ORIGINAL ARTICLE

# Immunoinformatic of novel self-amplifying mRNA vaccine lipid nanoparticle against SARS-CoV-2

Turmidzi Fath[1,2,3,4], Endang Winiati Bachtiar[3], Gulimiran Alitongbieke[1,4], Yutian Pan[1,4], Yuanqing Hu[1,4]*, Retno Widowati[2,5]*

[1]Engineering Technological Center of Mushroom Industry, Minnan Normal University, [4]School of Biological Science and Biotechnology, Minnan Normal University, Zhangzhou, Fujian 363000, China, [2]Department of Biology, Faculty of Biology, Universitas Nasional, [3]Department of Oral Biology and Oral Science Research Center, Faculty of Dentistry, Universitas Indonesia, [5]Center for Biotechnology Studies, Universitas Nasional, Jakarta, Indonesia

*J. Adv. Pharm. Technol. Res.*

## ABSTRACT

We developed innovative self-amplifying mRNA (sa-mRNA) vaccine based on the derivative of S and Nsp3 proteins, which are considered crucial adhering to human host cells. We performed B-cell, Major histocompatibility complex (MHC) I, and II epitope which were merged with the KK and GPGPG linker. We also incorporated 5′ cap sequence, Kozak sequence, replicase sequence, 3′/5′ UTR, and poly A tail within the vaccine structure. The vaccine structure was subsequently docked and run the molecular dynamic simulation with TLR7 molecules. As the results of immune response simulation, the immune response was accelerated drastically up to >10-fold for immunoglobulin, interferon-γ, interleukin-2, immunoglobulin M (IgM) + immunoglobulin G (IgG) isotype, IgM isotype, and IgG1 isotype in secondary and tertiary dose, whereas natural killer cells, macrophages, and dendritic cells showed relatively high concentrations after the first dose. As our finding, the IgM + IgG, IgG1 + IgG2, and IgM level (induced by sa-mRNA vaccine) ensued three times with two-fold increase in days 25, and 50, then decreased after days 70–150. However, 150–350 days demonstrated constantly in the range of 20,000–21,000.

**Key words:** COVID-19, lipid nanoparticles, mRNA vaccine, SARS-CoV-2, self-amplifying mRNA

## INTRODUCTION

Currently, the available commercialized vaccines for COVID-19 prevention were developed by AstraZeneca, Moderna, Pfizer, Sinopharm, and Sinovac Biotech. These vaccines are used globally with various formulas. The vaccine formulas commonly used for vaccine design include lipid nanoparticle (LNP) nucleoside modified mRNA-encoded protein spike (Moderna and Pfizer), inactivate virus (Sinopharm and Sinovac), and recombinant vaccine (AstraZeneca and Johnson and Johnson).[1]

In this study, we focused on developing and designing different models of vaccine candidates using self-amplifying mRNA (sa-mRNA). The sa-mRNA design bases are derived from Spike glycoprotein (S) and Nsp3 because they are predicted to be crucial adhering into human host cells and induced high immunogenicity.[2] This vaccine will be analyzed using bioinformatics tools, which aim to predict and select the best sa-mRNA vaccine construction using

**Address for correspondence:**
Prof. Yuanqing Hu,
School of Biological Science and Biotechnology, Minnan Normal University, Zhangzhou, Fujian 363000, China.
E-mail: hyq1708@mnnu.edu.cn
Prof. Retno Widowati,
Department of Biology, Faculty of Biology, Universitas Nasional, Jakarta, Indonesia.
E-mail: retno.widowati@civitas.unas.ac.id

**Submitted:** 01-Sep-2023 **Revised:** 03-Jan-2024
**Accepted:** 01-Mar-2024 **Published:** 06-May-2024

Access this article online

Quick Response Code:

**Website:** www.japtr.org

**DOI:** 10.4103/JAPTR.JAPTR_424_23

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

**For reprints contact:** WKHLRPMedknow_reprints@wolterskluwer.com

**How to cite this article:** Fath T, Bachtiar EW, Alitongbieke G, Pan Y, Hu Y, Widowati R. Immunoinformatic of novel self-amplifying mRNA vaccine lipid nanoparticle against SARS-CoV-2. J Adv Pharm Technol Res 2024;15:91-8.

online servers and several software to ensure vaccine immunogenicity.

## MATERIALS AND METHODS

### Genomic of SARS-CoV-2

The complete genome of Indonesian (37 province) SAR-CoV-2 was retrieved from GISAID. Each province's sequence submitted at least four complete genomes of SARS-CoV-2 in 2021–2023. In total, 150 complete genomes of SAR-CoV-2 were merged into one file using MergeFasta software. The reference genome of SARS-CoV-2 was named NC_045512.2.

### Retrieving and visualization of genome mutations

The selected complete genome of Indonesia SARS-CoV-2 with less mutations which found in S and Nsp3 proteins was used to construct sa-mRNA vaccine (ISL14946059). The visualization of the selected genome from GISIAD was performed using two servers; CoVsurver: Mutation Analysis of hCoV-19 server and the Nextclade V2.6.1 server.

### Prediction of cytotoxic T lymphocyte epitope and MHC Class I binding

The selected sequence of S and Nsp3 proteins was submitted to MHC Class I IEDB server to predict the MHC Class I Binding. We used "recommended 2020.09 (NetMHCpan EL 4.1)" as the prediction tools in IEDB server. In addition, the MHC Class I Binding alleles were set into HLA allele reference sets following the recommended option of IEDB resources. The epitopes sequence were then evaluated using the VaxiJen ToxinPred, AllerTOP v. 2.0, and Major histocompatibility complex class I (MHC I) immunogenicity server.

### Prediction of helper T lymphocyte epitope and MHC Class II binding

The selected sequence of S and Nsp3 proteins were assessed using the consensus method of the MHC Class II IEDB server to identify the best binding for both. The smallest number of percentile ranks (<1 considered standard percentile ranks) and peptides with SMM align IC50 were selected in further investigation using the Vaxijen, ToxinPred, AllerTOP v. 2.0, interferon (IFN)-γ epitope, interleukin-4 (IL-4), and IL-10 server.

