

The Omicron Binding Mode: Contact Analysis and Dynamics of the Omicron Receptor Binding Domain in Complex with ACE2

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ABSTRACT: On 26 November 2021 the WHO classified the Omicron variant of the SARS-CoV-2 virus (B.1.1.529 lineage) a variant of concern (VOC)¹. The Omicron variant contains as many as 26 unique mutations of effects not yet determined². Out of its total of 34 Spike protein mutations, 15 are located on the receptor-binding domain (S-RBD)³ that directly contacts the ACE2 host receptor and is also a primary target for antibodies. Here, we studied the binding mode of the S-RBD domain of the Spike protein carrying the Omicron mutations and the globular domain of human ACE2 using molecular dynamics (MD) simulations. We identified new and key Omicron-specific interactions such as R⁴⁹³ (of mutation Q493R), which forms salt-bridges both with E³⁵ and D³⁸ of ACE2, Y⁵⁰¹ (N501Y), which forms an edge-to-face aromatic interaction with Y⁴¹, and Y⁵⁰⁵ (Y505H), which makes an H-bond with E³⁷ and K³⁵³. The glycan chains of ACE2 also bind differently in the WT and Omicron variants in response to the different charge-distribution of the surface of the Spike proteins. However, while the Omicron mutations considerably improve the overall electrostatic fit of the two interfaces, the total number of specific and favorable interactions between the two does not increase. The dynamics of the complexes are highly affected too, making the Omicron S-RBD:ACE2 complex more rigid; the two main interaction sites, Patch I and II, isolated in the WT complex, become connected in the Omicron complex through the alternating interaction of R⁴⁹³ and R⁴⁹⁸ with E³⁵, and D³⁸.

1. INTRODUCTION

Due to an unprecedented, focused effort of the scientific community, much has been discovered concerning the host invasion and replication cycle of SARS-CoV-2 coronaviruses⁴⁻¹¹. Since the outbreak of the COVID-19 pandemic (officially classified as such in March of 2020)¹², there has been a large diversification of the SARS-CoV-2 viral genome circulating worldwide. The most notable variants to date (Variants of Concern (VOCs)), the B.1.1.7 Alpha, B.1.351 Beta, P.1 Gamma, and B.1.617.2 Delta variants¹³⁻¹⁵, carried a moderate number of amino acid changes as compared to the original Wuhan variant. However, in the currently dominant B.1.1.529 Omicron virus 50 non-synonymous mutations were identified. The virus Spike protein, responsible for host receptor recognition, carries 34 mutations, 15 of which are located on the receptor binding domain (S-RBD), the outermost interacting edge of the Spike protein, which resulted in unparalleled increase in infection rate and immune evasion. Somewhat unexpectedly however, the Omicron mutations also reduced the effectiveness of Spike protein cleavage by furin (one of the key steps of the Spike-activation cascade) and of cell-cell fusion^{16,17}, also demonstrating a greatly varied effect on the efficiency of cell invasion of different cell lines¹⁶⁻¹⁹. Overall, the mutations seem to have created a virus that poses less severe clinical implications than the previous Delta variant²⁰. Omicron is the first VOC where infectivity and virulence are so clearly decoupled which underlines the need for a yet more detailed understanding of the mechanisms involved in host invasion by SARS-CoV-2.

The human angiotensin-converting enzyme 2 (ACE2) has been recognized as the most important receptor of SARS-CoV-2²¹⁻²³ thus, the structure and strength of the Spike:ACE2 complex is considered one of the major determinants of viral infectivity and symptom development (**Fig 1**). However, thus far markedly contradicting experimental results have emerged concerning the effectiveness of the Spike:ACE2 interaction in case of the Omicron variant^{17,24-28}, ranging between 3-fold increase and 15-fold decrease in affinity as compared to that of the original Wuhan form (WT).

The cryo-electron microscopy (cryo-EM) derived and crystal structure of the wild type Spike RBD (WT S-RBD):ACE2 complex was solved (PDB ID: 6M17 and 6M0J)^{29,30}, which show an $\sim 900 \text{ \AA}^2$ buried surface area upon complexation. The interaction surface, from the Spike protein's side consist of three loops^{31,32} (those of 474-485, 488-490, and 494-505) along with nearby supporting residues, which face the first two helices and the neighboring residues of ACE2 in the complex. The mobile loops, constituting the receptor binding motif of the S-RBD, are stabilized when bound to ACE2, forming several intermolecular H-bonds (Y⁴⁴⁹, Q⁴⁹², Q⁴⁹⁸), hydrophobic interactions (Y⁴⁷³, A⁴⁷⁵, F⁴⁸⁶), and salt bridges (K⁴¹⁷). No less than 10 of the 15 Omicron S-RBD mutations are located on this interface constituting nearly half of the interacting residues, suggesting that both conformation and dynamics might be largely affected by the changes.

