A Novel Multi-Epitopic Peptide Vaccine Candidate Against *Helicobacter pylori*: In-Silico Identification, Design, Cloning and Validation Through Molecular Dynamics

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Abstract

Helicobacter pylori is a highly potential pathogen to colonize in the human stomach. This bacterial strain is now alarming serious health concern all over the world. Combating through available drugs is a difficult task due to lack of appropriate common targets against genetically diverse strains. Therefore, the developments of effective targets vaccines require alternative strategies to eliminate the *H. pylori* infection. In this study, we developed a novel vaccine construct using B-cell derived T-cell epitopes from four target antigenic proteins (HpaA, FlaA, FlaB and Omp18), and found the induction of possible immune response using advanced immunoinformatics approaches. In order to boost immune system, we tagged adjuvant (50S ribosomal protein L7/L12) with a suitable linker at the N-terminus side of vaccine sequence. Protein–protein docking between human Toll like receptor 5 (TLR5) and vaccine construct help to predict the way of inductive signaling that leads to immune-response. The calculated negative score $(-151.4, +/-8.7)$ of molecular docking complex signify the best binding interface. Molecular dynamics simulation studies confirmed the proper docking between TLR5 and vaccine candidate. Moreover, Normal mode analysis (NMA) calculates the molecular motion of the docking complex. The low eigenvalue $(2.935e^{-05})$ indicates the stable and flexible molecular motion in the binding interaction side. Finally, in-silico cloning of vaccine candidate was performed using expression vector pET28b (+) with the optimized restriction sites.

Keywords *Helicobacter pylori* · B-cell · T-cell · In-silico · Novel vaccine · Molecular dynamics simulation

Introduction

The flagellated gram-negative bacterium *H. pylori* is a spiral-shaped, microaerophilic bacteria which colonizes in human gastric epithelial cell surface region (Amieva and El–Omar 2008; Meza et al. 2017). Approximately half of the global human population is suffering from chronic gastritis, among which maximum cases remains

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asymptomatic. However, 10% of those infection leads to severe peptic ulcer (gastric and duodenal), gastric adenocarcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma (Cover and Blaser 2009; Salama et al. 2013; Yamaoka 2018). As per report of Testerman and Morris (2014) gastric cancer kills over 700,000 people per year globally (Testerman and Morris 2014). The virulent surface proteins of this extracellular pathogen play a pivotal role in host–pathogen interactions and pathogenicity including flagella-driven motility in the gastric epithelial layer (Viala et al. 2004). Virulent factors of *H. pylori* cause chronic and progressive gastric mucosal inflammation, resulting in tissues necrosis, atrophy, lesions, intestinal metaplasia and subsequently stomach cancer (Khan et al. 2019). It was observed that the infection of *H. pylori* is increasing day by day globally (Fig. 1) (Hooi et al. 2017; Khoder et al. 2019; Zamani et al. 2018). Motility, adherence, colonization and several virulence factors are the key requirements of the gastrointestinal disease by *H. pylori*. This phenomenon is mainly facilitated by bacterial outer membrane proteins (OMPs) and flagellar proteins. HpaA and Omp18 are well characterized outer membrane proteins of *H. pylori* (Lundström et al. 2001; Voland et al. 2003). On the other hand, FlaA and FlaB

are mostly studied flagellar proteins (Allan et al. 2000; Josenhans et al. 1995).

Toll like receptors (TLRs) plays a crucial role in pathogen recognition, host–pathogen interaction and downstream signal regulation (Bhattacharya et al. 2020b; Netea et al. 2004). TLRs are most studied pathogen recognition receptors (PRRs), which recognizes specific antigenic epitope of surface proteins of the pathogen, known as a pathogen associated molecular patterns (PAMPs). However, in our study bacterial flagellin protein is most proper antagonist of TLR5 for pathogen recognition.

Previously, there is no effective antibiotic or drug-resistant medication available to eradicate *H. pylori* strain (Meza et al. 2017; Testerman and Morris 2014). So, designing of potential vaccine is an urgent need to combat *H. pylori* (Del Giudice et al. 2009). Among the large number of virulent proteins, identification of potential virulent proteins is the most challenging task for the development of effective vaccine candidate. Researchers are seeking an effective virulent protein to breakdown the virulence of *H. pylori* through vaccination. But, classical or conventional approach of vaccine candidate formulation is a time consume process and there are some limitations and drawbacks of conventional vaccinology, like in case of live attenuated or inactivated vaccines, which expose an
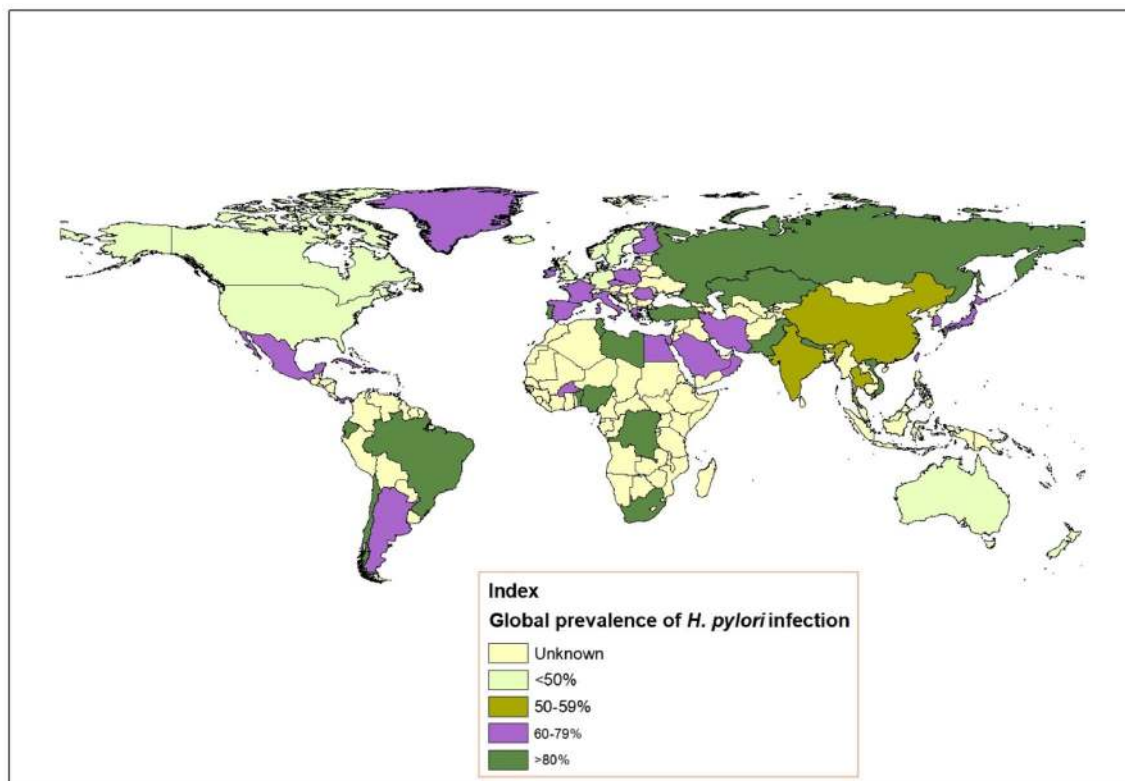

Fig. 1 The mapping of *H. pylori* infection from previous published data (Hooi et al. 2017; Khoder et al. 2019; Zamani et al. 2018)

individual with whole pathogens, is very nonspecific and there is also a risk of the attenuated pathogen reverting to full virulence. Therefore, scientists are now using modern immunoinformatics approach to design a vaccine against pathogenic infection (Bhattacharya et al. 2020a; Dhal et al. 2019; Pandey et al. 2018).