### Prediction of B-cell epitope

We picked BepiPred Linear Epitope Prediction 2.0 method to evaluate the B-cell epitope, and the result was compared using three recommended methods of B-cell epitope prediction (Emini Surface Accessibility Prediction, Karplus and Schulz Flexibility Prediction, and Parker Hydrophilicity prediction for the validation). In addition, we used Discotope, Elipro, and I-TASSER-MTD server to perform the three-dimensional (3D) structure of S and Nsp3 protein in PDB format. The selected epitope was subsequently evaluated using VaxiJen, ToxiPred, and AllerTOP servers.

### Prediction of population coverage

The MHC I and II epitopes (S and Nsp3 protein) were then examined to estimate the world population coverage using the IEDB server. More distributed population coverage of MHC I and II is better for the success of mRNA vaccine.

### Epitopes binding with T-cell and B-cell receptors

3D structures of selected epitopes of T-cell and B-cell were obtained in the PDB format using the APPTEST server. They were docked with the retrieved protein (PDB: 4U6Y and PDB: 1AQD), whereas B-cell epitopes were docked with PDB: 5IFH using the HADDOCK server.

### Designing of 5' cap sequence and self-amplifying mRNA vaccine

5' cap sequence structure was synthesized using the ACD/3D Viewer software. The epitopes (cytotoxic T lymphocyte [CTL], helper T lymphocyte [HTL], or B-cell) were inserted into the required linker. Furthermore, 5'm7Gcap, 150 nt of 5' UTR, Kozak sequence, replicase sequence, linker + each epitope (CTL, HTL, B-cell) +3' UTR and 120–150 nt of poly A tail were integrated within the vaccine construction. The optimized codon of replicase (EAAAK peptide linker flanking of the CDS) was also incorporated within the vaccine structure.

### Molecular docking of self-amplifying mRNA vaccine with human TLR-7

The sa-mRNA vaccine was docked with human TLR-7 (PDB ID: 7CYN) using the HADDOCK server and evaluated using PDBSum and ChemiraX for the interactions.

### Molecular dynamic

The molecular dynamic (MD) of sa-mRNA vaccine was examined using AMBER14 yet Another Scientific Artificial Reality Application by applying modified scripts. Its protocol was followed by trajectory analysis using md_analyse.mcr and md_play.mcr to investigate the results of Root mean square deviation (RMSD) and The root-mean-square fluctuation (RMSF).

### Simulation of immune response

The sa-mRNA vaccine sequence was simulated using the C-ImmSim server to perform an immune response through the sa-mRNA vaccine in the host cell for the assessment of immune simulation were referred to previous study.[3]

### Molecular docking of lipid nanoparticles with self-amplifying mRNA vaccine

The sa-mRNA vaccine candidate was optimized using the JCat server for codon sequence optimization. The optimal codon of sa-mRNA vaccine and LNP synthetic were docked using the HADDOCK server and visualized using ChemiraX.

## RESULTS AND DISCUSSION

We reconstructed the molecular phylogenetic tree with maximum-likelihood test and revealed three big clusters among the 150 sequences of SARS-CoV-2 [Figure 1a]. They were identified as the omicron variant and closely associated with the SARS-CoV-2 sequence from Bali (GISAID: ISL14946059), which was considered the source of virus transmission in Indonesian. In addition, ISL14946059 performed the rarest mutation in S, Nsp3 or even the whole genome of SARS-CoV-2 compared to the 150 sequences. The mutations of amino acid residues in S protein from the ISL14946059 sequence are presented in Figure 1b and c. This sequence was utilized as a representative template of SARS-CoV-2 vaccine.

In this study, the sa-mRNA vaccine was constructed with potential of short linear epitopes to bind with MHC I/II and B-cell, through high affinity interactions to robust the native immune production and peptide co-immune booster based on the corresponding epitope sequences (S or Nsp3) [Figure 2a-j]. The epitopes were nonallergen, nontoxic, and high immunogenicity/antigenicity [Table 1]. In addition, they showed acceptable flexibility, hydrophilic properties, and accessibility [Figures 3 and 4]. It becomes the fundamental strategies for our sa-mRNA vaccine to activate the MHC I/II and B-cell modulation based on their corresponding interaction with the receptors to elicit adaptive immunity.

The selected epitopes were affixed using KK (T-cell epitopes) and GPGPG (B-cell epitopes) [Figure 5b]. The addition of linker aims to maintain the conformation of the sa-mRNA vaccine, avoid junctional epitope formation owing to the interaction between two epitopes with adjacent positions and ensure that there were not found irrelevant immune responses. In addition, the EAAAK linkers were also embedded as bridges after the fundamental sequence of mRNA regulating the self-amplify mRNA (Kozak sequence and coding replicase sequence) with the B-cell epitope for protein folding and assembly correction of sa-mRNA vaccine. Moreover, the presence of Kozak sequence (gcc-gcc-R[A/G] cc) and coding sequence of replicase at the prior epitopes encoding RNA of S and Nsp3 efficiently encoded complex enzymes of self-amplify throughout the nucleus which was known as replicase polyprotein enzyme or versatile enzyme. They regulate self-amplifying antigenic encoding RNA sequence and increase the production of mRNA expression level.[4-6] Similarly, 5' cap sequence, α/β-globin (5' and 3' UTR), and poly A tail were added to serve translational recognition signal binding between ribosome unit with mRNA and trigger the immunogenic initiation of the host cell through IFN process [Figure 5a and b].[7,8] They also worked synergistically to increase the stability and efficiency of mRNA translation by approximately 1000 fold.[9]

Further, we validated the stability and efficiency of sa-mRNA vaccine by performing MD simulation. It was evident that the sa-mRNA vaccine binds with the TLR 7 in optimal energy (−275.10 kcal/mol) without any bias [Figure 5c and d]. It revealed six interaction types such as Van der Waals (PRO 83, TRY 128, HIS 282, GLY 424, VAL 425), unfavorable bump (ASP 89, HIS 90) conventional hydrogen bond (ASP 89, THR 127), carbon-hydrogen bond (ASP 84, HIS 80), Pi-Anion (ASP 89), and covalent

