Molecular dynamic and docking study of chemical structure of new corona viruses lineages of Omicron BA.n sub-variants (n=1-5); BA.4 or BA.5 strains exhibit the most concern

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ABSTRACT

The first dominant of Omicron-Covid-19 (BA.1) was produced around thirty mutations in its Spike protein in 2019. Quickly BA.1 became the dominant variant worldwide. Omicron is dangerous for public health concern due to its high infectivity and antibody evasion. Omicron has three lineages or sub variants, BA.1, BA.2, and BA.3. Among them, BA.1 is the currently prevailing sub variant. Omicron BA.1 has around 65 mutations on non-structure protein (NSP3), NSP4, NSP5, NSP6, NSP12, NSP14, S protein, envelope protein, membrane protein, and nucleus capsid proteins. BA.4 and BA.5 are two newly-designated Omicron lineages.

They are Omicron viruses with a new combination of mutations containing critical spike protein as a concern for human. In terms of their mutations, BA.4 and BA.5 share mutations across their genomes with both BA.1 and BA.2, but are most similar to BA.2. L452R that previously seen in Kappa, Delta, Epsilon variants and also F486V, and R493 can be seen in both BA.4 and BA.5 where differ from one another in mutations that are outside of the spike gene Data on BA.4 and BA.5, which were first detected in South Africa in early 2022, remain limited. But, these variants seem to spread more quickly than earlier versions of Omicron, such as BA.2, and may be better at dodging the immune system's defenses. By this work, we simulated the spike protein structures, along with peptide-like inhibitor structure of the 7QO7, 7WE9, 7WPC and 7DF4 structures including small-molecule inhibitors, via molecular dynamic and docking methods. Several genomes of various coronaviruses using BAST and MAFFT software have been evaluated.

Keywords: *Omicron; BA.4; BA.5; docking; Covid-19.*

1. INTRODUCTION

1.1 Delta variants and the history of Omicron mutation

The Delta variant consists of several mutations in the spike protein, where four of them are most important [1-10]. One of these is known as L452R, which was reported in March 2020 in Denmark firstly. This mutation has been shown more transmissible compared with wild-type strains and has also been related to reduced antibody influence and decrease neutralization during vaccination. The P681R's mutation has been related to chemical processes that might be enhanced transmissibility [3,4].

The D614G mutation first appeared in the US early in the pandemic. There is affirmation with a report of the Centers for Disease Prevention and Control (CDC), those variants with the above-mentioned mutations spread more quickly compared with other variants of COVID-19. T478K is a delta mutation that appeared in around 60% of occurrences in variant B.1.1.222, was first detected in Mexico City, and was included with high infectivity [10-18]. Public Health England stated Delta exhibits much more growth rating compared with Alpha through several statistical and experimental analyses. Recently, UK found that 62% of pandemic cases are delta [5-8]. Consequently, by decreasing the alpha pandemic, Delta cases are rising day by day [11,12]. Some variants of mutations that have been associated with changes in their receptors, antibodies generated against the previous infection, and reduced efficacy of treatments are listed in Table 1.

Diagnostic detection failures are listed in Table 2. Variants of concern might require one or more appropriate public health actions, such as notification to WHO under the International Health Regulations, reporting to CDC, local or regional efforts to control spread, increased testing, or research to determine the effectiveness of vaccines and treatments against the variant [13-15]. Based on the characteristics of the variant, additional considerations may include the development of new diagnostics or the modification of vaccines or treatments. Current variants of concern in the United States that are being closely monitored and characterized by federal agencies are included in the table below.

Table 1. Diagnostics, therapeutics or immune escape.

Mutated	Variants Name	Spike Protein Substitutions	WHO Label	First Identified
B.1.617.1	20A/S:154K	T95I, G142D, E154K, L452R, E484Q, D614G, P681R, Q1071H	Kappa	India – December 2020
B.1.429	20C/S:452R	S13I, W152C, L452R, D614G	Epsilon	United States-(California)
B.1.525	20A/S:484K	A67V, 69del, 70del, 144del, E484K, D614G, Q677H, F888L	Eta	United Kingdom/ Nigeria – December 2020
B.1.526	20C/S:484K	L5F, D80G , T95I, Y144, F157S, D253G, L452R, S477N, E484K, D614G, A701V, T859N, D950H, Q957R	Iota	United States (New York) – November 2020
P.2	20J	E484K, F565L, D614G, V1176F	Zeta	Brazil – April 2020
B.1.617.3	20A	T19R, G142D, L452R, E484Q, D614G, P681R, D950N		India – October 2020

