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The emerging Omicron variant spike mutation: the relative receptor-binding domain affinity and molecular dynamics

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Summary. – This study aims to fill a knowledge gap in our understanding of Omicron variant receptor-binding domain (RBD) interactions with host cell receptor, angiotensin-converting enzyme 2 (ACE2). Protein-protein docking, scoring, and filtration were all performed using the HDOCK server. A coarse-grained prediction of the changes in binding free energy caused by point mutations in Omicron RBD was requested from the Binding Affinity Changes upon Mutation (BeAtMuSiC) tools. GROMACS was utilized to perform molecular dynamics simulations (MD). Within the 15 mutations in Omicron RBD, several mutations have been linked to increased receptor affinity, immunological evasion, and inadequate antibody response. Wild-type (wt) SARS-CoV-2 and its Omicron variant have 92.27% identity. Nonetheless, Omicron RBD mutations resulted in a slight increase in the route mean square deviations (RMSD) of the Omicron structural model during protein-protein docking, as evidenced by RMSDs of 0.47 and 0.85 Å for the wt SARS-CoV-2 and Omicron RBD-ACE2 complexes, respectively. About five-point mutations had essentially an influence on binding free energy, namely G6D, S38L, N107K, E151A, and N158Y. The rest of the mutations were expected to reduce the binding affinity of Omicron RBD and ACE2. The MD simulation supports the hypothesis that Omicron RBD is more stably bound to ACE2 than wt SARS-CoV-2 RBD. Lower RMSD and greater radius of gyration (Rg) imply appropriate Omicron structure 3D folding and stability. However, the increased solvent accessible surface area (SASA) with a greater Omicron shape may have a different interaction with receptor binding and regulate virus entrance. Omicron RBD's mutations help it maintain its structural stability, compactness, ACE2 binding, and immune evasion.

Keywords: Omicron variant; SARS-CoV-2; COVID-19; phylogenetics

Introduction

There have been more than 577 million confirmed cases and more than 6.4 million deaths in the global coronavirus disease 2019 (COVID-19) pandemic caused by SARS-CoV-2 (WHO COVID-19 pandemic, https://covid19.who.int/, accessed on 2022/8/3). Despite extensive vaccination, the COVID-19 pandemic continues, as SARS-CoV-2 evolves into many forms. Since the November 24th, 2021 discovery of the novel SARS-CoV-2 variation of concern (VOC) Omicron in an immunocompromised patient in South Africa, the variant has quickly surpassed Delta as the most common lineage in the country and spread to over 40 countries (Wang and Cheng, 2022).

Omicron possesses a slew of previously identified mutations in other VOCs, including at least 32 mutations in the spike protein alone (compared to 16 mutations

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Abbreviations: COVID-19 = coronavirus disease 2019; RBD = receptor-binding domain; ACE2 = angiotensin-converting enzyme 2; NSP = nonstructural proteins; MD = molecular dynamic; RMSD = route mean square deviations; Rg = radius of gyration; SASA = solvent accessible surface area

in the already highly infectious Delta form), as well as mutations in other viral replication proteins including nonstructural proteins (NSP), NSP12 and NSP14 (Gao *et al*., 2022). It is possible that SARS-CoV-2 could generate crucial mutations to boost transmission and annul earlier vaccination under the influence of immunological selection (Wang and Cheng, 2022).

Various issues have been raised as a result of the Omicron variant's emergent character, including the source of emergence, the effect of Omicron mutations on vaccination response, the role of mutations on the regulation of host immunity, clinical data, and Omicron spreading potency and lethality. This research aims to 1) determine the impact of Omicron spike mutations on variations in binding free energy between Omicron and the wt SARS-CoV-2 RBD and the host cell receptor ACE2. In this regard, all mutations are studied, including those at the RBD-ACE2 interface and those that do not share the direct virus spike-cell receptor interface. 2) Omicron RBD protein-protein docking with the host ACE2 receptor, and changes in binding confirmation. 3) Wt SARS-CoV-2 and its Omicron variant MD simulation. The analysis criteria include binding stability, changes in protein surface area, protein compactness, and integrity of the 3D structure.

Materials and Methods

Retrieval and handling of Omicron data. The genomes of Omicron variants were retrieved from GISAID website (https://www.gisaid.org/) (Shu and McCauley, 2017). The spike sequence, conversion of DNA to protein, and allocation of RBD sequences were handled by in-house Geneious prime software (Kearse *et al*., 2012).

