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Omicron variant receptor-binding domain phylogenetics and molecular dynamics

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\textbf{ABSTRACT}

\textbf{Background:} We investigated the evolutionary relationships, mutations, antigenic epitopes, and structural dynamics of the receptor-binding domain (RBD) of SARS-CoV-2, Omicron and other recently evolved variants.

\textbf{Methods:} The RBD of SARS-CoV-2 and its Omicron, Alpha, Beta, Gamma, Delta, and Mu variants were subjected to pairwise sequence matrix evaluation, antigenic epitope prediction, and phylogenetic relationship and structural dynamics analyses.

\textbf{Results:} The Omicron RBD contained 13–15 amino acid mutations, of which 12 were new and three conserved with other variants. In addition, two mutations found in the Alpha, Beta, Gamma, and Mu variants were not found in the Omicron RBD. The ultrametric clustering unweighted pair group method with arithmetic mean identified Omicron as a novel monophyletic class, but the neighbor-joining method clustered Omicron with Alpha and Delta variants. In the SARS-CoV-2 RBD, five main antigenic epitopes were predicted, and these epitopes were conserved across all SARS-CoV-2 variants tested. Surprisingly, the additional mutations in the Omicron variant increased the size of the expected antigenic sites in two of these antigenic epitopes. Molecular dynamics (MD) simulations revealed higher root-mean-square deviation in the Omicron RBD, greater residue fluctuation at residues 32–42 and 140–160, and increased solvent-accessible surface area.

\textbf{Conclusions:} The Omicron RBD mutations indicate the variant is within a new phylogenetic class of SARS-CoV-2 and significantly impact RBD structure, conformation, and molecular dynamics. However, conserved anticipated antigenic sites may imply partial changes in receptor affinity and response to immune reactions. Omicron RBD binding with the angiotensin-converting enzyme 2 receptor was suggested to be weaker than the original SARS-CoV-2 binding in MD simulations.

\textbf{1. Introduction}

Following the discovery of SARS-CoV-2 in December 2019, several new variants have emerged, including the Alpha variant in the United Kingdom (B.1.1.7) \cite{1}, the Beta variant in South Africa (B.1.351) \cite{2}, the Gamma variant in Brazil (P.1) \cite{3}, California (B.1.429) \cite{4}, and New York (B.1.526) \cite{5}, the Delta variant in India (B.1.617.2) \cite{6}, and most recently, the Omicron variant (B.1.529) \cite{7}, discovered in Botswana. The creation of novel variants is expected to continue because the virus is subjected to higher immunological pressure as a greater proportion of the host population is vaccinated or acquires spontaneous infection immunity \cite{8}.

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initially doubled every two days. Furthermore, this variant also has a very high reinfection rate, 5.4 times higher than the Delta variant [10, 11]. Researchers have proposed various SARS-CoV-2 evolutionary routes, some of significant concern, based on the robust genomic and evolutionary examination of all variants emerging during the COVID-19 pandemic [10]. The assumptions of these proposed routes include structural constraints that prevent additional spike mutations, as well as insertions, deletions, point mutations and recombination events involving related viruses or even viruses from other genera [10].

A recent mutational scan of SARS-CoV-2 RBDs revealed constraints on angiotensin-converting enzyme 2 receptor (ACE2) folding and binding [12]. Although most of these mutations were detrimental to RBD expression and ACE2 binding, this binding was improved or maintained by multiple alterations, including those in the ACE2 interface residues that differed across coronaviruses related to SARS. However, current SARS-CoV-2 pandemic isolates do not appear to have been selected for ACE2-affinity-enhancing mutations [12].

Many unresolved questions remain about the Omicron variant's origin and its effect on vaccination response and host immunity, relevant clinical aspects, spread potency, and lethality. In this study, we examined the evolution of the Omicron RBD, a crucial component of virus-host interactions. We analyzed the antigenic epitopes of the SARS-CoV-2 variants Omicron, Alpha, Beta, Gamma, Delta, Mu, and SARS-CoV-2 USA to determine epitope differences. The structural stability and variation of RBD residues were also studied.

2. Materials and methods

2.1. Collection of input protein data

The genomes of SARS-CoV-2 variants were retrieved from the Global Initiative on Sharing All Influenza Data (GISAID) (https://www.gisaid.org/) [13]. Only genomes with high coverage were selected and saved as FASTA files. Table 1 provides information about the genomes used. The sequences were analyzed using CLC Genomics Workbench 12.0 (QIAGEN, Aarhus, Denmark) and Geneious Prime (Auckland, New Zealand) [14].