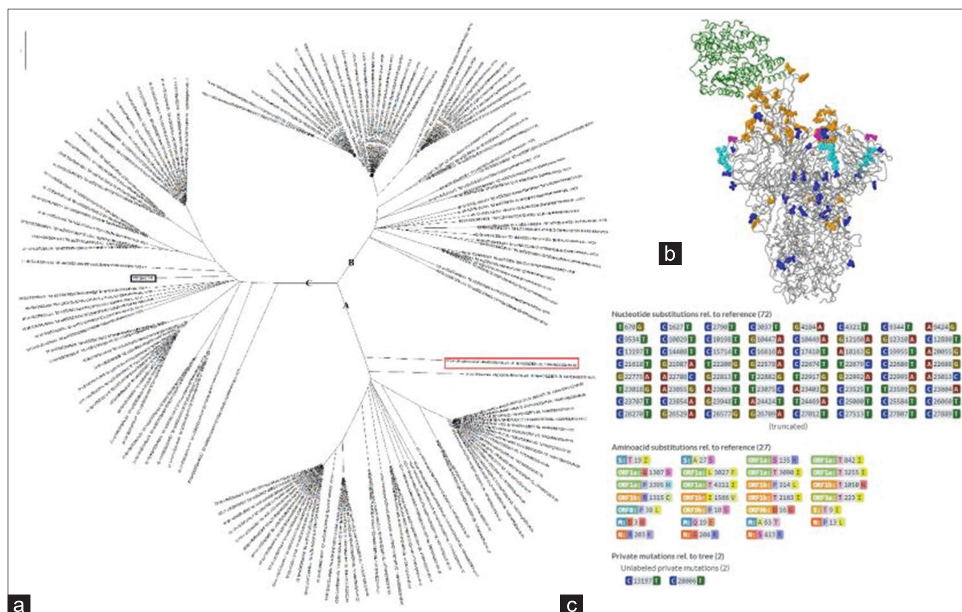


**Figure 1:** Genomic profile of SARS-CoV-2. Phylogenetic of 150 SARS-CoV-2 (a), S protein mutation of representative vaccine template in three-dimensional (3D) model (ISL14946059) (b), S protein mutation of representative vaccine template in 3D descriptives model (ISL14946059) (c)

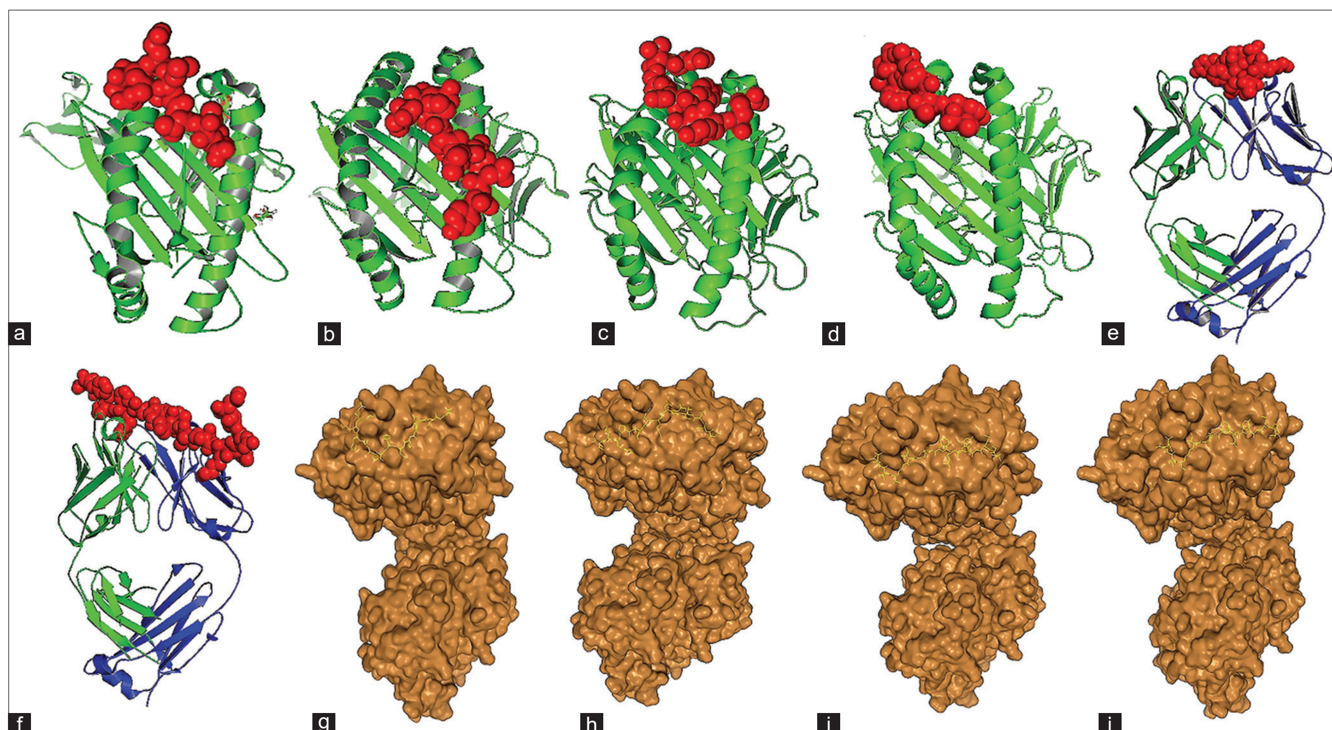


**Figure 2:** Binding epitopes of MHC I, II, and B-cell. ASRELKVTF versus HLA-A*02:01 (a), EIDPKLDNY versus HLA-A*02:01 (b), KVGGNYNYR versus HLA-A*02:01 (c), VVFLHVTYV versus HLA-A*02:01 (d), VAIDYKHYTPSFKKG versus HLA-DRB1 * 01:01 (e), DIDITFLKKDAPYIV versus HLA-DRB1 * 01:01 (f), TNFTISVTTEILPVS versus HLA-DRB1 * 01:01 (g), MQMAYRFNGIGVTQN versus HLA-DRB1 * 01:01 (h), SYKDWSYSGQ versus BcR (i), NFAPFFAFKCYGVSPTKLNDL versus BcR (j). The receptors above were available in PDB database (HLA-A 02 * 01/PDB: 4U6Y, HLA-DRB1 * 01:01/PDB: 1AQD, and BcR/PDB: 5IFH