Recently, both cryo-EM derived and crystal-structures of the Omicron S-RBD:ACE2 complex were also deposited into the PDB (ID: 7T9L³³, 7WBP and 7WBL²⁷), highlighting several changes on the interface. The relatively similar structures (the backbone trace of the interaction surfaces (see Methods) can be fitted with an RMSD of 0.46-0.73 Å) show an extensive H-bonding network comprised of D³⁸, Q⁴², and K³⁵³ from the ACE2 side, and Y⁴⁴⁹, S⁴⁹⁶, Y⁵⁰¹, and G⁵⁰² from the S-RBD side. Besides this, H-bonds are present between H³⁴-Y⁴⁵³, E³⁵-R⁴⁹³, Y⁴¹-T⁵⁰⁰, and Y⁸³-N⁴⁸⁷, along with a hydrophobic cluster created by F²⁸, L⁷⁹, M⁸² and Y⁸³ in ACE2, and F⁴⁸⁶ in S-RBD. Two other, smaller networks are formed between K³⁵³ from the ACE2 side, and G⁴⁹⁶ and G⁵⁰² from the S-RBD side, and also between Q²⁴ and Y⁸³ from the ACE2 side, and N⁴⁸⁷ from the S-RBD side. An H-bond between Y⁴¹-T⁵⁰⁰, and a salt bridge between D³⁰-K⁴¹⁷ are also present.

As both the Spike and ACE2 are glycoproteins³⁴, (contributing significantly to the immune evasion³⁵ of the former in the vicinity of the latter) it is important to consider the effects of the glycan-conjugated side-chains at the glycosylation sites. The extracellular domain of ACE2 has seven *N*-glycosylation sites, namely N⁵³, N⁹⁰, N¹⁰³, N³²², N⁴³², N⁵⁴⁶ and N⁶⁹⁰, out of which N⁵³, N⁹⁰, N¹⁰³ and N³²² are all capable of interacting with the S-RBD within the complex. In contrast, the S-RBD has only one glycosylation site, that of N³⁴³, approximately 26 Å away from the interface. It has been reported, that these glycosylated residues can both enhance^{36,37} (ACE2 N³²²) or weaken (ACE2 N⁹⁰) the interaction between the two proteins³⁸. Due to the large number of mutations on the Omicron RBD and the number and relative size of these glycan-modifications, it can be expected that the mutated residues exert some kind of effect on the glycans and *vice-versa*. However, in the cryo-EM and crystal-structures these glycan modifications are only partly visible due to their highly mobile nature.

Here we carried out MD simulations to assess the structural and dynamic effect of the Omicron mutations on the S-RBD:ACE2 complex, using fully glycosylated models of both the WT and the mutant variant (**Fig 1**). Such MD simulations have proved useful for clarifying the molecular details of the virus' behavior³⁹⁻⁴³. We found that while the Omicron mutations create a favorable change in overall electrostatics, the S-RBD:ACE2 complex is not strengthened due to steric un-complementarity at the crowded interaction surface. We also found that the dynamics of the complex is altered by the Omicron mutations. Through an anchoring H-bond network - located in the middle of the interaction surface, connecting the two previously recognized hot-spots, Patch I and II - the Omicron complex interface becomes more rigid, suggesting a larger loss in degrees of freedom and unfavorable $\Delta\Delta_{\text{bind}}S$.

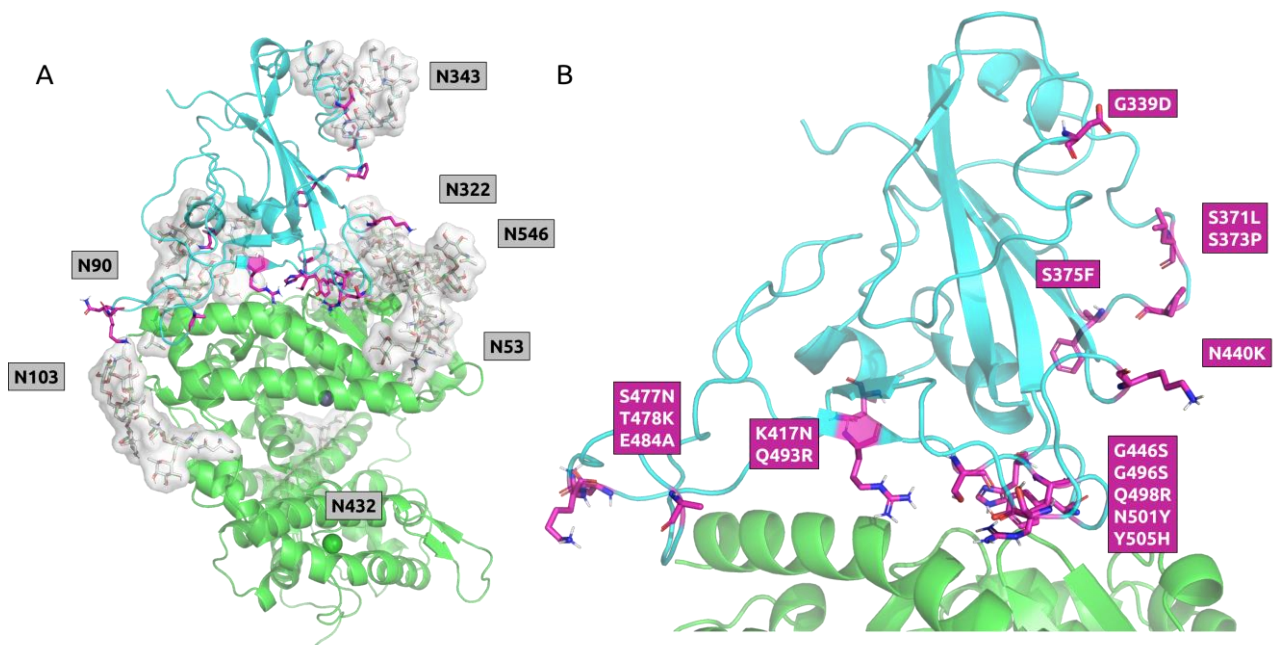


Figure 1. **A)** Structures of the Omicron S-RBD domain (cyan) bound to ACE2 protein (green) and all the 7 glyco-antennas associated (gray) with. **B)** All the 15 Omicron S-RBD specific mutation sites are depicted (magenta), of which 10 are located at the interface.

