Molecular dynamic simulation suggests stronger interaction of Omicron-spike with ACE2 than wild but weaker than Delta SARS-CoV-2 can be blocked by engineered S1-RBD fraction

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Abstract

The SARS-CoV-2 claimed millions of lives, globally. Occurring from Wuhan (wild type) in December, 2019, it constantly mutated to Omicron (B.1.1.529), the predecessor to Delta. Omicron having ~ 32 spike mutations has variable infectivity-multiplicity-immuno-invasive properties. Understanding of its mutational effect on ACE2-binding/disease severity and developing preventive/therapeutic strategies are important. The binding affinities of Wuhan/Delta/Omicron spikes (PDB/GISAID/SWISS-MODEL) were docked (HADDOCK2.4) with ACE2 and compared by competitive-docking (PRODIGY). The protein structural stability was verified by kinetic-data/Ramachandran-plot (Zlab/UMassMedBioinfo). After several trials, a 59 amino acid (453ARG-510VAL) peptide-cut (Expasy-server) of the wild-type spike RBD with some desired mutants (THR500SER/THR500GLY/THR500ALA/THR500CYS) was blindly/competitively docked (PyMOL-V2.2.2) to block the Omicron-ACE2 binding. We examined molecular dynamic simulation (iMOD-server, with 9000 cycles/300 k-heating/1 atm pressure for system equilibration for 50 ns-run) of ACE2 and two CUTs with different SARS-CoV-2 variants. The binding-affinity of Omicron-ACE2 is slightly higher than the rest two in competitive docking setup. During individual (1:1) docking, Omicron showed little higher than wild type but much weaker binding affinity than Delta. Competitive docking suggests ten H-bonding (1.3–2.4 Å) with highly favorable energy values/Van-der-Walls-force/Haddock score for more stable-binding of Omicron-RBD with ACE2. Blind docking of different CUTs (wild/mutants) and Omicron to ACE2 completely rejected the Omicron-RBD from ACE2-target. The best blocking/binding affinity of −16.4 and −13 kcal/mole were observed in the case of THR500SER and THR500GLY, respectively, with multiple H-bonding 1.9–2.2 Å. These are supported by the MD-simulation results. So, the spike binding affinities were Delta > Omicron > wild in 1:1 docking with ACE2. Considering the wild type is non-existing nowadays, Omicron showed less ACE2 binding properties. The 59 cut of spike-RBD and its mutant THR500SER/THR500GLY may be further screened as universal blockers of this virus.

Keywords SARS CoV-2 · Wild type · Delta and Omicron variant · Comparative affinity · Preventive blocking/docking · 59 amino acid RBD cut

Introduction

The SARS-CoV-2 pandemic has been surging all over the world for 2 years. According to the statistical report of the World Health Organization (WHO), more than 260 million confirmed cases and over five million deaths have been reported [1]. The original (Wuhan) severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first identified at the end of December 2019 and was followed by a wave that peaked in July 2020 and ended in September 2020. SARS-CoV-2 evolved quickly and a variety of variants emerged.
The WHO classifies them into three types: (a) variants of concern (VOCs), (b) variants of interest (VOIs), and (c) variants under monitoring (VUMs). The second wave, which was caused by the Beta (B.1.351/501Y.V2/20H) variant, peaked in January 2021 and ended in February 2021. The third wave, which peaked in July and ended in September 2021, was driven by Delta (B.1.617.2/478 K. V1/21A) variant [2]. In late November 2021, a new variant named Omicron (B.1.1.529/21 K) was designated as the fifth VOCs by the WHO. The emergence of the Omicron variant was first detected in South Africa, raising concerns based on at least 32 mutations in spike proteins. Many of which are located in the receptor binding domain (RBD). In addition, Omicron has 3 deletions and 1 insertion in the spike protein [3].

The effects of most of the remaining Omicron mutations are not known, resulting in a high level of uncertainty about how the full combination of deletions and mutations will affect viral behavior and susceptibility to natural and vaccine-mediated immunity. Several reports provide preliminary indications on more transmissibility, immune escape, and severity of the Omicron variant. But a recent report suggests, unlike previous variants, its less severe nature due to failure to penetrate the lung tissues in healthy individuals. But the nature of the severity in the co-morbid individuals is yet to be confirmed. It is also to be established the actual impact of Omicron in non-infected and non-vaccinated individuals. More data are needed to characterize the null or various factorial effects alone or in combination. Omicron has some deletions and more than 30 mutations, which overlap with those in the alpha, beta, gamma, or Delta VOCs [4]. N501Y increases binding to the ACE2 receptor to induce higher transmissibility [5] and the combination of N501Y and Q498R may increase binding affinity even more; however, other substitutions in the Omicron spike protein are expected to decrease binding to ACE2. Omicron does not infect cells deep in the lung tissues as readily as it does those in the upper airways, and after a few days, the concentration of virus in the lungs of animals infected with Omicron was at least ten times lower than that in rodents infected with other variants [6]. Other studies reveal that Omicron may be over 10 times more contagious than the original virus or about 2.8 times as infectious as the Delta variant [7].