**Table 1: T-lymphocyte (MHC I and II) and B-cell epitopes to their corresponding alleles**

| Remark/regions | Epitopes | Antigenicity | Allergenicity | Toxicity | MHC and B-cell binding alleles | MHC I immunogenicity | IFNγ/IL4/IL10 |
|---|---|---|---|---|---|---|---|
| MHC I | | | | | | | |
| Nsp3 | ASRELKVTF | 1.2900 | Nonallergen | Nontoxin | HLA-A*02:01 | 0.1278 | |
| | EIDPKLDNY | 1.6159 | | | | −0.01991 | |
| S | KVGGNYNYR | 1.5212 | | | | −0.52715 | |
| | VVFLHVTYV | 1.5122 | | | | −0.20946 | |
| MHC II | | | | | | | |
| Nsp3 | VAIDYKHYTPSFKKG | 1.3600 | | | HLA-DRB1*01:01 | | −/+/+ |
| | DIDITFLKKDAPYIV | 1.4995 | | | | | −/+/− |
| S | MQMAYRFNGIGVTQN | 1.3025 | | | | | −/+/+ |
| | TNFTISVTTEILPVS | 1.1691 | | | | | −/+/+ |
| B-cell | | | | | | | |
| Nsp3 | SYKDWSYSGQ | 1.6384 | | | BcR | | |
| S | NFAPFFAFKCYGVSPTKLNDL | 1.4472 | | | | | |

BcR: B-cell receptor, HLA-DRB: Human leukocyte antigen - heterodimer consisting of beta, MHC: Major histocompatibility complex

bond (TYR 128). In addition, we further explored these interactions based on the Ramachandran plot and amino acid interaction [Figure 6a and b]. Their stability related to the physical movement of atoms and molecules was indicated by an RMSD of ± 5.2 Å (22–40 ns), whereas the flexibility or efficiency was indicated by an RMSF of 10 Å (800–850 residues) [Figure 6c and d]. The target system for our vaccine must be based on TLR7 because it acts an immune response sensor to induce appropriate immunogenicity using sa-mRNA properties against the ssRNA virus, which is necessary to enhance immune response for early prevention.

Here, we design our vaccine, coated with LNPs, targeting muscle cells [Figure 7]. According to the sa-mRNA interaction with LNPs, it was revealed that the LNPs intact with sa-mRNA vaccine in ARG 28, ILE 223, THR 231, LYS 687, LYS 732, and LYS 742. It was considered the most beneficial

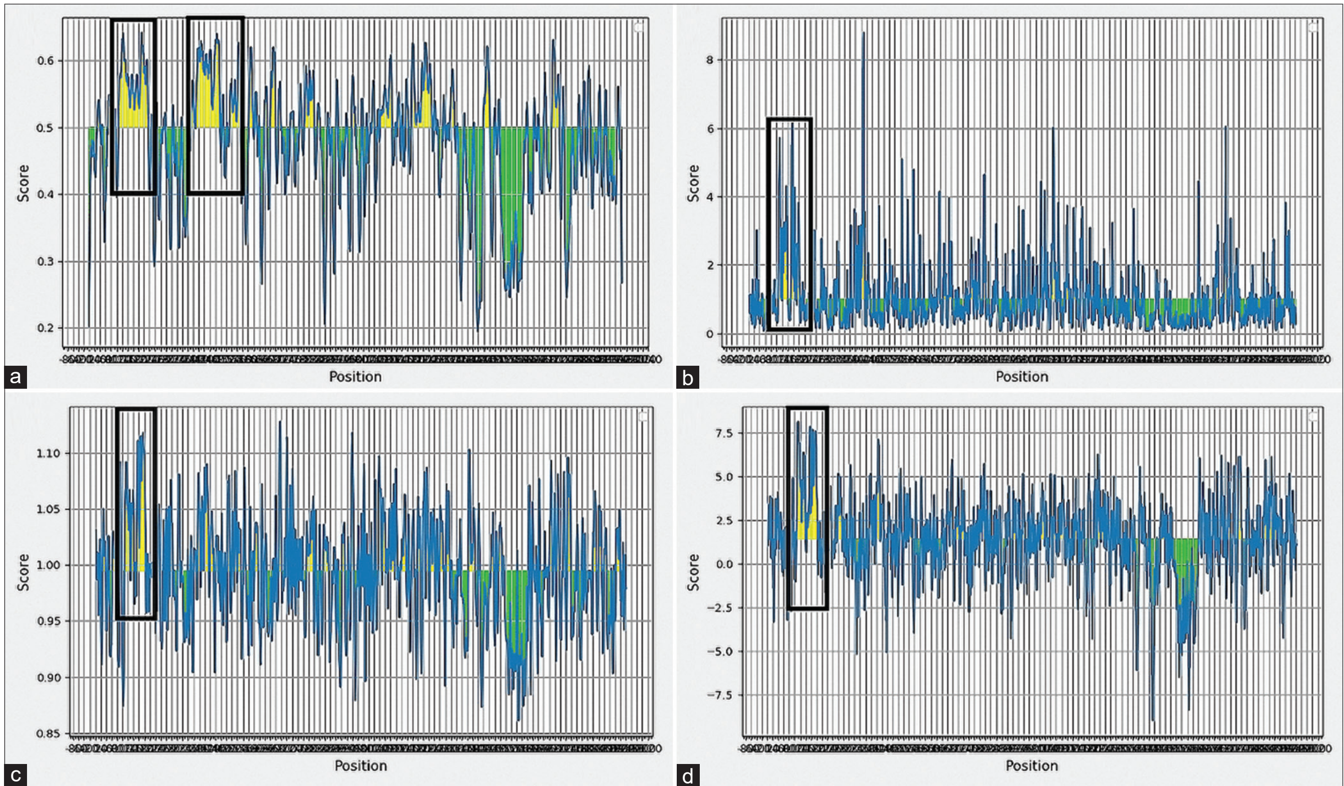


**Figure 3:** Nsp3 of B-cell prediction. BepiPred linear epitope 2.0 (a), Emini surface accessibility (b), Karplus and Schulz flexibility (c), Parker hydrophilicity (d)