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Emergence of the Omicron pandemic raises serious concerns due to preliminary reports of a significant growth advantage and potential immune escape compared to the Delta variant.The B.1.1.529 variant of the coronavirus was first detected in South Africa in November 2021[16-17]. Three separate peaks have specified the epidemiological condition that one of them belongs to the Delta variant. Immediate planning should be considered to increase healthcare capacity to treat the expected higher number of cases. Cases are also being detected through representative sampling in routine surveillance systems. This indicates that community transmission is already ongoing in EU countries and that further rapid increase in the number of Omicron cases is expected in the next months. Recently, infections have increased significantly, coinciding with the B.1.1.529 variant [18,19].

Table 2. Specification of SARS-CoV-2 Variants of Concern.

Mutated	Variants Name	Spike Protein Substitutions	WHO Label	First Identified
B.1.617.2	20A/S:478K	157del, R158G, L452R, T478K, D614G, P681R, D950N D614G, Q677H	Delta	India
B.1.351	20H/501.V2	241del, 242del, 243del, K417N, E484K, N501Y, D614G	Beta	South Africa
B.1.1.7	20I/501Y.V1	144del, E484K, S494P, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H,	Alpha	United Kingdom

This variant consists of numerous mutations, much more than the other variants. Preliminary observation predicts a high risk of reinfection with this variant compared with other variants[19,20]. Several cases of this variant appear to increasing in many parts of South Africa. Some other labs have exhibited that for one widely used PCR test, one of the three target genes (S gene target failure) is not detected due to the sequencing confirmation of this variant. Some other testing shows this variant has a faster rate of infection due to its growth advantage than the others [21,22]. Omicron has a concern position in itself due to dozens of mutations that can affect the way it behaves. Therefore, it needs to be further investigated for its potential impacts. In contrast to omicron variants, current vaccines offer protection against severe disease and death from Covid-19 variants, including Delta [15,18]. At the same time, this variant has an emerging situation [23-25].

Various mutations or combinations of them might change virus behavior. Omicron is of concern due to its several numbers of mutations that cause transmissibility and possible immune removal. In other words, humans got infected via it even if they have developed some natural immunity from previous Covid-19 infection [26,28]. At the same time, we must not forget that preventing the transmission of Delta should remain our priority parallel with the omicron problem. All preventive measures that are suitable for the Delta variant continue to be effective against Omicron, based on data so far [29-31]. Getting vaccinated with complete doses and taking all other preventive measures will decrease the risk of infection [32-47].

2. BA.n sub-variants (n=1-5)

2.1. Omicron BA.1, BA.2 (B.1.1.529.2) and BA.3

Omicron variant of severe acute respiratory syndrome coronavirus 2 has quickly replaced the Delta variant, which favors the variant with higher infectivity and stronger vaccine breakthrough capability. The initial Omicron wave was caused by the BA.1 strain, compared with ancestral strains, contains 30 substitutions, 6 deletions, and 3 insertions, which are largely clustered at the sites of interaction of potently neutralizing antibodies: the ACE2 interacting surface, around the N343 glycan. Omicron has three lineages or sub variants, BA.1, BA.2, and BA.3 [48-50].