Structure preparation. In this study, Omicron RBD was built utilizing the previously disclosed structure of wt SARS-CoV-2 RBD in conjunction with the human cell receptor ACE2. The RBD-ACE2 of wt SARS-CoV-2 was obtained from the Protein Data Bank (PDB ID 6m0j). In preparation for docking, water was removed, free forms of RBD and ACE2 were generated and energy was minimized. The Omicron RBD structure was built using the wt SARS-CoV-2 RBD using the SWISS-MODEL server (Waterhouse *et al*., 2018). The resulting RBD was used for template-based homology modeling on the server. The modeling procedure and confirmation of model quality were done as previously described (Kandeel *et al*., 2020, 2021a).

Protein-protein docking. The structure of the Omicron RBD and human ACE2 complex was built using HDOCK server, a hybrid protein-protein docking method (Yan *et al*., 2020). HDOCK uses a fast Fourier transform (FFT)-based search approach to sample all possible binding modes between the interacting partners (He *et al*., 2020). To process the structure by HDOCK program, the wt SARS-CoV-2 RBD and the host receptor ACE2 were used as a starting position. An iterative knowledge-based scoring approach called ITScorePP was used to evaluate all of the binding possibilities that were tested in the sample. The binding modes were sorted according to their binding energy levels at the end of the process, and all viable binding modes were downloaded for further investigation. In the docking computations for the RBD-ACE2, all of the default settings were utilized. In a summary, the grid spacing for 3D translational search was set to 1.2, the angle interval for rotational sampling was set to 15, and the PDB binding interface information for individual structures was automatically utilized during template-based modeling of individual structures.

SARS-CoV-2 spike-receptor interface mutational analysis. Requests for the effect of mutations on spike-RBD binding affinity were submitted to the BeAtMuSiC server (Dehouck *et al*., 2013). The method is based on a coarse-grained prediction of the binding free energy changes that result from point mutations. Mutation-induced changes to the stability of the complex can be predicted by using statistical probabilities that are derived from known protein structures. The input structures were the wt SARS-CoV-2 RBD-ACE2 (6m0j) or wt SARS-CoV-2 RBD spike-ACE2 (7df4) complexes and the Omicron RBD-ACE2 generated structure model.

Molecular dynamic (MD) simulations. To execute molecular dynamics simulations on the protein, GROMACS v. 2021 was used. The simulations made use of the nucleic AMBER94 forcefield and the AMBER99SB-ILDN protein. The protein system composed of 3036 atoms was immersed in a 1.2 nm cubic box containing 3-point model (TIP3P) water, and the complex was ionized using NaCl molecules and neutral pH conditions. Each simulated system was subjected to energy minimization using the steepest descent approach in a maximum of 2000 steps, followed by two stages of equilibration simulations with position restraints. At 310 K, the constant-temperature and constant-volume (NVT) ensemble was used for 100 ps, followed by the constant-temperature and constant-pressure ensemble (NPT). The MD simulation was run for 100 ns at 310 K with no position constraints. Periodic boundary conditions were applied to all directions. Bond limitations were modeled using the LINCS algorithm, with a time step of 2 fs. The PME technique was used to describe short-range nonbonded interactions with a twin-range cutoff of 0.8 nm and long-range electrostatic interactions with a Fourier grid spacing of 0.12 nm. To evaluate simulation results, various GROMACS utilities were utilized. Secondary structure was determined using the do_dssp tool, hydrogen bonds using the hbond tool, surface accessible area using the sasa tool, and gyration using the gyrate tool. The diagrams were created using Grace, and the frames were visualized using Pymol.