In this current study we selected four such antigenic proteins (HpaA, FlaA, FlaB and Omp18) for in-silico design of multi-epitope-based vaccine model. A comprehensive computational framework was used to identify antigenic common B -cell and T- cell epitope to construct a vaccine construct against deleterious *H. pylori*. HpaA and Omp18 proteins are surface localizing protein and a promising candidate for a vaccine against *H. pylori* infection (Carlsohn et al. 2006). Similarly, FlaA and FlaB protein of *H. pylori* flagellin also having virulence property (Gu 2017). This two protein are promising peptide based vaccine candidate against *H. pylori.* According to Song et al. flagellin is recognized by human toll-like receptor 5 (TLR5) as a pathogen invasion signal and subsequently evokes the immune response (Song et al. 2017). So FlaA and FlaB protein, a part of the flagellin protein, can recognized by TLR5 and also induces immune response a part of activity response against human TLR5.

In this work, we selected four antigenic proteins (HpaA, FlaA, FlaB and Omp18) from *H. pylori*. Using these antigenic protein common B -cell and T-cell epitopes was predicted and vaccine construct was developed. We have also characterized different properties of vaccine candidate such as secondary structure, antigenicity, allergenicity and physiochemical properties. We have also performed tertiary structure modeling and model validation of vaccine construct. The vaccine construct was docked with human TLR5 to understand the binding affinity. We profiled the thermodynamics stability, in-silico cloning and expression was performed using vaccine candidate. The present research aims to achieve potent multi epitopic peptide vaccine component against *H. pylori* to control its infection.

Materials and Methods

Collection of Proteins

Four proteins were selected for the development of computer aided multi-epitope peptide vaccine designing against *H. pylori*, including HpaA, FlaA, FlaB and Omp18, based on their role in *H. pylori* infection. The target protein sequences of *H. pylori* were retrieved as FASTA format from the NCBI database for further computational analyses to detect the antigenic peptide sequence for vaccine designing (Pruitt et al. 2007).

Prediction of B-Cell Epitopes

B lymphocytes cells are generating antibody and memory cells; herewith it turns active long-term immune responses against pathogens. For prediction of sequence-based linear B-cell epitope of the four target proteins, we employed Bepipred Linear Epitope Prediction 2.0 algorithm under the Immune Epitope Database (IEDB) online server (Jespersen et al. 2017; Kim et al. 2012). FASTA sequences of those targeted proteins were provided as input and selected all default parameters.

Identification of MHC-I and MHC-II Epitopes Within B-Cell Epitopes

T-cells play a pivotal role in stimulating both cytotoxic and helper T-cell mediated immune response (Saha et al. 2017). MHC-I and MHC-II class epitopes were selected from the previously retrieved. The Bcell epitope of target proteins were predicted using T-cell epitope prediction tool under the IEDB server (Patra et al. 2020). Here, we opted IEDB recommended 2.22 method for identifying MHC-I & MHC-II epitopes and chosen all repository. After successful identification of T-cell epitopes, we selected common epitope for B-cells, T-cells and both MHC molecules, which might serve as potential vaccine as it can induce both humoral and cell mediated immunity.

Construction of Vaccine Sequence

Common B-cell and T- cell epitopes are selected for final vaccine construction on the basis of high antigenicity and non-allergenicity nature. In order to establish final multi-epitopes-based vaccine construct, the selected MHC-I and MHC-II epitopes were linked together by AAY and GPGPG linkers, respectively. Finally, 50S ribosomal protein L7/L12 adjuvant is linked with N-terminus of the vaccine model with the help of EAAAK peptide linker to accelerate immune response.

Secondary Structure Prediction of Vaccine

PSIPRED 4.0 web server was used to predict the secondary structure of vaccine construct, as it delivered highly accurate result (Buchan and Jones 2019). This server has two feed-forward neural networks-based algorithms, which executes a study on output generated from PSI-BLAST and refine the secondary structure.

Evaluation of Antigenicity, Allergenicity and Physiochemical Properties of the Designed Vaccine

Analysis and evaluation of allergenic property is very much crucial for modulating any vaccine. Antigenic propensity of

vaccine model was evaluation by using VaxiJen v2.0 server and further validated by AntigenPro server (Doytchinova and Flower 2007; Magnan et al. 2010). For additional analysis, allergenicity was evaluated by performing AllergenFP v.1.0 server (Dimitrov et al. 2014). Protein-Sol webserver was used to determine the solubility of vaccine construct (Hebditch et al. 2017). Finally, various physicochemical properties of each designed vaccine constructs were determined by ExPASy ProtParam server (Gasteiger et al. 2005).

Tertiary Structure Modelling

Three-dimensional modelling of the vaccine construct is a critical step in epitope-based vaccine development (Roy et al. 2012; Willard et al. 2003). SPARKS-X; protein fold recognition web server was used to develop 3D structure of vaccine construct (Yang et al. 2011). The FASTA format of vaccine construct (amino acid sequence) was an input for the SPARKS-X. This server implements BLASTp for searching template for 3D structure prediction.

Model Validation

Predicted tertiary structure validation of amino acids chain is a crucial step in model building process. Subsequently, SPARKS-X provided protein model has to go through some validation servers such as (a) ProSA-web: calculates energy plot as well as Z score value that indicate overall quality score of target vaccine construct model (Wiederstein and Sippl 2007). (b) PROCHECK-server predicts the residue-by-residue stereochemical qualities validation of the vaccine model through Ramachandran plot (Laskowski et al. 1993).

Discontinuous B-Cell Epitope Prediction

Discontinuous B-cell epitopes of the vaccine construct was predicted through Ellipro tool of IEDB server (Ponomarenko et al. 2008). If the score appears as 0.8, it indicates 80% of the protein residues are inside the ellipsoids and 20% being outside. The clustering of discontinuous epitopes is subjected by distance R, which is measured in Å and more R indicates larger discontinuous epitope.

Molecular Docking Analysis and Binding Affinity Analysis

Molecular docking is very promising and most fundamental process to characterize the interaction and binding affinity between the vaccine constructs and the human Toll-like receptor 5 (TLR5). The crystal structure of human TLR5, downloaded from RCSB protein data bank (PDB ID: 3J0A) was used for molecular docking. Molecular docking was carried out with HADDOCK web server at the easy interface (De Vries et al. 2010). Prior of molecular docking, the active residues (directly involved in the interaction) and passive residues (surrounding residues) for both TLR5 and vaccine construct were determined using CPORT server (de Vries and Bonvin 2011).

The binding affinity of the docking complex or Gibbs free energy (ΔG) determinate the actual form of interaction pattern and dissociation constant (K_D) values of docked complex was predicted at 37 °C temperature by PRODIGY web server (Xue et al. 2016).

Molecular Dynamics Simulation of Vaccine Construct

The design vaccine component were completely energy-minimized by the default energy minimizer process of the NAMD (Nanoscale Molecular Dynamics). Software which uses conjugate with gradient and line search algorithms based on NVT ensemble at the pressure of 1.025 atm (Phillips et al. 2005). The molecular simulations were passed out by the CHARMM27 coordinate protein-based topology, and all atom force field with map correction with a time-step of 1 fs and periodic boundary conditions applied in all three dimensions (MacKerell et al. 2000). RMSF and RMSD plot of the docking complex showed its residue by residue fluctuation graph.

Normal Mode Analysis

The Normal Mode Analysis study was performed on the docking complex (TLR5-vaccine construct). Here, we opted iMODS online server to complete the NMA study (López-Blanco et al. 2014). iMODS was employed for investigation of the structural dynamics of the docking complex as well as determined the molecular motion. iMODs server is a customable, easy and user-friendly online server. This web server also generates complex deformability, B-factor, eigenvalues, variance, co-variance map and elastic network. The docked PDB files act as an input file and uploaded to the iMODS server and the results were displayed keeping all the parameters as default.

Codon Optimization and In-Silico Cloning

Codon optimization of vaccine construct was performed through online web server namely Java Codon Adaptation Tool (JCAT) for high-level expression of the vaccine sequence in *E. coli* K12 strain (Grote et al. 2005). We used pET28b(+) expression vector to execute in silico cloning of vaccine sequence(Bhattacharya et al. 2020c). This expression vector map and sequences was downloaded from "addgene" vector database (Kamens 2015). Here, SnapGene

5.1.7 restriction cloning tool was employed for finalizing in silico cloning (GSL Biotech LLC 2015).

