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DESIGNING MULTI-EPITOPE VACCINE FOR SARS-COV-2 *THROUGH IN-SILICO* TECHNIQUES

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ABSTRACT

The extensive blowout of COVID-19, an infectious disease caused by SARS-CoV-2, has resulted in millions of demises and has wreaked havoc on social, economic, and political systems all over the biosphere.

Vaccines are despairingly needed to prevent the spread of this disease in view of the fact that there is currently no viable medical treatment. In this article, we offer an in silico deep learning approach for possibility of multi-epitope vaccines prediction and design.

The virus is comparable to SARS-CoV and MERS-CoV, according to genome sequence research. Despite this, antiviral medications used to treat previous SARS-CoV and MERS-CoV infections have been found to be ineffective in controlling SARS-CoV-2. The need to discover a vaccine solution grows as the rate of infection and mortality from COVID-19 rises.

The immunogenicity, stability, safety, and vaccination potential of many epitope targets of SARSCoV-2's spike (S) protein are being explored. The vaccine designs described in this article show promise as vaccine candidates, but more in vitro and in vivo testing is required.

Keywords: SARS-CoV, MERS-CoV, Immunogenicity, Epitope targets, In-silico

INTRODUCTION

Coronaviruses are classified as Orthocoronaviridae (order Nidovirales, division Cornidovirineae, family Coronaviridae) and are further classified into four genera: alpha, beta, gamma, and delta CoV **[1]**. Because it infects animals, the alpha and beta CoV is of particular importance during the 2020 coronavirus pandemic. A new beta CoV, now known as SARS-CoV-2, was discovered in pneumonia patients in Wuhan, China, in December 2019. The virus was named SARS-CoV-2 by the International Committee for Taxonomy of Viruses, but the World Health Organization refers to it as COVID-19 **[2]**. SARS-Cov-2 is a more dangerous strain than SARS-CoV from 2002 or MERS-CoV from 2013. SARS-CoV and MERS-CoV are both coronaviruses that infected humans through zoonotic transmission and spread through close human contact. The SARS-CoV-2 genome shares approximately 82 percent of its sequence with SARS- and MERS-CoV, and more than 92 percent of its sequence identity for key enzymes and two structural proteins **[3]**. The pathogenicity of the three coronaviruses is shared due to their high degree of similarity.

Coronaviruses are viruses that are enclosed and have a positive single-stranded RNA genome. SARS coronaviruses contain the spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins. These glycoproteins aid in viral particle construction, replication, and release. The S protein is essential because it allows the virus to enter the host cell by attaching to the surface receptor of the host cell. S protein is composed of two subunits: S1 and S2. The S1 unit contains the receptor binding domain (RBD), which is required for attachment to host cell receptors, whereas the S2 unit contains additional domains required for cell fusion and intracellular trafficking **[4]**. The S protein stimulates cell entry specifically by interacting with angiotensin-converting enzyme 2 (ACE2) and host proteases that prime the S protein, such as transmembrane serine protease 2. (TMPRSS2) **[5, 6]**. TMPRSS2 activates the S protein and cleaves the ACE2 receptor, which allows the virus to attach to the host cell. Virion particles enter the host cell through endocytosis, and their genome binds to the host's ribosomes. When this occurs, proteolysis mediated by the proteases 3CLpro and PLpro is used to further process the translation of two co-terminal and large polyproteins. These mediators break down large polyproteins into smaller components for folding and packaging new virions, which aids in virion replication **[3]**.

SARS-CoV-2 enters the host primarily through the upper respiratory tract of alveolar epithelial type II cells, where its harmful effects are also visible, while the viral infection spreads throughout the host. The ACE2 and TMPRSS2 receptors are highly expressed in alveolar epithelial type II cells, according to several studies **[4]**.

The host immune system recognises the virus and/or its epitopes as the virus spreads and mounts an innate or adaptive immune response. Immune cells' pathogen recognition receptors (PRRs) and toll-like receptors (TLRs) 3, 7, and 8 recognise the virus and initiate interferon production (IFN). By evading innate immune cells, SARS-CoV-2 infection and replication dampens the response of the generated IFN. Pro-inflammatory cytokine production rises when monocytes, neutrophils, and adaptive immune cells are present. Th1 and Th17 cells infecting helper T cells with viral epitopes can cause cytokine storms, which can lead to pulmonary edoema or pneumonia. Cytotoxic T cells are drawn to the infection site to destroy any contaminated cells in the lungs. B cells, like T cells, detect viral proteins and produce more antibodies in response **[7]**.