Results and Discussion

The location of Omicron variant RBD mutations

In Omicron RBD, there were 15 mutations. Seven mutations were facing the solvent (G6D, S38L, S40P, S42F,

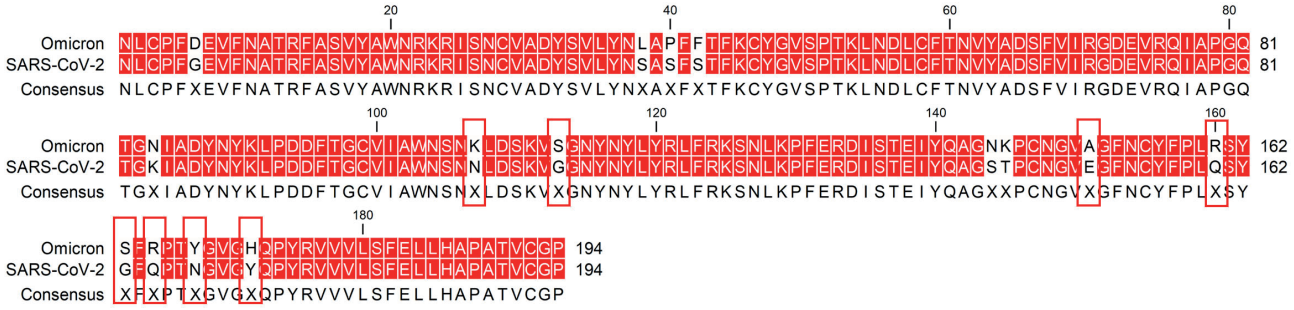

Fig. 1

Pairwise sequence alignment of RBD from wt SARS-CoV-2 and its Omicron variant
The conserved residues are highlighted in red. The mutations at RBD-ACE2 interface are enclosed in boxes.

N107K, S144N, and T145K) and eight mutations were at the RBD-ACE2 interface (K84N, G113S, E151A, G163S, N158Y Q160R, Q165R, and Y172H) (Fig. 1). The significant number of mutations found at the RBD necessitated additional research using mutational analysis of the wt SARS-CoV-2 RBD to determine its impact on binding free energy.

The significance of Omicron mutations

For attachment, fusion and internalization, the SARS-CoV-2 spike glycoprotein interacts with the human ACE2 receptor. As a result, most prophylactic drugs and vaccinations are designed to prevent attachment or entry steps by preventing or neutralizing spike and ACE2 interactions. Therefore, mutations in this part of the virus could be associated with changes in virus infectivity and interfere with the response to immunization. In the Omicron variant, several mutants as K84N, S144N, T145K, E151A, and N158Y were found to improve affinity with receptors or immune escape (Ho *et al.*, 2021; Li *et al.*, 2020; Starr *et al.*, 2020). A shift to basic residues was detected among the eight alterations at the Omicron

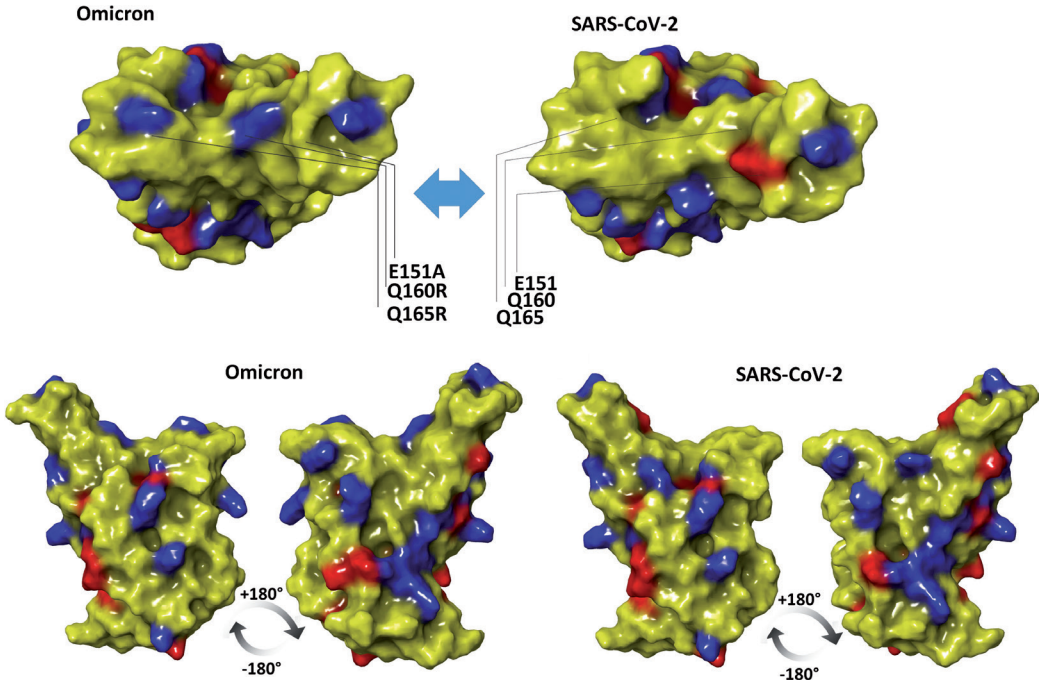

Fig. 2

Surface representation of wt SARS-CoV-2 and Omicron RBD
(a) The interface colored by charge in wt SARS-CoV-2 and Omicron RBD. Positive charge is represented by blue, whereas the negative charge is represented by red. **(b)** The solvent-facing surfaces of wt SARS-CoV-2 and Omicron RBD.