Result

Collection of Target Proteins

In this study, four proteins of *Helicobacter pylori* were screening for the possible vaccine construction. These proteins were: HpaA (accession number: ANH47002.1), FlaA (accession number: AAU21201.1), FlaB (accession number: AAU21202.1) and Omp18 (accession number: WP_171924268.1) were retrieved as FASTA format from NCBI database.

Prediction of B-Cell Epitopes

B-cell epitopes of four target protein of *Helicobacter pylori* were predicted different algorithms from Bepipred Linear Epitope Prediction 2.0 algorithm of IEDB server. IEDB predicted B-cell epitopes of varying length of each target proteins were identified and tabulated along with epitopes with their start position, sequence and length (Supplementary Table 1). The predicted B-cell epitopic regions of each target protein shown in supplementary Fig. 1.

Identification of MHC-I and MHC-II Epitopes Within B-Cell Epitopes

Common B-cell and T-cell epitopes are more applicable for vaccine designing, here each four-target protein of *H. pylori* have same number of MHC-I and MHC-II binding epitopes. In order to identification of MHC class I epitopes, we retrieved 9mer epitopic sequence. Similarly, for MHC-II epitopes identification we collected 15mer epitopic sequence. These two types of epitopic sequences were further used vaccine construction which is illustrated in Table 1 respectively.

Designing of Vaccine Construct

Common MHC-I and MHC-II epitopes were used to design vaccine constructs; here total 15 MHC class I and 16 MHC class II epitopes were filtered and linked them with AAY and GPGPG linker respectively. 50S ribosomal protein L7/L12 with (NCBI accession no. P0A7K2) as an adjuvant, was added to the amino terminus of the vaccine construct with linker EAAAK, graphical representation shown in Fig. 2. Lastly, all of the linked MHC-I, MHC-II and adjuvant considered as a final vaccine construct had a total of 632 amino acids making it ~ 64.367 kDa peptide and an Extinction coefficient of 25960 M^{-1} cm^{-1}.

Secondary Structure Prediction of Vaccine

PSIPRED v4.o assessed the secondary structure of the vaccine component, where server calculates the number of the amino acids in the alpha helix, beta-sheet and coil formation within the vaccine model. Vaccine model had a total of 207 (32.75%) amino acids in the alpha-helix formation, 62 (9.81%) amino acids in the beta-sheet formation, and 363 (57.43%) amino acids in the coil structure formation represented in Table 2 and PSIPRED generated cartoon structure shown in Fig. 3.

Evaluation of Antigenicity, Allergenicity and Physiochemical Properties of the Designed Vaccine

Antigenic propensity is an important parameter for designing vaccine construct that typically induce the humoral as well as cell mediated immune responses against the *H. pylori*. The predicted data recommend that our vaccine is antigenic with an antigenic probability score of 1.2317 and 0.918291predicted by VaxiJen v2.0 and ANTIGENPro servers, individually (Table 3).

AllergenFP v.1.0 server, concluded that the vaccine constructs as a non-allergenic sequence although the AllerTOP v.2.0 supported the vaccine non-allergenic nature. Therefore, the vaccine construct might proceed for further investigation. As per the Protein-Sol server, predicted scaled solubility of the vaccine construct is 0.668 (Fig. 4). The ExPASy protparam result of all other physiochemical property of vaccine construct listed within the Table 3.

Tertiary Structure Modelling

SPARKS-X sever predicted 3D protein architecture from amino acid sequences represents one of the most crucial steps in computational structural biology. The 3D structure of vaccine construct was visualized by PyMol software. The 3D structure of vaccine construct is represented in Fig. 5, where red color depicts helix, blue color depicts coiled structure and cyan color depicts beta strand.

Model Validation

In this section, we used two online web servers regarding validation of vaccine tertiary structure. ProSA and PROCHECK web servers simultaneously revealed the model quality as well as analysis of vaccine stereo-chemical properties. ProSA 'Z' score of vaccine construct is − 3.74 and energy plot represented in Fig. 6a and b, respectively. PROCHECK server analyse residue-by-residue localization in Ramachandran plot, as shown in Fig. 6c. According to PROCHECK, 80.0% of amino acids residues are placed

Table 1 A Selection of MHC-I epitopes (derived from B-cell epitopes) based on VaxiJen score > 1

Protein name	B-cell epitope sequence	MHC-I epitope sequence	MHC-I binding alleles	VaxiJen score
A				
HpaA	NPSVETKTQNDAKNQQPVQTHERMKTSSEHVTPLD-FNYPIHIVQAPQN	HVTPLDFNYP	HLA-A*0201 HLA-A*0205 HLA-B*2702 HLA-B*3501 HLA-B*3701 HLA-B*3801 HLA-B*0702	1.1099
	GYQVLRFQDEKALSAQDKR	QDEKALSAQ	HLA-A*1101 HLA-A*3101 HLA-B*2702 HLA-B*5801	1.1087
	PESNRVVHDFAVEVG	VVHDFAVEV	HLA-A*0201 HLA-A*0205 HLA-B*3701 HLA-B*4403 HLA-B*5301	1.3684
	YTYKHSNSGGLDSSNNIIHEALEKNKEDA	KHSNSGGLD	HLA-B*3902 HLA-B*5301 HLA-B*5401	2.2506
FlaA	NALKTSLERLSSGLRINKAAD	RLSSGLRIN	HLA-B*2702 HLA-B*2705 HLA-B*3901 HLA-B*5801	1.5169
	AAQDGQTTESRKAIQSD	QDGQTTESR	HLA-B*2702 HLA-B*5101 HLA-B*5801	2.9528
	VTGNFNANVKSASGANYNAVIASGNQSLGSGVT-TLRG	NANVKSASG	HLA-B*5401	2.2390
	VNNISITQVNVKAAESQIRDV	VNVKAAESQ	HLA-B*5801	1.9766
FlaB	QNNRDLSSSLEKLSSGLRINKAADD	KLSSGLRIN	HLA-A*0205 HLA-B*3901 HLA-B*5801	1.5519
	AQDGQTLESRRALQSDIQRLLEE	QDGQTLESR	HLA-A*3302 HLA-B*2702 HLA-B*3501 HLA-B*3902 HLA-B*5801	2.2153
	VRGIFDANVASAAGANANGAQAETNSQGIGAGVT-SLKGAM	NANGAQAET	HLA-B*5101 HLA-B*5103	2.4424
	ISVTQVNVKAAESQIRDVDF	VNVKAAESQ	HLA-B*5801	1.9766

Table 1 (continued)

Protein name	B-cell epitope sequence	MHC-I epitope sequence	MHC-I binding alleles	VaxiJen score
Omp18	HKMDNKTVAGDVSAKTVQTAPVTTEPAPEKEEP-KQEPAPVVEEKPAVESGT	GDVSAKTVQ	HLA-A*3302 HLA-B*5101 HLA-B*5102 HLA-B*5103 HLA-B*5201 HLA-B*5801	1.9177
	HKMDNKTVAGDVSAKTVQTAPVTTEPAPEKEEP-KQEPAPVVEEKPAVESGT	EKEEPKQEP	HLA-B*4403 HLA-B*0702	1.3706
	FDFDKYEIKESDQET	EIKESDQET	HLA-A*3302 HLA-B*3701 HLA-B*3901 HLA-B*3902 HLA-B*4403 HLA-B*5301	1.8008
	ISFGETKPKCTQKTRECYKEN	GETKPKCTQ	HLA-A*1101 HLA-A*3101 HLA-B*5401 HLA-B*5801	1.7641

