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Significance of the RBD mutations in the SARS-CoV-2 omicron: from spike opening to antibody escape and cell attachment†

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We computationally investigated the role of the omicron RBD mutations on its structure and interactions with the surrounding domains in the spike trimer as well as with ACE2. Our results suggest that, compared to WT and delta, the mutations in the omicron RBD facilitate a more efficient RBD “down” to “up” conformation as well as ACE2 attachment. These effects, combined with antibody evasion, may have contributed to its dominance over delta.

As SARS-CoV-2 continues to spread around the world, it's amassing mutations that occasionally lead to increased virulence and immune escape. The SARS-CoV-2 delta variant outpaced the alpha and beta variants, and recently the omicron variant, also known as B.1.1.529, quickly took over. Consternation about omicron put global health sectors on high alert due to its high transmissibility and resistance to the existing therapeutic antibodies or those produced by vaccines and prior infections.^{1–3} Its spread was so rapid that cases of B.1.1.529 were reported in >90 countries in less than a month.⁴ In just three weeks since its detection in the US, it became the most dominant variant (>70%) followed by the delta (~30%).⁵ Infections with the delta variant are still high worldwide and though more research is needed to ascertain exactly how the presence of omicron will affect the epidemiology of delta, early results indicate that the immunity developed after omicron infection is able to successfully neutralize delta.⁶ Therefore, there is a glimmer of hope that omicron will be able to displace the more severe delta and ultimately lessen the burden of COVID worldwide. However, new variants of omicron such as BA.1 and the “stealth” BA.2 have emerged, underscoring the need for caution and further research.⁷

The large number of spike protein mutations sets the omicron variant significantly apart from the other variants. Compared to the WT, the spike protein harbors more than 30 mutations, including 15 in the receptor binding domain (RBD) alone. The mutations in the RBD are – G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, and Y505H,⁸ compared to only 501Y in B.1.1.7, 417N, 484K, and 501Y in B.1.351, and 478K and 452R in B.1.617.2 (Fig. 1a). Specific mutations can give a variant an edge on the fitness landscape. For example, the P681R mutation in delta is believed to have increased its transmissibility by enhancing the spike protein cleavage.⁹ In the RBD, previous studies show that the mutations Q498R and N501Y increase the affinity to bind with the human receptor ACE2,¹⁰ whereas

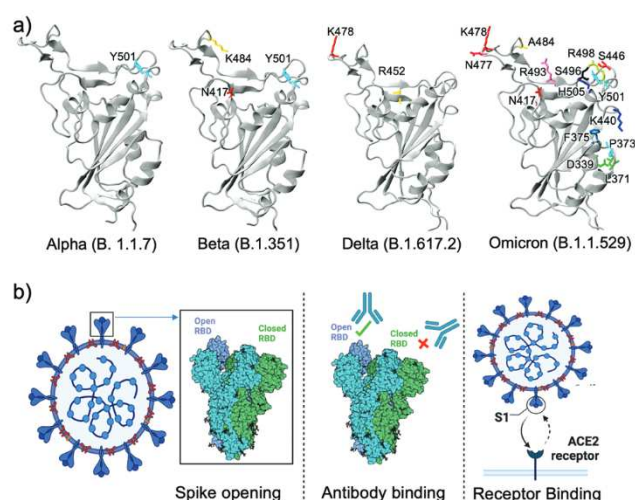


Fig. 1 (a) RBD structures of different variants. While alpha, beta, and delta variants have less than three mutations in the RBD, omicron has a remarkably large number of mutations. (b) Three different ways RBD mutations may contribute to the high transmissibility of a variant (created with BioRender.com).

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mutations in the RBD loop region, *e.g.* E484K and others, are found to be associated with immune evasion.¹¹

The transmissibility of a respiratory viral infection such as with SARS-CoV-2 is a complex process that involves a myriad of viral, host, and environmental factors¹² and there is a trade-off between the transmissibility and virulence of SARS-CoV-2.^{13,14} Considering only the RBD mutations, omicron seems to have optimized its ability to infect in three different ways: (1) RBD “down” to “up” opening, (2) antibody escape, and (3) ACE2 receptor binding. These three aspects of the RBD mutations are summarized in Fig. 1b. To investigate the consequences of the RBD mutations, we performed molecular dynamics (MD) simulations of various RBD systems for the WT, delta, and omicron variants. These include 1 μ s MD simulations of the RBD-only system for each of the WT, delta, and omicron, as well as 100 ns MD simulations of the RBD, together with the surrounding domains of the spike trimer, and a 100 ns simulation of the RBD-ACE2 complex. The details of the simulations, calculations, and analyses are given in the ESI,[†] including the set-ups and systems given in Table S1.

