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DESIGNING A MULTI-EPITOPE VACCINE AGAINST SARS-COV2: AN IMMUNOINFORMATIC APPROACH

Alamdari-palangi V.[a],
Dehghan Z. [a],
Kian M.[a],
Zonar S.[b],
Fallahi J.[a],
Sisakht M.[a],
Khajeh S.[a,]
Razban V.[a]

[a] Shiraz University of Medical Sciences, Shiraz, Iran
[b] Islamic Azad University, Tehran, Iran.

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РАЗРАБОТКА МУЛЬТИЭПИТОПНОЙ ВАКЦИНЫ ПРОТИВ SARS-COV2: ИММУНОИНФОРМАТИЧЕСКИЙ ПОДХОД

Аламдари-паланги В. [1],

Деган З. [1],

Киан М. [1],

Зонар С. [2],

Фаллахи Дж. [1],

Сисахт М. [1],

Хадже С. [1],

Разбан В. [1]

[1] Ширазский университет медицинских наук, Шираз, Иран

[2] Исламский университет Азад, Тегеран, Иран.

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Abstract

Background: An outbreak of SARS-CoV-2 in 2019 has brought a great challenge to public health and rapid identification of immune epitopes for designing an effective vaccine for different variants of SARS-CoV-2 is necessary at the time of the pandemic. Rational, rapid, and precise vaccine design, especially vaccine antigen identification and optimization by in silico methods of bioinformatics, structural biology, and immunoinformatic is critical to efficient vaccine development against the SARS-CoV-2 virus. The aim of this study was to develop a particular novel and effective vaccines vaccine using bioinformatics approaches and resources that can target B- and T-cell epitopes to combat SARS-CoV-2 infection.

Methods: The variants of SARS-CoV2 (Alpha, Beta, Delta, and Omicron strains) spike protein were selected for designing the vaccine. The B-cell, T-cell, and interferon-gamma-inducing epitopes were predicted. The beta-defensin-3 protein was selected as adjuvant and predicted epitopes were connected using suitable linkers. The vaccine's allergenicity, antigenicity, physicochemical characteristics, 2D and 3D structure modeling, and molecular docking were evaluated for the final construct.

Results: The *in-silico* results showed that the multi-epitope vaccine has a stable structure and can induce humoral and cellular immune responses against SARS-CoV2.

Conclusions: B-cell and T-cell epitopes on spike protein were identified and recommended for design and confirmation of in vivo evaluation for multi-epitope peptides as vaccines against SARS-CoV-2.

Keywords: COVID-19, SARS-CoV-2, Vaccine, Immunoinformatic, Epitope, T-cell epitopes, B-cell epitopes

Резюме

История вопроса: Вспышка SARS-CoV-2 инфекции в 2019 году стала серьезным вызовом для общественного здравоохранения, а быстрое описание иммунных эпитопов для разработки эффективной вакцины против различных вариантов SARS-CoV-2 необходимо в ходе пандемии. Рациональная, быстрая и точная разработка вакцины, особенно идентификация и оптимизация вакцинных антигенов с помощью методов *in silico* биоинформатики, структурной биологии и иммуноинформатики, имеет решающее значение для эффективной разработки вакцины против вируса SARS-CoV-2. Целью настоящего исследования была разработка новой и эффективной вакцины с использованием подходов и ресурсов биоинформатики, которая может содержать эпитопы B- и T-клеток для борьбы с инфекцией SARS-CoV-2.

Методы: варианты спайкового белка SARS-CoV2 (штаммы альфа, бета, дельта и омикрон) были выбраны для разработки вакцины и предсказания эпитопов, индуцирующих B-клетки, T-клетки и продукцию интерферона-гамма. Белок бета-дефензин-3 был выбран в качестве адъюванта, а предсказанные эпитопы были связаны с использованием разных линкеров. Для формирования окончательной конструкции были оценены аллергенность, антигенность, физико-химические характеристики вакцины, моделирование 2D и 3D структуры и молекулярная стыковка.

Результаты: Результаты *in-silico* анализа показали, что мультиэпитопная вакцина имеет стабильную структуру и может индуцировать гуморальный и клеточный иммунный ответ против вируса SARS-CoV2.

Выводы: были идентифицированы B-клеточные и T-клеточные эпитопы спайкового белка вируса SARS-CoV2, рекомендованные для разработки и подтверждения эффективности *in vivo* мультиэпитопных пептидов в качестве вакцин против вируса SARS-CoV-2.

Ключевые слова: COVID-19, SARS-CoV-2, вакцина, иммуноинформатика, эпитоп, Т-клеточные эпитопы, В-клеточные эпитопы

1. Introduction

Coronavirus Disease-19 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome-coronaviruses-2 (SARS-CoV-2), a highly pathogenic and transmissible coronavirus size with nearly 65–125 nm in diameter, which was first detected in Wuhan and on March 2020, the World Health Organization (WHO) declared COVID-19 a global pandemic (23, 26). The Coronaviridae family divides into four subgroups that are alpha (α), gamma (γ) CoVs, beta (β) and delta (δ). Among them, β and α CoVs subgroups are transmitted from animals to humans or zoonotic (8, 47). Sequencing and etiological investigations for a causative agent of the pandemic verified a novel coronavirus pertains to β coronavirus, which contains both MERS-CoV and SARS-CoV-2 (23). COVID-19 disease has affected nearly all of the countries with over 687 million cases and the number of deaths had reached almost 6.87 million worldwide by May 2, 2023 (36). COVID-19 disease exhibits a wide range of non-symptomatic and mild illnesses (fatigue, sore throat, cough, fever, muscle pain, and headache) to acute respiratory distress syndrome, pneumonia manifestations, hyperinflammatory states, and multi-organ collapse in severe cases (1, 3, 9). Thus, due to the rapid and global outbreak of SARS-CoV-2 along with numerous mutations of this virus, there is an urgent need to develop effective and safe new-generation vaccines against the SARS-CoV-2 virus (32, 37). Because of the lack of antigenic diversity, high costs, time-consuming antigen identification, and lack of antigenic diversity, traditional approaches in vaccination based on laboratory experiments for vaccine design and development are not enough (19, 21).

