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Design of a Peptide-Based Subunit Vaccine against Novel Coronavirus SARS-CoV-2

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Abstract

Coronavirus disease 2019 (COVID-19) is an emerging infectious disease that was first reported in Wuhan, China and has subsequently spread worldwide. In the absence of any antiviral or immunomodulatory therapies, the disease is spreading at an alarming rate. 5 to 10% of recovered patients in Wuhan test positive again; this suggest that for controlling COVID-19, vaccines may be better option than drugs. A clinical trial to evaluate an anti-COVID-19 vaccine has started recently. However, its efficacy and potency have to be evaluated and validated. As an alternative, we are presenting a first-of-its-kind, designed multi-peptide subunit based epitope vaccine against COVID-19. The vaccine construct comprise an adjuvant, CTL, HTL, and B-cell epitopes joined by linkers. The vaccine is non-toxic, non-allergenic, thermostable and immunogenic with the capability to elicit a humoral and cell-mediated immune response. The findings are validated with high-end computation-based methods. This unique vaccine is made up of 33 highly antigenic epitopes from three proteins that have a prominent role in host receptor recognition, viral entry, and pathogenicity. We advocate this vaccine must be synthesized and tested urgently as public health priority.

Keywords:

Adjuvant, COVID-19, Immunogenic epitopes, Peptide vaccine, Subunit vaccine, Molecular dynamics simulation.

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1 Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) belongs to the genus *Betacoronavirus* of the Coronaviridae family and was identified as the pathogen of Coronavirus disease 2019 (COVID-19) [1]. The epicenter of the COVID-19 coronavirus outbreak was central Chinese city of Wuhan, from where it spread globally. On 30th January 2020, the World Health Organization officially declared the COVID-19 epidemic as a public health emergency of international concern. Human to human transmission occurs through droplets, contact, and fomites. People with COVID-19 show symptoms of fever, cough, muscle aches, headache, and diarrhea. The principal feature of the severe disease is acute onset of hypoxemic respiratory failure with bilateral infiltrates.

The virus genome has been sequenced that allowed the development of diagnostic tests and research into vaccines and therapeutics [1,2]. A specific RT-PCR-based test has been developed that is in use for clinical diagnoses [3]. More than 100 clinical trials are currently underway to test novel and repurposed compounds against SARS-CoV-2 [4]. Certain drugs including hydroxychloroquine, chloroquine, and remdesivir are being tested in clinical trials [5-7]. One small study reported that a combination therapy of hydroxychloroquine with azithromycin reduced the detection of viral RNA compared to control [8,9]. A recent open-label trial with two protease inhibitors, lopinavir and ritonavir failed completely [10]. Several inactivated vaccines, viral vectored vaccines (adenovirus vector, ankara vector), nanoparticle-based vaccines, fusion-protein based vaccines, adjuvanted vaccines, recombinant protein, and DNA vaccines, as well as live-attenuated vaccines, are also being developed and tested, but these vaccines are many months away from the market [11-13]. A phase 1 clinical trial of an mRNA vaccine, called mRNA-1273, has started recently [14-16]. However, this is the first of several steps in the clinical trial process for evaluating the potential benefits of the vaccine.

The SARS-CoV-2 consists of single, positive-stranded RNA and four structural proteins: a spike glycoprotein (S), a membrane glycoprotein (M), an envelope protein (E), and a nucleocapsid protein (N) [17]. To enter the host cells, the virus uses a densely glycosylated spike protein that binds to the angiotensin-converting enzyme 2 (ACE2) receptor with high affinity [18,19]. Mutations in the receptor-binding domain (RBD) of the spike protein are the most variable part of the SARS-CoV-2 genome. Structural and biochemical studies suggest that the RBD has an ultra-high binding affinity to human ACE2 receptor [20]. Few groups have designed

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subunit vaccine against SARS-CoV-2, however, their workflow involved either use of single protein for vaccine design [21,22] or use only CTL epitopes without considering the importance of B-cell or HTL epitopes [23]. Some subunit-vaccines are also in preclinical trials [24,25]. Here, we focused on designing a multi-epitope-based subunit vaccine against SARS-CoV-2 using 33 highly antigenic epitopes. We believe that experimental evaluation may result in a novel and immunogenic vaccine that may confer protection against SARS-CoV-2 infection.

2 Methods

2.1 Screening of antigenic proteins

The protein sequences of SARS-CoV-2 were retrieved from the NCBI database (https://www.ncbi.nlm.nih.gov/nuccore/MN996531.1/) for subunit vaccine development (Table 1) [26]. Each of these proteins was screened for their average antigenic propensity using the antigenic peptides prediction tool (http://imed.med.ucm.es/Tools/antigenic.pl). Proteins with antigenic probability score of greater than 0.8 were considered for vaccine construction.

2.2 Prediction of helper T-lymphocyte (HTL), cytotoxic T-lymphocyte (CTL) and B-cell epitopes

The HTL epitopes for the selected SARS-CoV-2 proteins were predicted using the MHC-II epitope prediction tool from the Immune Epitope Database (IEDB, http://tools.iedb.org/mhcii/). Selected epitopes had the lowest percentile rank and IC_{50} values. Additionally, these epitopes were checked into the IFN epitope server (http://crdd.osdd.net/raghava/ifnepitope/) for capability to induce Th1 type immune response accompanied by IFN-ϒ production. CTL epitopes for the screened proteins were predicted using the NetCTL1.2 server (http://www.cbs.dtu.dk/services/NetCTL/). B-cell epitopes for the screened SARS-CoV-2 proteins were predicted using the ABCPred server (http://crdd.osdd.net/raghava/abcpred/). The prediction of the toxic/non-toxic nature of all the selected HTL, CTL and B-cell epitopes was checked using the ToxinPred module (http://crdd.osdd.net/raghava/toxinpred/multi_submit.php).

2.3 Construction of the multi-epitope subunit vaccine

The vaccine subunit was designed by adding an adjuvant, HTL, CTL and B-cell epitopes connected by specific linkers to provide effective separation of epitopes *in vivo*. EAAAK linker

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was used to join the adjuvant and HTL. Intra HTL, Intra CTL, and B-cell epitopes were joined using GPGPG, AAY, and KK, respectively. To enhance the immunogenicity of the vaccine construct, the TLR-3 agonist, human β-defensin 1 (Uniprot ID: P60022), was used as the adjuvant.