IN WHAT WAYS DOES SARS-COV-2 MANIFEST CLINICALLY IN HUMANS?

SARS-CoV-2 is a novel virus that has been linked to acute respiratory illness. COVID-19 spreads through the respiratory tract through droplets, respiratory secretions, contact surfaces, and direct human contact **[3, 7]**. The virus has a 1-14 day incubation period, but the majority of cases take 3-7 days. Patients with COVID-19 experience symptoms such as fever, lethargy, coughing, and flu-like symptoms **[8, 9]**. Severe difficulties are more common in the elderly and those with comorbidities. Acute respiratory distress syndrome (ARDS), respiratory failure, and other symptoms plagued those in critical condition.

POSSIBILITIES OF MULTI-EPITOPE VACCINE AGAINST SARS-COV-2

SARS-CoV-2, like SARS-CoV and MERS-CoV, causes severe respiratory illness and, in severe cases, death. The virus is extremely pathogenic due to possible adaptive changes in the SARS-CoV-2 genome, making pharmacological treatments and vaccines difficult to develop. Despite the difficulties, a number of pharmaceutical research firms are actively looking for vaccine candidates to combat COVID-19 infections. There is hope for vaccine development because phylogenetic analysis of various SARS-CoV-2 strains' glycoproteins reveals a close relationship, implying that a vaccine targeting a single strain could be effective **[4]**. One strategy for combating the SARS-CoV-2 outbreak is the development of multi-epitope vaccines. These vaccines, which are made up of a sequence of or overlapping peptides, are an excellent option for preventing and treating viral infections and cancer. 18th multi-epitope subunit vaccines, developed through immune-informatics and computational studies, are a revolutionary technique for the prevention and treatment of infectious diseases. Researchers can now isolate immunogenic and highly conserved epitopes from bacterial and viral antigens using this novel method. To generate a diverse set of vaccination candidates, CD4+ and CD8+ epitopes can be used alone or in combination **[10]**.

AN OVERVIEW OF THE PROS AND CONS OF MULTIPLE EPITOPE VACCINES

Multi-epitope vaccines have promise because they are cost-effective, extremely safe, take less time to make, are water soluble, use natural adjuvants, are durable under simple storage conditions, and have positive preclinical assessments, despite being a novel method of vaccine creation **[11, 12]**. They also outperform many wet lab trials because they don't require microbial culture **[10]**. Due to the lack of a full pathogen, the vaccination is extremely specific and stable, making it a safer option to pursue. Whole pathogen vaccinations have this disadvantage because they can cause autoimmune or severe allergic reactions **[13]**. A multi-epitope vaccine architecture differs from traditional vaccinations containing a complete pathogen or single-epitope vaccines in terms of design principles. They are primarily made up of cytotoxic T-cells, T helper cells, and B cell epitopes, all of which can simultaneously elicit powerful cellular and humeral immune responses. The inclusion of an adjuvant may improve the vaccine's immunogenicity and long-term immune responses. Furthermore, the use of several epitopes from various viral antigens broadens the range of viruses that can be targeted. Finally, because the components that drive these cascades aren't used, abnormal immune responses or negative consequences could be reduced by using a multi-epitope vaccine **[14]**. With these properties, the use of multi-epitope vaccines as a therapeutic agent against viral diseases, particularly SARS-CoV-2, could be a promising future research topic.

While promising for vaccine development, a multi-epitope vaccination method has a number of drawbacks, including identifying acceptable candidate antigenic epitopes and developing an effective delivery mechanism, which is often accomplished by adding an adjuvant to boost the immune response. Furthermore, peptides are weak immunogens that require the addition of adjuvants or a delivery mechanism to elicit an immune response. They are prone to enzymatic breakdown and are frequently misidentified by the entire outbred group. In the race to develop a vaccine for SARS-CoV-2, **[13]** mRNA vaccine candidates have outpaced peptide-based vaccine candidates.2. Despite the fact that mRNA vaccines are a new technique in vaccine development, they have demonstrated powerful immunity, inducing six strong CD8+ T cell responses as well as strong CD4+ T cell responses, unlike protein vaccines **[12]**.