SARS-CoV-2 is a single-stranded positive-sense RNA virus with a genome ranging approximately from 27 to 32 kilobases in size, which encodes many proteins including envelope (E), membrane (M), nucleocapsid (N), and spike (S) proteins as well as 16 non-structural proteins (NSP1 to NSP16), proteases (3C-like proteinase) and accessory protein chains (3, 51, 53). Among these -proteins, S and N proteins

have been shown to be immunogenic (25). The N protein is a multifunctional RNA-binding protein, that plays a role in viral RNA-protein (vRNP) assembly, promotion of RNA template switching, and packaging of the viral genome (25). The spike protein mediates the viral entry through its interaction with the human angiotensin-converting and also membrane fusion (45). All of the available COVID-19 vaccines including, RNA-based and adenovirus-based), mRNA-based, and inactivated viruses expose the S-protein to the host immune system to induce an immune response. However, with the advent of new COVID-19 strains and variants due to S-protein mutations, the available vaccines lose their effectiveness in preventing infection and hospitalization (5, 10, 22).

In the last decade, progress in bioinformatics and Artificial Intelligence has incredibly facilitated the development of efficient vaccines, especially in cases of rapid outbreaks and unknown pathogens (17, 33). Reverse Vaccinology, Antigen(s) Choice, Disclosure, and Optimization and Prediction of B Cell Epitopes and T Cell Epitope Prediction are Bioinformatic principles for efficient vaccine design (7, 24). Bioinformatics is a strong tool that processes large amounts of the available virus genome and its protein sequence information, thus, predicting presented epitopes and virus characteristics and significantly accelerating the progress of vaccine development (41, 49).

In this study, we apply an integrated knowledge of computational informatics, immunoinformatic, and modeling fields (in silico) for B-cell and T-cell epitope prediction of SARS-CoV-2s Spike SARS-CoV-2 Spike receptor-binding domain (RBD) and comparison in silico immunogenicity by applying bioinformatics methods to for the development of vaccines under a guide procedure against COVID-19.

2. Material and method

A workflow of the methods used for the epitope-based peptide vaccine prediction is depicted in Figure 1.

2.1. Strain identification and retrieval of the protein sequence

The variants of SARS-CoV2 (Alpha, Beta, Delta, and Omicron strains) spike protein were retrieved from the National Center for Biotechnology Information or NCBI (https://www.ncbi.nlm.nih.gov/) database. The sequence of spike protein (accession number: P0DTC2.1) retrieved of NCBI and all mutations shown on a sequence.

2.2. Prediction of B-cell epitopes

To predict B cell linear epitopes, the spike protein with all mutations was submitted to the ABCpred server (http://crdd.osdd.net/raghava/abcpred/). This server predicts B cell epitopes using an artificial neural network in an antigen sequence. ABCpred widely was used in disease diagnosis, allergy research, and vaccine design (42). Finally, epitopes with high scores were selected for future analyses. (Score>0.8)

2.3. Prediction of T-cell epitopes

The multi-epitope vaccines would be able to stimulate the immune response, comprised of epitopes cytotoxic T-cell and helper T-cell (12). For prediction of HTL epitopes (MHC II binding) and CTL epitopes (MHC I binding) was used IEDB tool (http://tools.iedb.org/). The IEDB tool was used from an Artificial Neural Network (ANN) for selecting MHC class I and class II epitopes. For the prediction of CD4+ helper T-lymphocyte (HTL) and CD8+ cytotoxic T-lymphocyte (CTL) epitopes, was used from all HLA reference sets, and finally, epitopes with low percentile ranks were used for future analyses.

2.4. Prediction of IFNλ inducing epitopes

IFN-gamma cytokine leads to the activation of the innate and adaptive immune system, therefore epitopes IFNλ inducing can enhance the immunogenic capacity of any vaccine. IFN epitope server

(http://crdd.osdd.net/raghava/ifnepitope/) was used for identifying epitopes that can produce IFNλ (16).

2.5. Construction of multi-epitope vaccine sequence

In this step, the epitopes with low percentile ranks and high scores were conjugated together to construct a vaccine. Human beta-defensin-3 was conjugated to N-ter epitopes as an adjuvant by the EAAAK linker. Adjutants have a key role in the immunogenicity and antigenicity of vaccines (30). The EAAAK linkers are used in vaccines to generate the bifunctional domains in fusion proteins (6). The AAY linker was used for connecting B-cell epitopes. These linkers have linkers effectiveness and efficiency which widely are used in the in-silico vaccine's design (44). Finally, HTL and CTL epitopes were also conjugated together by the GPGPG linker. The GPGPG linkers are used to generate the junctional epitopes and also enhance immune processing and presentation (40).

2.6. Evaluation of antigenicity, allergenicity, and physicochemical properties

The antigenicity and allergenicity of the multi-epitope vaccine was predicted using ANTIGENpro tool (http://scratch.proteomics.ics.uci.edu/) and AllergenFP v1.0 (https://ddg-pharmfac.net/AllergenFP/) servers. ANTIGENpro tool (http://scratch.proteomics.ics.uci.edu/) was used for antigenicity prediction. This server predicts 82% of the known protective antigens. This server identified protein antigenicity from a sequence with an accuracy of 56% **(31)**. For analysis of the physicochemical properties of the designed vaccine, the protein sequence was submitted to the ProtParam server (https://web.expasy.org/protparam/) to evaluate physicochemical properties such as number of amino acids, Molecular weight, Instability index, Aliphatic index, and Grand average of hydropathicity (GRAVY) **(18)**.

2.7. Analysis of cross-reactivity with proteome human

Comparative analysis of the designed vaccine with human proteome was performed in the protein Basic Local Alignment Search Tool (BLAST) module (blastP) (https://blast.ncbi.nlm.nih.gov/Blast.cgi) tool with *Homo sapiens* (taxid: 9606) and parameter default (29).