2. RESIDUE-SPECIFIC INTERACTIONS

To obtain directly comparable results, both simulations were started from the crystal structure of the WT S-RBD:ACE2 complex (PDB ID: 6M17)³⁰, introducing the Omicron mutations manually without changing the backbone structure of the complex. The obtained equilibrium ensemble of the two states show appreciable similarity to the experimentally determined structures of the corresponding complexes (with an RMSD of 1.43Å for the backbone trace

of the interaction surfaces of the two proteins when the WT calculated ensemble and the cryo-EM structure of 6M17 is compared, and 1.41 Å, 1.45 Å, and 1.33 Å for the same atom set of the calculated model set of the Omicron variant:ACE2 complex when compared to structures 7WBP, 7WBL and 7T9L, respectively) – which is especially significant in case of the simulation concerning Omicron variant, which achieved this degree of similarity despite being initiated from the structure of the WT-ACE2 complex.

Residue-residue contacts of the key interacting residue pairs of the simulated WT and Omicron RBD:ACE2 complexes are compared on **Fig S5-11**. From a global perspective, the interface of the Omicron S-RBD becomes more positively charged, when compared to its WT counterpart. The E484A mutation diminishes a negative charge, while the T478K, Q493R and Q498R mutations introduce positive charges at the interface, overall (also including the opposing effects of K417N and N440K mutations) a +4 electrostatic change is acquired. These alterations could have occurred to enhance the electrostatic attraction towards the ACE2 interface, which is predominantly negatively charged. The ACE2 helix 1 (H1, residues between 21-52) contains 6 negatively charged and only 2 positively charged residues, making it highly negative and therefore more easily targeted by the Omicron S-RBD interface (**Fig 2/C**, **Fig S12-14**).

The interacting residues of the two proteins can be divided into two independent hot-spots at either end of the contact surface – Patch I and II (following the nomenclature of Wanchao Yin *et al.*³³) – separated by a segment along which no specific interaction is formed, in case of the WT S-RBD:ACE2 complex. (**Fig 2/A** and **Fig 2/B**).

The Q493R Omicron mutation occurs at this critical spot and determines the flexibility of both Patch I and II contacts. The WT Q⁴⁹³ residue interacts with K³¹ and E³⁵, both within Patch I, in an anti-correlated manner. In fact, the Spearman correlation coefficient (SpR) between the Q⁴⁹³-K³¹ and the Q⁴⁹³-E³⁵ interaction distance is -0.39. In the Omicron variant R⁴⁹³ changes interaction partners: instead of contacting K³¹, it forms salt-bridges with E³⁵ in 70% of the time, and with D³⁸ in 30% of the time. In contrast to the WT, the SpR coefficient changes to -0.54 in case of the R⁴⁹³-E³⁵ and the R⁴⁹³-D³⁸ distance-pair, making these distances more dependent on each other. Considering all this, upon the Q493R mutation, a solitary Patch I interaction (Q⁴⁹³-K³¹, E³⁵) is replaced by an alternating bidentate E³⁵-R⁴⁹³-D³⁸ salt bridge network, linking Patch I and Patch II through the newly introduced guanidino group. This can partly restrict the movement of the S-RBD interface with respect to H1. Interestingly, when comparing the crystal- and cryo-EM structures of the of Omicron S- RBD:ACE2 complex (7WBP, 7WBL - neither of which is of sufficiently high resolution that would allow unequivocal assignment of sidechain conformations), it is this region that shows the greatest differences – with H³⁴, D³⁸ and R⁴⁹³ in characteristically different conformations in the two. Thus, the multi-state nature of this interaction hot-spot is reflected in the experimental results too.

In case of the Q498R mutation, again, a solitary Patch II interaction (Q⁴⁹⁸-Y⁴¹) is replaced by a loosely correlated bifurcated interaction (D³⁸-R⁴⁹⁸-Q⁴²), where the distance SpR is 0.34. However, since D³⁸ already participates in a salt bridge with R⁴⁹³, the R⁴⁹⁸-D³⁸ interaction only occurs in about 22% of the time. The distance SpR between R⁴⁹³-D³⁸ and R⁴⁹⁸-D³⁸ is -0.02, indicating that these distances can change independently of another. Nevertheless, this interaction further extends the anchoring of the Patches.

The N501Y change replaces a H-bond with Y⁴¹ of the WT S-RBD:ACE2 complex with a fixed σ - π interaction in the Omicron variant. This way, the partially polarized C ^{ϵ} (δ^-)-H(δ^+) bond of Y⁵⁰¹ can interact with the negatively charged π electron cloud of Y⁴¹. This mutation is also present in the Alpha, Beta, Gamma, and Mu variants³ and is considered stabilizing⁴⁴⁻⁴⁶. The Y505H mutation, which has not been observed before, replaces a stable Y⁵⁰⁵-R³⁹³ H-bond and a partial Y⁵⁰⁵-E³⁷ H-bond of the WT complex with the H⁵⁰⁵-E³⁷ and H⁵⁰⁵-K³⁵³ H-bond. These interactions are highly anti-correlated with a SpR of -0.72.