The Omicron variant, when combined with the H69/V70 deletion, the transmissibility, might be further increased [8]. H655Y is proximal to the furin cleavage site and may increase spike cleavage, which could also increase transmission. P681H has been shown to enhance spike cleavage, which could help transmission. This mutation is found in Alpha and an alternate mutation at this position (P681R) is found in Delta. Previous studies have clarified that D614G is associated with higher upper respiratory tract viral loads and younger age of patients [9–11]. So far, several drugs have been tested in COVID-19 for its mild, moderate, and severe conditions. And for preventive strategies, a significant number and types of vaccines have been used globally. Several reports reveal the sharp failure of the vaccines and that is demonstrated by the epidemiological or vaccinated patient’s sera neutralization data. The investigator examined its sensitivity to 9 monoclonal antibodies (mAbs) clinically approved or in development, and to antibodies present in 115 sera from COVID-19 vaccine recipients or convalescent individuals [12]. Omicron was totally or partially resistant to neutralization by all mAbs tested. Sera from Pfizer or AstraZeneca vaccine recipients, sampled 5 months after complete vaccination, barely inhibited Omicron [12]. Booster Pfizer dose as well as vaccination of previously infected individuals generated an anti-Omicron neutralizing response, with titers 6- to 23-fold lower against Omicron than against Delta. We noted that the activity of 17 of the 19 antibodies tested were either abolished or impaired, including ones currently authorized or approved for use in patients [13]. In addition, we also identified four new spike mutations (S371L, N440K, G446S, and Q493R) that confer greater antibody resistance to B.1.1.529 [13]. Omicron subsequently and swiftly replaced the circulating Delta and other variants. The genome surveillance data shows the comparison between predicted and observed fractions of Omicron, Delta, and other variants. Omicron was estimated to be 4.2 times (95% confidence interval (CI) 2.1, 9.1) greater than that of the Delta variant. It was found 3.3 times more transmissible than the Delta variant [14].

But it is evident that the hospitalization or the case fatality (CFR) is much lower in the Omicron infected cases. In earlier and also in recent periods, several angiotensin receptor blockers (ARBs) exhibited some protecting effects. In addition to the antihypertensive effects, these drugs manifested some anti-inflammatory effects also. The ARBs are found to be protective in severe acute respiratory syndrome caused by the virus. Moreover, in some cases, ACE-inhibitors and ARBs were also found to be associated with decreased mortality [15].

In this background, the blocking of the viral spike and the ACE2 binding was studied in the present study. A comparative in silico study was done to evaluate the ACE2 binding with Wuhan (wild type), Delta, and the Omicron variants of SARS CoV-2. Small peptides from the globally conserved spike variants were designed with or without mutations and docking effects were tested with ACE2 in the presence of the Omicron spike.

Materials and methods

Protein structure retrieval, prediction by analysis

The X-ray crystallography and electron microscopy structure were retrieved from RCSB Protein Data Bank (https://
ments were prepared using Pymol molecular visualization software [18]. After removal, receptor molecules were saved as a.pdb file for further analysis.

**Ramachandran plot analysis**

Ramachandran Plot server [19] was used for protein 3D quality structure assessment (https://zlab.umassmed.edu/bu/rama/). We observed the Ramachandran plot on the number of residues present in highly preferred regions and allowed regions; wild-type SARS-CoV2 94.5%, Delta 94.5%, and Omicron 94.402% were found as a very good quality structure. We also observe the Ramachandran plot of docked complexes and select the best structures (Table S4).

**Molecular docking studies**

**Surface topology calculation of proteins**

Protein pocket and cavity properties are characterized by the solvent accessibility factor. The second water molecule is restricted to entering after the first one occupies these areas. The CASTp (Computed Atlas of Surface Topography of Protein) (http://sts.bioe.uic.edu/castp/index.html?j_5e8c7bec25090) was used in the current study to characterize the pocket and cavity.