2.8. Secondary structure prediction and solvent accessibility analysis

For secondary structure prediction, the PRISPRED 4.0 tool (http://bioinf.cs.ucl.ac.uk/psipred/) was used with all the parameters default. PSIPRED predicts protein secondary structure based on position-specific iterated BLAST (psi-BLAST) for identification of significant homology with primary amino acid sequence (35, 54).

2.9. Tertiary structure prediction and validation of its

The tertiary model of the vaccine construct was prepared using the I-TASSER server (https://zhanggroup.org/I-TASSER/). This tool was used as an integrated platform based on multiple threading alignments and iterative structural assembly simulations for protein structure and function prediction (39). Finally, full atomic models of the query sequence with C-score and TM-score were generated. The best model was selected based on the C-score and TM-score further analysis. The refinement of the best model of the tertiary structure was used from PyProtModel software (48). The PDB file of structures input in the PROCHEK server for the analysis of the Ramachandran plot (https://saves.mbi.ucla.edu/results?job=1225021&p=procheck) (28).

2.10. B-cell conformational epitopes prediction

In order to predict the conformational epitopes, the tertiary structure of the vaccine protein was submitted to the IEDB Ellipro tool (http://tools.iedb.org/ellipro/) with the default setting. Ellipro identifies antibody conformational epitopes based on the shape, neighboring residue, and protrusion index (PI) of the protein (38).

2.11. Molecular docking

Toll-like receptors are important to generate potential immune response antiviral. In this study, the multi-epitope vaccine was docked with TLR3, 4, and 8. The structure of the TLR3 (PDB ID:1ZIW), TLR4 (PDB ID:3FXI), and TLR8 (PDB ID: 3W3M) were retrieved from the Protein Data Bank (https://www.rcsb.org/). The cluspro v2.0 server was used for docking vaccine protein (as a ligand) and TLRs (as a receptor) with the default server (https://cluspro.bu.edu/publications.php). The cluspro v2.0 is widely used for protein-protein docking. This tool has several advanced options including the removal of unstructured protein regions, construction of homo-multimers, accounting for pairwise distance restraints, consideration of small-angle X-ray scattering (SAXS) data, application of attraction or repulsion, and location of heparin-binding sites **(15, 27)**. Finally, the interaction complexes were visualized with YASARA software.

3. Results

3.1. Identification, Selection, and Retrieval of Spike Protein Sequence

The variants of SARS-CoV2 (Alpha, Beta, Delta, and Omicron strains) spike protein were retrieved from the NCBI database and shown on a sequence of the spike protein.

3.2. Prediction of B-cell linear and IFNλ inducing epitopes

The B-cell epitopes have a key role in antibody production by B lymphocytes and adaptive immunity. The linear B-cell and IFNλ inducing epitopes were predicted using the ABCpred server and IFN epitope server, respectively. This server predicts B-cell epitopes in an antigen sequence using an artificial neural network (machine-based technique). For each epitope, sequence and score were determined. The epitopes with a score>0.8 are listed in **Table 1**.

3.3. Prediction of CTL, HTL, and IFNλ inducing epitopes

The IEDB database and IFN epitope server were used for the prediction of T-cell and IFNλ epitopes, respectively. This tool widely was used for the prediction

and analysis of epitopes in humans, non-human primates, and other animal species. A total of six CTL epitopes and two HTL epitopes were predicted with strong binding affinity for multiple alleles. **Table 2** shows CTL and HTL epitopes extracted using the IEDB database.

3.4. Construction of multi-epitope vaccine

For the construction of the multi-epitope vaccine against SARS-CoV2, the appropriate epitopes were selected and joined by using proper linkers (AAY for linear B-cell epitopes and GPGPG for CTL and HTL epitopes). To enhance the immunogenicity of the multi-epitope vaccine, the HBD-2 (41 amino acids) adjuvant was connected to the N-ter of construct by the EAAAK linker. Altogether, the final multi-epitope vaccine has 244 amino acids and an adjuvant (41 amino acids). **(Figure 2)**

3.5. Evaluation of antigenicity, allergenicity, and physicochemical properties

The antigenicity, allergenicity, and physicochemical properties of the vaccine construct were evaluated using various servers. The multi-epitope vaccine has an immunogenic property with a value of 0.939130 predicted by ANTIGENpro. The AllergenFP v1.0 online tool suggested the vaccine construct as non-allergenic with the highest Tanimoto similarity index of 0.84. In addition, various physicochemical properties were calculated by the ProtParam server. The designed vaccine has 244 aa with a molecular weight of 25.824 kDa. The theoretical isoelectric point (pI) was calculated at 9.25. The instability index was 26.21, which was identified as a stable protein. The aliphatic index (63.65) identified the vaccine construct as a highly thermostable protein. The Grand average of hydropathicity (GRAVY) represented -0.452 for the vaccine construct that indicated multi-epitope vaccine as a hydrophilic protein.

3.6. Evaluation of cross-reactivity with human proteome

Analysis of cross-reaction vaccine construct with human proteome represented that selected epitopes have no crass-reactivity and homology with human proteome.

3.7. Secondary structure prediction and solvent accessibility analysis

The secondary structure analysis of 244 amino acids multi-epitopes vaccine using PRISPRED 4.0 tool (**Figure 3**).

3.8. Tertiary structure prediction and its validation

The 3-D protein model of the designed vaccine candidate (244 aa) was predicted using I-TASSER and was refined by PyProtModel software **(Figure 4a).** Ramachandran plot analysis of structure predicted by PROCHEK server (**Figure 4b)**

3.9. B-cell conformational epitopes prediction

The conformational B-cell epitopes were identified using the ElliPro tool. The three potential regions were determined as highlighted epitopes of multi-epitope vaccine with a score >0.7. **(Table 3 and Figure 5).**

3.10. Molecular docking

The ability interaction of the vaccine candidate and immune receptors has a key role in immune response. The designed vaccine represented a binding affinity to TLRs when docked by the ClusPro 2.0 server. This server showed the best binding affinity of vaccine construct -TLR3 with -1230.6 cal/mol. The docking of vaccine construct-TLR4 predicted good binding affinity with

-1560.8 cal/mol. The analysis of vaccine construct-TLR8 docking represented the best affinity binding with -1871.4 cal/mol. The visualization of the interactions was done using YASARA software. The interaction of the TLRs-vaccine constructs is shown in **Figures 6a, 6b, and 6c**.

