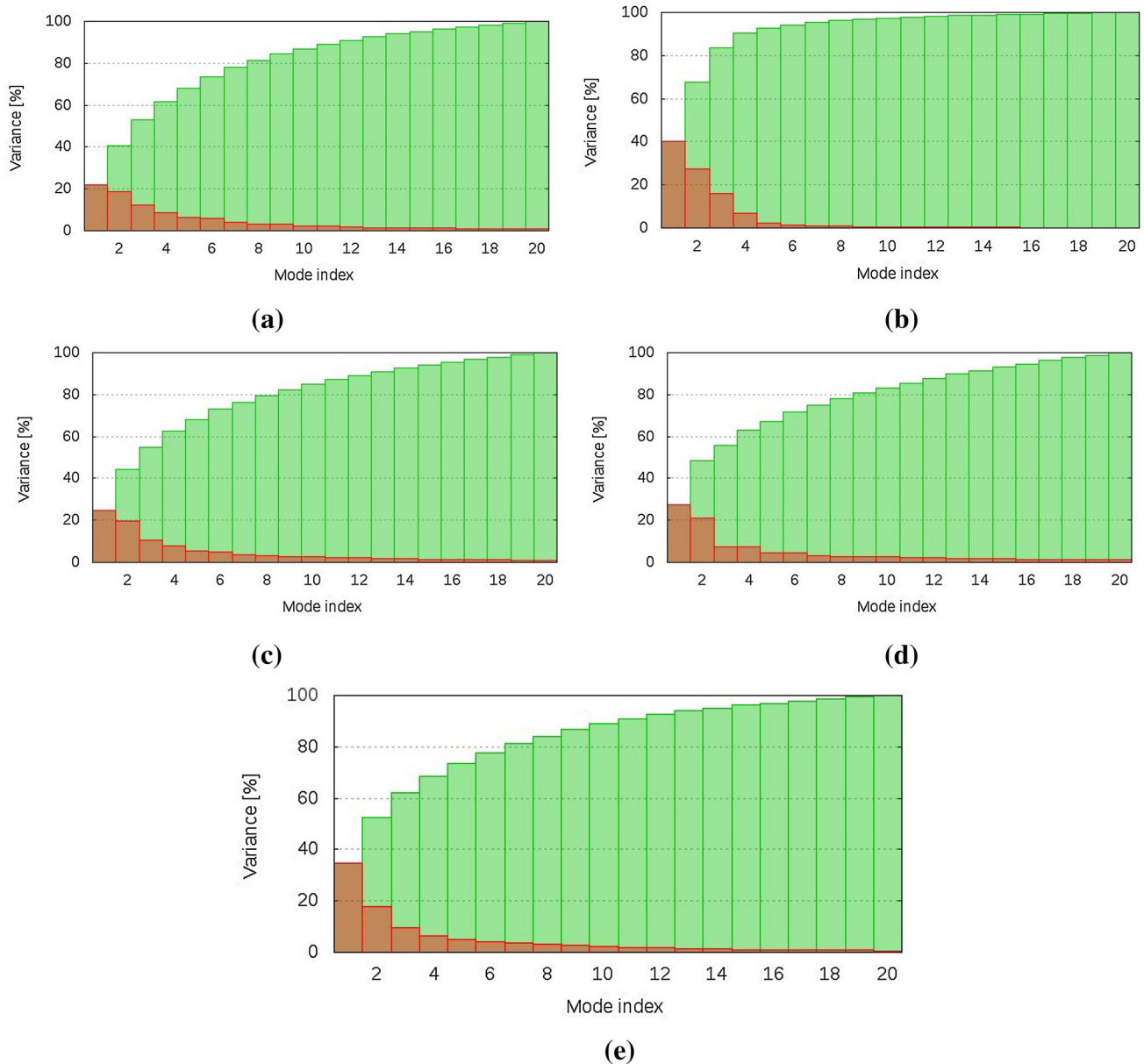
Antigenicity, allergenicity and toxicity of the constructed vaccine were also analyzed to ascertain these properties of the vaccine. It is expected that a worthy vaccine peptide must be capable of sensitizing an immune response, however

not able to cause either hypersensitive reaction within the host or toxins (Enayatkhani et al. 2020) to be certified safe for consumption. Also, it is considerable to note that not all epitopes projected have uniform ability to stimulate immune response notwithstanding its occurrence (Soria-Guerra et al. 2015). This vaccine prototype certifies all the requirements and hence can be endorsed as suitable.