receptor-binding motif. Three acidic residues were found in close proximity to the receptor at the receptor-binding motif, Y172H, Q160R, and Q165R (Fig. 2). At the interface of RBD-ACE2, three important mutations were found E151A, Q160R, and Q165R. More importantly, the formal mutation E151A has been associated with resistance and poor immunological response, while the latter two mutations Q160R and Q165R have been associated with stronger binding with the receptor, inferring higher infectivity (Greaney *et al*., 2021). The susceptibility to pseudotyped Omicron, as well as other variants like Alpha, Beta, Gamma, and Delta, was tested in serum samples from COVID-19 convalescent patients infected with the original SARS-CoV-2 strain. In comparison to 1.2–4.5 folds in other variations, the mean neutralization ED50 of these sera against Omicron was lowered by 8.4 times (Zhang *et al*., 2022). This shows that the Omicron version was able to evade the consolidated immunological response to SARS-CoV-2.

N158Y, E151K, S144N, and most importantly, Q156R were the mutations fixed for increased affinity binding (Zahradnik *et al*., 2021). A large number of mutational changes in Omicron result in a significant decrease in the neutralizing capacity of immunological serum and the failure of immune protection (Dejnirattisai *et al*., 2022). Though it appears to have a negative impact on vaccine efficacy and vaccine breakthroughs are expected, there are hopes for T-cell-mediated protection from severe disease in the event that vaccines do not fully fail. The combination of several mutations can increase the binding affinity several folds, for instance, Q498R in combination with N501Y mutation showed a 26-fold increased affinity (Dejnirattisai *et al*., 2022).

There are restrictions on ACE2 folding and binding, according to a recent SARS-CoV-2 RBD mutational screening (Starr *et al*., 2020). Multiple modifications, including those in the ACE2 interface residues that varied between coronaviruses connected to SARS, increased or maintained RBD production and ACE2 binding despite the fact that the majority of these mutations were harmful (Starr *et al*., 2020).

Table 1. List of mutations in Omicron RBD and their impact on the binding free energy ΔΔGBind (kcal/mol). The values were estimated by the BeAtMuSiC server

No. (spike)	No. (RBD)	ΔΔGBind (kcal/mol)	At the RBD/ACE2 interface.
G339D	G6D	-0.07	No
S371L	S38L	-0.08	No
S373P	S40P	0.32	No
S375F	S42F	0.25	No
K417N	K84N	0.27	Yes
N440K	N107K	-0.07	No
G446S	G113S	0.97	Yes
S477N	S144N	0.08	No
T478K	T145K	0.31	No
E484A	E151A	-0.02	Yes
Q493R	Q160R	0.71	Yes
G496S	G163S	0.36	Yes
Q498R	Q165R	0.55	Yes
N501Y	N158Y	-0.08	Yes
Y505H	Y172H	1.43	Yes

Protein-protein docking

Molecular modeling tools are gold standards in microbial evolution and drug discovery against selected molecular targets (Kandeel *et al*., 2021a,b; Mahmoud Kandeel Elsayed, 2021). Figure 3 shows the docking of Omicron RBD with ACE2 receptor. Wt SARS-CoV-2 and its Omicron variant have a 92.27% sequence identity. Nonetheless, mutations in Omicron RBD, as well as the high sequence identity of wt SARS-CoV-2 and its Omicron variant, resulted in a slight increase in RMSD of the Omicron structural model during protein-protein docking, as indicated by the wt SARS-CoV-2 and Omicron RBD-ACE2 complexes RMSD of 0.47 and 0.85 Å, respectively (Fig. 3b).