Sl. No	B-cell epitope sequence	MHC-II epitope sequence	MHC-II binding alleles	VaxiJen score
B				
HpaA	NPSVETKTQNDAKNQQPVQTHERMKTSSEHVTPLD-FNYPIHIVQAPQN	VQTHERMKTSSEHVT	HLA-DRB1*1302 HLA-DRB1*0901 HLA-DRB1*0701 HLA-DRB1*0301 HLA-DRB1*0405	1.2231
	NPSVETKTQNDAKNQQPVQTHERMKTSSEHVTPLD-FNYPIHIVQAPQN	PSVETKTQNDAKNQQ	HLA-DRB1*0802 HLA-DRB1*1302 HLA-DRB4*0101 HLA-DRB3*0101	1.6689
	GYQVLRFQDEKALSAQDKR	LRFQDEKALSAQDKR	HLA-DRB1*0401 HLA-DQA1*0401 HLA-DPA1*0103 HLA-DRB5*0101	1.1177
	YTYKHSNSGGLDSSNNIIHEALEKNKEDA	YTYKHSNSGGLDSSN	HLA-DRB1*0101 HLA-DQA1*0501 HLA-DRB1*0401 HLA-DRB3*0101 HLA-DRB1*0405	1.3437
FlaA	AAQDGQTTESRKAIQSD	QDGQTTESRKAIQSD	HLA-DQA1*0301 HLA-DQA1*0102 HLA-DQA1*0401 HLA-DRB1*0701 HLA-DQA1*0501	1.7622
	VTGNFNANVKSASGANYNAVIASGNQSLGSGVT-TLRG	ASGNQSLGSGVTTLR	HLA-DQA1*0301 HLA-DPA1*0301 HLA-DRB1*0405 HLA-DQA1*0501 HLA-DPA1*0201	1.3656
	VTGNFNANVKSASGANYNAVIASGNQSLGSGVT-TLRG	NANVKSASGANYNAV	HLA-DRB1*1101 HLA-DRB3*0101 HLA-DQA1*0301 HLA-DQA1*0501 HLA-DPA1*0103	1.6352
	VNNISITQVNVKAAESQIRDV	NISITQVNVKAAESQ	HLA-DQA1*0101 HLA-DRB3*0101	1.6160

Table 1 (continued)

Sl. No	B-cell epitope sequence	MHC-II epitope sequence	MHC-II binding alleles	VaxiJen score
FlaB	AQDGQTLESRRALQSDIQRLLEE	QDGQTLESRRALQSD	HLA-DRB1*1101 HLA-DQA1*0102 HLA-DRB1*0802 HLA-DRB1*0301 HLA-DRB1*0901	1.2748
	VRGIFDANVASAAGANANGAQAETNSQGIGAGVT-SLKGAM	ETNSQGIGAGVTSLK	HLA-DQA1*0102 HLA-DQA1*0301 HLA-DRB1*0101 HLA-DQA1*0401 HLA-DRB1*0301	1.7236
	VRGIFDANVASAAGANANGAQAETNSQGIGAGVT-SLKGAM	GANANGAQAETNSQG	HLA-DRB1*0401 HLA-DQA1*0501 HLA-DRB1*0405 HLA-DRB4*0101 HLA-DPA1*0103	2.3765
	ISVTQVNVKAAESQIRDVDF	QVNVKAAESQIRDVD	HLA-DPA1*0103 HLA-DQA1*0101 HLA-DRB1*1201 HLA-DRB1*1501 HLA-DPA1*0301	1.6015
Omp18	HKMDNKTVAGDVSAKTVQTAPVTTEPAPEKEEP-KQEPAPVVEEKPAVESGT	KMDNKTVAGDVSAKT	HLA-DQA1*0501 HLA-DRB1*0401 HLA-DRB1*0802 HLA-DRB3*0101 HLA-DRB1*0301	1.7244
	HKMDNKTVAGDVSAKTVQTAPVTTEPAPEKEEP-KQEPAPVVEEKPAVESGT	NKTVAGDVSAKTVQT	HLA-DQA1*0401 HLA-DRB1*0802 HLA-DQA1*0301 HLA-DRB1*0901 HLA-DRB1*1302	1.4458
	FDFDKYEIKESDQET	FDFDKYEIKESDQET	HLA-DPA1*0201 HLA-DPA1*0103 HLA-DRB1*0401 HLA-DRB1*0301 HLA-DRB4*0101	1.3271
	ISFGETKPKCTQKTRECYKEN	GETKPKCTQKTRECY	HLA-DRB1*0301 HLA-DRB1*1201 HLA-DRB1*1101 HLA-DRB1*0701 HLA-DRB5*0101	1.3419

in most favored region of Ramachandran plot, moreover, as all amino acid residues are tabulated in the Table 4.

Discontinuous B-Cell Epitope Prediction

ElliPro online web server was predicted discontinuous B-cell epitopes based on tertiary structure of vaccine construct. Five epitopes were predicted from the ElliPro server from which epitope with maximum prediction score 0.841 was nominated as a discontinuous epitope. 3D structure of predicted discontinuous epitopes were shown in Fig. 7.

Molecular Docking Analysis and Binding Affinity Analysis

HADDOCK clustered 163 structures in 7 cluster(s), which represents 81% of the water refined HADDOCK generated models. The top ranked cluster with the lowest HADDOCK score is the most significant one for docking analysis. The HADDOCK score of $-151.4 +/- 8.7$; indicates proper interaction between the vaccine construct and TLR5. The score of buried surface area (BSA) is $2692.6 +/- 99.3$ Å^2. It reflected close proximity and a less water-exposed on protein binding surface. Subsequently, RMSD plots determined the docking interaction which is represented in Fig. 9. The small
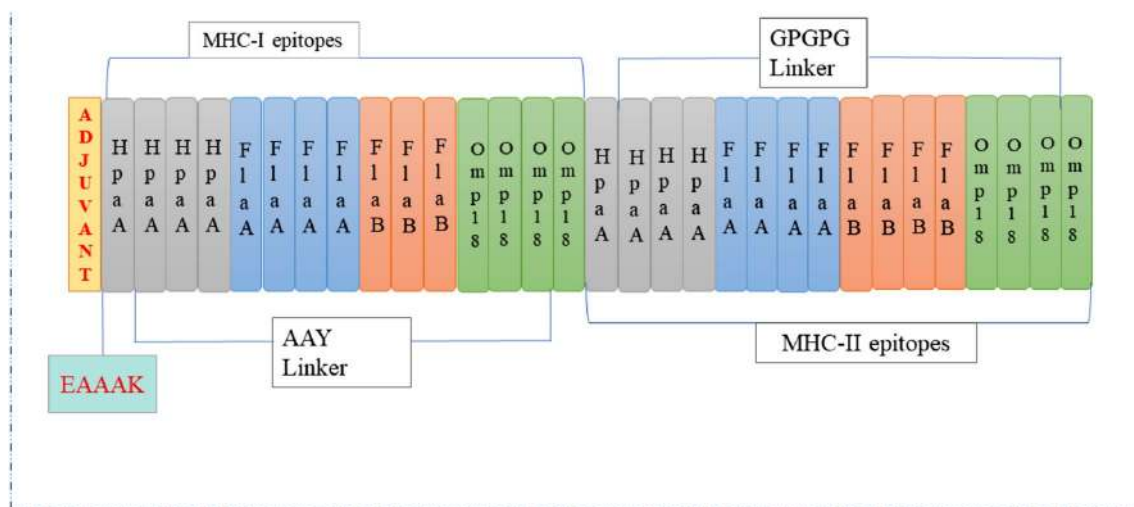
Fig. 2 Schematic diagram of final multi epitopic peptide vaccine construct

Table 2 Percentage and number of amino acids of secondary structure (Alpha helix, beta sheet and coil) present in vaccine construct

Candidate name	Alpha helix (percentage and number of amino acids)	Beta sheet (percentage and number of amino acids)	Coil structure (percentage and number of amino acids)
Vaccine construct (632 amino acids)	207(32.75%) amino acids	62 (9.81%) amino acids	363 (57.43%) amino acids

RMSD value of the docked complex specifies the formation of good quality docked. The HADDOCK calculated electrostatic energy, desolvation energy, restraints violation energy, van der waals energy and Z-Score values listed in the Table 5. The Fig. 8 show molecular docking between Human TLR5 and vaccine construct, and Table 6 represented the hydrogen bond between both protein molecules.