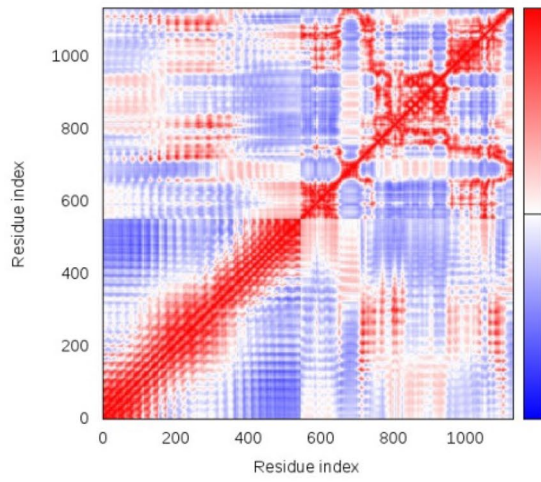
The molecular weight of 61.32 kDa generated is above the minimum guiding requirement of 40–50 kDa for vaccine transportation to the lymph node, because the outcome of ingested molecules is dependent on its size (Liu and Irvine 2015). This is necessary because particles with lower weight

**Fig. 12** Molecular dynamics simulation residual index result: **a** residual index of the vaccine–TLR2 interaction. **b** Residual index of the vaccine–TLR3 interaction. **c** Residual index of the vaccine–TLR4 interaction. **d** Residual index of the vaccine–TLR8 interaction. **e** Residual index of the vaccine–TLR9 interaction

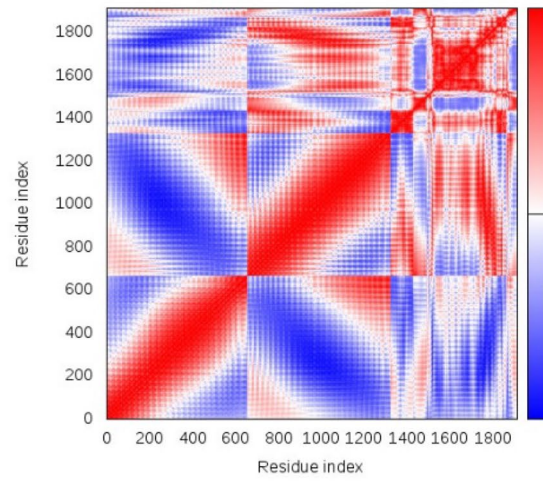
are easily displaced from the tissues by blood and thus prevent its access to the lymph node where T and B cells maturation takes place. The theoretical pI was also calculated by the server to be 6.70, indicating a slightly acidic and almost neutral protein. This implies that it is most likely somewhat soluble cytosolic protein and hydrophilic given the GRAVY



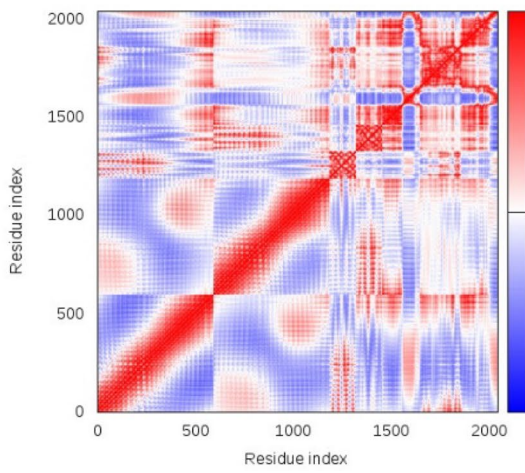
**Fig. 11** Molecular dynamics simulation variance result: **a** variance of the vaccine–TLR2 interaction. **b** Variance of the vaccine–TLR3 interaction. **c** Variance of the vaccine–TLR4 interaction. **d** Variance of the vaccine–TLR8 interaction. **e** Variance of the vaccine–TLR9 interaction