4. Discussion

The SARS-CoV-2 outbreak has been one of the most challenging infectious diseases in recent years, it was first found in Wuhan China in early December 2019 and spread rapidly around the world in a short time.

The wide spread of this viral infection brought many concerns and caused the death of a large number of people in the world. Most of these deaths were due to the unpreparedness of the health system and the lack of drugs to combat it. The use of some drugs such as Camostat, Chloroquine, Imatinib, Nafamostat, Hydroxychloroquine, Remdesivir, and Ivermectin for the treatment of severe cases of the disease as an emergency was approved by the US Food and Drug Administration (FDA) (11, 20). The most important way to protect from viral infections is to use vaccines. In the COVID-19 outbreak, on the one hand, the lack of safe and reliable vaccines and on the other hand, changes in virus variants caused many mortalities. Vaccines were released on the market in a short time, and on the other hand, the virus showed a new face after some time. Different variants of COVID-19 were formed and spread quickly and neutralized the effects of vaccines. Vaccines were made on different platforms (inactivated or attenuated virus, nucleic acid vaccines, recombinant proteins or synthetic peptides-based vaccines, and viral vector-based vaccines) (2, 4, 13, 43). COVID-19 enters into cells, especially lung cells, through spike (S) protein to ACE2 as its receptor. All vaccines against COVID-19 were mainly based on one virus epitope and were designed to prevent the binding of the spike protein to the ACE2 receptor and, as a result, prevent the virus from entering the cell (55).

Designing and producing multi-epitope peptides as antigens can be a way to vaccinate people to create immunity against different strains of a specific virus. In recent years, in silico methods have improved the design of epitope-based vaccines for infectious diseases and cancers, enhancing development and evaluation processes. Multi-epitope vaccines target immunodominant regions of pathogen proteins, making them effective against highly mutable RNA viruses. They offer

advantages such as safety, efficacy, cost-effectiveness, and ease of production. Effective vaccines should include both B-cell and T-cell epitopes to stimulate comprehensive immunity. B cell activation for antibody production is crucial for coronavirus immunity, along with CD8+ T cells for eliminating infected cells. These vaccines can elicit broad immune responses, highlighting their clinical potential (52).

Before COVID-19, a spike protein-based DNA vaccine was tested for anti-SARS immunity in 2008. The study by Julie E et al. found the vaccine to be well tolerated, with 80% of participants showing SARS-CoV-specific antibodies and all individuals having neutralizing antibodies. SARS-CoV-specific T4 CD4+ responses were observed in all cases, while T CD8+ responses were seen in 20% of participants (34).

Tourani, Mehdi, et al used bioinformatics tools to select suitable epitopes from the S protein, which were linked with appropriate linkers and combined with a TLR4 binding adjuvant to form a multi-epitope construct. Then 3D model of the construct was predicted, refined, and validated. The vaccine's properties, including antigenicity, allergenicity, solubility, and physicochemical characteristics, were assessed, along with the identification of B cell conformational epitopes and IFN-γ inducing regions. The effectiveness of the adjuvant and TLR4 binding was evaluated through docking studies, while the stability of the protein-protein complex was analyzed. The vaccine's coding sequence was optimized and sub-cloned into an expression vector using an in-silico approach, and the structure, energy, and stability of the coding mRNA were assessed. Their result showed ten continuous B cell epitopes, nine T helper epitopes, and eight CTL epitopes were identified, demonstrating that the multi-epitope vaccine is a stable and soluble protein capable of eliciting both humoral and cellular immunity without causing allergenicity in humans (1).

Shehata, Mahmoud M., et al carry out an in-silico predictions identified six B cell epitopes—QTGKIADYNYK, TEIYQASTPCNGVEG, LQSYGFQPT, IRGDEVRQIAPGQTGKIADYNYKLPD, FSQILPDPSKPSKRS, and PFAMQMAYRFNG—for their cross-reactivity with MHC-I and MHC-II T-cell binding epitopes. These were selected for vaccination in experimental animals due to their strong antigenic compatibility. The peptides were administered individually or in combinations to female Balb/c mice, resulting in the production of antibodies against SARS-CoV-2, specifically targeting peptides in the receptor binding domain and S2 region. Combination immunizations showed an additive effect compared to single peptide vaccinations. This study introduces new epitope-based peptide vaccine candidates against SARS-CoV-2 (46).

Dariushnejad et al carry out a computational analysis to predict the conserved epitopes of Spike and Nucleocapsid proteins from SARS-CoV-2 for the design of a novel coronavirus 2019 multi-epitope vaccineand. They used immunoinformatics techniques to identify and select potential conserved epitopes based on allergenicity, toxicity, antigenicity, and molecular docking. The selected epitope segments were linked with appropriate linkers and Maltese-bound protein (MBP) was added as an adjuvant to the vaccine structure. The secondary and tertiary structures of the multi-epitope vaccine were predicted using immunoinformatics algorithms, and these structures were refined and validated for optimal stability. To confirm the vaccine's efficacy, immunoinformatics evaluations, molecular docking, and molecular dynamics studies were conducted. Additionally, codon optimization and in silico cloning were performed to ensure effective expression of the vaccine in the target host. Their study indicated that designed vaccine has the potential to elicit immune responses against variants of SARS-CoV-2 (14).

Another study showed that prospective cytotoxic T lymphocyte (CTL) and helper T lymphocyte (HTL) vaccines against SARS-CoV-2 infection are expected to stimulate both cellular and humoral immune responses. The epitopes of the

designed multi-epitope vaccines (MEVs) are predicted to be applicable to a significant portion of the global human population (96.10%). Therefore, both MEVs could be evaluated in vivo as promising vaccine candidates against SARS-CoV-2 (50).