2.4 Immunogenicity and allergenicity prediction

The immunogenicity of the vaccine was determined using the VaxiJen server (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) and ANTIGENpro module of SCRATCH protein predictor (http://scratch.proteomics.ics.uci.edu/). The allergenicity of the vaccine was checked using AllerTOP v2.0 (http://www.ddg-pharmfac.net/AllerTOP/) and AlgPred Server (http://crdd.osdd.net/raghava/algpred/).

2.5 Determination of physiochemical properties

The physiochemical characteristics of the vaccine were determined using the ProtParam tool of the ExPASy database server (http://web.expasy.org/protparam/).

2.6 Structure prediction, validation, and docking with the receptor

The secondary structure of the subunit vaccine construct was predicted using PSIPred Protein Sequence Analysis Workbench (http://bioinf.cs.ucl.ac.uk/psipred/), while the tertiary structure was predicted by *de novo* structure prediction-based trRosetta modeling suite. trRosetta uses a deep residual neural network to predict the inter-residue distance and orientation distributions of the input sequence. Then it converts predicted distance and orientation distributions into smooth restraints to build 3D structure models-based on direct energy minimization. The model of the vaccine construct with the best TM-score was validated by PROCHEK v.3.5 (https://servicesn.mbi.ucla.edu/PROCHECK/) and ProSA (https://prosa.services.came.sbg.ac.at/prosa.php) web servers. Vaccine-receptor docking was performed by the ClusPro web server to determine the binding affinity of the vaccine with the TLR-3 receptor (PDB ID: 3ULV).

2.7 Molecular dynamics simulations

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Molecular dynamics (MD) simulation is an effective method to study the molecular interactions and dynamics of protein-protein complexes. We used it to evaluate the stability, binding mode, and dynamics of the TLR3-vaccine complex. The complex structure of the TLR3-vaccine was initially optimized using Schrödinger Maestro (Schrödinger Release 2016–4: Maestro, Schrödinger, New York) and subsequently utilized as the starting structure for MD simulations. The analysis from MD simulation was performed as described earlier [27].

2.8 Codon adaptation and *in-silico* cloning

Java Codon Adaptation Tool (JCAT) (http://www.jcat.de/) was used for codon optimization of the vaccine sequence to test high-level expression of the vaccine in *E. coli* strain K12. NEBcutter (http://nc2.neb.com/NEBcutter2/) was used for the selection of restriction enzyme cleavage sites, and the expression vector pET28a(+) was selected. *In silico* clone of the vaccine was designed using the SnapGene 1.1.3 restriction cloning tool.

3 Results

3.1 Screening of antigenic proteins

The amino acid sequence of the three SARS-CoV-2 proteins, namely, nucleocapsid protein, membrane glycoprotein, and surface spike glycoprotein, were retrieved from the NCBI database (Table 1). These proteins are known to have a prominent role in host receptor recognition, viral entry, and pathogenicity. The proteins with an antigenic score of greater than 0.8 (Table 1) were used further for the prediction of epitopes for subunit vaccine designing (Figure 1).

3.2 Prediction of HTL, BCL and CTL epitopes

Helper T-lymphocytes are the key players of the adaptive immune response. They are involved in the activation of B-cells and cytotoxic T cells for antibody production and killing infected target cells, respectively. All three proteins were subjected to the IEDB MHC-II epitope prediction module for HTL prediction. A total of six highest immunogenic epitopes of 15-mer were selected based on their percentile rank and IC_{50} values. Also, all these epitopes showed positive scores on IFNepitope server output (Table 2). B-cells are the main components of humoral immunity during the adaptive immune response that produces antibodies, which recognize antigens. Therefore, it was necessary to predict B-cell epitopes prior to vaccine

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designing. ABCpred was performed for predicting B-cell epitope, and a total of 9 epitopes with top scores from the three proteins were considered for the vaccine (Table 3).

CTL epitopes are important for inducing MHC-I cellular immune response by neutralizing virus-infected cells and damaged cells via releasing cytotoxic proteins like granzymes, perforins, etc. The CTL epitopes were predicted for all selected proteins using the NetCTL 1.2 server. Here, A2, A3 and B7 supertypes were considered for prediction as they cover at least 88.3% of the total ethnic population. Eighteen epitopes with a combined score of >0.75 were finally considered for the vaccine (Table 4). All the selected HTL, CTL and B-cell epitopes were subjected to the ToxinPred module to screen for their toxicity. Supplementary Table 1 shows that all epitopes selected for the vaccine were non-toxic.

3.3 Subunit vaccine designing

A total of 6 HTLs, 18 CTLs and 9 B-cell epitopes derived from the three proteins were used to design the subunit vaccine (566 amino acid residues) against SARS-CoV-2 (Supplementary Figure 1). The human β-defensin 1(68 amino acid residues) sequence was added as an adjuvant followed by the HTL, CTL and B-cell epitopes and linked by specific linkers.

3.4 Antigenicity and allergenicity prediction of constructed vaccine

An important parameter of vaccine designing is ensuring that the constructed vaccine is immunogenic to induce a humoral and/or cell-mediated immune response against the targeted virus. Our vaccine was found to be antigenic with a probability score of 0.513 and 0.732 predicted by VaxiJen v2.0 and ANTIGENPro servers, respectively. The allergenicity score was found to be –0.658 in AlgPRED prediction module. Additionally, the vaccine was also found to be non-allergen using AllerTOP v2.0.

3.5 Physiochemical characterization of designed vaccine

The designed vaccine construct is composed of 566 amino acids with a molecular weight of approximately 62.34 kDa. The theoretical pI was 10.21, suggesting the vaccine is significantly basic. The half-life of the vaccine was estimated to be 30 h in mammalian reticulocytes (*in vitro*), >20 h in yeast (*in vivo*) and >10 h in *E. coli (in vivo)*, suggesting that the construct is stable *in vivo*. The instability index was estimated to be 24.76, suggesting a stable protein. The computed

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aliphatic index and grand average of hydropathicity were found to be 77.79 and –0.187, respectively, suggesting that the vaccine is thermostable and hydrophilic, respectively.