ADJUVANTS

After epitope selection, adjuvants are commonly used in vaccines to increase immune response activation while also protecting the antigen from degradation and delivering the relevant antigen to the desired tissue. Because the vaccine design lacks a complete pathogen, adjuvants can be used to replace the natural danger signal generated by infection **[15]**. Among the suggested epitope targets discussed below, the use of beta-defensins as an adjuvant was a popular technique. Vaccines containing defensin adjuvants have previously been shown to stimulate the main innate antiviral immune response and mediate additional immunomodulatory effects against certain viruses, including coronaviruses **[16]**. Cholera Toxin B has also been studied as a protein adjuvant, with previous research indicating that it can boost systemic and mucosal immune responses **[14]**.

POTENTIAL EPITOPE TARGETS FOR THE SARS-COV-2 VIRUS

Multi-epitopic subunit vaccines have sparked interest due to their role in viral structural proteins eliciting a strong immune response. According to studies, humoral and cell-mediated immune responses protect against coronavirus S and N proteins **[6]**. Research on vaccine designs for SARS-CoV-2 has emerged as a new vaccine development strategy, with multi-epitopic subunit vaccines emerging as a new technique. Immuno-informatics and computer analysis are used to create these vaccine structures, with servers predicting epitopes and then selecting them based on antigenicity, toxicity, allergenicity, and cross-reactivity with human proteomes **[17]**. Linkers are used to connect the predicted epitopes. The vaccine design is then tested for validity and potency using bioinformatics techniques on a physiochemical, immunological, and structural level. Below is a list of potential epitope targets for vaccination candidates.