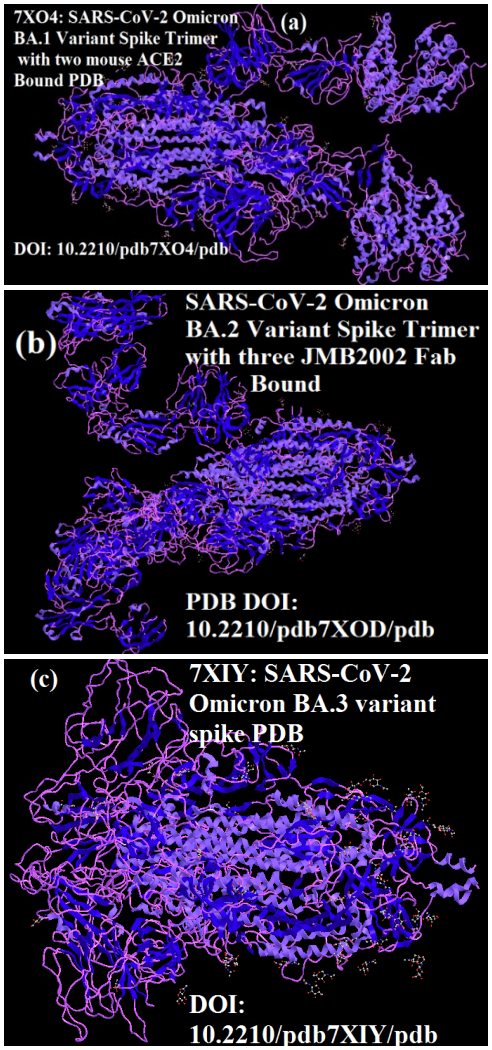
Among them, BA.1 is the currently prevailing sub variant. Omicron BA.1 has around 65 mutations on non-structure protein (NSP3), NSP4, NSP5, NSP6, NSP12, NSP14, S protein, envelope protein, membrane protein, and nucleus capsid proteins [51-54]. Among them, 30 mutations are on the spike (S) protein, the main antigenic target of antibodies generated by either infection or vaccination. BA.3 shares most of its mutations with BA.1 and BA.2 except for one. BA.2 is found in blood of patients originally infected by Omicron BA.1. BA.2 shares 32 mutations with BA.1, but has 28 distinct ones. The BA.2 strain which possesses a small transmission advantage, has become globally dominant. BA.3, reported in relatively few sequences compared with BA.1 and BA.2, appears to be a mosaic of BA.1 and BA.2 changes. On the RBD, BA.2 has four unique mutations and 12 shared with BA.1. BA.2 is inherently substantially more transmissible than BA.1 and capable of vaccine breakthrough. In contrast, the Delta variant has only two RBD mutations [55-59].

Although BA.2 did not cause bad patients than the original Omicron BA.1 strain, its reinfection is dangerous due to antibodies generated from the early Omicron BA.1. An important question is whether BA.2 or BA.3 will become a new dominating variant of concern. It is important to know whether BA.2 will become the next dominating strain for reinfection the world population or not [60-63] BA.3 shares most of its mutations with BA.1 and BA.2, except for one on NSP6. An accurate analysis of all main variants namely, Alpha, Beta, Gamma, Delta, Lambda, BA.1, BA.2, and BA.3, unveils that BA.2 is about 1.6 and 4.5 times as contagious as BA.1 and Delta, respectively. It is also 35% and 15-fold more capable than BA.1 and Delta, respectively, to escape current vaccines. Studies show that binding free energy between the SRBD and the ACE2 is proportional to the viral infectivity and selection more infectious variants were related to the Covid-19 transmission and evolution, including the occurrence of Alpha, Beta, Gamma, Delta, and Omicron variants. BA.3 virus and its infectivity, basically is a non-effect vaccination with an antibody resistance [64, 65].

Experimental data indicate that the Omicron BA.2 variant is about 1.6 times as infectious as BA.1 and about 4.3 times as contagious as the Delta variant. It also has a 35% higher potential than BA.1 to a non-effect vaccination. Therefore, it has been guessed that the Omicron BA.2 can be become one of the next dominating variants. The Delta RBD mutations cause approximately same setting for Omicron BA.1, BA.2, and BA.3 may lead to increase in their vaccine breakthrough capabilities. As such, BA.2 is more capable to escape existing vaccines than BA.1 and Delta variant. BA.2 over BA.1 has highest infectivity and highest antibody resistance and

ant vaccine potential in infecting the world population [66, 67]. Unfortunately, viral mutations are dangerous, particularly antibody-resistant ones for the BA.2 sub variant that might drive a new wave of infections in the world population. Since BA.3 sub variant s RBD mutations are the subsets of those of BA.1 and BA.2, the antibody Omicron mutations might be compromised (Figure 1) [67-69].

Figura 1. SARS-CoV-2 Omicron B. **A.** Variant spike. **B.** SARS-CoV-2 Omicron BA.2 variant spike. **C.** SARS-CoV-2 Omicron BA.3 variant spike.