3.6 Structure prediction and validation

The secondary structure was predicted using PESIPRED 4.0 server (Supplementary Figure 2). The tertiary structure of the vaccine was predicted using trRosetta modeling suite. The 3D model generated by trRosetta modeling was subjected to PROCHECK server, where Ramachandran plot statistics were generated. The output showed 98.4% residues were present in the favored region, 1.0% residues in the generously allowed region, and 0.6% residues in disallowed regions. Further, the Z-score plot and energy plot was generated by the ProSA web server. The calculated Z-score (–8.46) lies within the X-ray crystal structure range. The energy plot suggested that all the residues have low energy value in the modeled structure (Figure 2).

3.7 Vaccine-receptor docking

Vaccine-receptor docking was performed to evaluate the binding energy of the vaccine with its TLR-3 receptor. ClusPro analysis provided 30 vaccine-receptor complexes with respective energy scores. The lowest energy complex with binding energy of about –1491 $kJ.mol^{-1}$ was selected and subjected to MD simulation (Figure 3).

3.8 MD simulations

The binding modes, dynamics, and stability of the vaccine-TLR3 complex were evaluated using a 40 ns MD simulation study (Figure 4). The atomic level interaction between vaccine and TLR3 were determined and root mean square deviation (RMSD), root mean square fluctuation (RMSF), hydrogen bond and contact energy were calculated.

The RMSD data suggests that the receptor-vaccine complex was stabilized after about 20 ns till the end of the simulation (Figure 4). All the calculations were then done for the 20-40 ns MD simulation trajectory. Next, RMSF calculation, which gives information about the residue wise dynamics of a protein with respect to its initial position was done. An average RMSF value of 1.19 nm was observed for the complex (Figure 4). Our subsequent analysis was on hydrogen bonds that play very important role in stabilizing protein structure and recognition of other protein partners in a complex. The vaccine-receptor complex formed an average of 327 hydrogen

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bonds, suggesting the favourable intermolecular interactions between the vaccine protein and TLR3 receptor. The formation of a large number of hydrogen bonds and its stabilization during simulations reflect the specificity and selectivity of intermolecular interactions (Figure 4). In total, the complex formed about 5369 bonds during the 40 ns simulation run. Next, we further computed the contact energy for the vaccine-TLR3 complex and it was found that while the starting complex structure exhibited a total contact energy of –814.36 kcal.mol^{-1}, the stabilized complex exhibited a total contact energy of –935.68 kcal.mol^{-1}, showing the increased stability during MD simulations. Lastly, the detailed interactions between TLR3 and the vaccine protein was computed from the starting structure of MD simulations and the stabilized structure of the complex extracted from MD simulated trajectory (Figure 5 and Table 5). The higher total number of interactions in the stabilized complex suggest the stability and tighter binding of the vaccine with TLR-3.

3.9 Codon adaptation and *in silico* cloning

The codon optimization index ensures the relationship between codon usage and gene expression in a heterologous system. The JCAT output was further analyzed in NEBcutter and at the N- and C-terminal ends of the optimized vaccine sequence BamHI and NdeI restriction sites were added that are non-cutters for the vaccine construct but are present in the multiple cloning site of the selected expression vector pET28a(+). *In silico* clone was generated using SnapGene 1.1.3 restriction cloning tool that resulted in a cloned product of 7034 bp (Figure 6).

4 Discussion

Recent reports suggest that about 5%-10% of patients from Wuhan who had been tested positive earlier and then recovered from the disease are testing positive for the virus a second time. As a consequence, spread can be also caused by asymptomatic carriers [28,29]. These suggest that a vaccine is a better option for coronavirus management than drugs.
The efforts to produce a vaccine against coronavirus are moving at a rapid pace. Two candidate vaccines are in the Phase I clinical trials: i) An adenovirus type-5 vector based vaccine, and ii) a LNP-encapsulated mRNA vaccine. Studies evaluating the safety and immunogenicity of these vaccines are underway [30]. Additionally, 42 vaccine candidates are under preclinical evaluation [30]. Though these trials are underway, there are known situations that vaccines have failed.

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Recently few groups have tried designing subunit vaccine against SARS-CoV-2, however, their workflow involved either use of single protein for vaccine design [21,22] or use only CTL epitopes without considering the importance of B-cell or HTL epitopes [23]. We kept all of these points in our mind while designing the vaccine. Based on extensive bioinformatics analysis, we used three proteins to design a multi-epitope subunit vaccine against novel coronavirus SARS-CoV-2. These proteins are nucleocapsid protein (N), membrane glycoprotein (M), and the surface spike glycoprotein (S). The N protein is involved in packaging the viral genome into a helical ribonucleocapsid, and it plays a fundamental role during viral self-assembly [31]. The M protein is responsible for the assembly and immunogenicity of virus particles. The S protein mediates the entrance of the virus to human respiratory epithelial cells by interacting with cell surface receptor ACE2. The S protein has two regions: S1, for host cell receptor binding; and S2, for membrane fusion. The S protein is a key target for the development of vaccines, therapeutic antibodies, and diagnostics for coronavirus [13,32,33]. Our vaccine contains a suitable adjuvant, HTL, CTL and B-cell epitopes that are joined by suitable linkers. Furthermore, the epitopes were screened for their toxicity potential. The subunit vaccine was found to be thermostable, antigenic and non-allergenic. Molecular docking and MD simulation provided insights about the interaction, stability and dynamics of the vaccine-receptor complex. The data suggest constructive intermolecular interactions between the vaccine protein and TLR-3 receptor. Also, the *in-silico* cloning suggests the potential expression of the vaccine in microbial expression system, thereby, making it a potential vaccine against SARS-CoV-2 infection.

The subunit vaccines consist of only the antigenic portion of the pathogens that may directly elicit an immune response. Additionally, the vaccine does not utilize live pathogen, thus, reducing the risk of pathogenicity reversal. Hence, it can be used in immune-suppressed patients as well. These vaccines elicit long-lived immunity and have uncomplicated storage conditions. Thus, our multi-epitope-based subunit vaccine has a probability to show good protective efficacy and safety against SARS-CoV-2 infection in humans. We suggest the synthesis and experimental evaluation of this vaccine to determine its immunogenic potency.

Competing interests

The Authors have declared no competing interest.

Authors' contributions

PK and AKP carried out the experiments. PK and TT conceived the study and participated in its design and coordination. PK, AKP, KYJZ and TT analysed the data and drafted the manuscript. All authors read and approved the final manuscript.