In a study conducted at the India Institute of Technology Mandi, three structural proteins (S, N, and E) capable of generating a humoral and cell-mediated immune response were chosen for a multi-epitopic vaccine candidate. The IEDB, NetCTL, and IFNepitope servers were used to predict B lymphocyte, helper T cell, cytotoxic T cell, and IFN- epitopes. Using 48 high-scoring CTLs, four high-affinity HTLs, and four B-cell epitopes, a multi-epitopic vaccine was developed. These epitopes were linked using the CTL linker (AAY), HTL linker (GPGPG), and B epitope linker (KK). A -defensin-TLR-3 agonist was added as an adjuvant via an EAAAK linker at the vaccine's N-terminus to improve immunogenicity. The vaccine candidate's and human toll-like receptor-3 (TLR-3) binding stability was revealed through docking and molecular dynamic simulations. The N-terminal and middle regions of the vaccine construct were found to have stable interactions with TLR-3. The Ramachandran plot was used to verify the vaccine design, which revealed that 88 percent of the vaccine model's residues fell within the preferred and permitted areas. When compared to the RAMPAGE service, the results were nearly identical, with 90.7 percent of residues in the preferred and permitted areas. VaxiJen v2.0, with a score of 0.566, and ANTIGENPro, with a score of 0.845, both determined the vaccine to be antigenic. Researchers recommend further research to verify the multi-subunit vaccine through in vitro and in vivo investigations because the vaccine candidate is antigenic, non-allergenic, nontoxic, extremely stable, immunogenic in nature, and capable of producing INF-.14, By predicting B-cell and T-cell epitopes, researchers from the University of Lahore, the National University of Sciences and Technology, and the Khawaja Muhammad Safdar Medical College created three multi-epitopic peptide vaccine candidates **[10]**. The S1 and S2 domains of spike proteins were investigated, and T-cell and B-cell epitopes were used to select two of the three vaccine constructions. After that, linkers and adjuvants were used to simulate the epitopes that had been prioritised. 3D models were used to further investigate their physiochemical properties as well as possible interactions with ACE2, TLR2, TLR4, and HLA Superfamily alleles. In vaccination 1, four S1 domain epitopes were used. Three of the four epitopes were discovered in the N-terminal region of the S1 protein, while the fourth was discovered in the receptor binding domain (319-541). The specific positions of these four epitopes make them a fantastic potential target for antiviral treatments and vaccines because of the functions of the S1 subunit and RBD in viral entry into the host cell. Vaccine 2 was distinguished from vaccine 1 by the presence of both weak and strong epitopes. The 506QPYRVVVLSFELLHA520 epitope was implemented due to its presence in the RBD, while two weak epitopes from the S2 domain, 731MTKTSVDCTMYICGD745 and 733KTSVVTMY741, were used to test binding affinity. When docked with TLR2, TLR4, and ACE2, the two S2 weak epitopes bind with effectivity. Thr192, Val197, Lys186, Thr187, and Ser186 are linked to TLR4 by 31MTKTSVDCTMYICGD745; Lys186 has an affinity for the ACE2 receptor. It was discovered that the S2 epitope 33KTSVDCTMY741 overlapped with the other S2 epitope 31MTKTSVDCTMYICGD745. The third vaccination design was a modified version of vaccine 2 that included the adjuvant beta defensin and a new B-cell epitope 369YNSASFSTFKCYGVSPTKLNDLCFT 393. In previous studies, -defensin was shown to be a powerful adjuvant when conjugated with MERS-CoV antigens **[14]**. As previously reported in investigations, including this epitope and adjuvant enabled both a cellular and humoral immune response. TLRs and HLA superfamily alleles, as well as interactions between Cys93 and Phe94 from the B cell epitope and Arg8 and Glu96 from the BCR, were discovered to interact with vaccine 3. The role of spike proteins was mentioned in all of the potential vaccinations, as well as the possibility of vaccine delivery via MHC-I and MHC-II. The vaccine designs were validated using ERRAT analysis and Ramachandran plots. ERRAT scores for vaccines 1-3 were 74.1379, 67.5676, and 74.2574, respectively. According to the Ramachandran plot research, 97.1 percent of vaccine 1 residues were in the liked zone, 98.1 percent of vaccine 2 residues were in the liked zone, and 86.5 percent of vaccine 3 residues were in the liked zone. The Ramachandran plot values demonstrated that among the three vaccine constructions, vaccines 1 and 2 had the best structural quality. When compared to vaccines 2 and 3, the HADDOCK web server confirmed binding affinity and discovered that vaccination 1 had the best interaction capabilities. VaxiJen v2.0 was also used to assess the antigenic potential of the three vaccines. One-to-one antigenicity was determined for all three vaccinations: 0.883591, 0.946425, and 0.8853570. The vaccine candidates offered show promise as antiviral treatments based on these findings. Because they may hinder the viral interaction of ACE2 and spike glycoproteins, researchers recommend further research into these potential epitope possibilities through in vitro and in vivo experiments. Further research could provide light on the vaccine's interactions with the host cell, as well as the vaccine's role in the immunological response of the host.