The molecular docking between human ACE2 and different mutated spike proteins was individually and competitive blind docking through HADDOCK 2.4 web server [20] to check the highest and lowest binding affinity with ACE2 receptor. Also, blind docking was performed of selected mutated CUTs with ACE2 and Omicron variants were performed on HADDOCK 2.4 server. We only analyze the binding of CUT segments with ACE2 and Omicron because our previous study already checked the binding affinity of CUT segments with SARS-CoV2. HADDOCK is an integrative platform for the modeling of biomolecular complexes. It supports a large variety of input data and deals with a large class of modeling problems including protein–protein, protein–nucleic acids, and protein–ligand complexes, including multi-body assemblies. It also allows to define specific unambiguous distance restraints (e.g., from MS cross-links) and supports a variety of other experimental data including NMR residual dipolar couplings, pseudo contact shifts, and cryo-EM maps. It calculates the docking transformation between two molecules to get the best molecular interface complementary, which finds out the binding affinity between biomolecule complexes. HADDOCK analyzes the protein–protein docking from different angles through HADDOCK score, RMSD from the overall lowest-energy structure, Van der Waals energy, electrostatic energy, desolvation energy, restraints violation energy, buried surface area, and Z-score.
Docking result and binding affinity analysis of different docked molecules

Each sets of molecular docking were analyzed using PyMol molecular visualization software [18]. The presentation of docking structures and interactive bonds were represented through technical and transparent surface analysis mainly. To understand the binding between different docked molecules, each pairs was individually subjected to PRODIGY tool on HADDOCK server. PRODIGY was used to analyze the binding affinity of protein–protein and protein-small molecule complexes and also allows classifying crystallographic interfaces as biological or not [21]. The best structures for the docking result were accepted. Based on the previous study for complete displacement of nCOV2 spike protein from ACE2 RBD through the CUT segment, the highest binding affinity of CUTs with ACE2 RBD and Omicron variant, the best CUT was selected for further analysis. Binding affinity quantifies the binding strength of ligand to a protein.

Evaluation of H-bonding in a competitive docking analysis

Observation of the H-bonding length of amino acid residues between the ACE2 receptor and the original SARS-CoV-2 isolate Wuhan-Hu-1, the Delta mutant, and Omicron mutant demonstrated by PyMol-visualized system to recognize the H-bond length between amino acid residues short or long. The H-bond interaction in short CUT segments with ACE2 and Omicron spike protein was also observed.

Energetic and kinetic data analysis

During the generation of the HADDOCK score, cluster-size-based RMSD values and other bond energy values, i.e., Van der
Waals, electrostatic, and desolvation energy, were evaluated. Binding affinity ($\Delta G$ (kcal mol$^{-1}$)) dissociation constant [Kd (M) at 25 °C]) explains the strength and the affinity of the interactions.

**Molecular dynamic simulation study of different VOCs of SARS-CoV 2 with human ACE2 and two CUT sequences**

Based on the docking results, we performed molecular dynamic analysis studies on the selected lowest energy valued and best-posed docking complex. The MD simulations were carried out by iMod server (iMODS) (http://imods.chaconlab.org/) [22] at 300 K constant temperature, 1 atm constant pressure at molecular mechanics level. The iMod server gives a convenient interface for this enhanced normal mode analysis (NMA) methodology in inner coordinates. The web interface is very spontaneous and responsive to all major browsers and even to modern mobile appliances. Users can perform NMA or molecular dynamics to simulate feasible trajectories between two conformations and interactively explore in 3D the resulting structures, trajectories, animations, and even for large macromolecules. Finally, 50 ns molecular dynamic simulation was carried out for all the complexes, such as different VOCs of SARS-CoV 2 with ACE2 and two CUT 59 sequences (wild 59 and T500S) with Omicron variants.

For structural stability analysis for docking and simulations, providing an RMSD (root mean square deviation) or root-mean-square deviation is a standard measure of structural distance between two proteins. To simulate feasible transitions, the initial structure is iteratively deformed along the lowest modes while the root mean square deviation (RMSD) for a target structure is minimized. Two initial superimposition methods can be selected: either global [23] or local [24]. Whereas the former considers all atoms for the RMSD, the latter favors the overlap between most similar regions.

**Results and discussion**

In the current study, we are presenting by the in silico study the comparative analysis of binding/docking parameters of wild-type SARS CoV-2, Delta, and Omicron variants with human ACE2. We further analyzed the effects of the 59 amino acids of the RBD fraction of SARS-CoV-2 and its various mutants, THR500SER/GLY/ALA/CYS docking/blocking effects on ACE2 Omicron spike binding. Besides the individual spike variant binding with ACE2, comparative/combined binding of different spikes was also evaluated to predict which is more infective/transmissible. Our present results suggest a few considerations.