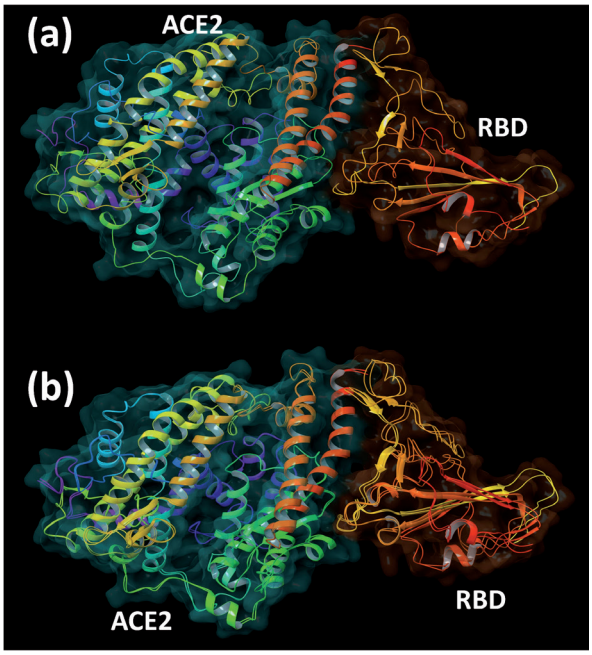

Fig. 3

Protein-protein docking of RBD and ACE2
(a) The produced model for RBD binding with ACE2. **(b)** Alignment of Omicron and wt SARS-CoV-2 RBD-ACE2 structures.

The mutations caused a slight increase in Omicron structure RMSD.

Analysis of mutations impact on the binding force by BeAtMuSiC server

Table 1 summarizes the mutations in Omicron structure and its calculated changes in the binding free energy estimated by the BeAtMuSiC server. The effect of point mutations on binding free energy was almost negative, with five variants related to negative ΔΔGBind, namely G6D, S38L, N107K, E151A, and N158Y, indicating improved binding strength. As a result of the mutations discovered in RBD, ten mutations were projected to decrease binding affinity and five mutations to increase the binding affinity of Omicron RBD and ACE2. While experimental data on Omicron infectivity continue to expand, recombinant Omicron proteins are currently being produced in order to determine their ACE2 binding affinity. A limitation of this technique is that it does not incorporate all probable mutations into a single estimate of affinity alterations. Rather, it examines the impact of specific alterations. As a result, an energy maturation experiment that includes all previously identified RBD mutations could reveal altered binding strength.

In order to examine the binding interactions between the human ACE2 protein and Omicron RBD, atomistic MD simulations have been performed (Lupala *et al.*, 2022). Researchers have found a stronger affinity between the human ACE2 protein and the Omicron RBD strain. By boosting hydrogen bonding contact and expanding hidden solvent accessible surface area, the