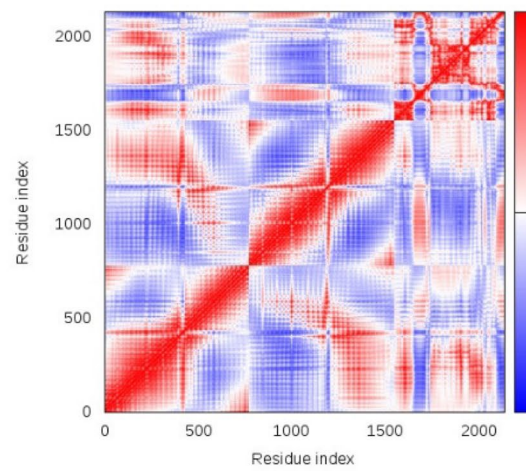
(a)



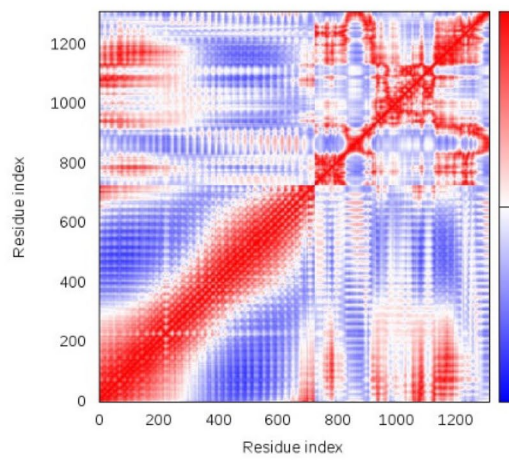
(b)



(c)