2.2. Omicron BA.4, BA.5

BA.4 and BA.5 are two newly-designated Omicron lineages. They are Omicron viruses with a new combination of mutations containing critical spike protein as a concern for human. In terms of their mutations, BA.4 and BA.5 share mutations across their genomes with both BA.1 and BA.2, but are most similar to BA.2. L452R that previously seen in Kappa, Delta, Epsilon variants and also F486V, and R493 can be seen in both BA.4 and BA.5 where differ from one another in mutations that are outside of the spike gene. Data on BA.4 and BA.5, which were first detected in South Africa in early 2022, remain limited. But these variants seem to spread more quickly than earlier versions of Omicron, such as BA.2, and may be better at dodging the immune system's defenses. So far, there is not much evidence that they cause more severe disease though more studies are needed. In April and May, the BA.4 and BA.5 sub-variants fueled a surge of cases in South Africa, despite widespread pre-existing immunity to the virus [70].

Currently, these variants have also been detected in other countries. This COVID-19 wave from Africa towards other places was basically due to 3 omicron lineages (BA.1, BA.2 and BA.3). In late 2021, BA.1 became a dominating variant which was then replaced by BA.2 [71, 72]. In addition, few new sub variants of Omicron have mutated such as BA.2.11 (France), BA.2.12.1 (USA) and BA.4/5 (South Africa) [73]. BA.4 and BA.5 as new lineages of Omicron have quickly replaced with old variants, reaching to an account of more than 45% of sequenced cases [74]. The BA.4 and BA.5 sequences extremely increased in April 2022 in South Africa [75], due to that BA.4 and BA.5 variants may be more transmissible than the other Omicron lineages [74].

Table 3. Omicron mutations in the RBD and NTD.

Omicron Variant	NTD	RBD
BA.1	A67V, T95I, G142D, N211I, Δ69-70, Δ143-145	G339D, S371L, s373P, s375F, K417N, N440K
BA.1.1	A67V, T95I, G142D, N211I, Δ69-70, Δ143-145	G339D, R346K, S371L, s373P, s375F, K417N,
BA.2	T191, Δ24-26, A27S, G142D, V213G	S371F, T376A, D405N, R408S, K417N, N440K, S477N, T478K, E484A, Q493R, Q498R, N501Y
BA.3	A67V, T95I, G142D, N211I, Δ69-70, Δ212	T376A, D405N, R408S, K417N, N440K, G446S
BA.4	Δ69-70, Δ24-26, A27S, G142D, V213G	G339D, S371F, T376A, D405N, R408S, K417N, L452R, S477N, T478K, E484A, Q493R, F486V
BA.5	T191, Δ24-26, A27S, G142D, V213G	S373P, S371F, T376A, D405N, R408S, K417N, N440K, L452R, T478K, E484A, Q493R, F486V

However, there is no registered data available yet regarding diseases intensity for BA.4 and BA.5 compared with other covide19 variants. The greatest proportion of these cases is recorded by South Africa; However BA.4 has also been detected in some part of European united countries, the USA and Denmark while BA.5 is detected in UK, Germany and Portugal. Hence, these variants may cause a significant overall increase in COVID-19 cases in the coming time owing to their predicted higher transmissibility. Omicron consists of BA.1, BA.2, BA.3, BA.4, BA.5 and descendent lineages as well as BA.1/BA.2 [76]. The BA.4 and BA.5 sub-variants produced by changing L452R and F486V mutations in S-protein (RBD) from BA.1. Although the BA.4 and BA.5, S-proteins are similar to BA.2 proteins, there are different in amino-acid substitutions of F486V and L452R [74]. In