PRODIGY provided binding affinity of Gibbs free energy (ΔG) and dissociation constant (Kd) values of docked complex, which was -11.7 kcal mol^{-1} and 5.5E-09 M, respectively (Table 5). The negative value of docking complex has indicated the thermodynamically stable docking structure.

Molecular Dynamics Simulation of Vaccine Construct

Molecular dynamics simulation is a computer-based simulation method for analyzing the physical movements of atoms and molecules. For the visualisation of the molecular structures and the trajectories, we used VMD1.9.3 software package (Humphrey et al. 1996). The trajectories were analysed by means of backbone carbon atom Cα fluctuations, atom distances, and dihedral angles through VMD program, as represented in Fig. 9a. Analyses of essential dynamics (ED), NAMD software based CHARMM topology file had been involve for structure fluctuation determination. In the

Fig. 9b represented our calculation of 1000 time step (TS) molecular dynamic simulation and analysed the overall vaccine structure was stable, it also flexible for binding properties accessed via B-factor and RMSD plot.

Normal Mode Analysis

Flexibility in biological macromolecules is a crucial property for interacting with substrate or protein–protein interactions machinery. Therefore, iMODs calculates the molecular motion as well as structural flexibility through NMA study that is incorporated with coordinates of the docked complex. The output of server directed the domains mobility represented by two coloured affine-arrows that indicates two clusters showing the NMA mobility (Fig. 10). The complex deformability mainly depends on the individual distortion of each residues (Cα atom), presented through coloured hinges in the chain (Fig. 11b). The server calculated eigenvalue as $2.935e^{-05}$, as represented in Fig. 11d. Moreover, eigenvalue and the variance are inversely related associated to each normal mode (Fig. 11c). The B-factor graph shows the average RMS, however B-factor plot represents the stable structure docked molecules shown in Fig. 11a. The covariance matrix represents with colours graph where, correlated, uncorrelated or anti-correlated motions, are represented

Fig. 3 Schematic representation of secondary structure prediction of the multi-epitope vaccine, secondary structure prediction result represents the arrangement of α-helix (32.75%) β-strands (9.81%) and coils (57.43%)
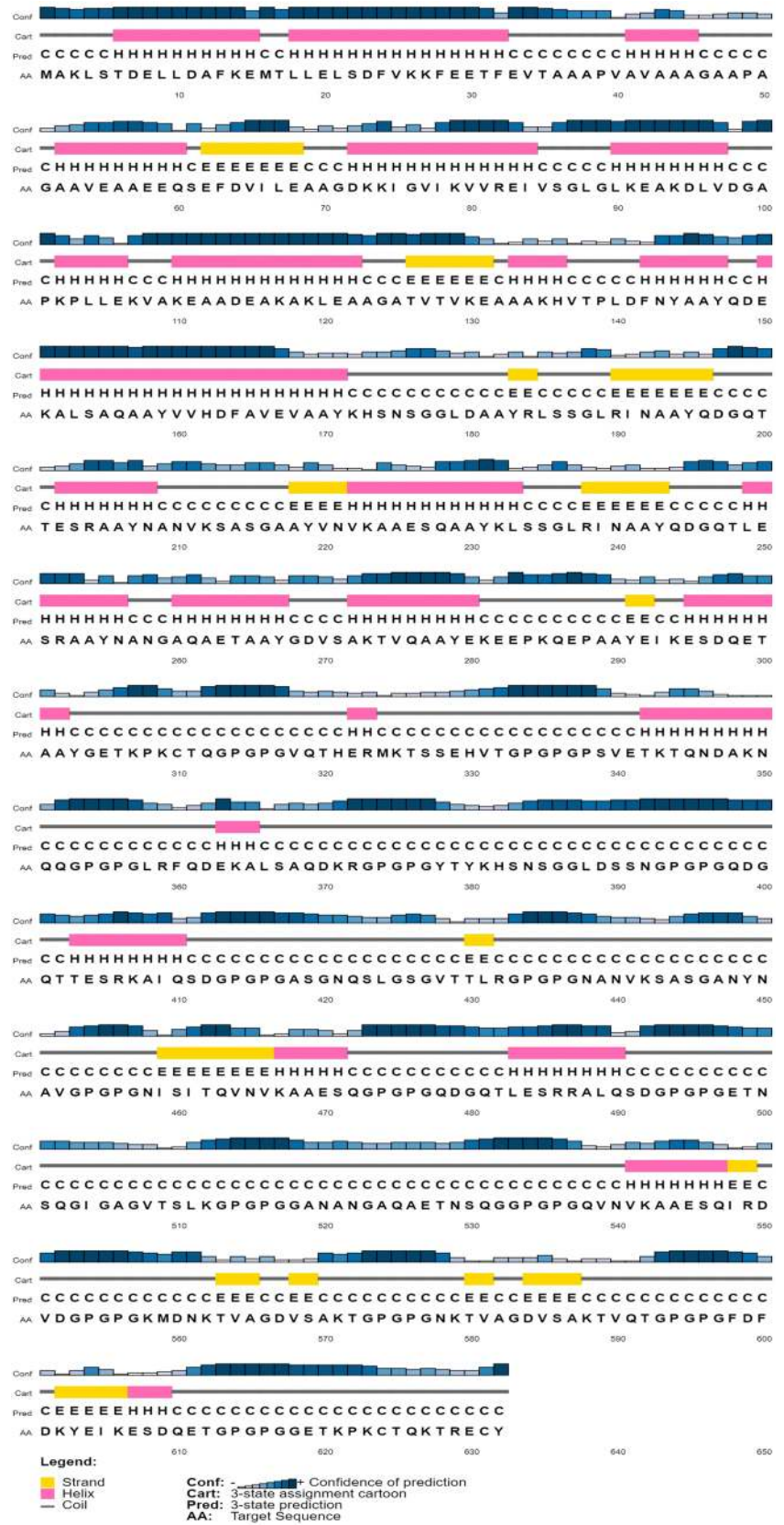
Table 3 Antigenicity, allergenicity, solubility, and physicochemical property assesment of the primary sequence of multi-epitope-based vaccine construct

Sl. No	Features	Assessment
1	Antigenicity	**1.2317** (Probable ANTIGEN) by VaxiJen v2.0 **0.918291** (Probable ANTIGEN) by ANTIGENPro
2	Allergenicity	Probable non-allergen (AllerTOP v.2.0) Probable non-allergen (AllergenFP v.1.0)
3	Solubility	0.668 (Soluble)
4	Number of amino acids	632
5	Molecular weight	64,366.48 Dalton
6	Theoretical Isoelectric point (pI)	5.12
7	Total number of atoms	8920
8	Formula	$C_{2764}H_{4392}N_{800}O_{957}S_7$
9	Estimated half-life	30 h (mammalian reticulocytes, in vitro) > 20 h (yeast, in vivo) > 10 h (*Escherichia coli*, in vivo)
10	Instability index	27.70 (Stable)
11	Aliphatic index	59.45
12	Grand average of hydropathicity (GRAVY)	− 0.646

Bold denotes that antigenic score is higher than 0.45 (threshold value)
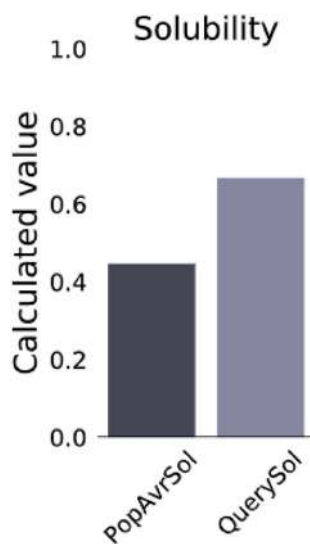
Fig. 4 The diagram shows vaccine solubility plot

Fig. 5 3-dimensional (3D) structure of final vaccine construct (orange color depicts helix, blue color depicts coiled structure and cyan color depicts beta strand) (Color figure online)

by red, white and blue colors, respectively (Fig. 11e). An elastic network model shows docked protein molecule (Cα) atoms are interconnected with "springs" of certain strengths (the darker greys representation of the stiffer springs).