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References

[1] P. Zhou, X.L. Yang, X.G. Wang, B. Hu, L. Zhang, W. Zhang, H.R. Si, Y. Zhu, B. Li, C.L. Huang, H.D. Chen, J. Chen, Y. Luo, H. Guo, R.D. Jiang, M.Q. Liu, Y. Chen, X.R. Shen, X. Wang, X.S. Zheng, K. Zhao, Q.J. Chen, F. Deng, L.L. Liu, B. Yan, F.X. Zhan, Y.Y. Wang, G.F. Xiao, Z.L. Shi, A pneumonia outbreak associated with a new coronavirus of probable bat origin, Nature 579 (2020) 270-273. 10.1038/s41586-020-2012-7.
[2] K. Andersen, A. Rambaut, W.I. Lipkin, E.C. Holmes, R.F. Garry, The proximal origin of SARS-CoV-2, Nat Med (2020). 10.1038/s41591-020-0820-9.
[3] L.L. Poon, O.K. Chan Kh Fau - Wong, W.C. Wong Ok Fau - Yam, K.Y. Yam Wc Fau - Yuen, Y. Yuen Ky Fau - Guan, Y.M.D. Guan Y Fau - Lo, J.S.M. Lo Ym Fau - Peiris, J.S. Peiris, Early diagnosis of SARS coronavirus infection by real time RT-PCR, J Clin Virol. 28 (2003) 233-238.
[4] Y. Zhou, Y. Hou, J. Shen, Y. Huang, W. Martin, F. Cheng, Network-based drug repurposing for novel coronavirus 2019-nCoV/SARS-CoV-2, Cell Discov 6 (2020) 14. 10.1038/s41421-020-0153-3.
[5] M.A. Martinez, Compounds with therapeutic potential against novel respiratory 2019 coronavirus, Antimicrob Agents Chemother (2020). 10.1128/aac.00399-20.
[6] J. Liu, R. Cao, M. Xu, X. Wang, H. Zhang, H. Hu, Y. Li, Z. Hu, W. Zhong, M. Wang, Hydroxychloroquine, a less toxic derivative of chloroquine, is effective in inhibiting SARS-CoV-2 infection in vitro, Cell Discov 6 (2020) 16. 10.1038/s41421-020-0156-0.
[7] M. Wang, R. Cao, L. Zhang, X. Yang, J. Liu, M. Xu, Z. Shi, Z. Hu, W. Zhong, G. Xiao, Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro, Cell Res 30 (2020) 269-271. 10.1038/s41422-020-0282-0.
[8] Anonymous, Information for clinicians on therapeutic options for COVID-19 patients, https://www.cdc.gov/coronavirus/2019-ncov/hcp/therapeutic-options.html, 2020.
[9] P. Gautret, J.-C. Lagier, P. Parola, V.T. Hoang, L. Meddeb, M. Mailhe, B. Doudier, J. Courjon, V.r. Giordanengo, V.E. Vieira, H.T. Dupont, S.p. HonorÃ©, P. Colson, E. ChabriÃ¨re, B. La Scola, J.-M. Rolain, P. Brouqui, D. Raoult, Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial, Int J Antimicrob Agents (2020) 105949.
[10] B. Cao, Y. Wang, D. Wen, W. Liu, J. Wang, G. Fan, L. Ruan, B. Song, Y. Cai, M. Wei, X. Li, J. Xia, N. Chen, J. Xiang, T. Yu, T. Bai, X. Xie, L. Zhang, C. Li, Y. Yuan, H. Chen, H. Li, H. Huang, S. Tu, F. Gong, Y. Liu, Y. Wei, C. Dong, F. Zhou, X. Gu, J. Xu, Z. Liu, Y. Zhang, H. Li, L. Shang, K. Wang, K. Li, X. Zhou, X. Dong, Z. Qu, S. Lu, X. Hu, S. Ruan, S. Luo, J. Wu, L.

Peng, F. Cheng, L. Pan, J. Zou, C. Jia, J. Wang, X. Liu, S. Wang, X. Wu, Q. Ge, J. He, H. Zhan, F. Qiu, L. Guo, C. Huang, T. Jaki, F.G. Hayden, P.W. Horby, D. Zhang, C. Wang, A Trial of Lopinavir-Ritonavir in Adults Hospitalized with Severe Covid-19, N Engl J Med (2020). 10.1056/NEJMoa2001282.
[11] W.-H. Chen, U. Strych, P.J. Hotez, M.E. Bottazzi, The SARS-CoV-2 vaccine pipeline: an overview, Curr Trop Med Rep (2020). 10.1007/s40475-020-00201-6.
[12] R.L. Roper, K.E. Rehm, SARS vaccines: where are we?, Expert Rev Vaccines 8 (2009) 887-898.
[13] E. Prompetchara, C. Ketloy, T. Palaga, Immune responses in COVID-19 and potential vaccines: Lessons learned from SARS and MERS epidemic, Asian Pac J Allergy Immunol 38 (2020) 1-9. 10.12932/ap-200220-0772.
[14] Anonymous, Moderna's work on a potential vaccine against COVID-19, https://www.modernatx.com/modernas-work-potential-vaccine-against-covid-19, 2020.
[15] Anonymous, NIH clinical trial of investigational vaccine for COVID-19 begins, https://www.nih.gov/news-events/news-releases/nih-clinical-trial-investigational-vaccine-covid-19-begins, 2020.
[16] J. Hodgson, The pandemic pipeline, Nat Biotechnol (2020).
[17] A.R. Fehr, S. Perlman, Coronaviruses: an overview of their replication and pathogenesis, Methods Mol Biol 1282 (2015) 1-23. 10.1007/978-1-4939-2438-7_1.
[18] F. Li, W. Li, M. Farzan, S.C. Harrison, Structure of SARS coronavirus spike receptor-binding domain complexed with receptor, Science 309 (2005) 1864-1868. 10.1126/science.1116480.
[19] R. Yan, Y. Zhang, Y. Li, L. Xia, Y. Guo, Q. Zhou, Structural basis for the recognition of the SARS-CoV-2 by full-length human ACE2, Science (2020). 10.1126/science.abb2762.
[20] W. Tai, L. He, X. Zhang, J. Pu, D. Voronin, S. Jiang, Y. Zhou, L. Du, Characterization of the receptor-binding domain (RBD) of 2019 novel coronavirus: implication for development of RBD protein as a viral attachment inhibitor and vaccine, Cell Mol Immunol (2020). 10.1038/s41423-020-0400-4. .
[21] M.I. Abdelmageed, A.H. Abdelmoneim, M.I. Mustafa, N.M. Elfadol, N.S. Murshed, S.W. Shantier, A.M. Makhawi, Design of multi epitope-based peptide vaccine against E protein of human 2019-nCoV: An immunoinformatics approach, bioRxiv (2020). 10.1101/2020.02.04.934232.
[22] M. Bhattacharya, A.R. Sharma, P. Patra, P. Ghosh, G. Sharma, B.C. Patra, S.-S. Lee, C. Chakraborty, Development of epitope-based peptide vaccine against novel coronavirus 2019 (SARS-COV-2): Immunoinformatics approach, J Med Virol n/a (2020) 1-14. 10.1002/jmv.25736.
[23] M. Seema, T Cell Epitope-Based Vaccine Design for Pandemic Novel Coronavirus 2019-nCoV, ChemRxiv (2020). 10.26434/chemrxiv.12029523.v1.
[24] S. Mukerjee, The first coronavirus drug candidate is set for testing in China, 2020 (accessed 29 Mar 2020).
[25] Clover initiates development of recombinant subunit-trimer vaccine for Wuhan coronavirus, 2020 (accessed 29 Mar 2020).
[26] P. Zhou, X.-L. Yang, X.-G. Wang, B. Hu, L. Zhang, W. Zhang, H.-R. Si, Y. Zhu, B. Li, C.-L. Huang, H.-D. Chen, J. Chen, Y. Luo, H. Guo, R.-D. Jiang, M.-Q. Liu, Y. Chen, X.-R. Shen, X. Wang, X.-S. Zheng, K. Zhao, Q.-J. Chen, F. Deng, L.-L. Liu, B. Yan, F.-X. Zhan, Y.-Y.