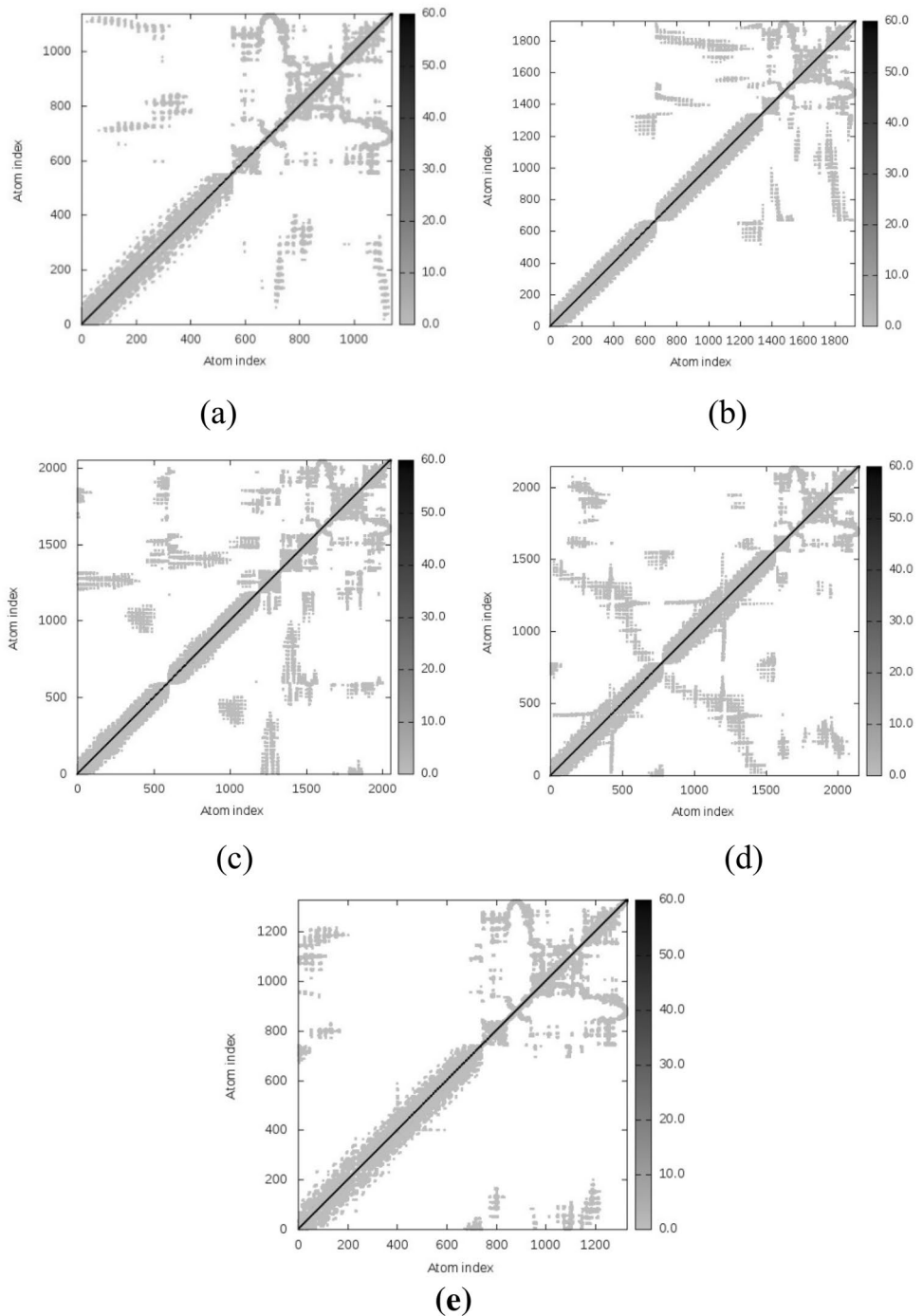


(d)



(e)

**Fig. 13** Molecular dynamics simulation elastic network result: **a** elastic network of the vaccine–TLR2 interaction. **b** Elastic network of the vaccine–TLR3 interaction. **c** Elastic network of the vaccine–TLR4 interaction. **d** Elastic network of the vaccine–TLR8 interaction. **e** Elastic network of the vaccine–TLR9 interaction