### Table 1  H-bonding patterns and bond length during different competitive binding of SARS-CoV-2 variants: wild (Wuhan), Delta, and Omicron spikes with human ACE2

| Competitive HADDOCK | ACE2 | Wild SARS-COV-2 | H-bond length (Å) |
|---------------------|------|-----------------|-------------------|
| 1. ACE2 + WILD SARS-COV-2 + Omicron | TYR83 | TYR489 | 2.4 |
|                     | GLN24 | AS487 | 2.5 |
|                     | LYS31 | TYR453 | 2.3 |
|                     | GLU35 | ARG403 | 2.7, 1.5 |
|                     | LYS353 | ASP405 | 2.1 |
|                     | ASP38 | TYR505 | 2.7 |
|                     | LYS68 | GLY496 | 1.7 |
|                     | LYS68 | ASN501 | 1.7 |
| ACE2 Omicron | GLU87 | ARG498 | 1.6, 2.0 |
| ACE2 Delta | LYS478 | 2.2, 1.8 |
| GLU75 | ASN487 | 1.6, 2.5 |
| GLU75 | TYR489 | 1.7 |
| 2. ACE2 + DELTA + Omicron | ACE2 Delta | H-bond length (Å) |
|                     | GLN42 | ASP571 | 2.4 |
|                     | ASP38 | ARG567 | 2.5, 1.6, 1.9 |
|                     | LYS353 | GLN563 | 2.1 |
| ACE2 Delta | H-bond length (Å) |
| 3. ACE2 + DELTA + Omicron + WILD SARS-COV-2 | GLU329 | LYS558 | 1.5 |
|                     | TYR41 | GLN563 | 2.5 |
|                     | TYR83 | LYS462 | 1.7 |
|                     | SER19 | GLU471 | 2.1 |
| ACE2 Omicron | H-bond length (Å) |
| GLU87 | ARG498 | 1.6, 2 |
| LYS112 | ALA372 | 2.1 |
SARS-COV2 and Omicron spike proteins binding with human ACE2 receptor

The spike glycoprotein of both variants initially binds to the angiotensin converting enzyme 2 (ACE2) present at the host cell surface and then the viral entry gradually proceeds. This ACE2 attachment site remains folded until it reaches the receptor. Just before ACE2 attachment in SARS-CoV2 spike, the protein’s flexible part becomes unfolded and the attachment site becomes exposed [25]. Individual docking of ACE2 and three different spike protein results were observed (Fig. 1). According to the competitive multi-docking studies between ACE2-SARS-CoV2–Omicron showed the highest docking score of $-194.5 \pm 5.1$. The cumulative energy calculation of Van der Waals energy, desolvation energy, and electrostatic energy represented the negative value of $-98.0 \pm 9.9$, $-50.9 \pm 13.4$, and $-315.3 \pm 105.1$, respectively (Table S1).

Electrostatic energy represents the potential energy of a system placed within the time-invariant electric field [26] where the positive value indicates the repulsion and the negative value indicates the surface attraction between two molecules [27]. Here, SARS-CoV-2 showed higher binding affinity with ACE2 with a value $-9 \Delta G$ (kcal mol$^{-1}$) and it also showed some dissociation constant value of 2.50E-07 (Kd(M) at 25 °C). In contrast, molecular docking with ACE2 and Omicron showed comparable binding affinity of $-7.4 \Delta G$ (kcal mol$^{-1}$) (Table S2) at the binding site. The active site of ACE2 comprises different amino acids, i.e., TYR83, GLN24, LYS31, GLU35, LYS353, ASP38, and LYS68, with which interaction of TYR489, AS487, TYR453, ARG403, ASP405, TYR505, GLY496, and ASN501 different amino acids of SARS-CoV2 binding sites were observed, respectively (Fig. 2; Table 1).

The active site of ACE2 comprises different amino acids, i.e., ASP67 and GLU75, with which interaction of LYS478, and ACE2 receptor indicated with blue. On the other hand, Omicron variant does not interact with ACE2 receptor. Delta variant interact with ACE2 receptor in 3 different amino acid positions which was indicated in table form.
ASN487, and TYR489 different amino acids of Omicron variant binding sites were observed, respectively (Fig. 2; Table 1). At the surface of ACE2 and spike glycoprotein interaction, all the amino acids interact with each other with the formation of H-bonds (Table 1).

Delta and Omicron spike proteins binding with human ACE2 receptor

Molecular docking with Delta variant and Omicron variant with the ACE2 receptor revealed the highest docking scores of $-137.6 \pm 12.1$. The calculation of Van der Waals energy, desolvation energy, and electrostatic energy represented the negative value of $-80.4 \pm 4.0$, $-31.8 \pm 2.9$, and $-284.8 \pm 64.1$, respectively (Table S1). Here, DELTA variant showed higher binding affinity with ACE2 with a value $-9.7 \Delta G$ (kcal mol$^{-1}$) and it also showed some dissociation constant value of 7.70E-08 (Kd(M) at 25 °C) (Table S2). Contrarily, Omicron variant did not show any binding affinity against ACE2 in the presence of DELTA. It could be possible that the 15 mutations of RBD Omicron are not evenly distributed in RBD, but rather crowed in its RBM with 10 residues, viz., N440K, G446S, S477N, T478K, E484A, Q493K, G496S, Q498R, N501Y, and Y505H [28]. For that reason, Omicron variant has lower binding affinity than DELTA.