Wang, G.-F. Xiao, Z.-L. Shi, A pneumonia outbreak associated with a new coronavirus of probable bat origin, Nature 579 (2020) 270-273.
[27] P. Kalita, D.L. Lyngdoh, A.K. Padhi, H. Shukla, T. Tripathi, Development of multi-epitope driven subunit vaccine against Fasciola gigantica using immunoinformatics approach, Int J of Biol Macromol 138 (2019) 224-233.
[28] E. Feng, A. Cheng, Mystery In Wuhan: Recovered coronavirus patients test negative then positive, https://www.npr.org/sections/goatsandsoda/2020/03/27/822407626/mystery-in-wuhan-recovered-coronavirus-patients-test-negative-then-positive, 2020 (accessed 27 Mar 2020).
[29] H.D. Beau, Some recovered coronavirus patients in Wuhan are testing positive again, https://science.slashdot.org/story/20/03/27/2014216/some-recovered-coronavirus-patients-in-wuhan-are-testing-positive-again, 2020 (accessed 27 Mar 2020).
[30] WHO, DRAFT landscape of COVID-19 candidate vaccines – 21 March 2020, Geneva Switzerland, 2020.
[31] C.K. Chang, M.-h. Chen Cm Fau - Chiang, Y.-l. Chiang Mh Fau - Hsu, T.-h. Hsu Yl Fau - Huang, T.H. Huang, Transient oligomerization of the SARS-CoV N protein--implication for virus ribonucleoprotein packaging, PLoS One 8 (2013).
[32] L. Du, Y. He, Y. Zhou, S. Liu, B.J. Zheng, S. Jiang, The spike protein of SARS-CoV--a target for vaccine and therapeutic development, Nat Rev Microbiol 7 (2009) 226-236. 10.1038/nrmicro2090.
[33] S.S. Al-Amri, A.T. Abbas, L.A. Siddiq, A. Alghamdi, M.A. Sanki, M.K. Al-Muhanna, R.Y. Alhabbab, E.I. Azhar, X. Li, A.M. Hashem, Immunogenicity of candidate MERS-CoV DNA vaccines based on the spike protein, Sci Rep 7 (2017) 44875. 10.1038/srep44875.

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Table 1. Antigenicity score of the selected proteins.

Proteins	Accession no.	Predicted Order of Antigenicity
Nucleocapsid protein	QHR63298.1	0.9871
Membrane glycoprotein	QHR63293.1	1.0532
Surface spike glycoprotein	QHR63290.1	1.0412

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Table 2. Predicted HTL specific epitopes and their percentile rank obtained from IEDB.

S. No.	Protein name	Epitope	Percentile rank	SMM align IC_{50} (nM)	Allele	IFN-γ Inducer score
1.	Nucleocapsid protein	GTWLTYTGAIKLDDK	0.58	15	HLA-DRB1*07:01	3
		AALALLLLDRLNQLE	0.61	17	HLA-DRB4*01:01	1
2.	Membrane glycoprotein	NRFLYIIKLIFLWLL	0.12	31	HLA-DRB4*01:01	1
3.	Surface spike glycoprotein	EFVFKNIDGYFKIYS	0.17	10	HLA-DRB5*01:01	2
		ITRFQTLLALHRSYL	0.26	2	HLA-DRB5*01:01	3
		ATRFASVYAWNRKRI	0.49	10	HLA-DRB5*01:01	0.56

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Table 3. Predicted B-cell binding epitopes with their probable score and start position.

Sl. No.	Protein Name	Epitope	Position	Score
1	Nucleocapsid protein	TRRIRGGDGKMKDLSP	91	0.94
		KSAAEASKKPRQKRTA	249	0.93
		EGALNTPKDHIGTRNP	136	0.93
2	Membrane glycoprotein	RSMWSFNPETNILLNV	107	0.89
		SFRLFARTRSMWSFNP	99	0.88
3	Surface spike glycoprotein	YACWHHSIGFDYVYNP	6149	0.96
		VVKIYCPACHNSEVGP	365	0.96
		TLKGGAPTKVTFGDDT	814	0.95
		TSRYWEPEFYEAMYTP	5304	0.94

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Table 4. Predicted CTL epitopes for A2, A3, B7 super types.