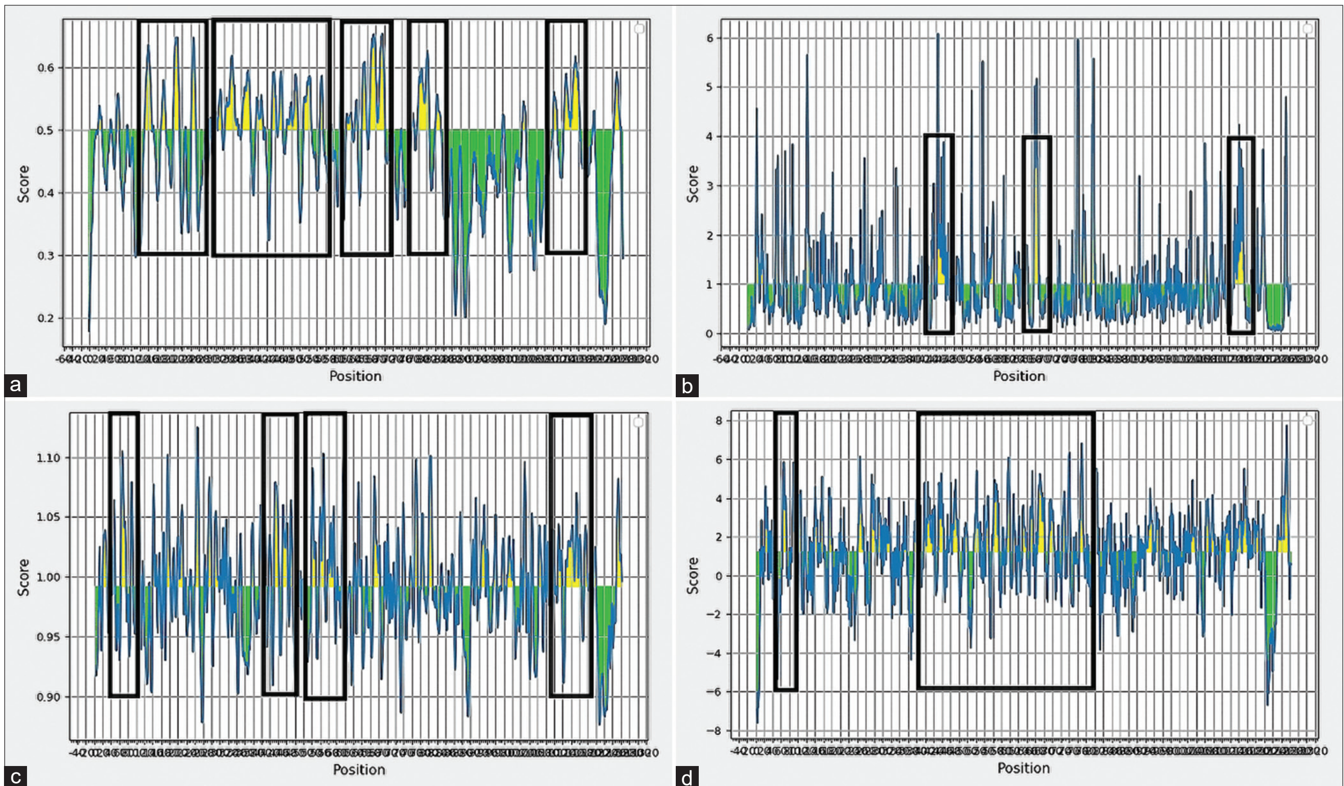


**Figure 4:** Spike glycoprotein of B-cell prediction. BepiPred linear epitope 2.0 (a), Emini surface accessibility (b), Karplus and Schulz flexibility (c), Parker hydrophilicity (d)