2.2. RBD Retrieval and sequence alignment

The RBD protein sequence was determined using the protein sequence tools in the CLC genomics program. The proteins were aligned using the protein alignment wizard with highly accurate alignment settings, including gap cost settings of a ten-gap open cost and a one-gap extension cost. A pairwise comparison matrix was produced to compare the sequences retrieved. After computing the differences, identity percentages, gaps, and mutations, the identity matrix was generated.

2.3. Phylogenetic analyses

The neighbor-joining (NJ) and the unweighted pair group with arithmetic mean (UPGMA) methods were used to construct the phylogenetic tree [15], which was then analyzed for its evolutionary relationships. The CLC genomics software was used by applying the default parameters. The Jones–Taylor–Thornton (JTT) or Whelan and Goldman (WAG) models were used to measure distances. The

![Table 1](https://example.com/table1.png)

The SARS-CoV-2 variants included in the RBD analyses.

| Variant          | Database Access ID |
|------------------|--------------------|
| Omicron          | EPI_ISL_6640916    |
| Alpha            | EPI_ISL_6756515    |
| Beta             | EPI_ISL_5416540    |
| Gamma            | EPI_ISL_6228367    |
| Delta            | EPI_ISL_6832166    |
| Mu               | EPI_ISL_4470504    |
| GH490R           | EPI_ISL_6887009    |

Fig. 1. Multiple sequence alignment of RBD from SARS-CoV-2 and its Omicron, Alpha, Beta, Gamma, Delta, and Mu variants. The mutated residues are highlighted in red.
neighbor-joining approach was subjected to 100 bootstrap resampling replicates.

2.4. RBD antigenic epitope analysis

The potential antigenic epitopes in the studied variants were searched using the EMBOSS antigenic prediction tool [16], in which the antigenic determination relies on a semi-empirical approach based on the physicochemical properties of amino acid residues and their occurrence frequencies in experimentally determined segmental epitopes. The minimal length of the antigenic region was set to six. The output format was set to EMBOSS motif.

2.5. Simulations of molecular dynamics

The GROMACS (v. 2021) and Desmond software packages were used to execute molecular dynamic (MD) simulations on the proteins.

Table 2
List of Omicron RBD mutations. The mutations are numbered according to the full spike protein length or the RBD residue number. The mutant residue is assigned as new or conserved if found in another SARS-CoV-2 variant.

| Mutation in Omicron (full spike numbering) | Mutation in Omicron (RBD residue no.) | New/conserved mutation | Conserved with other virus |
|-------------------------------------------|---------------------------------------|------------------------|---------------------------|
| G339D                                     | G6D                                  | New                    |                           |
| S371L                                     | S36L                                 | New                    |                           |
| S373P                                     | S40P                                 | New                    |                           |
| S375F                                     | S42F                                 | New                    |                           |
| K417N                                     | K84N                                 | Conserved              | Mu variant, Beta variant  |
| N440K                                     | N107K                                | New                    |                           |
| G446S                                     | G113S                                | New                    |                           |
| S477N                                     | S144N                                | New                    |                           |
| T578K                                     | T145K                                | Conserved              | SARS-CoV-2, Delta variant |
| E484A                                     | E151A                                | New                    |                           |
| Q493R                                     | Q160R                                | New                    |                           |
| G496S                                     | G163S                                | New                    |                           |
| Q498R                                     | Q165R                                | New                    |                           |
| N501Y                                     | N168Y                                | Conserved              | Alpha variant, Beta variant, Gamma variant, Mu variant |
| Y505H                                     | Y172H                                | New                    |                           |

Fig. 2. Pairwise comparative matrix of Omicron and SARS-CoV-2 variants. A) The upper diagonal panel shows the differences in amino acid content. The lower diagonal panel depicts percent identity. B) The upper diagonal panel shows the gaps in the sequence alignment. The lower diagonal panel provides the residues’ identities.
3. Results and discussion

3.1. Omicron RBD alignment

The RBD sequence of the first complete Omicron genome isolated in Botswana (accession no. EPI_ISL_6640916) was aligned with the RBD sequences of SARS-CoV-2 and its Alpha, Beta, Gamma, Delta, and Mu variants (Fig. 1). The alignment process revealed conserved and new mutations (summarized in Table 2). Fifteen Omicron RBD amino acids were mutated, 12 of which were unique to Omicron and three conserved with other variants. The conserved variants were K84N (also in Mu and Beta variants), T145K (SARS-CoV-2 and the Delta variant), and N168Y (Alpha, Beta, Gamma, and Mu variants). The 12 new mutations were G6D, S38L, S40P, S42F, N107K, G113S, S144N, E151A, Q160R, G163S, Q165R, and Y172H. In addition, two mutations identified in the RBDs of SARS-CoV-2 variants were not found in Omicron: R13K (Mu) and K145T (Alpha, Beta, Gamma, and Mu) (Fig. 1).