The active site of ACE2 comprises different amino acids, i.e., GLN42, ASP38, and LYS353, with which interaction of ASP571, ARG567, and GLN563 different amino acids at the Delta binding sites were observed, respectively (Fig. 3; Table 1).

Fig. 4 The superposition of the docked complexes of wild-type SARS-CoV-2, Delta, and Omicron spike proteins binding with human ACE2 receptor was presented. (a) The enlarged panel on the right side shows the interacting amino acid residues in colored sticks in which Delta is in green color and ACE2 receptor indicated with blue; (b) the left side shows the interacting amino acid residues in colored sticks in which Omicron variant is in pink and ACE2 receptor is in blue. Wild-type SARS-CoV-2 totally blocked by other two types of variant and do not interact with ACE2 at binding site. Delta variant interact with ACE2 receptor in 4 different amino acid positions and Omicron variant interact with ACE2 receptor in 2 different amino acid positions which was indicated in table form. So, the binding comparison between 3 spike glycoproteins with ACE2 receptor and Delta variant has highest binding positions with receptor than other 2 types of spike glycoproteins.
### Table 2

Data of HADDOCK competitive binding and blocking of human ACE2 binding by the wild SARS-CoV-2 spike RBD fraction of 59 amino acid and its different mutants, i.e., TYR500SER, TYR500ALA, TYR500GLY, and TYR500CYS