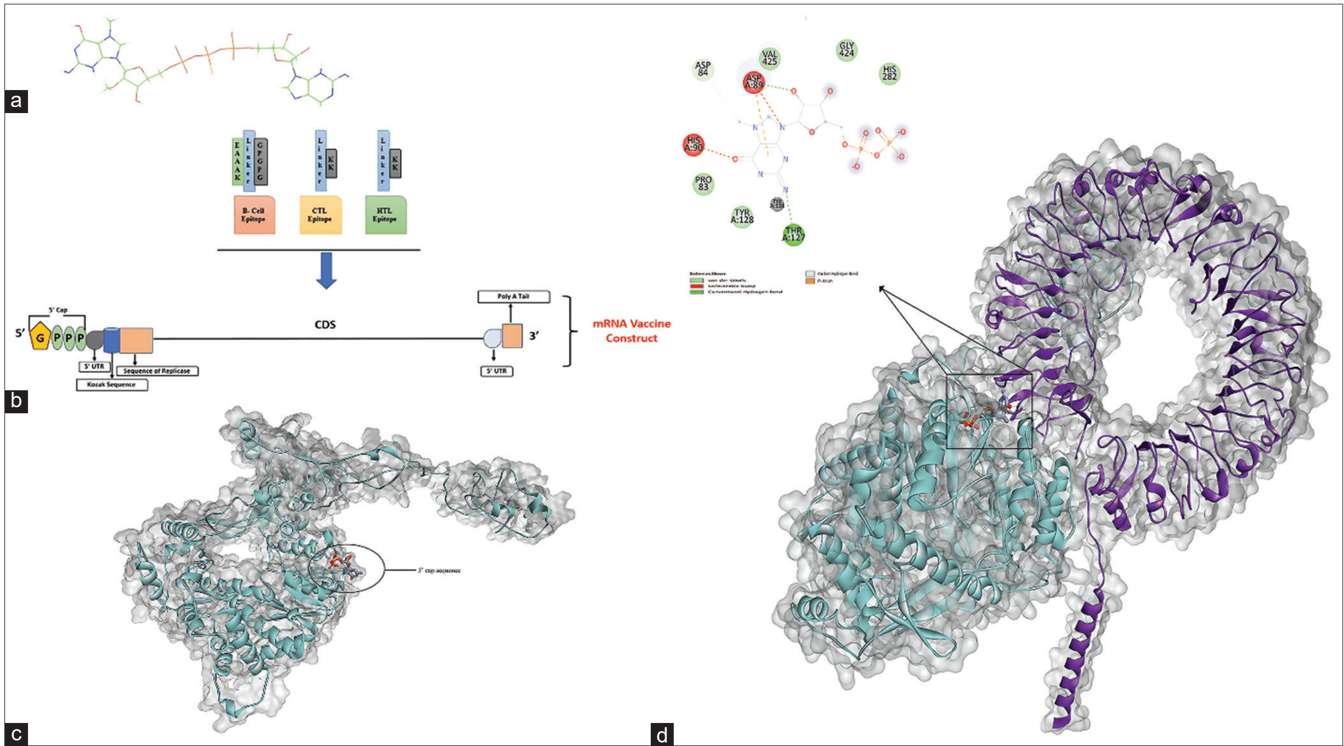


**Figure 5:** Design and the properties of self-amplifying mRNA (sa-mRNA) vaccine. Structure of 5' cap sequence (a), sa-mRNA vaccine construction against SARS-CoV-2 (b), Three-dimensional structure of sa-mRNA vaccine (c), Molecular docking of sa-mRNA vaccine with TLR7 (PDB: 7CYN) by HDOCK (−275.90 kcal/mol) (d)

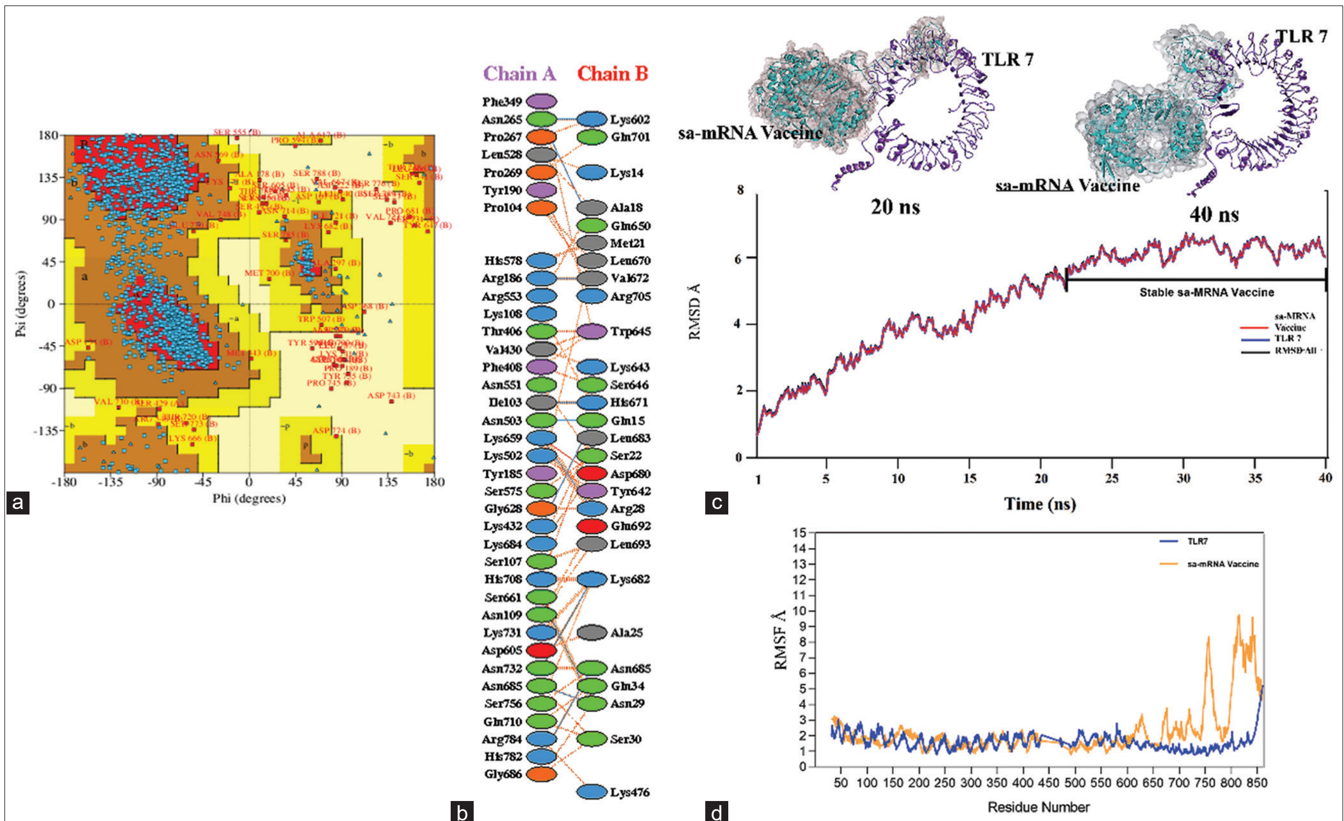


**Figure 6:** Self-amplifying mRNA (sa-mRNA) vaccine residue and molecular dynamic simulation plot. Ramachandran plot (a), Amino acid interaction between sa-mRNA vaccine-TLR7 (PDB: 7CYN) by PDBSum (b), Root mean square deviation (RMSD's) plot of sa-mRNA vaccine (c), The root-mean-square fluctuation (RMSF's) plot of sa-mRNA vaccine (d). sa-mRNA: Self-amplifying mRNA