Interestingly, not all Omicron mutations lead to improvement of the local interaction network. In the WT complex the S-RBD N⁴⁴⁰ and G⁴⁴⁶ interact with N⁵³ and Q⁴², respectively, while within the Omicron complex the mutated residues K⁴⁴⁰ and S⁴⁴⁶ make no contacts with these or any other residues of ACE2. Overall, in the central region of the interaction surface the introduction of the Omicron mutations fortifies the inter-chain contacts, but it also shifts the distribution of the total number of interchain H-bonds (at each snapshot), decreasing the average from 10.7 (SD: 2.2) seen in case of the WT complex to 9.9 (SD: 1.9) (**Fig S17**). In order to assess the binding free energy difference of the two systems, we used the EMBL-EBI PISA server to estimate the solvation free energy gain upon formation of the complexes (see the Materials and Methods section). For the WT complex this resulted in a -8.1 kcal/mol value (SD: 1.3 kcal/mol), while for the Omicron mutant complex this resulted in a less favorable -6.2 kcal/mol value (SD: 1.3 kcal/mol).

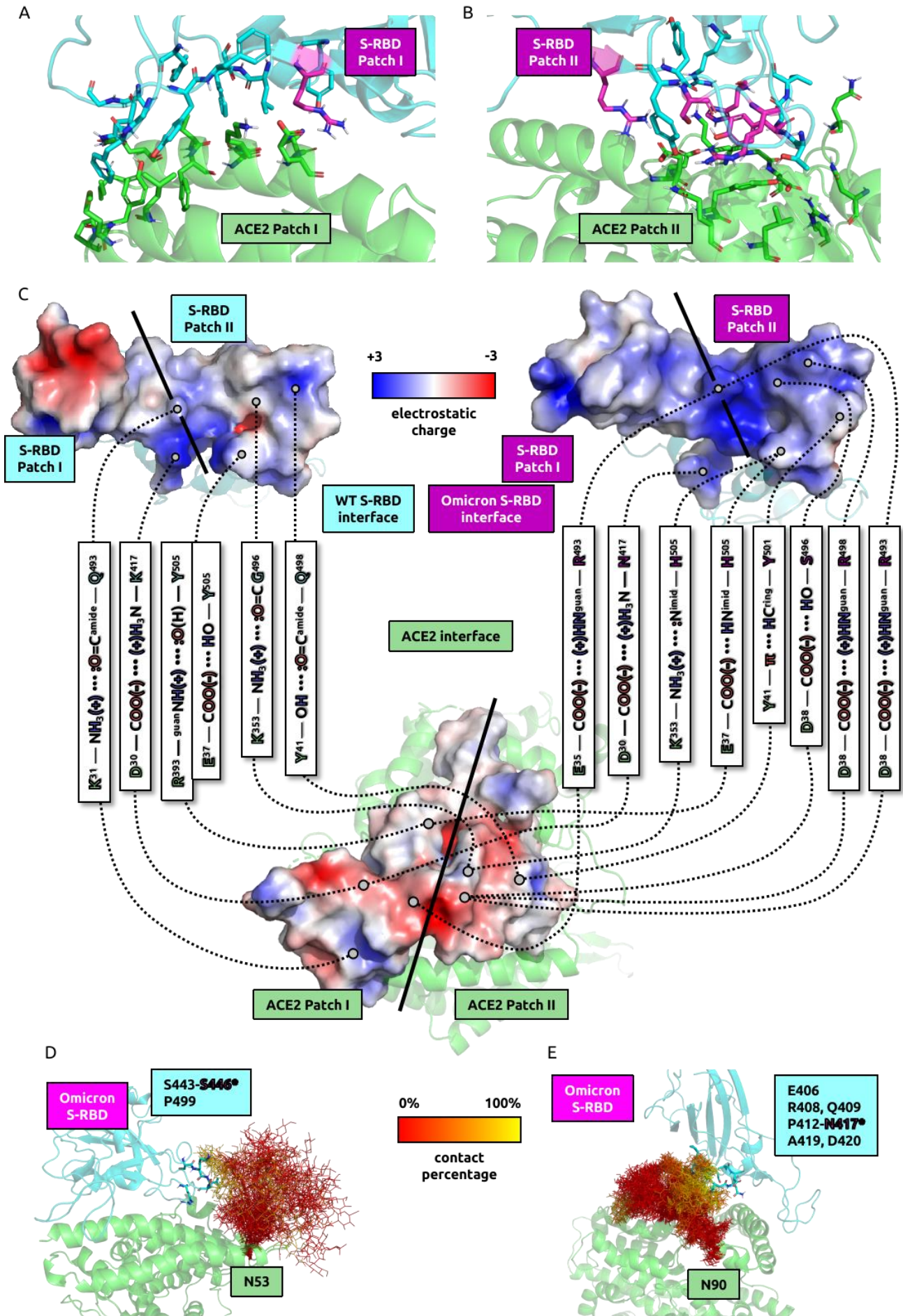


Figure 2. Patch I (A) and II (B) of the Omicron S-RBD:ACE2 interaction surface (shown on the central structure of the most populated cluster of the MD simulation). Patch I consists of residues 21-35 in H1, and residues 79-84, present in the C-terminal part of helix 2 (H2, residues 56-84) of ACE2, and 453-456, 473-476, and 485-493 of S-RBD. Patch II