| HADDOCK parameters | Cluster name | HADDOCK score | Cluster size | RMSD from the overall lowest-energy structure | Van der Waals energy | Electrostatic energy | Desolvation energy | Restraints violation energy | Buried surface area | Z-score |
|--------------------|--------------|---------------|--------------|-----------------------------------------------|----------------------|----------------------|---------------------|--------------------------|---------------------|---------|
| 1. ACE2 + WILD 59 + OMICRON | Cluster 2 | $-175.1 \pm 13.8$ | 17 | 46.8±4.0 | $-116.1 \pm 18.8$ | $-272.1 \pm 60.5$ | $-64.7 \pm 7.0$ | 601.6±148.7 | 3631.3±165.7 | $-0.8$ |
| | Cluster 3 | $-167.8 \pm 7.1$ | 11 | 32.9±7.3 | $-125.2 \pm 17.0$ | $-375.9 \pm 92.9$ | $-40.0 \pm 9.0$ | 725.6±193.6 | 3809.2±250.7 | $-0.5$ |
| | Cluster 1 | $-166.2 \pm 16.2$ | 23 | 27.8±10.7 | $-114.7 \pm 10.6$ | $-363.6 \pm 66.9$ | $-44.3 \pm 10.2$ | 655.3±52.1 | 3666.6±171.6 | $-0.5$ |
| | Cluster 4 | $-1007 \pm 17.4$ | 4 | 53.3±1.6 | $-82.2 \pm 6.5$ | $-298.1 \pm 59.5$ | $-51.6 \pm 6.7$ | 926.7±71.5 | 2918.6±117.4 | 1.7 |
| 2. ACE2 + MUTANT 59 + OMICRON | Cluster 1 | $-163.1 \pm 13.8$ | 15 | 23.5±6.1 | $-121.9 \pm 15.2$ | $-247.0 \pm 65.0$ | $-49.6 \pm 2.6$ | 579.9±99.6 | 3408.9±185.0 | $-1$ |
| | Cluster 3 | $-155.8 \pm 8.8$ | 8 | 27.4±13.2 | $-108.9 \pm 10.8$ | $-332.9 \pm 53.8$ | $-38.6 \pm 8.8$ | 582.4±120.0 | 3462.3±105.0 | $-0.5$ |
| | Cluster 2 | $-154.6 \pm 21.0$ | 9 | 19.3±3.2 | $-105.4 \pm 18.2$ | $-226.9 \pm 50.7$ | $-55.2 \pm 5.0$ | 514.7±120.1 | 3315.5±259.2 | $-0.5$ |
| | Cluster 5 | $-145.3 \pm 4.2$ | 4 | 21.2±7.7 | $-97.1 \pm 15.5$ | $-263.4 \pm 88.1$ | $-51.1 \pm 6.0$ | 556.0±106.3 | 3120.4±252.1 | 0.2 |
| 3. ACE2 + T500GLY + OMICRON | Cluster 1 | $-163.6 \pm 9.4$ | 5 | 26.4±9.7 | $-100.6 \pm 11.3$ | $-293.0 \pm 87.5$ | $-50.0 \pm 7.5$ | 455.7±28.7 | 3450.5±189.1 | $-1.6$ |
| | Cluster 5 | $-154.4 \pm 9.5$ | 5 | 31.2±3.2 | $-113.3 \pm 140$ | $-369.7 \pm 131.2$ | $-45.4 \pm 15.5$ | 781.8±162.4 | 3409.0±134.9 | $-0.6$ |
| | Cluster 3 | $-151.5 \pm 2.1$ | 7 | 44.2±6.7 | $-115.9 \pm 137$ | $-214.2 \pm 30.8$ | $-56.1 \pm 6.5$ | 632.8±115.7 | 3684.8±273.1 | $-0.3$ |
| | Cluster 2 | $-145.4 \pm 10.3$ | 7 | 32.4±5.3 | $-95.1 \pm 7.8$ | $-402.7 \pm 63.6$ | $-52.3 \pm 8.4$ | 824.6±230.0 | 3449.7±164.2 | 0.3 |
| 4. ACE2 + T500ALA + OMICRON | Cluster 1 | $-174.0 \pm 11.9$ | 13 | 46.1±5.2 | $-106.4 \pm 8.5$ | $-314.5 \pm 99.0$ | $-58.8 \pm 10.4$ | 541.6±270.0 | 3543.3±178.1 | $-1.4$ |
| | Cluster 3 | $-161.5 \pm 9.0$ | 9 | 32.4±4.2 | $-121.1 \pm 11.7$ | $-322.9 \pm 73.0$ | $-41.3 \pm 6.2$ | 655.1±680.0 | 3491.0±222.5 | $-0.8$ |
| | Cluster 4 | $-149.5 \pm 8.8$ | 7 | 37.0±3.2 | $-110.0 \pm 153$ | $-275.9 \pm 65.1$ | $-47.6 \pm 5.6$ | 633.0±94.5 | 3535.8±404.0 | $-0.2$ |
| | Cluster 2 | $-147.3 \pm 16.3$ | 10 | 35.6±8.8 | $-108.1 \pm 142$ | $-343.2 \pm 60.4$ | $-34.0 \pm 6.3$ | 635.6±121.4 | 3588.2±105.4 | $-0.1$ |
| | Cluster 3 | $-131.1 \pm 13.9$ | 6 | 38.1±0.6 | $-82.8 \pm 14.4$ | $-441.0 \pm 54.2$ | $-42.3 \pm 5.4$ | 821.9±121.8 | 3335.9±42.5 | 0.7 |
| | Cluster 6 | $-108.5 \pm 32.7$ | 4 | 39.5±5.9 | $-91.2 \pm 9.1$ | $-298.1 \pm 81.1$ | $-40.8 \pm 5.2$ | 828.5±152.9 | 2915.9±195.8 | 1.7 |
| 5. ACE2 + T500CYS + OMICRON | Cluster 5 | $-172.2 \pm 26.4$ | 4 | 8.6±6.4 | $-106.5 \pm 137$ | $-391.1 \pm 95.5$ | $-46.1 \pm 10.8$ | 585.7±119.2 | 3817.6±377.4 | $-1$ |
| | Cluster 1 | $-162.4 \pm 15.5$ | 10 | 27.6±9.0 | $-108.3 \pm 17.5$ | $-303.4 \pm 130.3$ | $-54.3 \pm 14.5$ | 608.6±74.4 | 3530.5±284.7 | $-0.7$ |
| | Cluster 2 | $-159.7 \pm 16.9$ | 9 | 27.3±3.4 | $-105.4 \pm 4.9$ | $-324.2 \pm 52.7$ | $-45.9 \pm 6.8$ | 564.0±147.4 | 3345.2±152.7 | $-0.6$ |
| | Cluster 3 | $-127.2 \pm 19.2$ | 5 | 28.0±7.1 | $-96.8 \pm 5.2$ | $-315.1 \pm 71.0$ | $-36.7 \pm 6.0$ | 694.1±113.2 | 3411.4±349.6 | 0.5 |
| | Cluster 4 | $-93.5 \pm 32.7$ | 4 | 23.2±6.7 | $-92.1 \pm 15.1$ | $-249.0 \pm 45.7$ | $-34.6 \pm 7.1$ | 829.2±110.1 | 2651.1±368.2 | 1.7 |
Table 1). The H-bond length is also observed between amino acid residues (Table 1). On the other hand, molecular docking with ACE2 and Omicron showed no binding affinity and no binding occurs (Table S2).