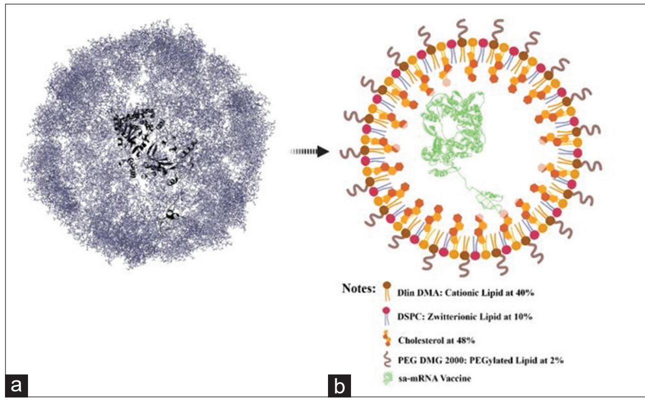


**Figure 7:** The encapsulated of lipid nanoparticle for self-amplifying-mRNA vaccine profile (−249.00 kcal/mol). Three-dimensional structure (a), Two-dimensional structure (b). sa-mRNA: self-amplifying-mRNA

due to the process that does not need to pass through the nucleus of the host cell. Finally, as shown in Figure 8a-n, upon examining the immune response simulation of our sa-mRNA vaccine with three interval doses (4 weeks between each interval dose), the immunoglobulin G1 (IgG1) + IgG2, immunoglobulin M (IgM), and IgM + IgG increased two-fold for secondary and tertiary responses [Figure 8a]. The level of IFN-γ, IL-10, and IL-2 was also higher following repeated exposure, indicating the induction of immune response from the sa-mRNA vaccine. The increase in the level of IFN-γ cytokines greatly affected the IgM + IgG, IgM and IgG1 isotypes switching, thereby encouraging the humoral immune response production.

To find out the dynamic immune response following secondary and tertiary doses, we investigated the pattern of immune response at prevaccine and postvaccine stages. The result showed that prevaccine could trigger the rising immune. Hence, we argued that the presented immune response of T-cell and B-cell in prevaccine phase (first dose) observed to have own lasting effect in early week to 1-month and bestow proper proxy of magnitude for next injection vaccine doses in immune rising issues. Thus, the rate of secondary and tertiary immune response drastically accelerated up to > 10-fold [Figure 8a and e]. This might have occurred regularly following the administration of the vaccine injected. In terms of vaccination with the sa-mRNA vaccine, the quantity of antibodies or the immune responses could reduce over time in immunocompromised systems. However, the substantial amount of the humoral or cellular immunity would be in the range of < 20,000 ng/L [Figure 8a and b].

Comparing the immune production of sa-mRNA vaccine with other vaccines (peptides and non-sa-mRNA) using C-ImmSim server by previous studies,[10,11] we observed that their immunes count level of IgM + IgG, IgG1 + IgG2, and IgM ensued at a time (in day 13 for peptides vaccine, day 5 for non-sa-mRNA vaccine), appeared to decreased in day

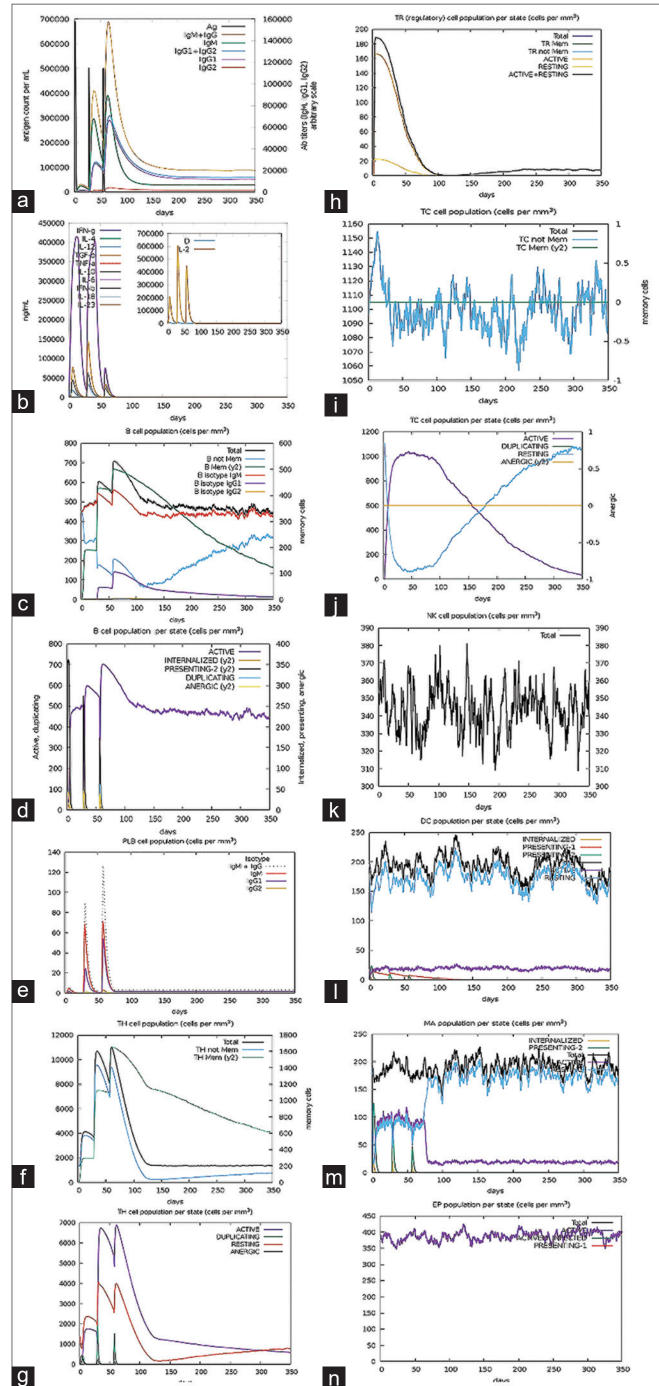


**Figure 8:** Self-amplifying-mRNA vaccine immune response. Antigen and immunoglobulin profile (a), Cytokine concentration (b), B-lymphocytes (c), B-lymphocytes population per entity-state (d), Plasma B-lymphocytes count sub-divided perisotype (e), CD4 T-helper lymphocytes count (f), CD4 T-helper lymphocytes count sub-divided per entity-state (g), CD4 T-regulatory lymphocytes count (h), CD8 T-cytotoxic lymphocytes count (i), CD8 T-cytotoxic lymphocytes count per entitystate (j), Natural killer cells (total count) (k), Dendritic cells (l), Macrophages (m), Epithelial cells (n)