Considering the mutated variants of COVID-19, we designed a peptide based on changed epitopes of important variants that can create immunity against different virus strains. This multi-epitope peptide was evaluated for the activation of B and T cells. It has no cross-reactivity and homology with human proteome and docking data also represented a binding affinity to TLRs. Synthesis of this peptide and conducting in vivo studies can clarify the result of this design.

5. Study Limitations

The study uses computational and immunoinformatic methods to design the vaccine. While these methods are powerful, they are based on algorithms and models that may not fully capture the complexity of biological systems. Therefore, the predictions made by these methods need to be validated experimentally. The designed vaccine's safety, antigenicity, immunogenicity, and stability were evaluated using various physicochemical, allergenic, and antigenic characteristics. However, these evaluations are based on computational predictions and have not been confirmed through in vitro or in vivo experiments. The study focuses on the design of a multi-epitope vaccine based on cytotoxic T-lymphocyte and helper T-lymphocyte epitopes. Other aspects of vaccine development, such as production, delivery, and potential side effects, are not addressed. The study assumes that the designed vaccine will stimulate an effective immune response. However, the actual immune response can vary greatly among individuals due to factors such as age, genetic background, and health status. Hence, in silico vaccine designs must be rigorously evaluated through in vivo studies and clinical trials to ensure they are safe, effective, and suitable for human use. This multi-stage evaluation is essential

for advancing public health and ensuring that vaccines provide reliable protection against diseases.

6. Conclusion

COVID-19 outbreak was a bitter reality in human history, which still causes the death of some people in the world every day. As of 2 August 2023, there have been 768,983,095 confirmed cases of COVID-19, including 6,953,743 deaths, reported to WHO (https://covid19.who.int/). It showed how important the existence of specialized drugs and prediction for treatment methods in similar infections is very important. The timely supply of safe and reliable vaccines without side effects gained great value. The design and confirmation of in vivo evaluation for multi-epitope peptides as vaccines can be helpful for disease control in viral epidemics such as COVID-19.

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ТАБЛИЦЫ

Table 1: Prediction of linear B-cell and IFNλ inducing epitopes of SARS-CoV2 spike protein.

Epitopes	Score	IFNλ
PQIITTHNTFVSGNCD	0.96	-
TEIYQAGNKPCNGVKG	0.91	+
GRDIDDTTDAVRDPQT	0.88	-
KVSGNYNYRYRLFRKS	0.87	+
EVSQIAPGQTGNIAD	0.87	+
SYQTQTKSHRRARSVA	0.82	+
GREPEGLPQGFSALEP	0.81	-

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Table 2: Prediction T-cell and IFNλ inducing epitopes of SARS-CoV2 spike protein.

Epitopes	Alleles	IFNλ
	CTL epitopes	
RSYSFRPTY	HLA-A*30:02, HLA-A*32:01, HLA-A*30:01, HLA-B*57:01, HLA-B*15:01, HLA-B*58:01, HLA-A*01:01, HLA-B*35:01,…	+
VLYQGVNCT	HLA-A*02:03, HLA-A*02:01, HLA-A*02:06, HLA-B*15:01, HLA-A*32:01, HLA-B*08:01, HLA-A*68:02, …	-
IPINFTISV	HLA-B*51:01, HLA-B*35:01, HLA-B*53:01, HLA-B*07:02, HLA-A*68:02, HLA-B*08:01, HLA-A*26:01,…	-
VLNDIFARL	HLA-A*02:03, HLA-A*02:01, HLA-A*02:06, HLA-A*32:01, HLA-A*68:02, HLA-B*08:01, HLA-A*23:01,…	-
SQCVNFRTR	HLA-A*31:01, HLA-A*31:01, HLA-A*33:01, HLA-A*33:01, HLA-A*30:01, HLA-A*68:01, HLA-A*11:01, …	+
KRFANPVLPF	HLA-A*23:01, HLA-A*24:02, HLA-A*32:01, HLA-A*30:02, HLA-B*15:01, HLA-B*58:01, HLA-B*40:01, HLA-B*57:01, HLA-B*35:01,…	-
	HTL epitope	
VENLVAYSNNSIAI	HLA-DRB1*15:01, HLA-DRB1*15:01, HLA-DRB1*13:02, HLA-DRB1*13:02,…	-

KLQNVVNHNAQALNT	HLA-DRB3*02:02, HLA-DRB1*13:02, HLA-DRB1*08:02, HLA-DQA1*01:02/DQB1*06:02,…	-

Table 3: The conformational B-cell epitopes of vaccine construct and their position.

	Residues	Number of residues	Score
1	A:T168, A:Y169, A:G172, A:P173, A:G174, A:V175, A:L176, A:Y177, A:Q178, A:G179, A:V180, A:N181, A:C182, A:P199, A:Y209, A:S210, A:N211, A:N212, A:S213, A:I214, A:A215, A:I216, A:P217, A:I218, A:N219, A:F220, A:T221, A:I222, A:S223, A:V224, A:G225, A:P226, A:G227, A:P228, A:G229, A:K230, A:L231, A:Q232, A:N233, A:V234, A:V235, A:N236, A:H237, A:N238, A:A239, A:Q240, A:A241, A:L242, A:N243, A:T244	50	0.741
2	A:F123, A:V124, A:S125, A:G126, A:N127, A:C128, A:D129, A:A130	8	0.738
3	A:Y113, A:P114, A:I116, A:I117, A:T118, A:T119, A:H120, A:N121, A:T122	9	0.708

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РИСУНКИ

Figure 1: Schematic workflow of in silico prediction and evaluation of the peptide based multi-epitope vaccine.