The binding of the RBD with the ACE2 receptor requires the RBD to be in the “up” conformation from “down” in the prefusion state of the spike trimer. RBD's successful attachment to ACE2 is also facilitated by the spike protein's flexibility provided by the three hinges in the stalk domain (residues 1140–1234),^{15,16} which is highly conserved in different variants. Several structures of the spike trimer solved with or without ACE2, including the structure with the three RBDs in the down conformation and the structure with one RBD in the up-conformation,^{17–20} provide insights into the mechanism of the cell attachment of SARS-CoV-2. The RBDs in the “down” conformation are held together by symmetrically arranged, centrally clustered, interdomain hydrogen bonds,²¹ which break during the RBD “up” conformation. In order to calculate the inter-domain hydrogen bonds formed with a RBD in a closed-form spike trimer, we prepared a simulation system for a RBD surrounded by the interacting domains of the spike trimer using the full spike trimer structure.²² To minimize the computational time, we prepared a truncated trimer including only the domains that directly interact with one of the RBDs (here, we chose the RBD of chain A), as shown in Fig. 2 and Fig. S1 (ESI[†]). These domains include residues 330–530 of chain A (RBD), 16–530 and 968–1000 of chain B, and 330–530 and 968–1000 of chain C. The domains surrounding the RBD of chain A are harmonically restrained by applying harmonic forces to all C $_{\alpha}$ atoms that are > 12 Å away from the RBD of chain A. This allows flexibility of the RBD in the trimer but maintains the integrity of the domains mimicking the full trimer. We performed 100 ns simulations for the WT and omicron and calculated the hydrogen bonds for the last 50 ns of the trajectories. To confirm that the RBD hydrogen bonding is adequately represented by the truncated system, we calculated and compared the % hydrogen bonds for the closed-form RBD to the simulation of the full system performed by the Amaro Lab.²² The RBD hydrogen bonding pattern has good agreement between the truncated trimer and the full trimer.

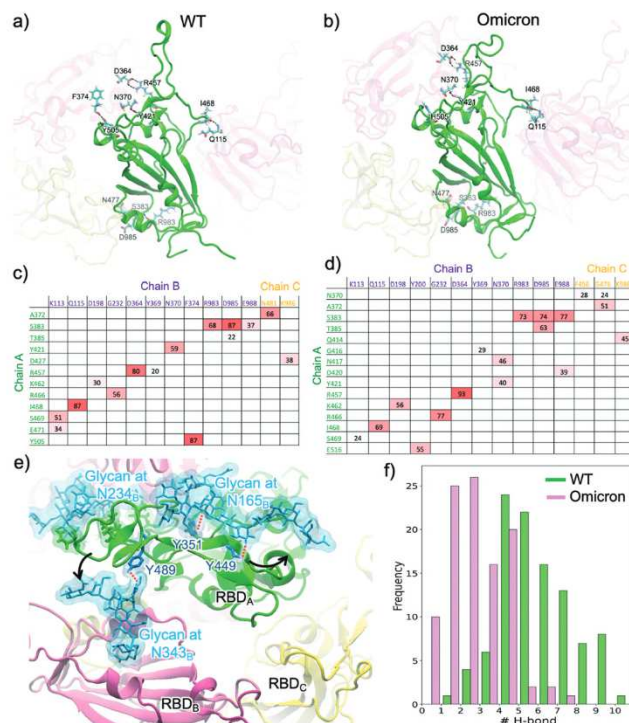


Fig. 2 Major hydrogen bonds formed between the RBD of chain A (green) and the surrounding domains in the closed-form spike trimer for (a) WT and (b) omicron. Additional interactions are shown in Fig. S1 (ESI[†]) (from different views). Hydrogen-bond pairs and % occupancies for the (c) WT and (d) omicron, with the color scale from red (maximum) to white (minimum). (e) The locations of three glycans N165, N234, and N343 in the RBD of chain B that interact directly with the RBD of chain A. The RBD_B glycans-RBD_A hydrogen-bonds (red dotted lines) break to make way for the RBD opening. (f) Histogram of the hydrogen-bonds made by RBD_B glycans at N165, N234, and N343 with RBD_A, though majority of the contribution comes from N165 and N343.