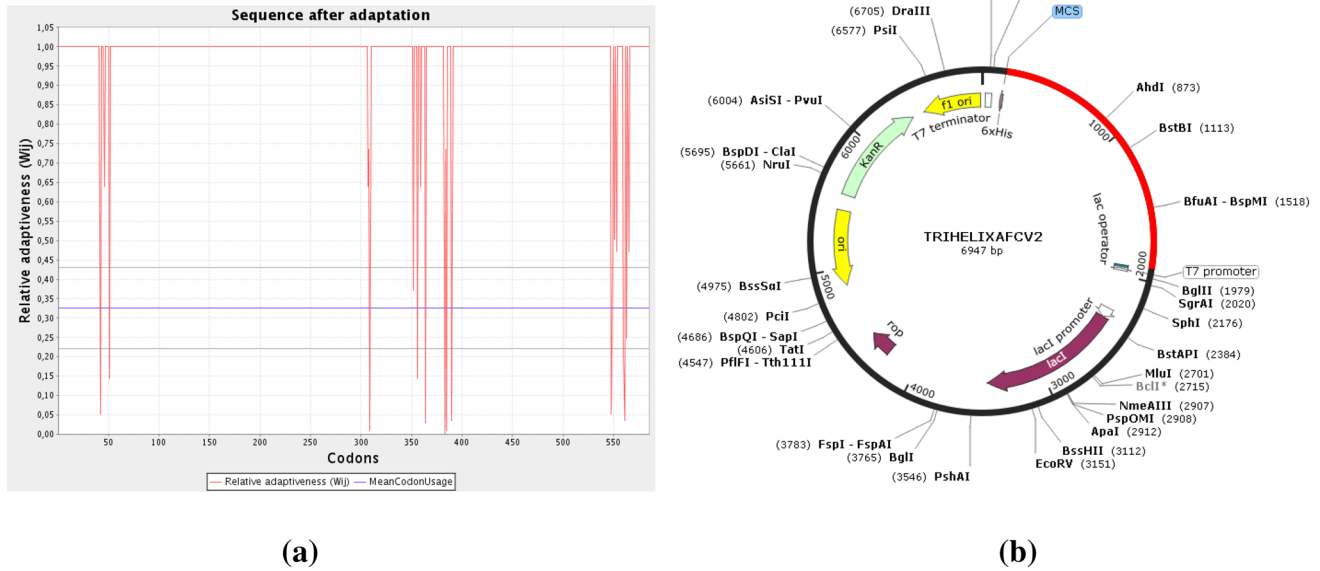


score ( $-0.261$ ) obtained (Mohanta et al. 2019). Hence it is water-soluble. The total number of harmful residues on the vaccine construct was predicted to be 33 while that of positive residue is 31. The molecular formula of the construct is  $C_{2740}H_{4107}O_{838}S_{15}$ , and the total number of atoms present in the construct is 8441. The estimated half-life is 4.4 h,  $> 20$  h and  $> 10$  h in mammalian reticulocytes (in vitro), yeast (in vivo) and *E. coli* (in vivo) respectively. The half-life value designates the required time for the antibody

to wane in vivo. The construct was found to be stable as inferred from its instability index 26.76 as any value above 40 indicates unstable (Gouripur et al. 2016). Aliphatic index of 65.78 all suggesting a thermostable structure as described by Sivakumar (2010).

The secondary structure of the vaccine revealed 20% Alpha Helix, 24.79% Extended Strand, 5.3% Beta Turn and 49.91% Random Coil (Fig. 2). Alpha helix is a dominant property of protein structure. The result showed that





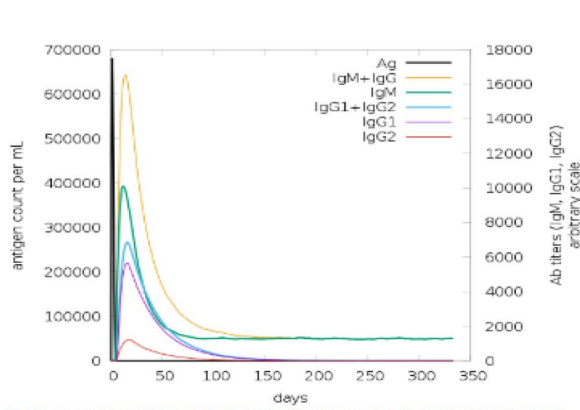
**Fig. 14** Codon adaptation and in silico cloning: **a** graph of sequence adaptation, **b** in silico cloning for the adapted vaccine sequence into pET28A (+) vector to ensure the vaccine protein expression in microbial system