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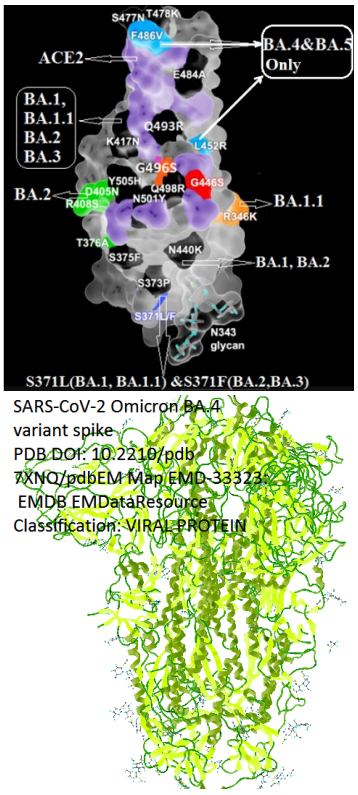
addition, BA.4 and BA.5 lineages have their ability to evade immune responses. A research exhibits that the BA.2.12.1, sub-lineage has the ability to evade antibodies triggered via previous infection with Omicron and vaccination [77]. BA.4 and BA.5 have similar S sequences and has been arisen from BA.2. They contain several mutations in the RBD, especially, the mutation Q493R with mutations L452R and F486V (Table3). In table 1 the S protein mutations of Omicron BA.1, BA.1.1, BA.2, BA.3, and BA.4/5 with NTD and RBD boundaries have been compared together. Q493R is a general mutation in all Omicron lineages, that is translated in BA.4 & BA.5, it can be seen in table3, including those are common to BA.1 and BA.1.1.

Figura 2. Omicron BA.n sub-variants (n=1-5).



In addition, there is no changing of the Q493R in BA.4 and BA.5 compared with Q493 as can be seen in the Wuhan strain. It is notable, the two additional mutations in the RBD exhibit most problem in terms of antibody escape. L452R is a chemically radical change and is one of the pair of changes in Delta RBD L452R and T478K are also found in Epsilon and BA.2.11. F486 is one of the sites, which escape from various Monoclonal antibodies (mAbs) [78]. The change F486V in both BA.4 &BA.5 also causes a reduction in the bulk of the hydrophobic side chain as in F486L[78,79]. Nutalai reported that several mAbs complete knockout of neutralizing activity against Delta [80]. Since BA.1 and BA.2 harbor only one (T478K) of the 2 Delta RBD mutations, while BA.4/5 also harbor L452R, it can be expected all five of these L452-directed mAbs to be knocked out on BA.4/5[80, 81] (Figure2).

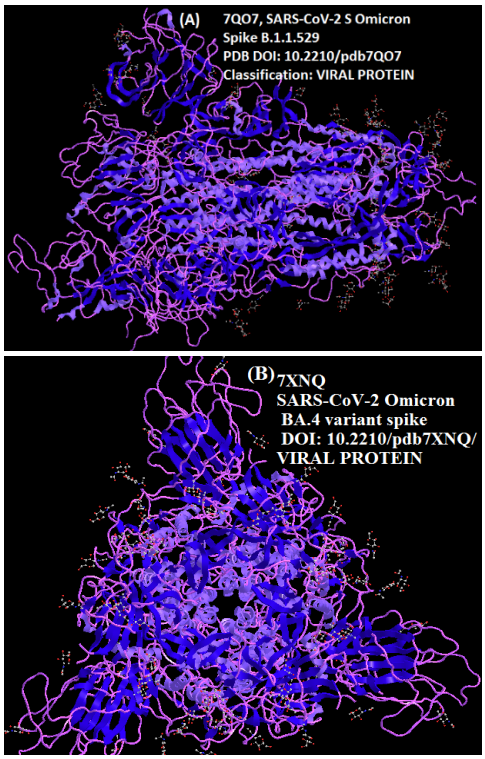
3. MATERIALS AND METHODS

3.1. 7QO7, 7WE9, 7WPC and 7DF4 structures

All computational calculations were done using the Gaussian, Hyper Chem, Chem office, Charmm, and Schrodinger packages. BA.4 and BA.5 spike proteins, along with peptide like inhibitors molecules of the SARS-CoV-2 spike glycolprotein (7QO7, SARS-CoV-2 S Omicron Spike B.1.1.529, *https://doi.org/10.2210/pdb7QO7*; 7XNQSARS-CoV-2 Omicron BA.4 variant spike, https://doi.org/10.2210/pdb7XNQ; 7WE9, SARS-CoV-2 *https://doi.org/10.2210/pdb7WE9*; SARS-CoV-2 omicron BA.2 variant spike, *https://doi.org/10.2210/pdb7XIX*).

Omicron variant spike protein in complex including small-molecules of inhibitor (Figure 1 and Figure 3).

Figura 3. (A): SARS-CoV-2 S Omicron Spike B.1.1.529, (B): SARS-CoV-2 Omicron BA.4.