We show in Fig. 2 the major hydrogen bonds that an RBD forms with its surrounding domains for both the WT and omicron. The % occupancies for the major hydrogen bonds for both the WT and omicron are given in the matrices in Fig. 2c and d. Major hydrogen bond interactions with % occupancy > 80% include R457(A)–D364(B), Y505(A)–F374(B), S383(A)–D985(B), and I468(A)–Q115(B). Residue S383 of the RBD makes three stable hydrogen bonds with the helix domain comprised of residues 968–1000 of chain B. However, only a few, weak interactions are observed between the RBD and the helix domain of chain C. The helix domains lie just below the RBD, as shown in Fig. 2 and Fig. S1 (ESI[†]). We also calculated the % hydrogen bonds (Fig. 2d) for the omicron RBD displayed in Fig. 2b. Most of the major hydrogen bonds found in the WT are also present in omicron, including R457(A)–D364(B), S383(A)–R983(B), S383(A)–D985(B), S383(A)–E988(B), I468(A)–Q115(B), K462(A)–D198(B), and E516(A)–Y200(B). However, one of the major WT hydrogen bonds with nearly 90% occupancy, Y505(A)–F374(B), is lost in omicron due to the Y505H mutation. In addition, the polar to hydrophobic mutation S371L also abrogates minor hydrogen-bonding with multiple residues. When the Y505(A)–F374(B) hydrogen bond is broken,

the RBD slightly repositions to make relatively weaker but new hydrogen bonds, mostly with chain C residues. The new interactions in omicron include N370(A)–F456(C), N370(A)–G476(C), A372(A)–G476(C), Q414(A)–K986(C), and D420(A)–E988(B). The loss of these hydrogen bond interactions in omicron appears to be compensated by the increase in the % hydrogen bond or new hydrogen bonds. Therefore, it's difficult to assess the stability of the RBD based on the total % hydrogen bonds alone. However, the RBD opening from the closed form trimer may still be affected for the following reasons. With the Y505(A)–F374(B) hydrogen bond in the WT, the RBD of chain A is held in a position slightly away from these residues, which are in the RBD and the helix domain of chain C. These new interactions can still form in the WT if the Y505(A)–F374(B) hydrogen bond is broken and *vice versa*. Therefore, our analysis shows that compared to the omicron RBD, the WT RBD is more protected from opening from the closed-form trimer due to the possibility of either being held by Y505(A)–F374(B) or the additional interactions with chain C, whereas the omicron RBD lacks the Y505(A)–F374(B) interaction. In addition to the interdomain residue interactions, our results suggest that the glycan interactions (Fig. 2e) are also weaker in omicron. Glycan-gating is shown to play a crucial role in the opening of the RBD to the “up” conformation.²² As shown in Fig. 2f, the chain B RBD glycans (mostly at N165 and N343) form 2.3 ± 1.5 hydrogen bonds on average in omicron vs. 5.5 ± 1.9 in WT. We note that the interactions of RBD with the neighboring domains are transient and may change as the RBD shifts. While we investigated the interactions in the closed-form state, further work is needed to determine exactly how these mutations affect the RBD opening along the opening pathway.

Once the RBD springs out from the “down” conformation, it is vulnerable to antibody detection and binding due to the loss of shielding by glycans.²² Due to mutation-induced changes in both the residue type as well as the RBD structure, antibodies elicited with prior infections or vaccines may not be able to optimally bind to the RBD. In an earlier work, we showed that the changes in the delta RBD structure, including in the receptor-binding motif (RBM) loop segment, cause some antibodies to be ineffective²³ at binding the RBD. With the significant number of mutations in the omicron RBD, such effects can be extensive. To explore the structural changes in the omicron RBD, we performed 1 μ s simulation of the RBD-only system for omicron (PDB ID 7T9L) and compared with that of WT^{24,25} and we find significant differences in the RBD structure for the isolated RBD (*i.e.*, not complexed with ACE2). Specifically, the motif consisting of residues 364 to 375, which contains the mutations S371L, S373P, and S375F, shows an extensive structural change (Fig. 3a). All these three mutations are from polar to hydrophobic and this causes the motif to realign and make non-specific interactions with F342, A435, and W436 in the hydrophobic pocket (Fig. S2 and Movie S1, ESI†). As shown in Fig. 3a, the distance between the C α atoms of residues 371 and 375 in the motif is relatively stable in WT, whereas it separates significantly in omicron. Both of these residues are binding sites for antibodies (*e.g.* RBD–Ab