A previous study examined the effect of mutations on RBD affinity...
and discovered that, like SARS-CoV-1, approximately 46% of single amino acid mutations in SARS-CoV-2 maintained high affinities to ACE2 receptors [12]. Affinity-boosting mutations are particularly common at RBD sites Q493, Q498, and N501. Although these SARS-CoV-2 residues are involved in a dense network of polar contacts with ACE2, mutations at these three sites are tolerated, and even slight changes in polarity will not affect the binding affinity [12]. In a wide-scale analysis of the effects of mutations on RBD–ACE2 affinity, approximately 84.3% of mutations did not affect binding affinity, and only 3.8% of mutations decreased the binding strength [17]. Furthermore, only the N501Y mutation has been linked to lower free energy values, implying stronger affinity [17].

The determined structure of the SARS-CoV-2 RBD–ACE2 interaction [18] revealed a salt bridge between RBD K417 and ACE2 D30. The mutation was expected to also affect a number of hydrogen bonds. In the Omicron RBD, these residues were mutated to K417N, G446S, Q493R, N501Y, and Y505H. These residues are linked to the SARS-CoV-2 RBD and ACE2 through hydrogen bonding.

3.2. Pairwise comparison matrix

The pairwise comparison matrix revealed that the largest number of amino acid mutations (13–15) were identified in the Omicron variant (Fig. 2). The other variants differed from SARS-CoV-2 by only one to a maximum of six mutations. Furthermore, there were no gaps, amino acid insertions, or deletions among the RBD variants.

3.3. Phylogenetics of the Omicron variant RBD

The phylogenetic analysis of the Omicron variant RBD is provided in Fig. 3 (NJ/JTT, Fig. 3A; NJ/WAG, Fig. 3B; UPGMA/JTT, Fig. 3C; UPGMA/WAG, Fig. 3D). The UPGMA method positioned Omicron in a new monophyletic class separate from other variants. In contrast, the NJ
method classified Omicron RBD with SARS-CoV-2 and the Alpha and Delta variants in a common node.

Bioinformatics and phylogenetic analyses are gold standards in the study of microbial evolution and the development of novel therapeutics targeting specific biological targets [19–22]; we used a variety of these techniques to gain insights into the evolution of the Omicron variant RBD. Two methods we used to construct a phylogenetic tree are UPGMA and NJ. In the former, the mutation rate is ignored in favor of the assumption that all lineages change at the same rate. Therefore, the distance between two points determines the structure of the tree. In contrast, the NJ technique accounts for the mutation rate. It does not assume that all lineages evolve at the same rate. This allowed the clustering of Omicron RBD with the most closely related variants in the alignment matrix.

The Omicron RBD protein represents a novel monophyletic class, similar to our recent finding regarding the Omicron genome, the outcome of UPGMA analysis [23]. In this context, in terms of the fraction of shared nucleotides, the Omicron variant is the most phylogenetically distant from the SARS-CoV-2 Wuhan-Hu-1 and Gamma variants [24].

Researchers have offered several hypotheses to explain the likely emergence of Omicron. One hypothesis is that the new variant was introduced during the winter wave in some southern African countries and circulated among chronically infected patients. Its introduction was not reported due to reduced genomic sequencing efforts in those countries. Subsequent spike protein mutations may have increased the protein’s ability to connect with the ACE2 receptor on host cells. Because of Africa’s low vaccination rate, the Omicron variant may have more readily circulated [25]. The nature of the Omicron RBD might indicate that the variant represents a new class with complex factors interacting during viral evolution.