Codon Optimization and In-Silico Cloning

In silico cloning was performed to understand the expression of our developed multi epitopic vaccine candidate on *E. coli* hosts. Java Codon Adaptation (JCat) tool optimized our construct for the expression *in E. coli* (K12 strain). Additionally, we selected two filter parameters to evade unnecessary error, those are (a) avoiding rho-independent transcription terminators, (b) avoid prokaryotic ribosome binding sites. The CAI value of optimized vaccine construct is 0.971788060 (range between 0.8 and 1.0), and an optimal range of GC content is 53.1118 (range between 30 and 70%), representing the strong probability of protein expression. In the following step, we incorporate two restricted endonucleases (XhoI and XbaI) in the both ends
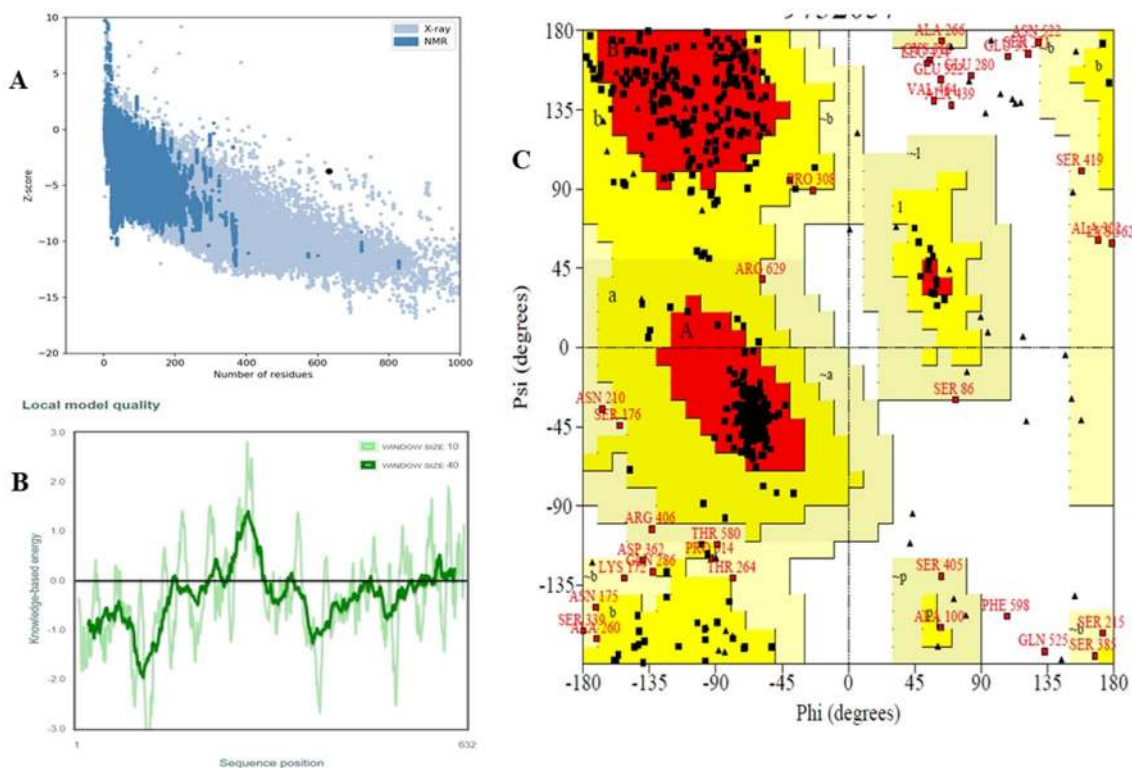
Fig. 6 Vaccine model validation **a** ProSA validation of predicted structure by Z score; **b** energy plot for structure validation; **c** Ramachandran plot

Table 4 Distribution of amino acid residues showing in Ramachandran plot

Type of amino acid residues	No. of amino acid residues	Percentage
Most favoured region [A, B, L]	404	80.0%
Additional allowed region [a, b, l, p]	69	13.7%
Generously allowed region [~ a, ~ b, ~ l, ~ p]	21	4.2%
Disallowed region	11	2.2%
Total Number of non-glycine and non-proline residues	505	100%
End-residues except glycine and proline	2	–
Glycine residues	83	–
Proline residues	42	–
Total number of residues	632	

of vaccine construct to assist the cloning process. Lastly, the vaccine construct was cloned within the pET28b (+) vector with the help of SnapGene software (Fig. 12). The clone was 7.094 kb long.

Discussion

H. pylori infection is increasing day by day in developed as well as developing countries. The different studies indicate chronic infection may cause of gastric carcinoma (Correa 1995; Leung et al. 2004; Nomura et al. 1991). Additional

infection with *H. pylori* leads to chronic gastritis, multifocal atrophic gastritis, occasionally gastric dysplasia, lesions and rarely intestinal metaplasia (Correa 1995). Till now, no safe and effective vaccine candidate is not available for treating *H. pylori* infection.

The current study is based on the development of a multi-epitope-based peptide vaccine against *H. pylori*. The main advantage of the epitope-based vaccine is that it can generate specific immune responses against epitopes and enhance binding affinity with the target receptor molecules and in turn avoid unfavorable non-epitopes selection (Bhattacharya et al. 2020d; Moise et al. 2015; Terry et al. 2015). The selected
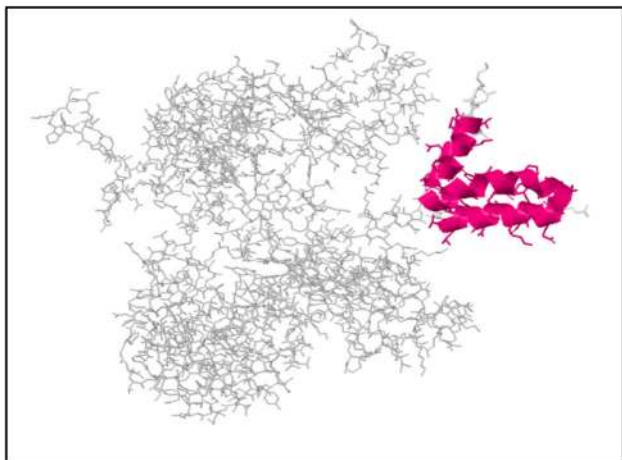
Fig. 7 Magenta colour helix structure showed discontinuous B-cell epitopes in the 3D model of multi-epitope vaccine (Color figure online)
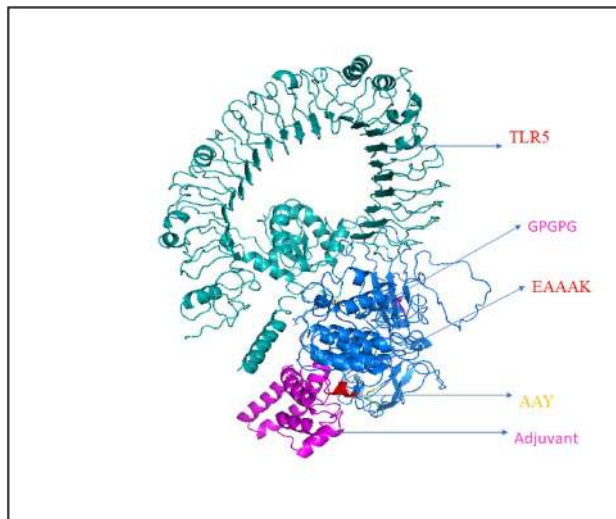

Fig. 8 The interaction pattern of designed vaccine construct with human TLR5

common epitopes elicits the immune-response with the target molecules for the next step of cellular cascades (Zhou et al. 2009). However, in the past Meza et al. reported multi epitope-based peptide (MEBP) vaccine candidates against *H. pylori* using reverse vaccinology approach (Meza et al. 2017). They predicted both B and T-cell epitopes of four virulent protein (FliD, Urease B, VacA and CagA) for designing a MEBP vaccine candidate against *H. pylori*. Lately, Khan et al. did the same and predicted B-cell and T-cell epitopes from different virulent protein (CagA, OipA, GroEL and VacA) and planned a MEBP vaccine against *H. pylori* (Khan et al. 2019).