random coil dominated the structure followed by alpha-helix and extended strand, which is consistent with the report of Gouripur et al. (2016). Tertiary structure with the highest c-score was selected because of the higher the score, the better the structure. The tertiary structure was further refined, and 86.8% Ramachandran favoured region was obtained. Subsequently, the refined structure was validated to acquire 89.2% residue in the select region; thus, a better structure was obtained after validation favouring a higher percentage of the protein residue. ProSA-web validated the refined structure with resultant Z score of  $-2.93$  (Fig. 4d) and ERRAT with a quality score of 74.4, revealing adequacy in the overall structure of the validated vaccine. The strength of the tertiary was predicted using Disulfide by Design 2 server. The understanding of the existing interaction of the vaccine construct (ligand) with TLRs is necessary because they are sensors that stimulate the natural host's immunity via the pathogen-associated molecular pattern (PAMP) (Steven et al. 2016). The TLR signaling pathway plays a vital role in various host immune defence mechanisms. For immunotherapeutic development, this pathway modulation has been identified as a drug target for many antibacterial or antiviral drug during the development (Chakraborty et al. 2020c). The TLRs (2, 3, 4, 8, 9) analysis was performed by ClusPro server, and the best receptor-ligand model was selected (Table 2, Fig. 5) based on the binding energy weight for the balanced coefficient. TLR 2 is reported to be expressed on antigen-presenting cell (APC) and its immune modulation capacity affects the T cells, B cells, natural killer (NK) cells, granulocytes and epithelial cells directly (Basto and Leitao

2014) which subsequently instigate these cells for the swift response. TLR 4 has a strong affinity for RSO 9 and is associated in human antiviral reaction stimulating the primary immune response of the host (Carty and Bowie 2010). TLR 9 controls the viral replication through the induction of type 1 interferon (IFN) (Duthie et al. 2011). The vaccine-TLR compound was examined to establish its residual stability, function and deformability region (Figs. 6, 7), B-factor, variance, residual index and the elastic network and eigenvalue was predicted. The eigenvalue is proportional to the required energy for structure deformation, which is more comfortable with low eigenvalue.

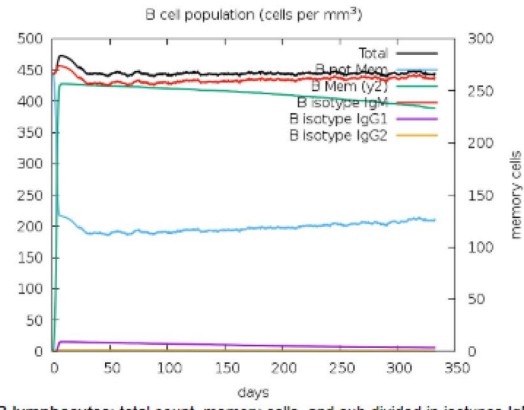
Recombinant protein expression within a suitable vector is vital to understand its biochemistry and functionality. The solubility of the protein as earlier predicted was observed in *E. coli*. Codon acclimatization was carried out in the *E. coli* expression system, to prevent the nominal rate of expression by non-adapting genes. Non adapting gene can result from differences in codon usage by host and organism. The vaccine proteins were inputted into *E. coli* k12 strain (JCAT server). Predicted GC content was 55.67% which is an immune stimulator and similar to the output of Sayed et al. (2020) codon Adaptation Index (CAI) of 0.93. Restriction enzyme XhoI (158) and XbaI (335) was the selected cloning site (Fig. 13). The expression of the codon was satisfactory.

Serological evaluation is a requirement to determine the immune reaction of the vaccine candidate (Sayed et al. 2020). The immune model results (Fig. 14) are consistent with characteristic immune responses. Immunoglobulin M (IgM) depicts existing infection while IgG presence is



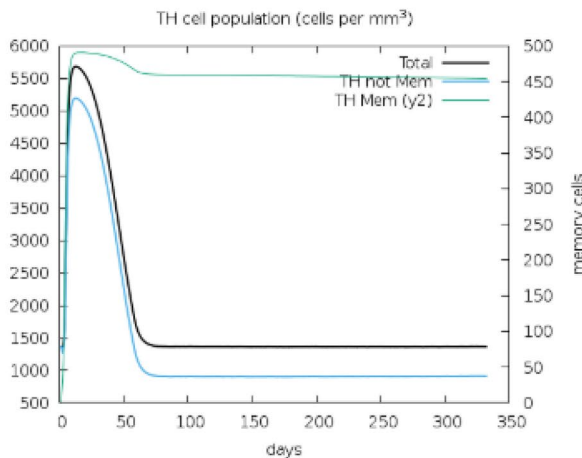
**Antigen and immunoglobulins.** Antibodies are sub-divided per isotype.