S. No.	Protein Name	Supertype	Epitopes	Score
1	Nucleocapsid protein	A2	LLLDRLNQL	1.2648
			GMSRIGMEV	1.0266
		A3	KSAAEASKK	1.4421
			KTFPPTEPK	1.4314
		B7	FPRGQGVPI	1.6470
			KPRQKRTAT	1.6339
2	Membrane glycoprotein	A2	GLMWLSYFI	1.3055
			FVLAAVYRI	1.2094
		A3	LSYFIASFR	1.4994
			RIAGHHLGR	1.2901
		B7	LPKEITVAT	1.1745
			RLFARTRSM	0.9882
3	Surface spike glycoprotein	A2	YLQPRTFLL	1.5152
			KIADYNYKL	1.4347
		A3	RLFRKSNLK	1.7563
			GVYFASTEK	1.4615
		B7	SPRRARSVA	1.5619
			IPTNFTISV	1.5619

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Table 5. Details of the interactions occurred in the vaccine-receptor complex during molecular dynamics simulation.

Type of interactions	Initial structure before simulation	Stabilized structure after simulation
VdW interactions	40	28
Proximal interactions	2136	2245
Polar contacts	69	69
Hydrogen bonds	47	45
Aromatic contacts	9	16
Hydrophobic contacts	77	108
Carbonyl interactions	7	5
Total number of interactions	**2385**	**2516**

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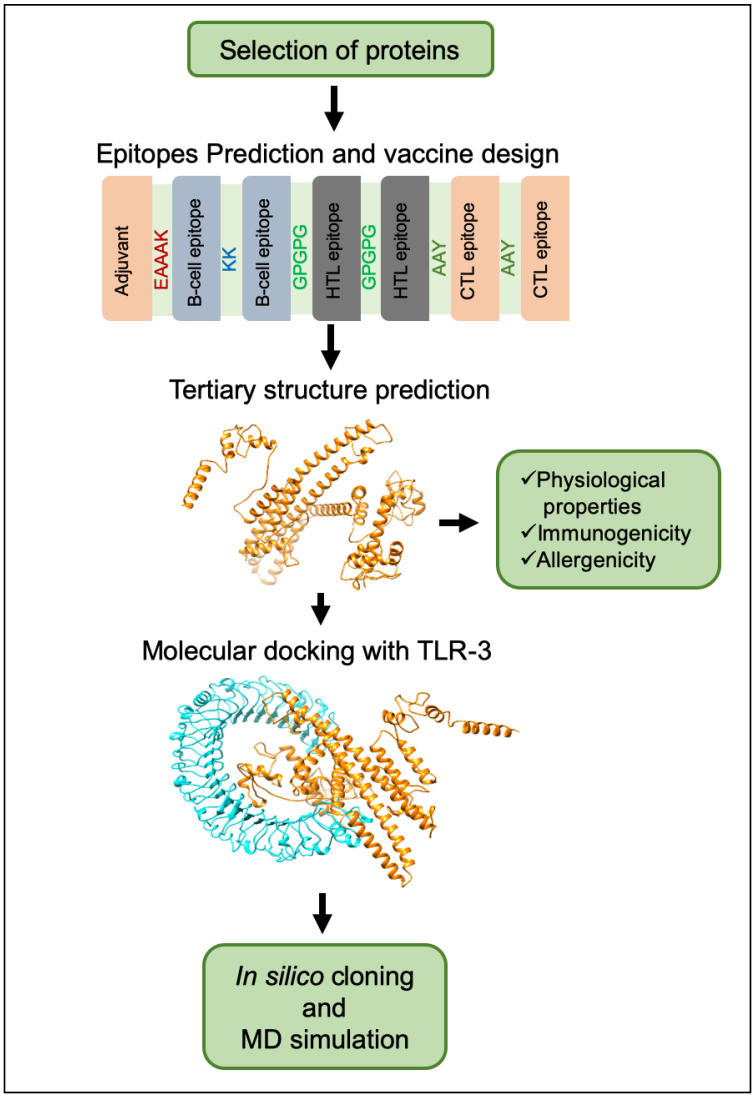

Figure 1: Schematic representation of the multi-epitope subunit vaccine candidate designing using B-cell, CTL and HTL epitopes.

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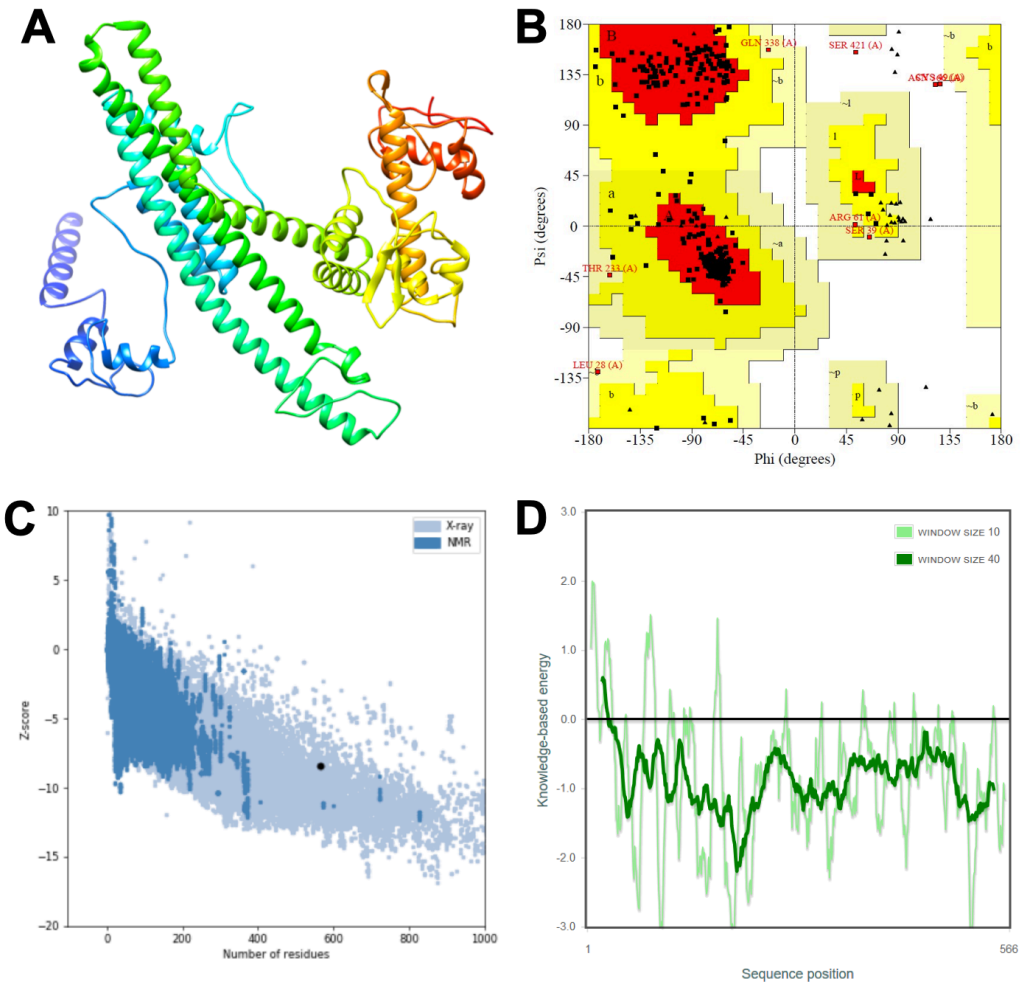