Another study centred entirely at Garden City University focused on a high-capacity multi-epitope vaccine candidate that targeted the spike glycoprotein due to its role in stimulating cytotoxic T lymphocytes (CTL), helper T lymphocytes (HTL), and interferon- (IFN-). A linear vaccine was created using GPGPG linkers to connect seven CTL, eight HTL, and three IFN-epitopes. The GPGPG linkers were chosen because of their ability to improve solubility and make nearby domain names approachable. To enhance a durable immunological response, an EAAAK linker was used to connect the N-terminal of the Cholera Toxin B (CTB) adjuvant to the N-terminal. Previous studies have shown that this specific adjuvant has the ability to improve both mucosal and systemic immunity to respiratory syncytial virus when used in conjunction with nasal vaccination **[14]**. Using the Ramachandran plot, Z-rating, and ERRAT evaluation, the vaccine assembly's structural quality was analysed and verified. The Ramachandran plot revealed that 96.4% of the residues were contained inside the required region, indicating that the quality of the vaccine assembly had improved. The linear vaccination received a Z-rating of -eight.1, indicating that it fell within a range of ratings for proteins of similar length. The vaccine assembly received a score of 74.2947 in the ERRAT test, implying that the predicted shape is now valid. Figure four contains a list of all validation strategies. eight Molecular docking to TLR 2, TLR4, MHC I receptor, and MHC II receptor confirmed binding affinity. With low HADDOCK scores, all binding affinities were determined to be powerful. VaxiJen v2.zero evaluated the vaccine assembly to be antigenic with a rating of zero.5107. The proposed vaccination was tested using in silico immune stimulation, and the results revealed that the vaccine was capable of eliciting exact immunological responses necessary for the antigen to be eliminated on secondary exposure. This study's computational analysis revealed that the vaccine was structurally sound, antigenic, and immunogenic, indicating that it might be used to combat SARS-CoV-2. Finally, another study including Guangxi University and Government College University Faisalabad (GCUF) focused on the SARS-CoV-2 spike glycoprotein, with B-mobileular and T-mobileular epitopes predicted using the ABCpred and IEDB consensus methods, respectively. VaxiJen v2.zero and Allergen FPv1.zero were then used to test the antigenicity and allergenicity of these chosen epitopes. The vaccination was narrowed down to three CTL epitopes (S1 and M2), six HTL epitopes (E3 and M3), and four B-mobileular epitopes after further epitope analysis (S3 and M1). With the use of an EAAAK linker, -defensin was once again employed as an adjuvant and bound to the vaccine assembly's N-terminal. The use of -defensin was determined due to its extremely short length of 45 amino acids, as well as its ability to act as an immunomodulator and antibacterial agent. Following that, AAY, GPGPG, and KK linkers were sent to the CTL, HTL, and B mobileular epitopes, respectively. These linkers improve the vaccine assembly's stability, folding, and expression. The shape of the assembly was tested and found to be non-allergenic, antigenic (zero.6737), and non-toxic. A Ramachandran plot revealed that 89.4 percent of the residues fell inside the required region, with a z-rating of -4.8 and an ERRAT rating of 82.4561, proving the validity and high-quality of the assemble's form. Binding affinity was discovered by molecular docking to have strong connections between the vaccine assembly and TLR3. All of the data points to a multi-epitopic subunit vaccine form that is very remarkable and has the potential to elicit a robust immune response **[18]**. The outcomes of the vaccine assembly on SARS-CoV-2 should be investigated further in vitro and in vivo tests.

CONCLUSION:

While the Food and Drug Administration (FDA) has not yet licenced a vaccination for COVID-19 infections, numerous vaccine candidates are now being tested in clinical studies. There is continuous interest in researching multi-epitope fractional monetary unit vaccinations, which can often produce an immunological response. Researchers can select matter epitope targets for vaccine constructions using immunoinformatics and machine learning techniques. Furthermore, multi-epitopic vaccine designs are revealing multiple subunit targets of SARS-CoV-2, in addition to being cost-effective, safe, time-saving, stable, victimisation natural adjuvants, and having satisfactory diagnostic evaluations. Analyses of those targets have revealed that they have promising promise as SARS-CoV-2 vaccine candidates. Multi-epitopic vaccines, which are starting to show up in vaccine candidate trials, need more testing to see how well they work with alternative antiviral agents like desoxyribonucleic acid recombinant and messenger RNA vaccines. Studies currently support in vitro and in vivo testing to further observe vaccine constructions in the goal of moving forward with vaccine development and clinical trials. Despite the fact that mRNA vaccines have been at the forefront of recent SARS-CoV-2 vaccine development, multi-epitopic vaccines still hold promise in future immunisation trials. Though these vaccines may not be considered further in relation to SARS-CoV-2, studies show that future research could lead to a viable strategy in the hunt for immunity in other infectious diseases.

REFERENCES:

[1] Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y, Ren R, Leung KS, Lau EH, Wong JY, Xing X, Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia, New England Journal of Medicine, 382, 2020, 1199–207.