**(a)**



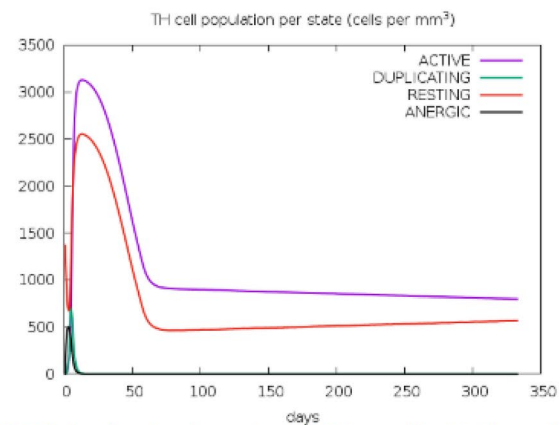
**B lymphocytes: total count, memory cells, and sub-divided in isotypes IgM, IgG1 and IgG2.**

**(b)**



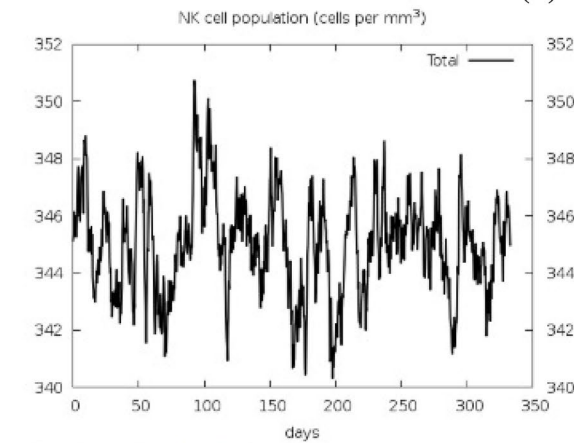
**CD4 T-helper lymphocytes count.** The plot shows total and memory counts.

**(c)**



**CD4 T-helper lymphocytes count sub-divided per entity-state (i.e., active, resting, anergic and duplicating).**

**(d)**



**Natural Killer cells (total count).**

**(e)**

**Fig. 15** C-ImmSim presentation of in silico immune simulation of the projected vaccine peptide. **a** Immunoglobulin production in response to antigen injection; specific subclasses are indicated as coloured

peaks. **b** The evolution of B-cell populations after the injection. **c** The population of T-helper cells after injection. **d** The evolution of T-helper cells. **e** The population of Natural Killer cells after injection

indicative of past infection and most serum antiviral activities are controlled by IgG antibodies CD4 T-helper lymphocytes and natural killer (NK) cells were observable with lasting memory B-cells for several months. NK cells differentiate between normal and abnormal cells; they interact with the constituents of the immune system such as the dendritic cells (DC), B-cells and the T lymphocytes. The NK cells have regulatory potentials hence help develop and coordinate the acquired immunity (Zingoni et al. 2009). It was observed that following exposure to the antigen, an increase in the immune secretions was sustained for many months. Elevated TH cell concentrations were noticed, which indicates immunoglobulin secretion that supports a humoral antibody. Besides, the active, duplicating and the resting stage of the immune response was projected. The half-life is implying the stability and potency of the vaccine in vivo.

## Conclusion

The panacea to the pandemic is of utmost importance, and it is required that a safe, readily available and cheap solution be developed. Reverse vaccinology approach of a subunit vaccine has proven to be highly effective against many harmful infections in time past. Science has evolved, making process time, and cost-effective at this stage. The projected vaccine promises to be efficacious, howbeit, in vivo analyses is required to validate and proof that it is safe.

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## Compliance with ethical standards

**Conflict of interest** The authors declare no conflict of interest.

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