comprises the 37-45 (part of H1) and 353-357 segments, along with N³³⁰ of ACE2, and residues 493-505 of S-RBD, along with Y⁴⁴⁹. Mutation sites are highlighted with magenta. It can be seen that R⁴⁹³ can interact with both Patch I and Patch II residues (mostly with E³⁵ and D³⁸). **C**) The Poisson-Boltzmann electrostatic surfaces of the WT S-RBD interface, the Omicron S-RBD interface, and the ACE2 interface. The surfaces are color-coded with a blue-white-red spectrum ranging from charge +3 to -3. Residue-residue interactions are denoted by dashed lines and their corresponding text-boxes. Patches are separated with tilted, bold lines also indicating their mode of superposition on ACE2. **D**) and **E**) Glycan ensembles of N⁵³ and N⁹⁰, respectively. The glycan-atoms are color-coded with a red-yellow spectrum, ranging from 0% to 100% according to their contact frequencies with the corresponding glycan's S-RBD contact residues.

3. GLYCAN-SPECIFIC INTERACTIONS

Both the S-RBD and the ACE2 contain *N*-glycosylation sites. Some of the glycan-modifications at these sites are sufficiently long to directly interact with the other protein partner and cause differences in the binding mode and dynamics. These modifications show micro-heterogeneity, thus for the simulations we considered the most abundant glycan-modification at each site⁴⁷ (**Fig S15**).

Geometrically, the glycans at positions 53, 90, 103, and 322 on the ACE2, and 343 on the S-RBD are the ones capable of interacting with residues and glycans from the other chain. Despite this, only the N⁵³, N⁹⁰ and N¹⁰³ ACE2 glycans form contacts with the S-RBD. The glycan at N³²² of the Spike is bound to the homo-chain N⁵⁴⁶ glycan in both considered systems, keeping it away from the S-RBD residues. The N³⁴³ glycan on the S-RBD also forms homo-chain contacts, far (residues 364-371) from the S-RBD:ACE2 interface.

The glycan N¹⁰³ of ACE2 only shows contact with 2 residues from the S-RBD, namely F⁴⁸⁶ and N⁴⁸⁷ in the Omicron complex, and no contacts in the WT complex. In case of N⁵³ we obtained similar results for the WT and the Omicron S-RBD:ACE2 complex. The common contacting amino acids are S⁴⁴³, K⁴⁴⁴, V⁴⁴⁵, and P⁴⁹⁹. The residues N⁴³⁹, N⁴⁴⁰, L⁴⁴¹, D⁴⁴², and Q⁴⁹⁸ only contact with N⁵³ in the WT complex, while S⁴⁴⁶ only contacts in case of the Omicron complex, slightly differentiating the behavior of this glycan in the two cases. The main contacting sugar subunit is the terminal *N*-acetyl glucosamine (NAcGlc) on the C⁶-branch of the central mannose subunit. The C³-OH and C⁴-OH groups of this NAcGlc are closer than 4.0 Å to the contacting residues for the whole length of the trajectory examined, forming H-bonds with the amide groups of these residues.

The glycan chains of N⁹⁰ behave radically different in the two complexes; they share only one common contacting residue, namely D⁴²⁰, and bind to different patches on the S-RBD. Since in our simulation this glycan contains a sialic acid subunit, which is present in a deprotonated form, the large change in the S-RBD electrostatics possibly resulted in the relocation of its binding region. In the WT complex it is bound to D⁴²⁰-Y⁴²¹, F⁴⁵⁶-R⁴⁵⁷, S⁴⁵⁹-L⁴⁶¹, I⁴⁷²-G⁴⁷⁶, C⁴⁸⁰ and its main contacting sugar subunits are ones on the C³-branch *i.e.*, the one terminating in the sialic acid moiety. These contacts are realized through H-bonds of the sugar OH-groups and the *N*-acetyl groups of the NAcGlc and sialic acid, but not through the negatively charged carboxylate, which faces towards the bulk solvent. In contrast, in the Omicron complex the N⁹⁰-glycan of ACE2 binds to the residues R⁴⁰⁸-Q⁴⁰⁹, P⁴¹²-N⁴¹⁷, A⁴¹⁹ and D⁴²⁰ of the Spike-protein. While in the WT complex only the C³-branch contacted with the S-RBD, in this case both the C³-, and the C⁶-branches remain proximal to these residues. The subunits responsible for these contacts are the sialic acid (from the C³-branch) and the terminal galactose (from the C⁶-branch). Besides the typical sugar-OH...O=C-residue and sugar-(H)O...HN-residue interactions, the carboxylate of the sialic acid is in a H-bond with Q⁴¹⁴ in one of the trajectories, and also stays close to R⁴⁰⁸, although it does not make a direct contact with it. In the other trajectory the sialic acid moiety participates in intra-chain interactions around the residues Q⁵⁵², N⁵⁵⁶, and R⁵⁵⁹ of the Spike protein.

4. GLOBAL DYNAMICS

For the analysis of the complexes' global movements, first we applied principal component analysis (PCA) along the trajectory structures. Schematic representation of the first three principal movements for the WT and the Omicron complex can be seen on **Fig 3** and **Fig S19**.