Model of novel coronavirus spike-receptor-binding domain complexes with its receptors ACE2 (7WPC The second RBD of SARS-CoV-2 Omicron Variant in complexes with RBD-ACE2, DOI: 10.2210/pdb; 7DF4, SARS-CoV-2 S-ACE2 complex, DOI: 10.2210/pdb7DF4;) were designed and simulated from related PDB of SARS-Cov-2 main protease (Figure 4).

The majorities of those structures are bound with small molecules and are suitable for the drug discovery approach. Proteins were provided in protein preparation wizard, H atoms have been added, and H2O molecules beyond 6Å of the binding sites were removed. The chains and loops were designed using the prime module.

Table 4. Potential of Neutralization of COVID-19 vaccines against Omicron variant.

Type of Vaccination	Neutralization Assay	Efficacy against omicron After 2nd dose	Days after booster	Increased omicron BA.1 neutralization after booster
Zhang et al. [90]	Pseudo virus Neutralization test	Vs. ancestral strain 8 ↓×BNT162b2	6- 69	10↑× BNT162b2
Garcia beltran [91]	Pseudo virus Neutralization Assay	Vs. ancestral strain 43 ↓×BNT162b2 mRNA 1273: ↓× 122	<90	27↑× BNT162b2 mRNA-1273: ↑× 19
Haveri et al. [92]	Pseudo virus Neutralization Assay	Vs. ancestral strain 19.7 ↓ ×BNT162b2	28	38.4↑× BNT162b2
Nemet et al. [93]	Live virus Neutralization Assay	Vs. ancestral strain 14.9 ↓ ×BNT162b2	25	96.9↑× BNT162b2
Gruell H et al. [94]	Pseudo virus Neutralization Assay	Vs. ancestral strain 68.2 ↓ ×BNT162b2	21	132.8↑× BNT162b2
Yu et al. [95]	Pseudo virus Neutralization Assay	Vs. ancestral strain 20.1 ↓×BBIBP-CorV	28	3.3 ↑×BBIBP -CorV
Muik et al. [96]	Pseudo virus Neutralization Assay	Vs. ancestral strain 22.8 ↓ ×BNT162b2	28	23.4↑× BNT162b2
Edara et al. [97]	Live virus Focus reduction	None of the vaccination had neutralizing anti body titer	7-28	90% of the subject retained nAb titer

3.2. Docking and free energy calculations

BIOVIA-2020's Docking software, chem3D, Hyper Chem, Rasmol, VMD and Charmm software have been applied for all optimization and docking calculation. The grids of 20& 19Å were produced over the co-crystallized peptide-like inhibitors. Re-docking of the co-crystallized molecules was accomplished for evaluating the docking protocols. The docked systems were based on crystal structures for calculating the root mean square deviation. The re-docking of structures and compounds pose with 1.10Å and 0.70Å RMSD, respectively have been also done.

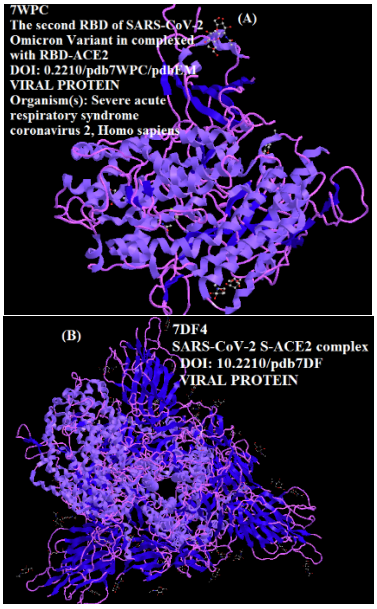
Lower RMSD demonstrates that our docking methodologies are adequate and can be applied to search small molecule inhibitors. Docking was done in 3 different modes, virtual screening followed by standard-precision (SP) and extra-precision (XP) docking using the Glide program.

3.3. MD simulations

Molecular dynamics modeling for polypeptide-ligands structures were accomplished using the above mentioned software. The OPLS and Charmm force fields were applied for modeling the interactions of the protein-small molecules. Long-range electrostatic forces were estimated using the Particle-mesh E-wald (PME) software with a grid spacing of 0.75 Å.

Nose-Hoover thermometry and Martyna-Tobias-Klein method were applied for maintaining the temperature and constant pressure, respectively. The formula of motion was considered using the multi-run RESPA by 3.0 fs time steps for bonded and non-bonded interactions within a low cutoff. An outer time step of 5.0 fs was used for non-bonded forces beyond the cutoff.