Now, in this work we selected different four antigenic proteins (HpaA, FlaA, FlaB and Omp18) to design a MEBP vaccine through modern immunoinformatics approach. For the development of both humoral and

Table 6 Hydrogen Bond (distance, Å) between TLR5 and vaccine construct

Vaccine construct (Atom)	TLR5 (Atom)	Distance (Å)
O:12,705	H:16,357	3.28
H:15,306	H:12,133	4.46
H:15,363	H:20,751	3.42
H:15,997	H:20,775	4.46
H:16,342	C:12,415	4.94
H:4343	O:11,787	2.49
O:4896	H:21,124	3.91

cytotoxic immune response, T-cell epitopes derived from B-cell epitopes were identified in this study. Afterwards, by using several web tools, both MHC-I and MHC-II

Table 5 The statistical analysis of docking complex (TLR5 and vaccine construct)

Vaccine construct-TLR5	
HADDOCK score (a.u)	$-151.4 +/- 8.7$
Cluster size	8
RMSD from the overall lowest-energy structure	$1.4 +/- 0.8$
Van der Waals energy (kcal mol^{-1})	$-78.3 +/- 1.3$
Electrostatic energy (kcal mol^{-1})	$-306.4 +/- 34.1$
Desolvation energy (kcal mol^{-1})	$-12.2 +/- 3.8$
Restraints violation energy (kcal mol^{-1})	$3.8 +/- 2.1$
Buried surface area (Å^2)	$2692.6 +/- 99.3$
Z-score	-1.8
PRODIGY binding score	Gibbs free energy (ΔG) -11.7 kcal mol^{-1} and dissociation constant (Kd) 5.5E-09 M

Negative HADDOCK score signifies strong protein interaction which is expressed in arbitrary units a.u) and binding affinity score
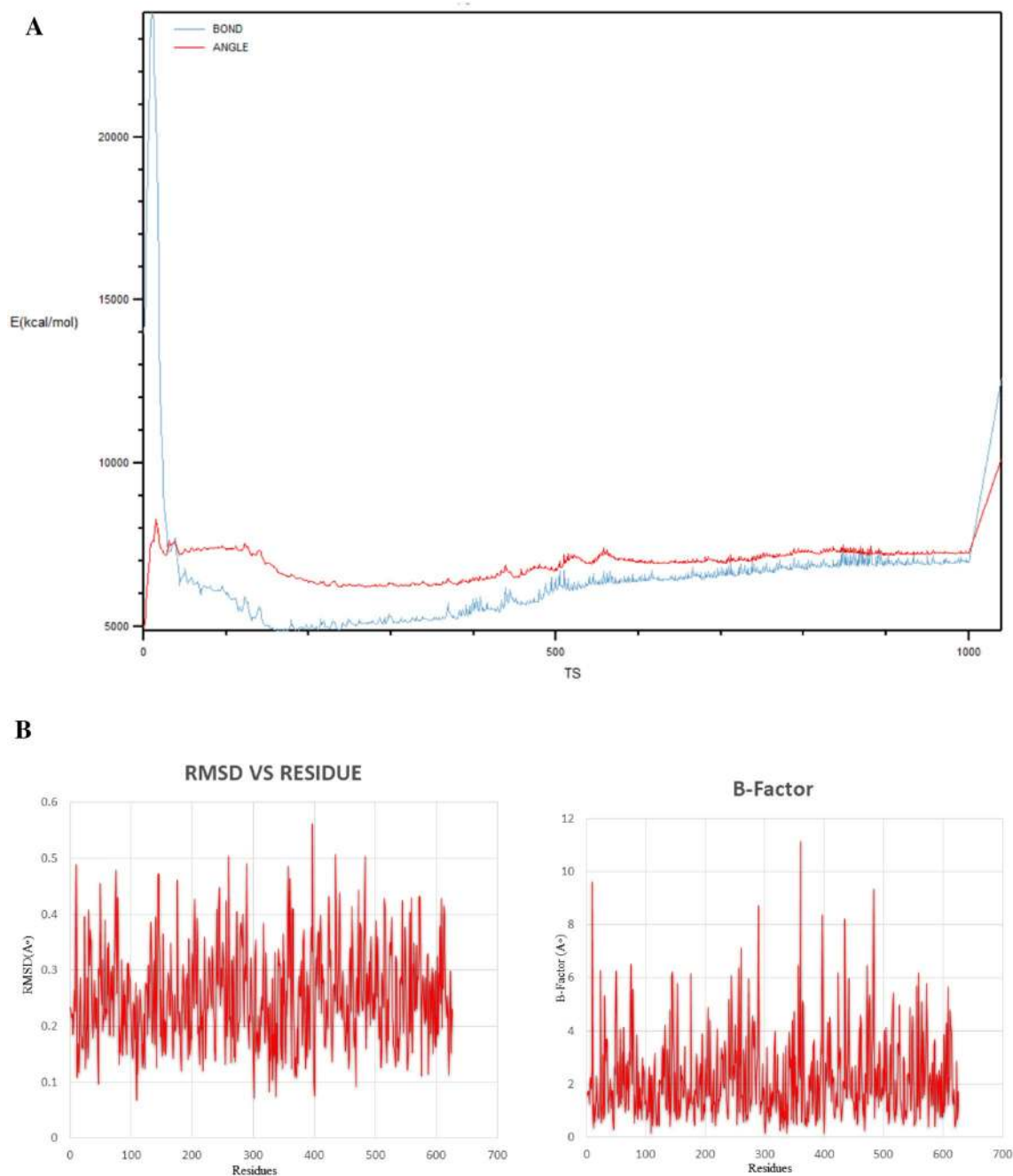
Fig. 9 a Molecular dynamics simulation of vaccine construct (through Cα fluctuations, atom distances, and dihedral angles). **b** B-factor plot analysis along with RMSD graphical plot

epitopes were predicted successfully. Subsequently, we have predicted common B-cell and T-cell epitope and joined with appropriate peptide linkers for formulation of this vaccine construct. An adjuvant 50S ribosomal protein L7/L12 was added at N terminal of the vaccine to boost up

immunogenicity at cellular level which was used by other researcher for vaccine development (Shey et al. 2019).

In this study the 3D structure of vaccine candidate developed through SPARKS-X web server, and its validated through ProSA and PROCHECK web-server. Subsequently the Z score, energy plot was evaluated to
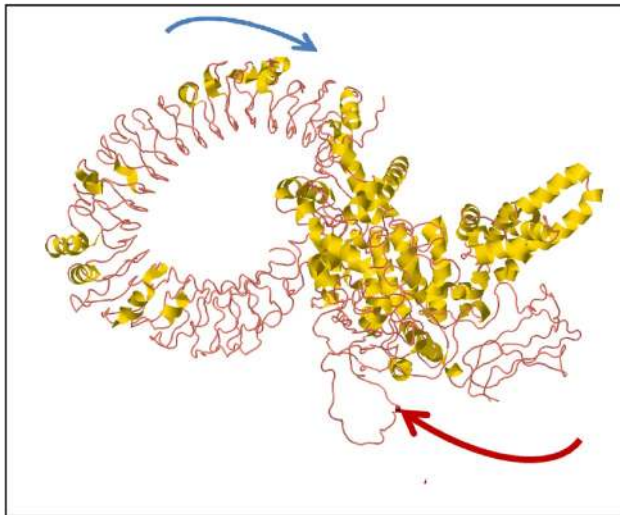
Fig. 10 Molecular mobility assessed through NMA of docked complex. (Two coloured affine-arrows showing the domains mobility)

complexes. Subsequently, the binding affinity of the designed vaccine with the TLR5 we passed through the PRODIGY server for calculating exact binding affinity score (Gibbs free energy (ΔG) is − 11.7 kcal mol^{-1} and dissociation constant (K_D) is 5.5E-09 M). The stability of the vaccine structure was accessed through molecular dynamics simulation with the help of NAMD software package, it generated NAMD plot to show the molecular flexibility and stability. Whereas, NMA study performed to analyzed the molecular mobility, comparative deformability. The eigenvalue was noted as $2.935e^{-05}$ which indicates the better flexibility of the docking complex. Finally, the SnapGene cloning tool was employed to amplify the desired vaccine sequence within the pET28b (+) expression vector.