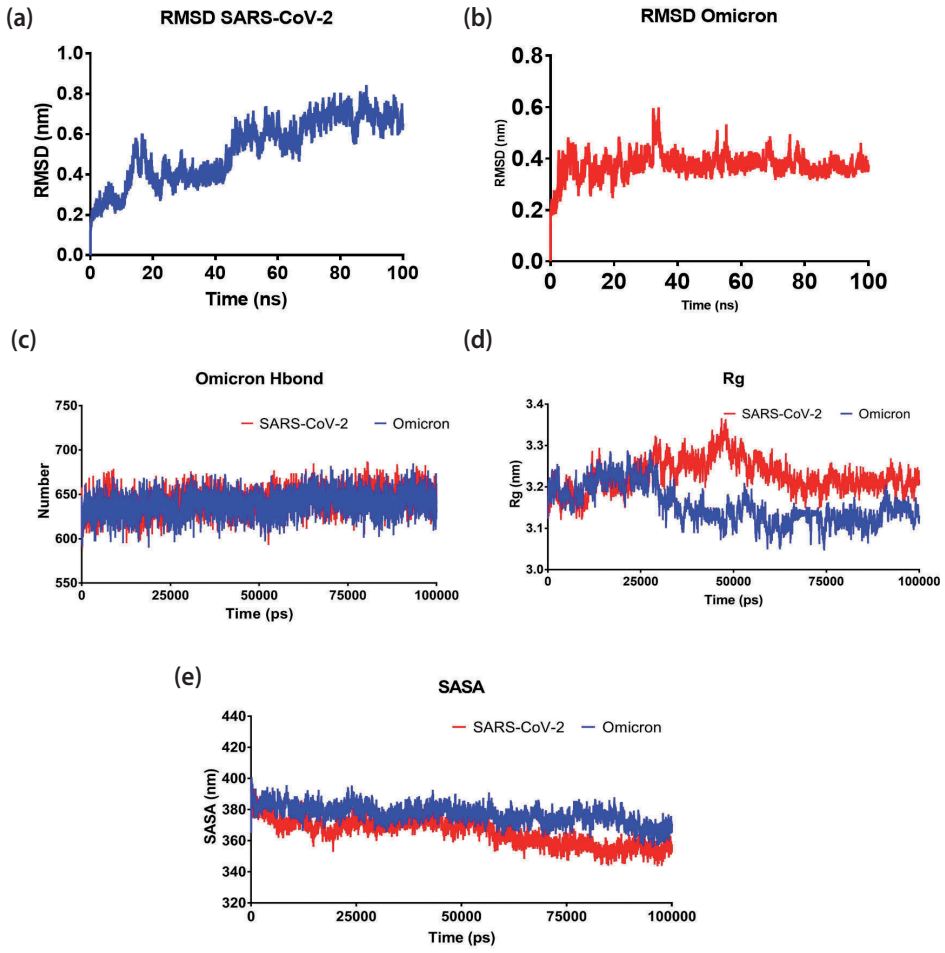

Fig. 4

MD simulations of RBD

(a) The RMSD of wt SARS-CoV-2 RBD-ACE2 complex during 100 ns MD simulation. **(b)** The RMSD of Omicron RBD-ACE2 complex during 100 ns MD simulation. **(c)** The hydrogen bonds of wt SARS-CoV-2 and Omicron RBD-ACE2 complex during 100 ns MD simulation. **(d)** The Rg of wt SARS-CoV-2 and Omicron SARS-CoV-2 and Omicron RBD-ACE2 complex during 100 ns MD simulation. **(e)** The SASA of wt SARS-CoV-2 and Omicron SARS-CoV-2 and Omicron RBD-ACE2 complex during 100 ns MD simulation.

alterations at the ACE2-RBD interface improve tight binding.

Comparison of wt SARS-CoV-2 and Omicron RBD-ACE2 stability and compactness

After 100 ns of MD simulation, it was evident that the Omicron RBD-ACE2 complex had more stable binding than the wt SARS-CoV-2. The RMSD of the alpha carbon atom of the RBD-ACE2 complexes is shown in Fig. 4a,b. The Omicron structure exhibits a smaller RMSD and gained stability faster than the wt SARS-CoV-2 structure, as seen in the image. The average RMSD for Omicron and wt SARS-CoV-2 structures were 0.357 and 0.523 nm, respectively. This displays Omicron's reduced RMSD value and greater stability. During the simulation, both structures formed nearly identical hydrogen bonds (Fig. 4c). The Omicron RBD-ACE2 complex had lower Rg values (Fig. 4d), indicating that both structures are compact and that the Omicron structure has greater structure compactness. During the simulation, the Omicron SASA structure was slightly larger than the wt SARS-CoV-2 structure, as a result of the Omicron mutations (Fig. 4e). Owing to a large number of mutations in Omicron RBD, SASA of wt SARS-CoV-2 structure cannot be compared with the Omicron structure.

The wt SARS-CoV-2 RBD and ACE2 receptors have been studied using computational modeling and dynamic simulations. The Omicron RBD and ACE2-RBD complexes according to the MD simulation were well-defined and stable (Kandeel and El-Deeb, 2022; Wang *et al*., 2020).

Conclusion

Several assumptions were made to predict the Omicron variant's likely emerging pattern, one of which was, that spike mutations boosted the spike's ability to attach to the ACE2 receptor on the host cells. The Omicron variant binding has suggested a stable binding with ACE2 in this work using several methods. Within 15 mutations, only five mutations had negative free energy and support the strong binding, albeit 10 other mutations negatively contributed to the binding. The MD simulation supports the premise that Omicron RBD is more likely to show stable structures than wt SARS-CoV-2 RBD with ACE2. Within these parameters, lower RMSD and higher Rg indicate the proper Omicron structure 3D folding and stability. The higher Omicron structure SASA might also suggest altered interaction with receptor binding and modulation of the virus entry process.

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