Figura 4. (A): Omicron variant in complex with RBD-ACE2; (B) SARS-CoV-2 S-ACE2 Complex.



3.4. Simulations for interactions between the CoV2-RBD and the ACE2

It can be discussed widely about the charged residues for many of the fraction and binding interface of CoV2-RBD and the ACE2. Moreover, electrostatic interactions have critical points for a complex formation. Distances among the two mentioned proteins are a key at the binding interfaces that identified for the three representative models (Figure 5 and Table 5).

Figura 5. (A): Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein ;(B): Structure of novel coronavirus spike receptor-binding domain complexes with its receptor ACE2.

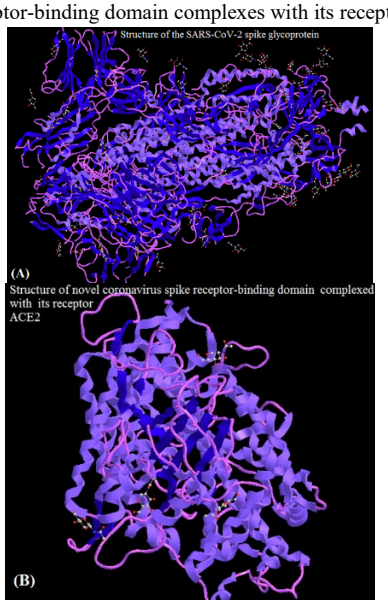


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Table 5. Anti-SARS-CoV-2 S protein RBD neutralizing anti-body and Nucleocapsid anti-body.

Molecule	Cat. No.	Species	Host	Product Description
	AC2-H52H8	Human	HEK293	Human ACE2 /ACEH Protein, His Tag (MALS verified)
	AC2-R5246	Rat	HEK293	Rat ACE2 / ACEH Protein, His Tag (MALS verified)
	AC2-M5248	Mouse	HEK293	Mouse ACE2 / ACEH Protein, His Tag (MALS verified)
	AC2-P5248	Paguma larvata	HEK293	Paguma larvata ACE2 / ACEH Protein, His Tag
	NUN-V52H3	HCoV-OC43	HEK293	HCoV-OC43 Nucleocapsid protein, His Tag
S1 protein	S1N-C52H3	SARS-CoV-2	HEK293	SARS-CoV-2 (COVID-19) S1 protein, His Tag
	S1N-S52H5	SARS	HEK293	SARS S1 protein, His Tag (MALS verified)
	S1N-C52H4	SARS-CoV-2	HEK293	SARS-CoV-2 (COVID-19) S1 protein, His Tag (MALS verified)
	S1N-C5255	SARS-CoV-2	HEK293	SARS-CoV-2 (COVID-19) S1 protein, Fc Tag
	S1N-C5257	SARS-CoV-2	HEK293	SARS-CoV-2 (COVID-19) S1 protein, Mouse IgG2a Fc Tag
S2 protein S-protein RBD	S2N-C52H5	SARS-CoV-2	HEK293	SARS-CoV-2 (COVID-19) S2 protein, His Tag
	SPD-C5255	SARS-CoV-2	HEK293	SARS-CoV-2 (COVID-19) S protein RBD, Fc Tag (MALS verified)
	SPD-S52H6	SARS	HEK293	SARS S protein RBD, His Tag (MALS verified)
	SPD-C5259	SARS-CoV-2	HEK293	SARS-CoV-2 (COVID-19) S protein RBD, Mouse IgG2a Fc Tag
	SPD-S52H5	SARS-CoV-2	HEK293	SARS-CoV-2 (COVID-19) S protein RBD (N354D), His Tag
	SPD-S52H7	SARS-CoV-2	HEK293	SARS-CoV-2 (COVID-19) S protein RBD (W436R), His Tag
	SPD-S52H8	SARS-CoV-2	HEK293	SARS-CoV-2 (COVID-19) S protein RBD (R408I), His Tag
	SPD-C52H4	SARS-CoV-2	HEK293	SARS-CoV-2 (COVID-19) S protein RBD (G476S), His Tag
S1-protein CTD	S1D-C52H3	SARS-CoV-2	HEK293	SARS-CoV-2 (COVID-19) S1 protein CTD, His Tag