Movement along the first principal axis in the WT complex results in a sliding/rocking motion of the S-RBD interface on the ACE2 interface. While a large portion (residues 497-505) of the S-RBD Patch II moves in unison with the ACE2 interface, Patch I rotates towards H2 with H1 as its axis of rotation. This indicates that Patch I is a loosely bound interface compared to Patch II. In the movements along the second and third principal axes we cannot observe such uncorrelated motion between the S-RBD and the ACE2 side interfaces *i.e.*, the motions of the S-RBD patches are highly correlated with the motions of the ACE2 patches. In contrast, the Omicron complex shows a different movement pattern. In the first principal movement the whole interface of the S-RBD, as well as the Patch II side of the ACE2 interface, with H³⁴ and E³⁵ from Patch I, move together without much internal deformation. This movement is mainly dominated by the opening-closing behavior of the ACE2 enzymatic active site. During the motion along the second principal axis, the whole RBD rotates around an axis perpendicular to the plane of the interface, which remains fairly

rigid, while in the third principal motion a deformative motion occurs, in which the Patch I part of the S-RBD moves together with the *N*-terminal of H1 with Patch II remaining relatively motionless.

In order to segment the whole complex into parts that stay approximately undeformed along the trajectory, we calculated the RMSD of each $C\alpha$ - $C\alpha$ distance, measured with respect to its average distance. This RMSD matrix was then used in a DBSCAN⁴⁸ clustering algorithm as a precomputed metric to segment the protein into parts, whose internal elements ($C\alpha$ atoms) show small distance variance (**Fig 3**).

In case of the WT complex this resulted in 18 clusters (counting the outliers, as a cluster). The full S-RBD interface aggregates into one cluster, while the ACE2 interface is segmented into 4 different clusters: the whole H1 and its connector loop (residues 21-54), the *N*-terminal part of H2 (residues 55-63), the *C*-terminal part of H2 (residues 65-82), and the anti-parallel β -strand pair containing the hotspot K³⁵³ (residues 345-360). The fact that the whole interface is segmented into 5 relatively large parts suggests a dynamically changing inter-chain residue-interaction environment. In contrast, in case of the Omicron complex (17 clusters in total), the interface is segmented into 2 parts only; one, containing the full S-RBD interface, as well as the aforementioned β -strand pair and another, containing both H1 and H2 of ACE2. Since we used the same DBSCAN parameters for both runs, these results suggest differences in the dynamics of the two complex interfaces.

The two complexes also differ in their relative interface mobilities; we calculated the root mean squared fluctuations (RMSFs) for each atom and compared the median value of the ACE2-contacting S-RBD residues' backbone RMSF to the full backbone median RMSF. In case of the WT complex, the mobility of the interface is 11% higher than the average full backbone mobility, whereas the interface of the Omicron complex is 9% less mobile than the average of the full backbone. This tendency can also be observed in the B-factors of the crystal structures 6M17 (WT complex) and 7WBP (Omicron complex); the WT interface backbone shows a moderate 3.9% increase in the median B-factor with respect to the median B-factor of the whole backbone, while the Omicron interface backbone median B-factor decreased by 21.5%.

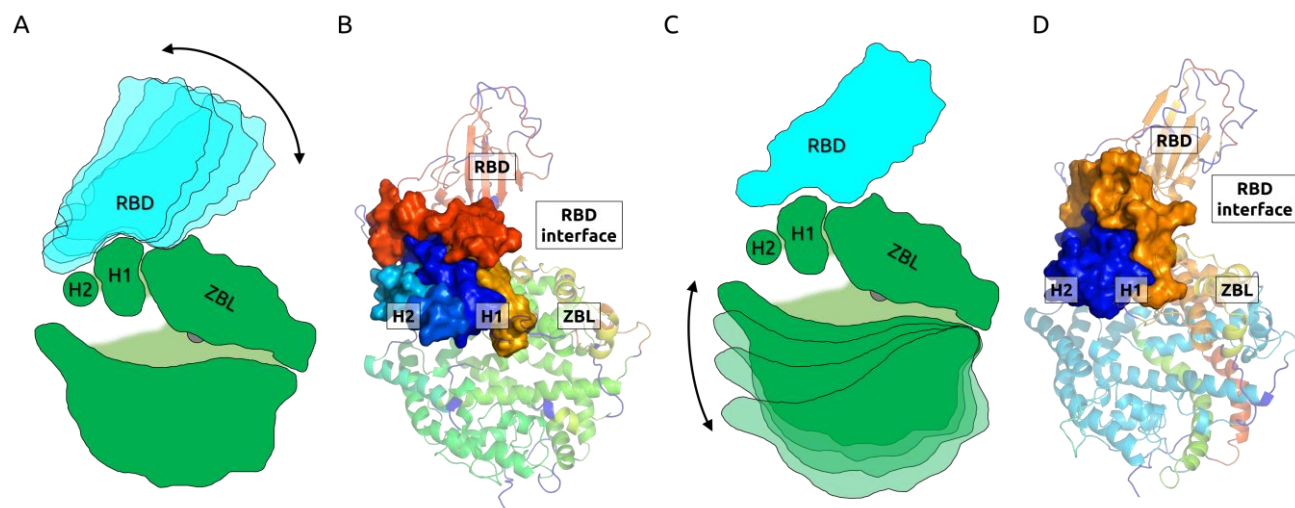


Figure 3. Principal component analysis and rigid body segmentation of the WT and the Omicron complexes. Panel A) and C) show the movement of the WT and the Omicron complexes along the first principal axis, respectively. While the WT S-RBD interface can move relatively freely on H1 in a sliding manner, this type of movement cannot be observed for the Omicron complex. This difference in interface dynamics is also apparent when the structures are segmented into rigid parts, shown in panel B) for the WT, and D) for the Omicron complex. In case of the WT complex, the interface is segmented into 5 different rigid parts (shown as 5 differently colored surfaces), indicating several mobile elements, while the Omicron interface is segmented into 2 rigid parts only.