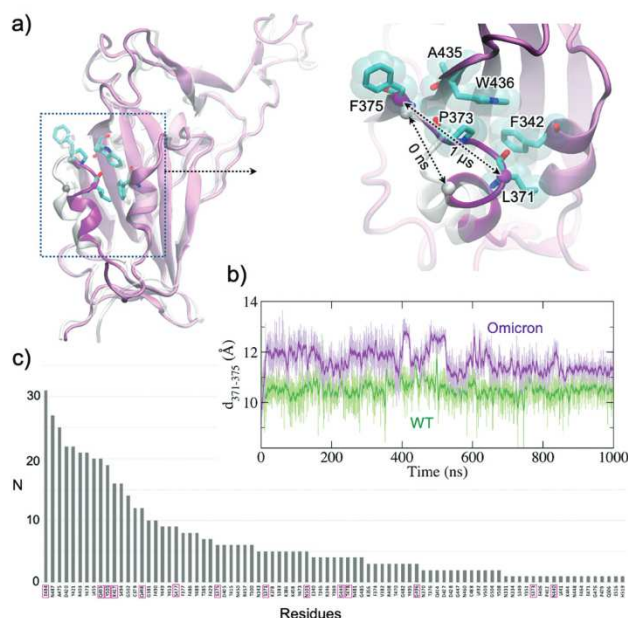


Fig. 3 (a) Structural changes in the omicron RBD motif containing the mutations S371L, S373P, and S375F (motif highlighted in bright purple), which form a hydrophobic cluster (right). (b) The C α –C α distance between residues 371 and 375 showing the difference in WT vs. omicron. (c) Number of times the RBD residues found to hydrogen bond with the antibodies in 105 RBD–Ab complexes from the Protein Data Bank. The mutated residues in omicron are highlighted in purple boxes along the x-axis.

complexes 7KN5 and 7M7B). This separation in the antibody-binding region can reduce or abolish the binding of the antibodies specific to these sites. Subtle structure changes in other sites in the RBD may also affect antibody binding, allowing the omicron RBD to escape antibody detection. Fig. 3b shows the antibody-binding sites identified from the RBD–antibody complexes available in the Protein Data Bank. Almost all mutations in the omicron RBD are located in important antibody-binding sites (residues indicated by purple boxes) and therefore can directly affect the binding of antibodies specific to the WT and other variants.

To explore the antigenic shifts due to the mutations, we first identified the RBD epitopes using various MHC-I and MHC-II prediction methods as well as sequence and structure-based B-Cell epitope prediction methods as described in the ESI† and used a consensus approach²⁶ to select the epitopes for further analysis. The consensus epitopes that contain the mutations in the RBD are given in Table S2 (ESI†), and all of the predicted epitopes (sequence-based and structure-based) are listed in Tables S3–S6 (ESI†). We calculated the antigenicity of the mutated sequences using VaxiJen²⁷ and compared with the corresponding WT sequence to assess the antigenic characteristic introduced by the mutation. Most of the epitopes listed in Table S2 (ESI†) have similar antigenicity after mutations in alpha, beta, delta, or omicron. However, three epitopes in omicron (E2, E3, and E9 in Table S2, ESI†) are found to have significantly increased antigenicity compared to the WT. These

omicron epitopes include 370-NLAPFFTFK-378 involving the mutations S371L, S373P, and S375F, 372-APFFTFKCY-380 involving the mutations S373P and S375F, and 483-VAGFNCYFPLR-493 involving the mutations E484A and Q493R. The antigenicity of E2 is 1.34 for omicron vs. 0.12 for WT. A similar increase is observed for epitope E3, which overlaps with E2. Similarly, the antigenicity of E9 is 1.23 for omicron vs. 0.56 for WT. The locations of these epitopes are shown in Fig. S3 (ESI†). While the mutation-induced antigenic shifts render reduced sensitivity for the WT-specific antibodies, the increased antigenicity in the omicron epitopes suggests a more potent immune response from these epitopes.