Conclusion

In the summary, we demonstrated a reverse vaccinology approach to design and developed a MEBP vaccine which might be elicited simultaneous cellular and humoral immune responses in humans. The constructed vaccine was gone through the antigenicity and allergenicity assessment server, and concluded that our vaccine construct showed high antigenicity and non-allergenic nature. 3D structures of vaccine construct show its binding affinity with human TLR5 receptor and elicit the cellular responses. Though
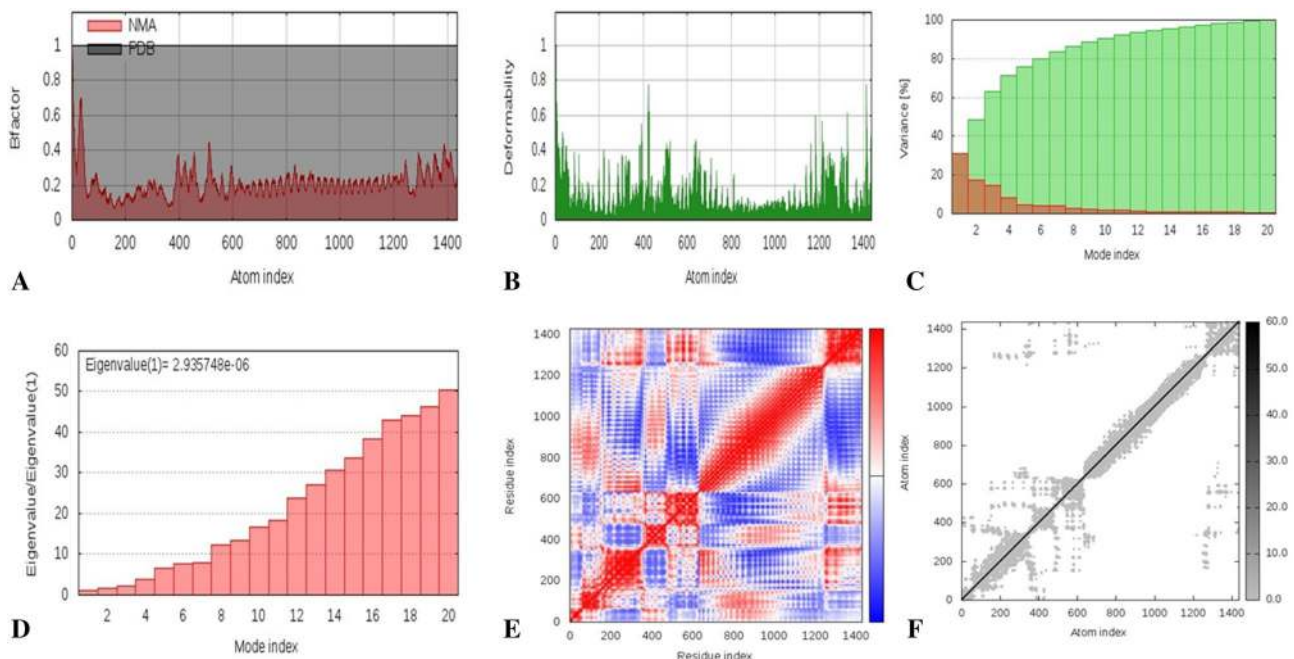
understand its proper folding. Ramachandran plot of the amino acid residues of vaccine candidate was studied. Whereas, we predicted negative value through Z score analysis which indicates its reliable structure quality.

HADDOCK server demonstrated the binding interaction of the design vaccine candidate and TLR5, and calculated HADDOCK score is noted as − 151.4 +/− 8.7. It indicates proper interaction between the protein–protein

Fig. 11 Results of iMODS **a** B-factor; **b** deformability plot; **c** variance plot; **d** eigenvalue; **e** covariance matrix analysis; **f** elastic network model
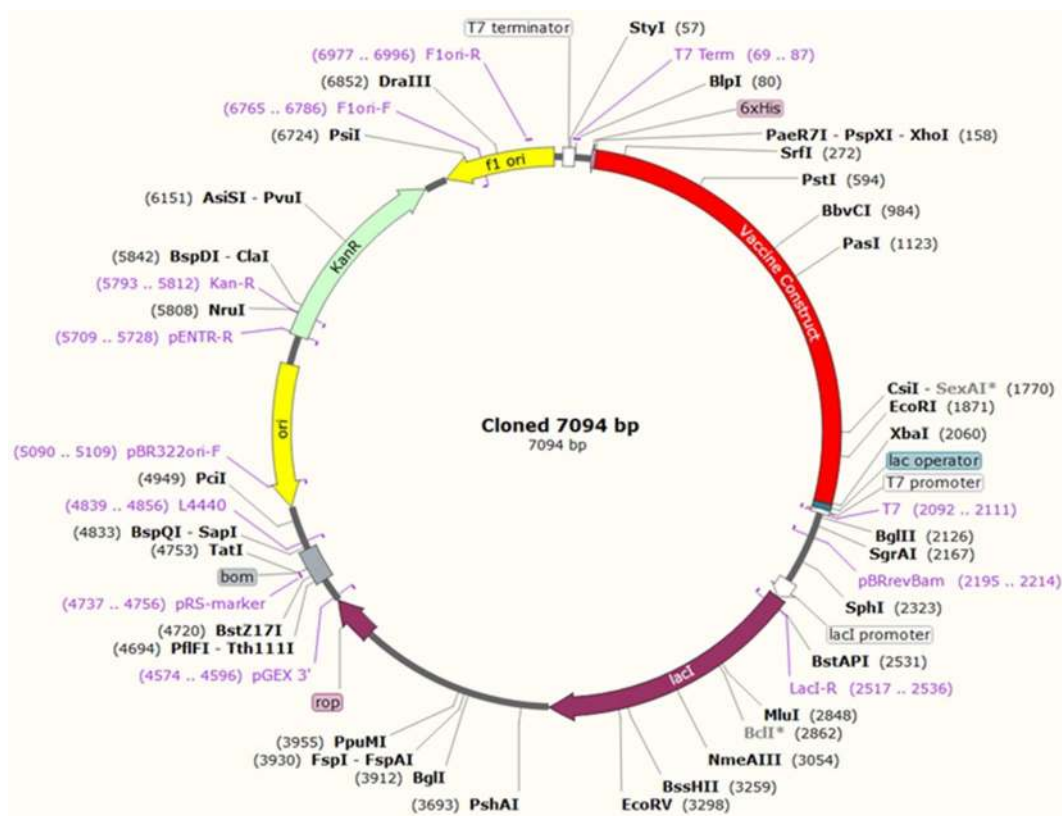
Fig. 12 In silico cloning of vaccine construct expressed in the *E. coli* host

we found immunogenic response in our study through in-silico approaches but there is a strong need of in-vitro and in-vivo studies to validate the effectively, immunogenicity and most vital host safety to vaccine structure before human administration.

Compliance with Ethical Standards

Conflict of interest The authors declare no competing interests.

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