[2] Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S *et al*., SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor, Cell, 181, 2020, 271-280.e8. https://doi.org/10.1016/j.cell.2020.02.052

[3] Naqvi AAT, Fatima K, Mohammad T, Fatima U, Singh IK, Singh A *et al*., Insights into SARS-CoV-2 genome, structure, evolution, pathogenesis and therapies: Structural genomics approach. Biochimica et Biophysica Acta - Molecular Basis of Disease, 1866, 2020, 165878. https://doi.org/10.1016/j.bbadis.2020.165878

[4] Wiersinga WJ, Rhodes A, Cheng AC, Peacock SJ and Prescott HC, Pathophysiology, Transmission, Diagnosis, and Treatment of Coronavirus Disease (COVID-19): A Review. JAMA - Journal of the American Medical Association, 324, 2020, 782–93. https://doi.org/10.1001/jama.2020.12839

[5] Fenner F, Ginsberg HS, Maurin J. and Valenta V. International committee on taxonomy of viruses. Intervirology. 1974, 199. https://doi.org/10.1159/000149756

[6] Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y *et al*., Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. The Lancet, 395, 2020, 497–506. https://doi.org/10.1016/S0140-6736(20)30183-5

[7] Mahmoud IS, Jarrar YB, Alshaer W and Ismail, S. SARS-CoV-2 entry in host cells-multiple targets for treatment and prevention. Biochimie, 175, 2020, 93–8. https://doi.org/10.1016/j.biochi.2020.05.012

[8] Kumar A, Kumar P, Saumya KU, Kapuganti SK, Bhardwaj T and Giri R. Exploring the SARS-CoV-2 structural proteins for multi-epitope vaccine development: an in-silico approach. Expert Review of Vaccines, Advance online publication. 19, 2020, 887–98. https://doi.org/10.1080/14760584.2020.1813576

[9] Yang J, Wang W, Chen Z, Lu S, Yang F, Bi Z *et al*., A vaccine targeting the RBD of the S protein of SARS-CoV-2 induces protective immunity. Nature, 586, 2020, 572–7. https://doi.org/10.1038/s41586-020-2599-8

[10] Kar T, Narsaria U, Basak S, Deb D, Castiglione F, Mueller DM *et al*., A candidate multi-epitope vaccine against SARS-CoV-2. Scientific Reports, 10, 2020, 10895. https://doi.org/10.1038/s41598-020-67749-1

[11] Tahir Ul Qamar M, Shahid F, Aslam S, Ashfaq UA, Aslam S, Fatima I *et al*., Reverse vaccinology assisted designing of multiepitope-based subunit vaccine against SARS-CoV-2. Infectious Diseases of Poverty, 9, 2020, 132.

https://doi.org/10.1186/s40249-020-00752-w

[12] Goumari MM, Farhani I, Nezafat N and Mahmoodi S, Multi-Epitope Vaccines (MEVs), as a Novel Strategy Against Infectious Diseases. Current Proteomics, 17, 2019, 354–64. https://doi.org/10.2174/1570164617666190919120140

[13] Sanami S, Zandi M, Pourhossein B, Mobini GR, Safaei M, Abed A *et al*., Design of a multi-epitope vaccine against SARS-CoV-2 using immunoinformatics approach. International Journal of Biological Macromolecules, 164, 2020, 871–83. https://doi.org/10.1016/j.ijbiomac.2020.07.117

[14] Shah VK, Firmal P, Alam A, Ganguly D and Chattopadhyay S, Overview of Immune Response During SARS-CoV-2 Infection: Lessons from the Past. Frontiers in Immunology, 11, 2020. https://doi.org/10.3389/fimmu.2020.01949

[15] Naz A, Shahid F, Butt TT, Awan FM, Ali A and Malik A, Designing Multi-Epitope Vaccines to Combat Emerging Coronavirus Disease 2019 (COVID-19) by Employing Immuno-Informatics Approach. Frontiers in Immunology, 11, 2020. https://doi.org/10.3389/fimmu.2020.01663

[16] Kim J, Yang YL, Jang SH and Jang YS, Human β-defensin 2 plays a regulatory role in innate antiviral immunity and is capable of potentiating the induction of antigen-specific immunity. Virology Journal, 15, 2018. https://doi.org/10.1186/s12985-018-1035-2

[17] Skwarczynski M and Toth I, Peptide-based synthetic vaccines. Chemical Science, 7, 2016, 842–54. https://doi.org/10.1039/c5sc03892h

[18] Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT and Veesler D, Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. Cell, 181, 2020, 281-292.e6. https://doi.org/10.1016/j.cell.2020.02.058