5. DISCUSSION

In this study, we analyzed the behavior of the fully glycosylated S-RBD:ACE2 complex both in case of the WT and the Omicron structure *via* MD simulations. We found several structural and dynamical changes of high significance. The overall electrostatic distribution of the S-RBD surface is decidedly more positive in case of the Omicron variant, that can help the virus to identify the predominantly negatively charged ACE2 interface and initialize the ACE2 binding process. However, our results indicate that ~~after the initial rearrangement of the side chains and convergence~~, the resulting Omicron S-RBD:ACE2 complex will not be more stable than that of the WT complex, especially since the reduction of flexibility of surface loops of the S-RBD ~~also~~ is also unfavorable via the entropic term.

Experimental evaluation of the affinity of the Omicron variant toward ACE2 remains surprisingly eluding, different methods deriving strikingly contrasting results, ranging from a decided increase to significant reduction as compared to

that of the WT^{17,24-28}. Previous theoretical studies overwhelmingly predicted stronger association between the RBD of the Omicron variant and ACE2⁴⁹⁻⁵³, based on calculated binding energies without the analysis of the dynamic properties of the complex. Deep mutational scanning results⁵⁴ indicate near unchanged binding potential for the Omicron variant: the N440K, S477N, N501Y single mutations increased, S375F, K417N, G496S, Y505H changes decreased, while the other Omicron mutations (introduced one-by-one) did not exert significant effect on ACE2 binding efficiency. Thus, we can conclude that the drastically increased infectivity and quick spread of the Omicron variant cannot be explained (at least not alone) through more optimal binding with the ACE2.

In the Omicron variant, beside those of the RBD domain, mutations appear both in the inner architecture of the tightly packed S2 helices, and also in the vicinity of the S1/S2 proteolysis site: H655Y in spatial proximity, N679K next to the O-glycosylated⁵⁵ T⁶⁷⁸ and P681H directly preceding the polybasic -RRAR- (residues 682-685) recognition motif of the furin cleavage site, that prompts the first proteolysis (of the R⁶⁸⁵-S⁶⁸⁶ peptide bond) of the activation cascade of the Spike protein. These changes have the capacity of altering the flexibility and accessibility of this site, which results in reduced effectiveness of the cleavage^{16,17}, but could also alter the affinity of the spike protein toward neuropilin-1 receptors that also bind to this specific motif. Neuropilin-1 receptors are abundant in the respiratory and nasal passages most easily accessed by the virus, and were shown to potentiate ACE2 mediated cell entry as well as functioning as possible independent receptors for SARS-CoV-2 viruses^{56,57}. It is tempting to speculate that the greater transmissibility of the Omicron variant could - at least partially - be explained by enhanced interaction with neuropilin-1 receptors due to the mutations near the S1/S2 proteolysis site. Alternatively, it seems the Omicron variant might also use the endosomal pathway for host cell entry, as suggested by a recent study¹⁹. Either way, the picture that emerges, based also on our results, is that while the Omicron mutations improve the overall electrostatic complementarity of the S-RBD and the human ACE2 receptor, the optimized residue-residue contacts especially in the middle of the interaction surface result in an unfavorably rigidified complex - thus the fast spread and great infectivity of the Omicron SARS-CoV-2 virus cannot be attributed this interaction. The conflicting aspects of the binding process between the S-RBD and ACE2 that we uncovered might explain the differing experimental results concerning the affinity of the two, and also suggest that mutations in the Patch II region of S-RBD (residues 493-505) that do not participate in antibody binding might recover the virus's affinity toward human ACE2 without the loss of its evasion mechanism, and produce a both highly infectious and severe variant.

MATERIALS AND METHODS

For MD simulations the 6M17 PDB was used as a starting structure. Glycan modifications were built on ACE2 at residues N⁵³, N⁹⁰, N¹⁰³, N³²², N⁴³², and N⁵⁴⁶, while on the RBD at N³⁴³ using the most abundant glycan-modifications published⁴⁷. The globular domain of ACE2 is built from two relatively rigid lobes that maintain an open conformation in the *apo* state, but close upon the physiological substrates of the enzyme⁵⁸ when they're bound to the active site. The architecture of the lobes is organized around ion binding sites: a Zn²⁺-ion in the lobe also containing the S-RBD binding surface, and a Cl⁻-ion in center of the other. Both ions are required for efficient catalysis⁵⁹. The structural chloride ion is coordinated by residues W¹⁶⁵, R¹⁶⁹, W⁴⁷⁸, K⁴⁸¹, D⁴⁹⁹, while the zinc ion is clamped by H³⁷⁴, E³⁷⁵, H³⁷⁸, and E⁴⁰². In case of the zinc ion (Zn²⁺) a harmonic potential for the H³⁷⁴:Nε2 - Zn bond and the H³⁷⁸:Nε2 - Zn bond was applied with an equilibrium bond length of 0.229 nm and a force constant of 22'000 kJ*mol⁻¹*nm⁻². The H³⁷⁴:Nε2 - Zn - H³⁷⁸:Nε2 angle was also penalized⁶⁰ with a harmonic potential with an equilibrium angle of 88.4° and a force constant of 60 kJ*mol⁻¹*nm⁻². The structure for the Omicron variant RBD was constructed from the above PDB file, using the Schrödinger Maestro 2022.1 software. The MD simulations were carried out by using the GROMACS 2021.4 software⁶¹⁻⁶³. The proteins were solvated in water with an OPC water model⁶⁴ and sodium and chloride ions were added to electrostatically neutralize the system, in a final concentration of 0.15 M. The forcefield employed here was AMBER-ff99SBildnp-star⁶⁵.