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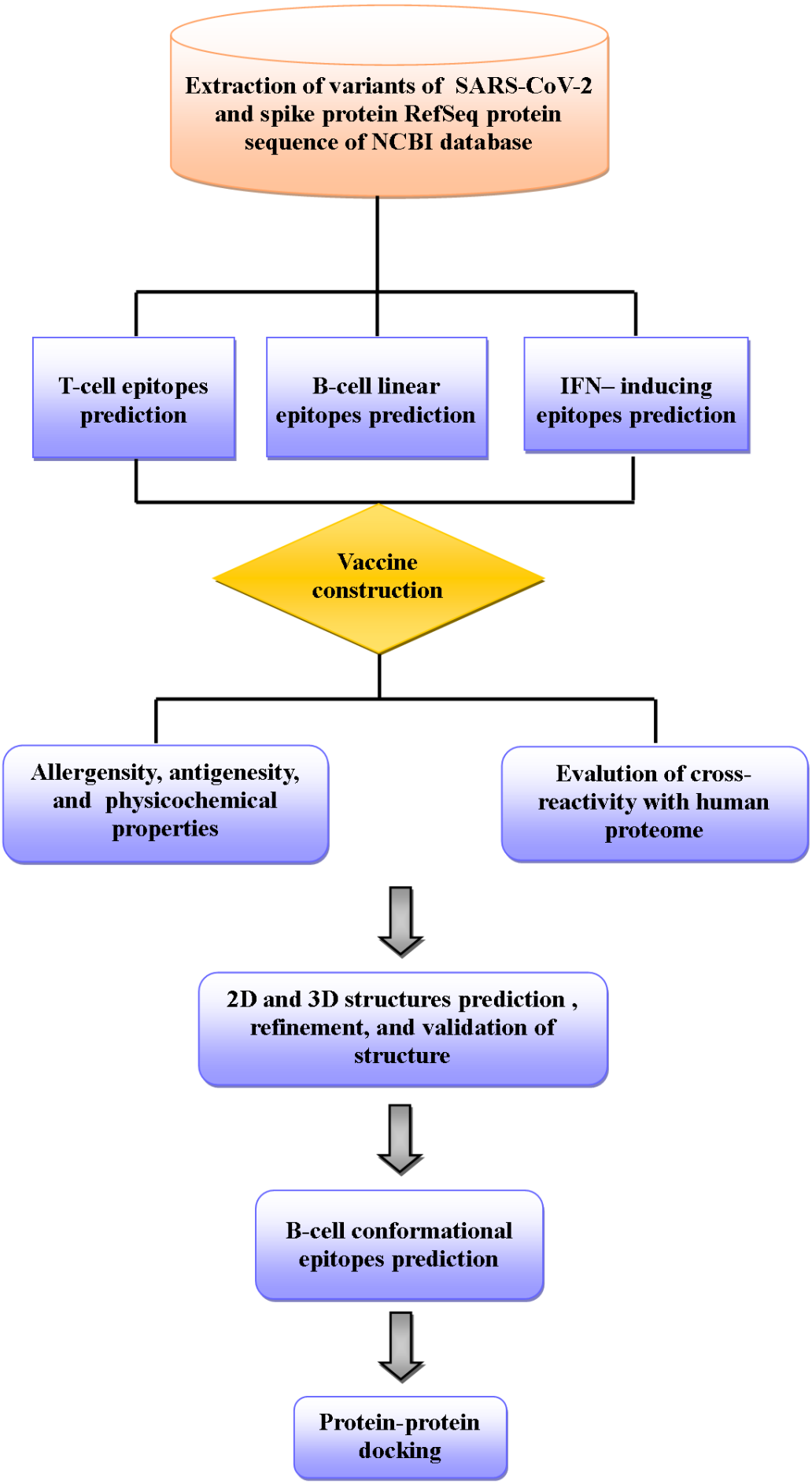

Figure 2: Final construct of the multi-epitope vaccine.

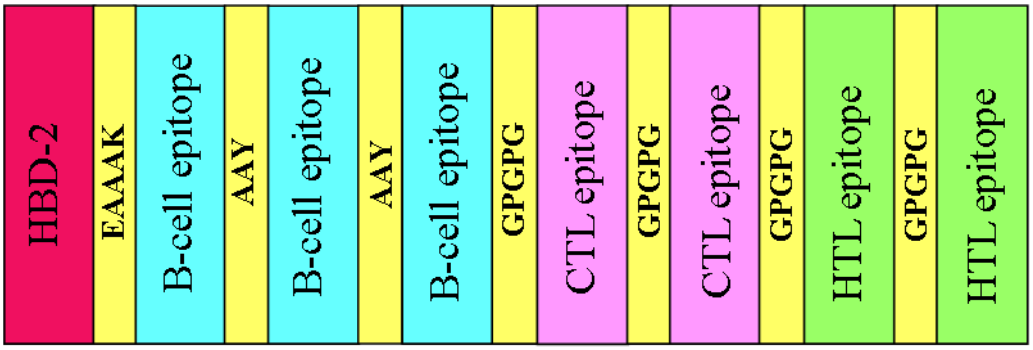

Figure 3: Secondary structure analysis of multi-epitope vaccine using PSIPRED server.