**Comparative study between 3 spike proteins binding with human ACE2 receptor**

According to the docking results between ACE2, Delta, Omicron, and wild-type SARS-CoV-2, it showed the highest docking score value of $-250.8 \pm 19.4$. The calculation of Van der Waals energy, desolvation energy, and electrostatic energy represented the negative values of $-157.9 \pm 1.4$, $-451.1 \pm 46.0$, and $-63.6 \pm 7.8$, respectively (Table S1). The binding affinity of DELTA and Omicron with ACE2 was $-7.1$ and $-9.9$, respectively (Table S2). But molecular docking with ACE2 and wild-type SARS-CoV2 showed no binding affinity. From the above two results, we analyze that Delta has a stronger binding affinity with ACE2 than wild-type SARS-CoV-2 and Omicron. When we studied MD stimulation of ACE2 binding affinity with 3 different strains together, Omicron variant showed comparable binding affinity with human ACE2 in comparison with Delta strain.

According to Bloom et al. [29], it was found that 9 RBD Omicron mutations (S371L, S373P, S375F, K417N, G446S, E484A, G496S, Q498R, Y505H) should decrease the binding affinity to ACE2 while the other 6 mutations (G339D, N440K, S477N, T478K, Q493K, N501Y) should increase the binding affinity, resulting in a challenge of predicting its transmissibility and potential immune evasion risk. The binding site of ACE2 comprises different amino acids, i.e., GLU329, TYR41, TYR83, and SER19, with which interaction of LYS558, GLN563, LYS462, and GLU471 different amino acids of Delta binding sites were observed, respectively (Fig. 4; Table 1). The binding site of ACE2 comprises different amino acids, i.e., GLU87 and LYS112, with which interaction of ARG498 and ALA372 different amino acids at the Omicron binding sites were observed,

![Fig. 5](image-url) The molecular docking of ACE2, Omicron variant, and mutant 59 cut. (A and B) The enlarged panel on the left side shows the interacting amino acid residues in colored sticks in which ACE2 is in blue and Omicron variant is in orange; (C, D, E) the enlarged panel on right side shows the interacting amino acid residues in which Omicron is in orange and mutant 59 cut is in green.
respectively (Fig. 4; Table 1). The H-bond length is also observed between amino acid residues (Table 1).

**Measurement of binding affinity and kinetics of different CUT segment**

Here, we investigate the blind docking of wild 59, T500S, T500C, T500G, and T500A interaction with ACE2 and Omicron showed the docking score value of $-175.1 \pm 13.8$, $-163.1 \pm 13.8$, $-172.2 \pm 26.8$, $-163.6 \pm 9.4$, and $-174.0 \pm 11.9$, respectively (Table 2). According to binding affinity result, the binding affinity between wild 59 segments and Omicron variant was $-12.5 \Delta G$ (kcal mol$^{-1}$), T500S and Omicron variant $-16.4 \Delta G$ (kcal mol$^{-1}$), T500C and Omicron variant $-12.2 \Delta G$ (kcal mol$^{-1}$), T500G and Omicron variant $-13 \Delta G$ (kcal mol$^{-1}$), and T500A and Omicron variant $-12.3 \Delta G$ (kcal mol$^{-1}$) (Table S2).

Among the mutated CUT segment, we showed that the best binding affinity between CUTs and spike protein was $-16.4 \Delta G$ (kcal mol$^{-1}$) because the lower energy and higher negative value means the higher stability of the complex. Due to the sequence shorting, it may be that T500S cut have higher binding affinity than 84 cut segments. So, we choose only T500S cut segment for further analysis and wild 59 cut segment as a control. After analyzing the H-bond pattern, T500S cut showed 6 interactions with Omicron spike protein at RBD site ranging from 1.9 to 2.1 Å, whereas wild 59 cut showed 5 interactions ranging from 1.9 to 2.8 Å. The binding affinity values and H-bond interactions of T500S were comparatively higher than the wild 59 cut.

The interaction site of T500S cut comprises different amino acids ASN137, TYR 185, VAL 187, ASN 164, and SER 154 with which interaction of ASN450, TYR501, PRO499, CYS488, GLY485, and ALA484 different amino acids of Omicron binding sites were observed, respectively (Fig. 5). Our previous study analyzed the CUT segments of 84 amino acids after molecular docking with ACE2 receptor and SARS-CoV-2; the docked structures were either partial or complete interactive distortions of spike glycoprotein from ACE2 receptor were observed. But when we observed the docking structure of T500S cut segment, Omicron, and ACE2, T500S cut binds at RBD site of Omicron and ACE2 binds at a different position of Omicron not in the RBD active site. The docking structure of WILD59 segment Omicron and ACE2 was shown WILD59 segment binds at RBD site instead of the ACE2 receptor (Fig. 6).