The majority of those residues are preserved for our simulation. The same models can be accomplished for the SARS-RBD/ACE2 complexes. Interestingly, the SARS-RBD match in CoV2-RBD did not form close with the ACE2 in related simulations. It is worthwhile to mention that the sequence identity between CoV2-RBD and SARS-RBD is low in this loop region, suggesting the loop region might be partially responsible for the difference in the receptor binding. The H-bonds among the CoV2-RBD and ACE2 can be extracted using VMD program. It can be discussed that the number of hydrogen bonds fluctuated over time. Similar trends can be observed in the other simulations, suggesting that the binding became stronger as the simulation progressed.

3. RESULTS AND DISCUSSION

3.1. Capsule formation.

Number of hydrogen bonds fluctuated over time. Similar trends can be observed in the other active inhibitor of HIV-1 protease, were found effective in treating COVID-19 disease. Data from the docking calculations were analyzing by the molecular modeling software [99,100]. We accomplished 50 ns molecular dynamic modeling (MD) of all 7QO7, 7WE9, 7WPC and 7DF4 structures to get insight into the binding cavities with a classical molecular dynamics method with water molecules applied as molecular indexes. These kinds of strategies are supposed to provide the highly detailed pictures of protein's interior dynamics. These small molecules tracking approaches were applied for determining the accessibilities of the pockets of the active sites in those macromolecules, and also the local distribution approaches were applied for providing information about an overall distribution of related solvents in the protein interior.

To properly examine the flexibilities of both activated sites, we applied the AQUA-DUCT (AQ) software for analyzing the water molecules flow through the cavities in a 20 ns time step. Effectiveness and outbreak rate as a target for vaccine and therapeutic development has been an important factor for anti-drugs. RBD of the S protein has been proposed as a promising target for the development of specific anti-bodies and vaccines. Although the same host receptors are used by different H-CoVs, they frequently target different binding sites on the host receptor. For this reason, specific RBD-active sites anti-bodies or vaccines inevitably lack broad spectrum activities against coronavirus infection. The delay time among emerging human CoVid-19 outbreaks and the development of new prophylactic treatments or vaccines are of concern. From fifteen mutations (RBD), nine of them are located in the Omicron Spike region in the virus's main entry receptor, the human angiotensin-converting enzyme (ACE2). Mutations within the RBD can potentially provide an evolutionary advantage by strengthening the viruses ACE2-RBD binding. Omicron is the root of the mutations for the high infectivity of Delta variant, and some estimates have indicated that Omicron BA.1 is three to six times more infectious than previous variants. However, transmission and reproductive number of SARS-CoV-2 viruses depend on various items such as social distancing, housing, ventilation, super spreading events and vaccination rates. A considerable behavior of Omicron is that it comprises three distinct sub-lineages (BA.1, BA.2, and BA.3) that were discovered near simultaneously.

Subsequently, two other broad sub lineages have been confirmed, BA.4, BA.5, as well as many sub-lineages within BA.1 and BA.2. Initially, BA.1 was the most percentage of the omicron sub-lineage where detected in many countries; however, BA.2 is overtaking BA.1 as the dominant variant globally. Although BA.1 and BA.2 emerge various mutations, but each of them has unique mutations by themselves; BA.2 has additional 8 unique mutations that there

are no emerge in BA.1 and also there are no 13 mutations from BA.1. In addition, mutations in BA.2, BA.4 and BA.5 have the mutations 69- 70del, L452R, F486V and wild type amino acid at position Q493. BA.4 and BA.5 have similar mutational patterns in the 5′ genome region yet exhibit divergence in the 3′ region. performance, well-defined, homogeneous shapes, and hand-pressed resistant

4. CONCLUSIONS

Up to now, the reasons of these types mutation in spike protein of Omicron are unknown. Invistgating and analysising of related sequences have not explained a certain mechanism in those sequences between Omicron and its closest relatives. Beside these subjects, pathway to the emergence of Omicron is also unclear. Evolutionary analysis did not reveal any special mutational reason that could suggest that it how emerge from the Alpha, Beta, Delta or Gamma variants. From Covid-19 emerging up to now, enormously high number of mutations observed in Omicron appears that compared with other SARSCoV-2 variants has raised a theory that the environment in which Omicron evolved may differ from other known VOCs.

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