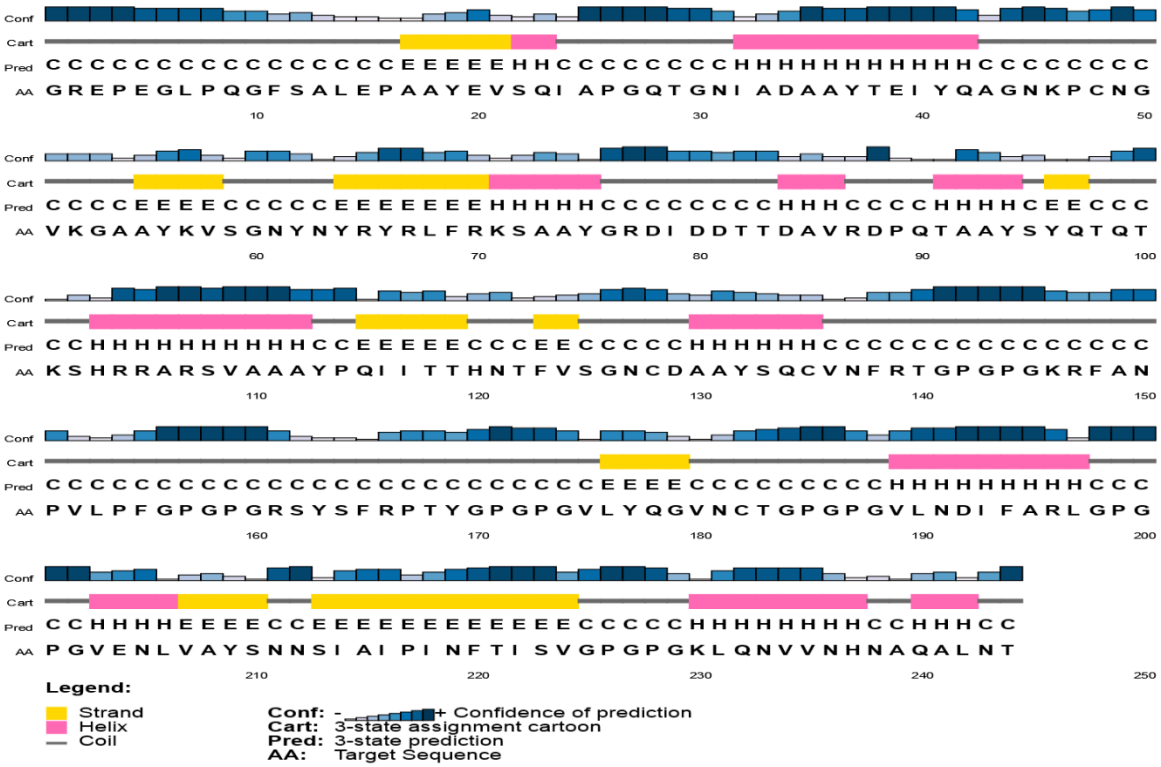

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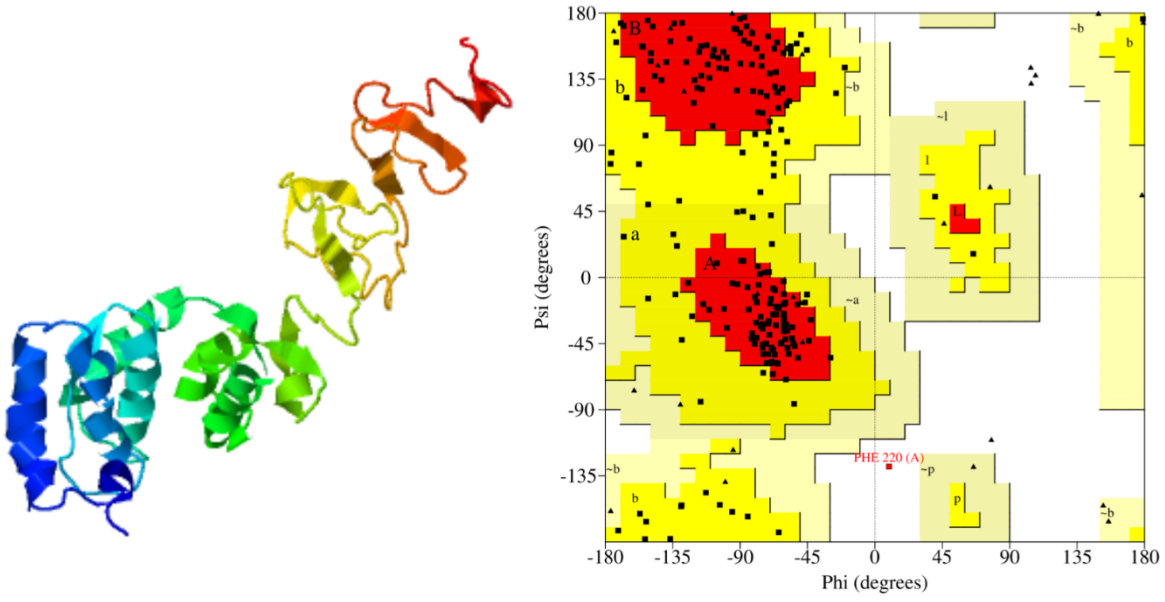
Figure 4: a) Tertiary structure of final vaccine construct refinement by PyProtModel software, **b)** Ramachandran plot analysis of structure predicted by PROCHEK server.



a **b**

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Figure 5: The three conformational B-cell epitopes predicted by the ElliPro tool in the multi-epitope vaccine