**Molecular dynamics simulation**

The result of molecular dynamics simulation and normal mode analysis (NMA) of different docked complexes of ACE2 and different variants of SARS-CoV 2 is illustrated in Fig. 7. The simulation study was conducted to determine the movements of protein molecules. The main-chain deformability graph of the three complexes is shown in S-Figure:

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![Fig. 6 The molecular docking of ACE2, Omicron variant, and wild 59 cut was presented. (A and B) The enlarged panel on the left side shows the interacting amino acid residues in colored sticks in which ACE2 is in blue and Omicron variant is in orange; (C, D, E, F) the enlarged panel on right side shows the interacting amino acid residues in which Omicron is in orange and Wild 59 cut is in red](image-url)
Fig. 7 The results of molecular dynamics simulation of different variant of SARS-CoV-2 and ACE2 docked complex. A, B RMSD plot and B-factor graph of wild-type SARS-CoV2 and ACE2; C, D RMSD plot and B-factor graph of Delta and ACE; E, F RMSD plot and B-factor graph of Omicron and ACE2.
(1) illustrating the peaks which represent the regions of the protein with deformability. The locations with hinge regions have high deformability. The B-factor is a measure for flexibility in a protein and quantifies the uncertainty of each. The B-factor values calculated by normal mode analysis are proportional to the root mean square shown in Fig. 7. B-factor values quantify the uncertainty of each atom. The B-factor graph gives a clear visualization of the relation of the docked complex between the NMA and the PDB sector. The main-chain deformability graph of the three complexes is shown in S-Figure: (2) illustrating the peaks which represent the regions of the protein with deformability. The locations with hinge regions have high deformability. The covariance matrix between the pairs of residues is shown in S-Figure: (3) indicating their correlations (correlated motion indicated by red color; uncorrelated motion indicated by white color; anti-correlated motion indicated by blue color). So, all our docking complexes are in stable form.

We also examined the molecular dynamic simulation of wild 59 CUTs and T500S 59 CUTs with Omicron variant is illustrated in Fig. 8. The B-factor is a measure for flexibility in a protein and quantifies the uncertainty of each. The B-factor values calculated by normal mode analysis are proportional to the root mean square shown in Fig. 8. B-factor values quantify the uncertainty of each atom. The B-factor graph gives a clear visualization of the relation of the docked complex between the NMA and the PDB sector. The main-chain deformability graph of the three complexes is shown in S-Figure: (2) illustrating the peaks which represent the regions of the protein with deformability. The locations with hinge regions have high deformability. The covariance matrix between the pairs of residues is shown in S-Figure: (3) indicating their correlations (correlated motion indicated by red color; uncorrelated motion indicated by white color; anti-correlated motion indicated by blue color). So, all our docking complexes are in stable form.

The Omicron RBD shows weaker binding affinity than the currently dominant Delta variant to human ACE2 [28]. Here, we experimentally measured how all amino acid mutations to the RBD affect expression of folded protein and its affinity for ACE2. Most mutations are deleterious for RBD expression and ACE2 binding. But a substantial number of mutations are well tolerated or even enhance ACE2 binding, including ACE2 interface residues that vary across
SARS-related coronaviruses. However, we find no evidence that these ACE2-affinity-enhancing mutations have been selected in current SARS-CoV-2 pandemic isolates [30].

The report reveals that infection with Omicron caused an enhancement of Delta virus neutralization, which increased 4.4-fold. This may result in decreased ability of Delta to re-infect those individuals [31]. Omicron mutations enhance infectivity and reduce antibody neutralization of SARS-CoV-2 virus-like particles [32]. Mutations in the RBD of the SARS-CoV-2 Omicron variant result in stronger binding to human ACE2 receptor [33].

In conclusion, the present 59 amino acid RBD cut fraction was found to be a good blocker of the spike-ACE2 binding. Moreover, introduced mutations especially T500S and T500G were highly potent with very high binding affinity to block the ACE2 and Omicron spike binding. The present investigation may explain that Delta variant is significantly stronger than the wild type and Omicron, while there is no significant difference between wild type and Omicron but more research is required to explore its less ability to penetrate the lung tissue. In this case, lung TMPRSS2 may have some role in viral internalization. In regard to the last 2 years’ strategies, global vaccination, and natural infection, the disease outcome and severity of different new variants have become less predictable.

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Author contribution SM-hypothesis study design, critical analysis, and reviewing. DS-experimentation and first draft preparation.

Data availability All data are available upon reasonable requests.

Declarations

Conflict of interest The authors declare no competing interests.

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