25 (peptides vaccine) and day 15 (non-sa-mRNA vaccine). Whereas the level of IgM + IgG, IgG1 + IgG2, and IgM in our sa-mRNA vaccine ensued three times with two-fold

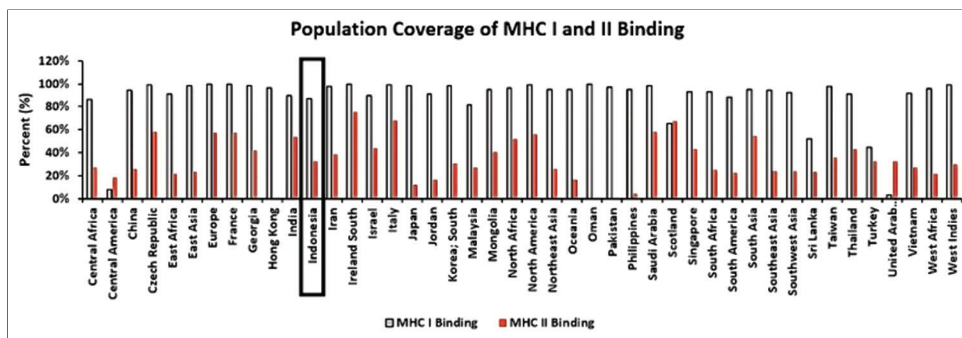


**Figure 9:** Population coverage of MHC Class I/II

increase in days 25 and 50. The IgM + IgG of sa-mRNA vaccine decreased after days 70–150. As our finding, the IgM + IgG production of sa-mRNA vaccine from days 150–350 demonstrated constantly in range of 20,000–21,000 comparing to other (<15,000). The different levels between the sa-mRNA with peptide and non-sa-mRNA vaccine was mediated by the presence of Kozak sequence and coding sequence of replicase, acting to replicate the carrying of incorporated encoding RNA sequence (S, Nsp3) for about $10^4$ fold per ± hour.[12]

In addition, we observed a moderate level of cytokine, such as IL-4 in every dose's response. However, it showed similar results in dose 1 and 2. In contrast, NKs, macrophages, dendritic cells showed higher levels following secondary or tertiary doses. According to the population coverage, the sa-mRNA vaccine proposes high potential efficacy against SARS-CoV-2. Moreover, it could cover 97% of the population worldwide and 86.79% of population in Indonesia [Figure 9]. Good vaccine efficacy should in arrange of at least 70% to prevent an epidemic and of at least 80% to largely extinguish an epidemic without any other measures.[13]

## CONCLUSION

sa-mRNA vaccine could produce specific immune responses based on the molecular properties activating response involved in the vaccine construction. This vaccine is safe, nonallergenic, and nontoxic. It can be vaccine candidate with high population coverage, particularly in Indonesia (86.79%).

### Financial support and sponsorship

This work was supported by the Project of Fujian Natural Science Foundation of China (grant number 2021J01995, 2021J05201) and Center for Biotechnology Studies Universitas Nasional Grant (02P/K/PKB/2022).

### Conflicts of interest

There are no conflicts of interest.

## REFERENCES

1. Devi YD, Goswami HB, Konwar S, Doley C, Dolley A, Devi A, *et al.* Immunoinformatics mapping of potential epitopes in SARS-CoV-2 structural proteins. PLoS One 2021;16:e0258645.
2. Ong E, Wong MU, Huffman A, He Y. COVID-19 coronavirus vaccine design using reverse vaccinology and machine learning. Front Immunol 2020;11:1581.
3. Bhattacharya M, Sharma AR, Ghosh P, Patra P, Patra BC, Lee SS, *et al.* Bioengineering of novel non-replicating mRNA (NRM) and self-amplifying mRNA (SAM) vaccine candidates against SARS-CoV-2 using immunoinformatics approach. Mol Biotechnol 2022;64:510-25.
4. Beissert T, Koste L, Perkovic M, Walzer KC, Erbar S, Selmi A, *et al.* Improvement of *in vivo* expression of genes delivered by self-amplifying RNA using *Vaccinia virus* immune evasion proteins. Hum Gene Ther 2017;28:1138-46.
5. Garmory HS, Brown KA, Titball RW. DNA vaccines: Improving expression of antigens. Genet Vaccines Ther 2003;1:2.
6. Shan S. Development and evaluation of DNA vaccines in chickens against a wild bird H6N2 avian influenza virus from Western Australia. Murdoch University; 2010 into Maruggi G, Zhang C, Li J, Ulmer JB, Yu D. mRNA as a transformative technology for vaccine development to control infectious diseases. Molecular Therapy 2019;27:757-72.
7. Liu T, Liang Y, Huang L. Development and delivery systems of mRNA vaccines. Front Bioeng Biotechnol 2021;9:718753.
8. To KK, Cho WC. An overview of rational design of mRNA-based therapeutics and vaccines. Expert Opin Drug Discov 2021;16:1307-17.
9. Malone RW, Felgner PL, Verma IM. Cationic liposome-mediated RNA transfection. Proc Natl Acad Sci U S A 1989;86:6077-81.
10. Dong R, Chu Z, Yu F, Zha Y. Contriving multi-epitope subunit of vaccine for COVID-19: Immunoinformatics approaches. Front Immunol 2020;11:1784.
11. Oladipo EK, Adeniyi MO, Ogunlowo MT, Irewolede BA, Adekanola VO, Oluseyi GS, *et al.* Bioinformatics designing and molecular modelling of a universal mRNA vaccine for SARS-CoV-2 infection. Vaccines (Basel) 2022;10:2107.
12. Kidmose RT, Vasiliev NN, Chetverin AB, Andersen GR, Knudsen CR. Structure of the qbeta replicase, an RNA-dependent RNA polymerase consisting of viral and host proteins. Proc Natl Acad Sci U S A 2010;107:10884-9.
13. Bartsch SM, O'Shea KJ, Ferguson MC, Bottazzi ME, Wedlock PT, Strych U, *et al.* Vaccine efficacy needed for a COVID-19 coronavirus vaccine to prevent or stop an epidemic as the sole intervention. Am J Prev Med 2020;59:493-503.