3.4. The content of predicted antigenic epitopes

The top five epitopes predicted by the EMBOSS antigenic tool are provided in Table 3. In the SARS-CoV-2 USA isolate, residues 171–191, 25–39, 152–164, 54–71, and 96–103 (in descending score order) were the predicted antigenic epitopes. The top five epitopes were conserved between the Omicron variant and the SARS-CoV-2 USA isolate (Fig. 4). Surprisingly, the additional mutations observed in the Omicron variant enlarged the expected antigenic sites in two of these antigenic epitopes. The N168Y mutation in the Omicron variant was predicted to enlarge the first antigenic epitope by four residues at its N-terminus. Furthermore, due to two additional mutations (S40P and S42F) in the Omicron variant, the second epitope length was predicted to extend from 15 residues in SARS-CoV-2 to 27 residues in Omicron (Fig. 5). In SARS-CoV-2 and the Alpha, Beta, Gamma, Delta, and Mu variants, the lengths of the first and second epitopes were consistently conserved. The anticipated length increase, in this case, was unique to the Omicron variant.

Multiple epitopes were revealed after applying neutralizing antibodies m396 and 80R [18]. Most of the epitopes were found in SARS-CoV-2 and Omicron RBD conserved areas. The novel Omicron RBD mutations S42F, G113S, Q160R, G163S, Q165R, and Y172H, however, interrupted the resolved epitopes. In a recent study, despite many mutations in Omicron, only one low-prevalence CD8+ T-cell epitope (T95I) from the spike protein contained a single amino acid change in this population [26]. There were no further mutations linked to the previously discovered epitopes. These findings show that almost all anti-SARS-CoV-2 CD8+ T-cell responses should be able to recognize Omicron and that SARS-CoV-2 has not yet generated significant T-cell escape mutations [26]. This result suggests that Omicron RBD may not have a role in immune response modulation.

It was recently found that despite its greater infectivity and transmissibility, the Omicron variant has evolved to have higher antigenicity and decreased pathogenicity in humans. This was determined by the low number of nonself stretches of short constituent sequences (NSC) in both the Omicron and Delta proteomes; however, the number of NSCs was much higher in the RBD of the Omicron spike sequence than in the Delta spike sequence [27]. These NSCs assist in identifying and escaping the immune response. The changes in these amino acids were also part of the altered epitope content in the emerging Omicron variant.

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N440K and T478K were two mutations (both to lysine) from polar to...
positively-charged and larger side-chain residues. These types of modifications may affect the binding affinity between an RBD and an antibody, either by altering protein surface charges or limiting tighter antibody connections [24].

3.5. MD simulations

To compare the results, we used two software packages to run MD simulations. The SARS-CoV-2 structure appeared to be more stable than the Omicron RBD after the 100-ns simulation, as evidenced by lower RMSD results using both software packages (Fig. 6). In the Omicron RBD, the average RMSD was 0.33 nm, compared to only 0.27 nm in the SARS-CoV-2 RBD.

The RMSF profile was nearly identical in SARS-CoV-2 and Omicron RBD, with a notable rise in RMSF values for residues 32–42, which coincides with three novel mutations in the Omicron RBD. These RMSF variations can be overlooked as they occur in a flexible loop that does not interface with the host receptors. The most prominent increase in Fig. 6. The RMSD of SARS-CoV-2 and Omicron RBD during the 100-ns MD simulation. A) SARS-CoV-2 and Omicron RBD RMSD implemented by GROMACS software analysis. B) SARS-CoV-2 and Omicron RBD RMSD values implemented by Desmond software analysis.

RMSF was noticed in the residue range 140–160 (Fig. 7).

The low radius of gyration (Rg) suggested the general compactness of both SARS-CoV-2 and Omicron RBD (Fig. 8). Surprisingly, the solvent-accessible surface area (SASA) was higher for the Omicron RBD than the SARS-CoV-2 RBD (Fig. 9). As SASA alterations can be interpreted as resulting from conformational changes caused by protein mutations [28], the mutated residues in the Omicron RBD had resulted in increased SASA. The hydrogen bond analysis (Fig. 10) revealed nearly identical profiles, except that the Omicron RBD had an average of 192 hydrogen bonds, six more than the SARS-CoV-2 RBD.

4. Conclusions

It is critical to explore the Omicron RBD molecular structure changes resulting from RBD mutations. According to the pairwise comparison matrix, the Omicron variant had the highest number of mutations from SARS-CoV-2 and is the first variant in a new monophyletic class. The creation of a new class is supported by 12 novel mutations identified in the Omicron RBD. These mutations extended the length of the conserved
antigenic epitopes and affected the protein behavior during MD simulation, especially SASA and RMSF. These findings can assist in determining the link between the identified Omicron RBD mutations and RBD structural alterations, changes in potential antigenic composition, and stability of the variant RBD.

Data availability statement
All data are within the manuscript.

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Conflict of interest disclosure
None.

Ethics approval statement
Not apply.