Figure 2: Tertiary structure model prediction and its validation (A) 3D model obtained for the multi-subunit vaccine protein. (B) Ramachandran plot showing the presence of amino acid residues in favoured, allowed and outlier region. (C) ProSA-web z-score plot for predicted the 3D structure and (D) Energy plot for all residues in the predicted structure.

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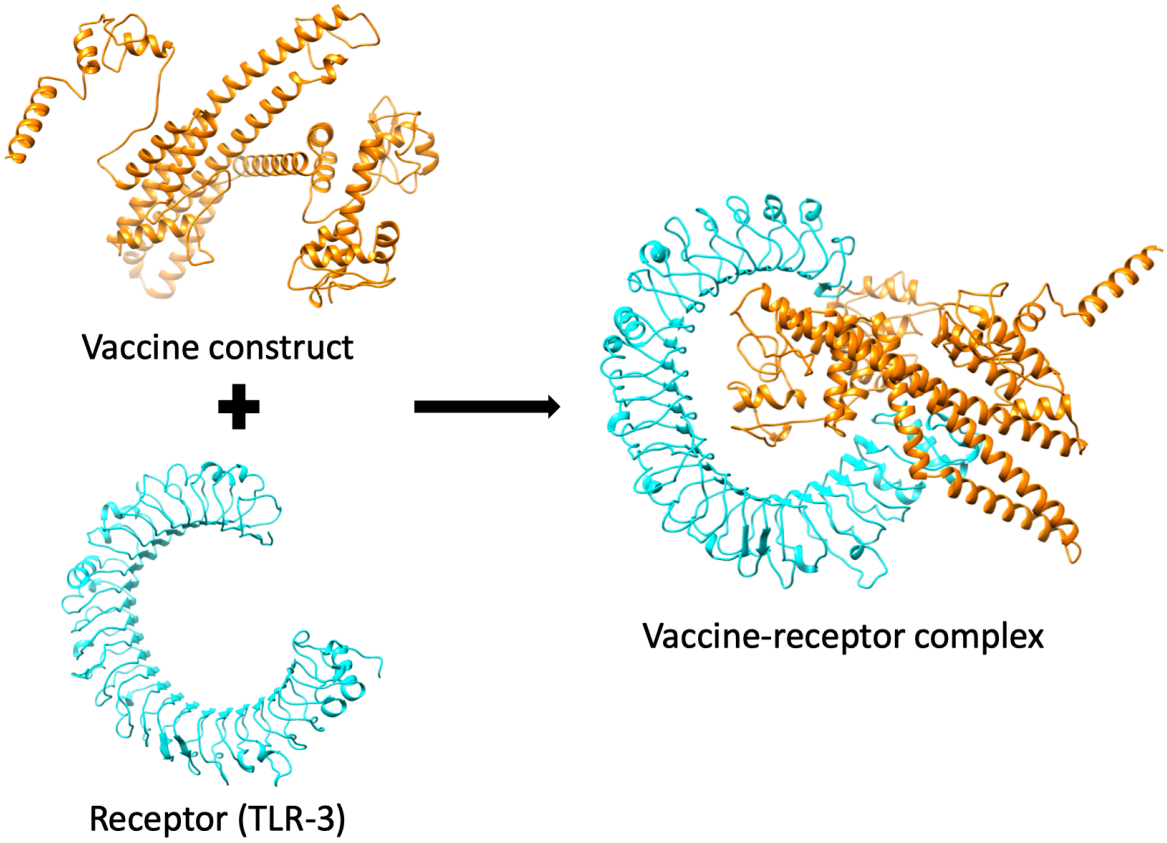

Figure 3: Stable interaction of between the vaccine construct and TLR3 after docking. The vaccine construct is shown in orange while the TLR-3 is shown in cyan.

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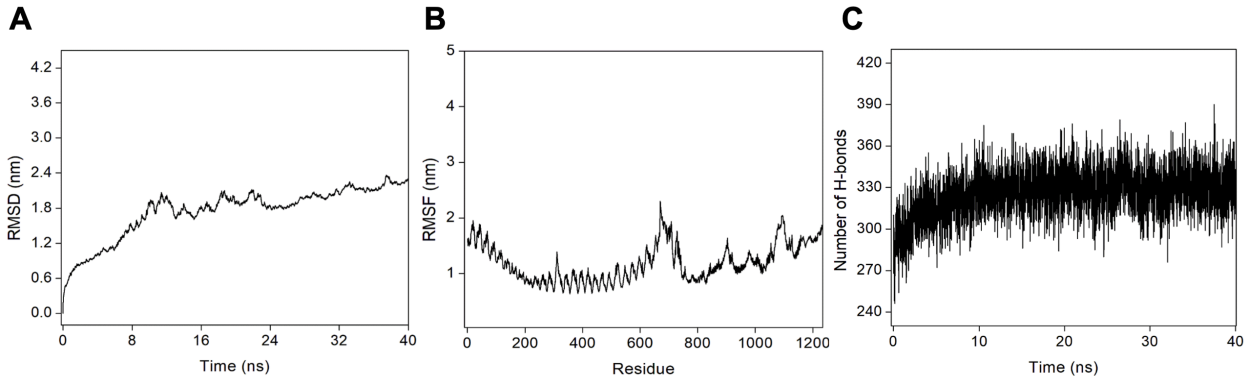

Figure 4: MD simulation of vaccine-receptor complex. (A) RMSD for the amino acid backbone of the vaccine-receptor complex, (B) RMSF of amino acids side chain of the vaccine-TLR3 complex and (C) Number of hydrogen bonds formed during the course of MD simulation trajectory.