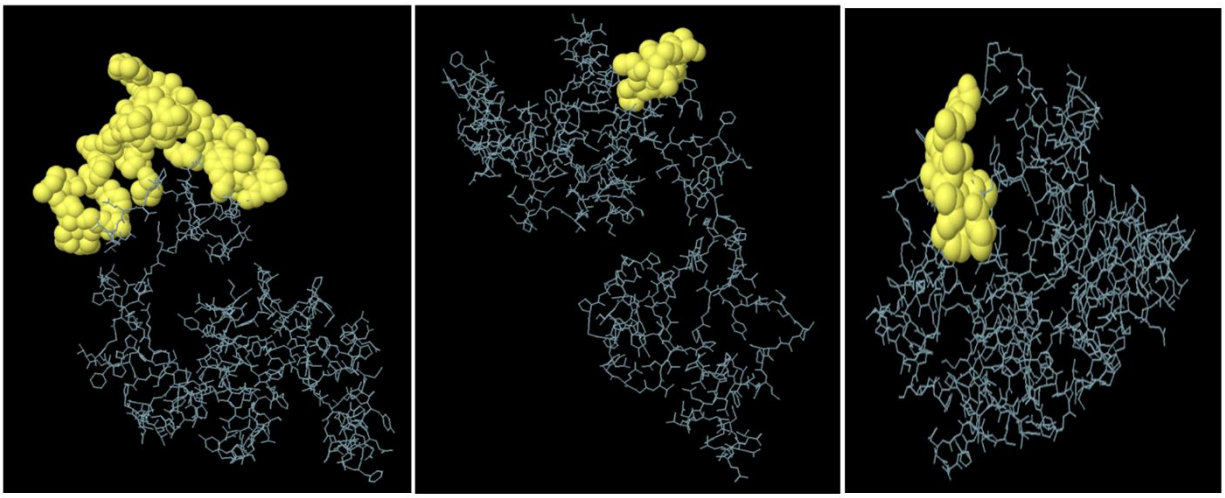
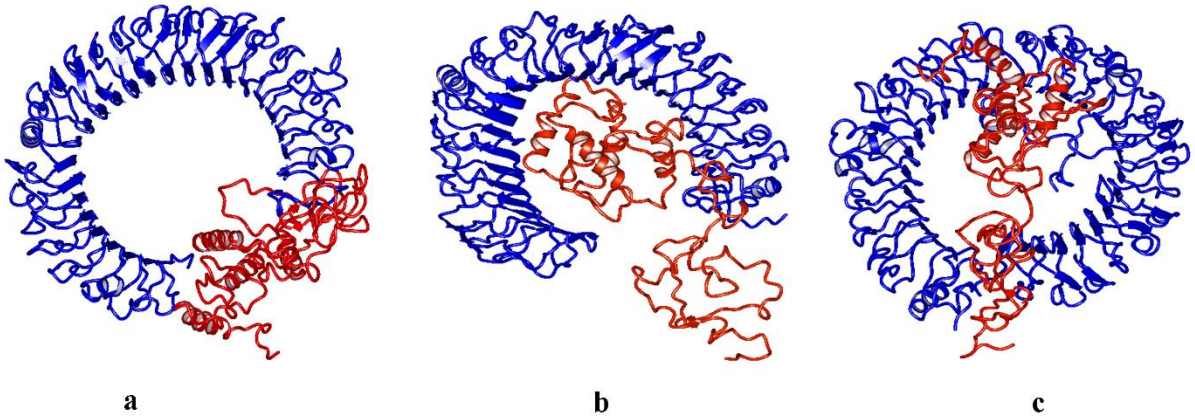


Figure 6: Molecular docking between multi-epitope vaccine and with TLR3, 4, and 8 (a-c, respectively)



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ТИТУЛЬНЫЙ ЛИСТ_МЕТАДАННЫЕ

Блок 1. Информация об авторе ответственном за переписку

Vahid Razban, PhD, Associate Professor, Department of Molecular Medicine, School of Advanced Medical Sciences and Technologies, Shiraz University of Medical Sciences, Shiraz, Iran (*corresponding author*).

Вахид Разбан, кандидат наук, доцент кафедры молекулярной медицины Школы передовых медицинских наук и технологий Ширазского университета медицинских наук, Шираз, Иран (автор, ответственный за переписку).

Блок 2. Информация об авторах

Vahab Alamdari-palangi, MSc, PhD Candidate, Department of Molecular Medicine, Shiraz University of Medical Sciences, Shiraz, Fars, Iran;
Вахаб Аламдари-паланги, магистр наук, соискатель, кафедра молекулярной медицины, Ширазский университет медицинских наук, Шираз, Фарс, Иран;

Zeinab Dehghan, PhD, Researcher, Department of Comparative Biomedical Sciences, School of Advanced Medical Sciences and Technologies, Shiraz University of Medical Sciences, Shiraz, Iran.
Зейнаб Дехган, кандидат наук, научный сотрудник кафедры сравнительных биомедицинских наук, Школа передовых медицинских наук и технологий, Ширазский университет медицинских наук, Шираз, Иран.

Mehdi Kian, DVM, PhD Candidate, Department of Comparative Biomedical Sciences, School of Advanced Medical Sciences and Technologies, Shiraz University of Medical Sciences, Shiraz, Iran.

Мехди Киан, доктор ветеринарной медицины, соискатель, кафедра сравнительных биомедицинских наук, Школа передовых медицинских наук и технологий, Медицинский университет Шираз, Шираз, Иран.

Saghar Zonar, MSc, Researcher, Department of Biology, Sciences and Research Branch, Islamic Azad University, Tehran, Iran.
Сагар Зонар, магистр наук, научный сотрудник, кафедра биологии, отделение наук и исследований, Исламский университет Азад, Тегеран, Иран.

Jafar Fallahi, PhD, Assistant Professor, Department of Molecular Medicine, School of Advanced Medical Sciences and Technologies, Shiraz University of Medical Sciences, Shiraz, Iran.
Джафар Фаллахи, кандидат наук, доцент, кафедра молекулярной медицины, Школа передовых медицинских наук и технологий, Медицинский университет Шираз, Шираз, Иран.

Mohsen Sisakht, PhD, Researcher, Department of Molecular Medicine, School of Advanced Medical Sciences and Technologies, Shiraz University of Medical Sciences, Shiraz, Iran.
Мохсен Сисахт, кандидат наук, научный сотрудник, кафедра молекулярной медицины, Школа передовых медицинских наук и технологий, Ширазский университет медицинских наук, Шираз, Иран.

Sahar Khajeh, PhD, Assistant Professor, Bone and Joint Diseases Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.
Сахар Хаджех, кандидат наук, доцент, Центр исследований заболеваний костей и суставов, Ширазский университет медицинских наук, Шираз, Иран.

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Блок 3. Метаданные статьи

DESIGNING A MULTI-EPITOPE VACCINE AGAINST SARS-COV2: AN IMMUNOINFORMATIC APPROACH

РАЗРАБОТКА МУЛЬТИЭПИТОПНОЙ ВАКЦИНЫ ПРОТИВ SARS-COV2: ИММУНОИНФОРМАТИЧЕСКИЙ ПОДХОД

Сокращенное название статьи для верхнего колонтитула:

A MULTI-EPITOPE VACCINE AGAINST SARS-COV2

МУЛЬТИЭПИТОПНАЯ ВАКЦИНА ПРОТИВ SARS-COV2

Keywords: COVID-19, SARS-CoV-2, Vaccine, Immunoinformatic, Epitope, T-cell epitopes, B-cell epitopes.

Ключевые слова: COVID-19, SARS-CoV-2, вакцина, иммуноинформатика, эпитоп, эпитопы Т-клеток, эпитопы В-клеток.

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