Patient consent statement
Not apply.
Fig. 10. The hydrogen bonds of SARS-CoV-2 and Omicron RBDs during the 100-ns MD simulation calculated by GROMACS software.

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References

[1] D.J. Grint, K. Wing, C. Houllihan, H.P. Gibbs, S.J.W. Evans, E. Williamson, H.J. McDonald, K. Bhaskaran, D. Evans, A.J. Walker, G. Hickman, E. Nightingale, A. Schultz, C.T. Rentsch, C. Baten, J. Cockburn, H.J. Curtis, C.E. Morton, S. Bacon, S. Davy, A.Y.S. Wong, A. Mehrkar, L. Tomlinson, J.J. Douglas, R. Mathur, B. MacKenna, P. Ingelby, R. Croker, J. Parry, S. Harper, N.J. DeVito, W. Hulme, J. Tazare, L. Smeeth, B. Goldacre, R.M. Eggo, Severity of SARS-CoV-2 infection: a nationwide retrospective electronic health records study in England, MedRxiv, 2020.

[2] H. Tegally, E. Wilkinson, M. Giovannetti, A. Irzanzadeh, V. Fonseca, J. Giandhari, D. Doolabh, S. Pillay, E.J. San, R. Sabarinathan, V.S. Kumar, Genomic characterisation of an alpha variant (B.1.1.529) of SARS-CoV-2, Emerging in the New York Region Detected by Software Utility Created to Query Multiple Spike Mutations in South Africa, MedRxiv, 2020.

[3] N.R. Faria, L.M. Claro, D.M. Franco, P.S. Andrade, T.M. Coletti, C.A. Silva, F.C. Sales, E.R. Manuli, R.S. Aguiar, Omicron variant (B.1.1.529) of SARS-CoV-2, a global urgent public health alert, J. Med. Virol. 94 (2022) 105633.

[4] C. Romenshurg, Cluster Analysis for Researchers, Lulu. Com, 2004.

[5] A.S. Kolaskar, P.C. Tongoaonkar, A semi-empirical method for prediction of antigenic determinants on protein antigens, FEBS Lett. 276 (1990) 172–174.

[6] S. Teng, A. Sobitan, R. Rhaodesh, D. Lia, Q. Tang, Systemic effects of immune mutations on SARS-CoV-2 spike glycoprotein stability and receptor-binding affinity, Brief Bioinform 22 (2021) 1239–1253.

[7] J. Lan, J. Ge, J. Yu, S. Shan, H. Zhou, S. Fan, Q. Zhang, X. Shi, Q. Wang, L. Zhang, X. Wang, Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor, Nature 581 (2020) 215–220.

[8] M. Kandeel, K. Albusadah, S.H. Alyada, I. Albakhdaisim, A. Marzok, Influenza from human, camel and farm animals: comparative bioinformatics and molecular dynamics studies, Pakistan J. Zool. 54 (2022) 65–73.

[9] M. Kandeel, M. Yamamoto, B.K. Park, A. Al-Taher, MERS-CoV Inhibitor Peptides, United States Patent Office, 2021. Patent no. US10975126B1.

[10] Mahmoud Kandeel Elsayed, Hyung-Joo Kwon, Mohammed Al-Nazawi, Abdulla Al-Taher, MERS-CoV Inhibitor Peptides, United States Patent Office, 2021. Patent no. US10975126B1.

[11] Mahmoud Kandeel, Byoung Kwon Park, Mohamed A Morsy, Kathariagita N. Venugopala, Kentoaro Oh-hashi, Mohammed Al-Nazawi, Hyung-Joo Kwon, Virtual screening and inhibition of Middle East respiratory syndrome coronavirus replication by targeting papain-like protease, Dr. Sulaiman Al Habib Med. J. 3 (4) (2021) 179–187.

[12] J.A. Marsh, S.A. Teichmann, Relative solvent accessible surface area predicts protein conformational changes upon binding, Structure 19 (2011) 859–867.

[13] A. Cooper, S. Markowitz, C. Duran, T. Thierer, B. Ashton, P. Meintjes, A. Purcell, L. Piccoli, M.S. Pizzuto, A.C. Walls, M.S. Diamond, A. Telenti, H. Chu, A. Gori, A. Riva, O. Giannini, A. Ceschi, P. Ferrari, P.E. Cipp, The remarkable evolutionary plasticity of coronaviruses by mutation and recombination: insights for the COVID-19 pandemic and the future evolutionary paths of SARS-CoV-2, Viruses (2022) 14.