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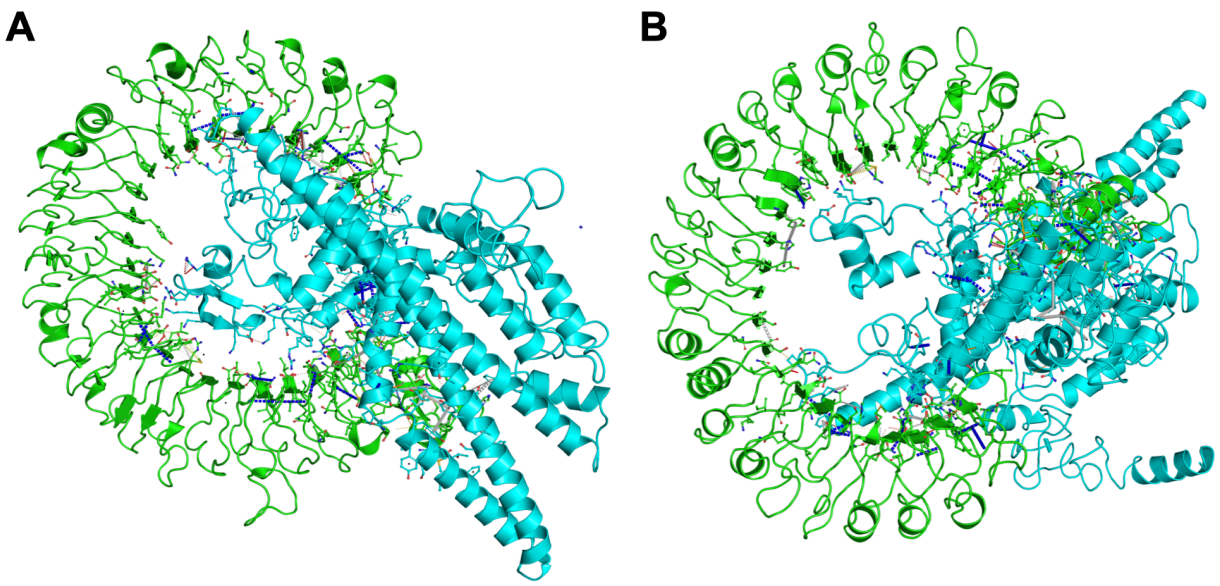

Figure 5: Key interactions obtained from (A) the initial complex structure of TLR3 and vaccine complex, (B) the stabilized TLR3 vaccine complex obtained from MD simulation. TLR3 receptor is shown in green color and the vaccine is shown in cyan color in both panels. Van der Waals interactions, proximal interactions, polar contacts, hydrogen bonds, aromatic contacts, hydrophobic contacts, carbonyl interactions and amide-amide interactions are shown in yellow, grey, red, white dashed, white long-dashed, green dashed, black-white dashed and in blue dashed lines respectively.

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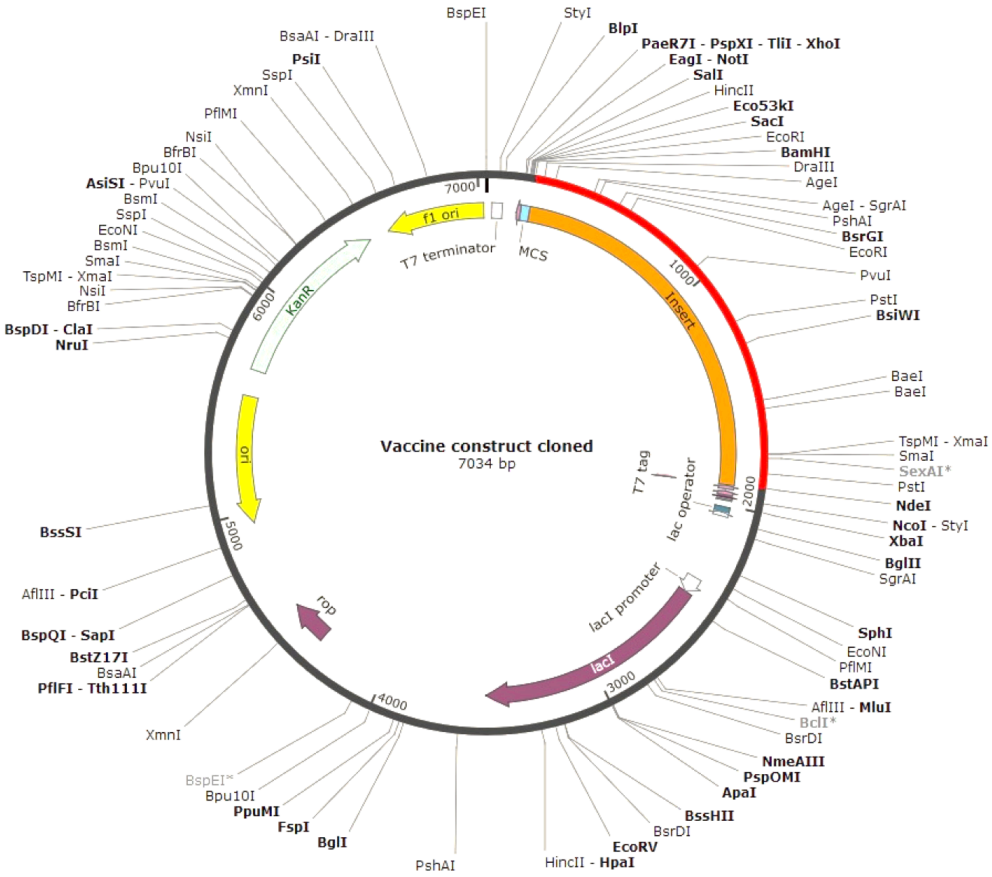

Figure 6: *In silico* cloning map showing the insert of vaccine protein specific optimized codons (red) into the pET28a(+) expression vector.