Parameters i.e., force-constants, ~~equilibrium~~ equilibrium bond-lengths, and angles for the glycan-modifications were ~~obtained from the~~ based on the DNA-and existing RNA sugar-parametrizations of the force-field, while partial charges were calculated from B3LYP/6-31G** optimized wave functions of the sugar units, ~~ESP-charges~~ using the electrostatic potential fitted charge calculation protocol (ESP charges) (Schrödinger Jaguar software using Jaguar of the Schrödinger Suite (Schrödinger, LLC, New York, NY 2021)). Glycan-topologies were built by hand using a standard text editor, while their initial structures were built using Schrödinger Maestro. Before the production MD, several equilibration steps were performed; first, using a steepest descent integrator with position restraints of 1000, 500, 100, and then 0 kJ*mol⁻¹*nm⁻² and a maximal force tolerance of 50 kJ*mol⁻¹*nm⁻¹. Next, an NVT equilibration was performed using the leap-frog integrator for 50'000 steps with a 2 fs step-size with position restraints of 1000, 500, 100, and 0 kJ*mol⁻¹*nm⁻² again at 310 K. Finally, an unconstrained NPT step was included to allow introduction of pressure. From the starting structures thus obtained 2000 ns production runs were started. In case of the Omicron S-RBD:ACE2 complex two independent runs were produced to make our analyses more robust against the initial conditions.

For the investigation of the residue-residue contacts in both the WT and Omicron complexes, we first clustered the extended contact group of the interaction surface, comprised of residues 21-52, 76-84, 315-326, 348-358, and 382-394 in ACE2, and 403-506 of S-RBD using the *gmx cluster* command with the *gromos* method and a distance cutoff of 1.5 Å.

The cluster centers of these clusters served as representative structures for further comprehensive investigations. To compare the interface structures, we created LigPlots⁶⁶ representing the measured WT S-RBD:ACE2 interface (Fig S2/A), the measured Omicron S-RBD:ACE2 interface (Fig S2/B), and also the cluster centers of their simulated counterparts (Fig S3 and Fig S4). We also collected the main contact partners for the residues at the mutation sites in both the WT and the Omicron complex. More precisely, at a given snapshot we call a residue R_c contacting with a residue R_m at the mutation site, if any of R_c 's atoms is closer to R_m than 4 Å. Then, we only considered R_c s that contacted with R_m for more than 15% of the time along the trajectory. For these residues we measured the distances between atoms that can potentially participate in residue-residue interactions, like H-bonds, salt-bridges, and aromatic interactions. These distance measurements were only kept, if they were closer than 3.5 Å for more than 15% of the time along the trajectory. The histograms for these measurements are collected in Fig S5-11. The SpR values were calculated using these distances with the 1.7.1 version of *scipy* in Python 3.8. This way, the time-sorted ranks of the distance-value pairs were correlated, which is independent on the linearity/non-linearity of their relationship and only depends on the monotonicity of the change.

When considering the effects of the glycan modifications, it is important to keep in mind, that the chosen glycans are samples from a larger distribution. The conditions we applied for selecting a glycan-residue contact were equivalent to that used in case of residue-residue contacts i.e., the residue must be closer than 4.0 Å to the corresponding glycan for more than 15% of the full analyzed trajectory time.

PCA analysis of the trajectories was carried out using the *gmx covar* and *gmx ana eig* built-in functions of GROMACS on the MainChain+C β atom-groups. We analyzed three converged 500 ns length trajectory slices, one from the WT trajectory, and two from the two Omicron trajectories. In order to visualize the movement of the proteins along these *eigen*-axes, for each *eigen*-axis we extracted the two structures along the trajectory, for which the coordinate along the given axis is maximal and minimal. Then, we visualized the transition from one structure to the other using linear interpolation.

For the rigid body segmentation the *scikit-learn* 1.0.1, *numpy* 1.20.3, and *mdanalysis* 2.0.0⁶⁷ Python 3.8 compatible packages were used. For each frame the C α -C α Euclidean distance-matrix was constructed, and then the Welford online-algorithm was used for the calculation of the standard deviation matrix. This matrix was provided as a precomputed metric to the DBSCAN algorithm. In both cases (for the WT and the Omicron complex) we used an epsilon value of 0.2 nm and a *min_samples* value of 5 in the DBSCAN algorithm. The same trajectory slices were used for the rigid body segmentation algorithm as for the PCA.

Binding energy was estimated using the PISA server^{68,69} to calculate a cluster weighted average of $\Delta_{\text{bind}}G$ (cluster mid-structures were evaluated with PISA and considered with the weight derived from the population ratio of the given cluster). In case of the WT complex the first 7 cluster mid-structures were used with a total population coverage of 75%, while in case of the Omicron complex we used 5 clusters with 78% coverage.

ABPS⁷⁰ surfaces and molecular visualizations were made using the PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC.


DATA AND SOFTWARE AVAILABILITY

Supporting Information is available free of charge at [future link]. Additional data i.e., results of the trajectory clustering, results of and script, as well as usage for the rigid body segmentation, and movies depicting the movements along the eigenaxes are available here: [future link].

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. The authors declare no competing financial interest.

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