[14] E. Cameroni, J.E. Bowen, L.E. Rosen, C.S. Zepeda, K. Culap, D. Pinto, L.A. Vardilargan, A. De Marco, J. Di Iulio, F. Zatta, H. Kaiser, J. Noack, N. Farhat, N. Czuochowska, C. Havenar-Daughton, K.R. Sprouse, J.R. Dillen, A.E. Powell, A. Chen, C. Maher, L. Yin, D. Sun, L. Soriaga, J. Bassi, C. Cohen, M. Amoutzias, N. Nikolaidis, E. Tryfonopoulou, K. Chlichlia, P. Markoulatos, S.G. Oliver, The remarkable evolutionary plasticity of coronaviruses by mutation and recombination: insights for the COVID-19 pandemic and the future evolutionary paths of SARS-CoV-2, Viruses (2022) 14.

[15] G.D. Amoutzias, N.R. Faria, I.M. Claro, D. Candido, L.M. Franco, P.S. Andrade, T.M. Coletti, C. Silva, F.C. Sales, E.R. Manuli, R.S. Aguiar, Genomic characterisation of an alpha variant (B.1.1.529) of SARS-CoV-2, a global urgent public health alert, J. Med. Virol. 94 (2022) 105633.

[16] C. Romenshurg, Cluster Analysis for Researchers, Lulu. Com, 2004.

[17] A.S. Kolaskar, P.C. Tongoaonkar, A semi-empirical method for prediction of antigenic determinants on protein antigens, FEBS Lett. 276 (1990) 172–174.

[18] S. Teng, A. Sobitan, R. Rhaodesh, D. Lia, Q. Tang, Systemic effects of immune mutations on SARS-CoV-2 spike glycoprotein stability and receptor-binding affinity, Brief Bioinform 22 (2021) 1239–1253.

[19] J. Lan, J. Ge, J. Yu, S. Shan, H. Zhou, S. Fan, Q. Zhang, X. Shi, Q. Wang, L. Zhang, X. Wang, Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor, Nature 581 (2020) 215–220.

[20] M. Kandeel, K. Albusadah, S.H. Alyada, I. AlBokhadaim, S. Alhojaily, M. Almarzok, Influenza from human, camel and farm animals: comparative bioinformatics and molecular dynamics studies, Pakistan J. Zool. 54 (2022) 65–73.

[21] Mahmoud Kandeel Elsayed, Hyung-Joo Kwon, Mohammed Al-Nazawi, Abdulla Al-Taher, MERS-CoV Inhibitor Peptides, United States Patent Office, 2021. Patent no. US10975126B1.

[22] Mahmoud Kandeel, Byoung Kwon Park, Mohamed A Morsy, Kathariagita N. Venugopala, Kentoaro Oh-hashi, Mohammed Al-Nazawi, Hyung-Joo Kwon, Virtual screening and inhibition of Middle East respiratory syndrome coronavirus replication by targeting papain-like protease, Dr. Sulaiman Al Habib Med. J. 3 (4) (2021) 179–187.

[23] A.S. Kolaskar, P.C. Tongoaonkar, A semi-empirical method for prediction of antigenic determinants on protein antigens, FEBS Lett. 276 (1990) 172–174.

[24] S. Teng, A. Sobitan, R. Rhaodesh, D. Lia, Q. Tang, Systemic effects of immune mutations on SARS-CoV-2 spike glycoprotein stability and receptor-binding affinity, Brief Bioinform 22 (2021) 1239–1253.

[25] J.M. Otaki, W. Nakasone, M. Nakamura, Nonself mutations in the spike protein conformational changes upon binding, Structure 19 (2011) 859–867.

[26] A.D. Redd, A. Nardin, H. Kared, E.M. Bloch, B. Abel, A. Pekoz, O. Laeyendecker, K. Kupferschmidt, Where did Omicron come from? Science (New York, N.Y.) 374 (2021) 1179.

[27] J.A. Marsh, S.A. Teichmann, Relative solvent accessible surface area predicts protein conformational changes upon binding, Structure 19 (2011) 859–867.

[28] J.A. Marsh, S.A. Teichmann, Relative solvent accessible surface area predicts protein conformational changes upon binding, Structure 19 (2011) 859–867.

[29] J.R. Pulliam, C. van Schalkwyk, N. Govedier, A. von Gotthberg, C. Cohen, M.J. Groome, J. Dushoff, K. Misiana, H. Moultrie, Increased risk of SARS-CoV-2 infection associated with emergence of the omicron variant in South Africa, Science 376 (2022), 105633.