The mutations in the RBM region can directly affect the binding affinity of the RBD to bind ACE2. The RBD binding with ACE2 in different SARS-CoV-2 variants has been a topic of intense research.^{23,24,28–33} Kim *et al.* showed differences in the force required to dissociate the RBD from ACE2 for different variants of concern (alpha, beta, gamma, and delta).³⁴ With 10 mutations in the RBM region of omicron, the effects on ACE2 attachment can be significant compared to other variants. Early results of Wu *et al.* suggested that omicron RBD–ACE2 interactions are weaker than in delta.³⁵ However, recent cryo-EM structure-based analysis of the RBD–ACE2 complexes of both the omicron and delta variants shows that the omicron RBD–ACE2 interface has better optimized interactions than delta.³²

Effort has been made to assess the binding affinity of RBD and ACE2 by launching an online tool.³⁶ In this study, to investigate the effects of the omicron mutations on the RBD attachment to the ACE2 receptor, we performed an MD simulation of the omicron RBD–ACE2 complex (PDB ID: 7T9L)³⁷ and compared the RBD–ACE2 interfacial interactions with those of WT (PDB: 7A92)³⁸ and delta (PDB ID: 7W9I).³⁹ The short MD simulations provide the dynamic nature of the interactions and allow us to calculate the probability (% occupancy) of each hydrogen bond. As shown in Fig. 4a–d, the occupancy of the inter-protein hydrogen bond in omicron is noticeably higher than in delta suggesting a much stronger ACE2-binding in

omicron. The major RBD–ACE2 hydrogen-bond pairs in omicron with >70% occupancy include Y453–H34, G502–K353, N487–Y83, T500–D355, and R493–E35, and H505–K353 and N477–S19 with >50% occupancy. In contrast, delta has only two interactions (N487–Y83 and G502–K353) with >70% occupancy and four (K417–D30, Q493–E35, Y505–E37, and T500–D355) with >50% occupancy, suggesting relatively weaker interfacial interactions in delta compared to omicron. This is consistent with a recent work of Genovese *et al.*⁴⁰ in which the interfacial interactions in the RBD–ACE2 complex were analyzed using *ab initio* and quantum mechanical calculations. Lupala *et al.*⁴¹ also found a significantly increased binding affinity of the omicron RBD to ACE2, compared to the delta RBD, suggesting an increase in infectivity. We compared the RBD–ACE2 hydrogen bonding with that in WT (Fig. S4, ESI†) and observed additional unique hydrogen bonds (*e.g.* A475–S19) in omicron compared to WT. Since the presence of glycans at the interface can enhance the RBD–ACE2 interactions,⁴² we calculated the hydrogen bonding with glycan at N90 at the interface and found that omicron has a relatively stronger interaction with 80% hydrogen bond occupancy in omicron vs. 56% in delta.

In addition to the increased hydrogen-bonding at the interface, the stability of the complex is also displayed by the dynamic network analysis.⁴³ We used the last 50 ns of the trajectory for the RBD–ACE2 complexes of the delta and omicron variants to calculate the dynamic network communities as shown in Fig. S5 (ESI†). We performed the community analysis by partitioning the network into subnetworks (community). A community is a collection of nodes (amino acids) that are connected with edges (connections). The communities that span across the RBD–ACE2 interface are shown in Fig. 4e and f and the corresponding residues are shown in Fig. S5 (ESI†). There are a total of 28 connections that occur between the RBD and ACE2 in omicron, compared to 20 in delta. This suggests a better binding of the RBD to ACE2 with increased interactions that stabilize the RBD–ACE2 complex in omicron. While the network analysis and hydrogen-bond analysis provide a general assessment of the RBD–ACE2 binding, free-energy calculations with potential mean-force⁴⁴ or work done with steering force³⁴ are needed to compute more reliable binding affinities.

Overall, our analyses show that the omicron RBD shows a higher ACE2 binding affinity than the delta RBD. Unlike other variants, omicron has RBD mutations Y505H and S371L that lie in the region interacting with the surrounding domains in the closed-form spike trimer and this can affect the RBD opening. Increased ACE2 affinity and potentially easier and more efficient RBD opening, combined with antibody evasion due to mutation-induced antigenic shifts, provide the omicron strain with a significant increase in the probability for successful cellular attachment and this may contribute to its dominance over delta. Other factors such as ACE2 accessibility of the RBD due to spike mutations affecting the hinge flexibility,^{45–47} altered TMPRSS2 usage in cleaving S1/S2⁴⁸ as well as post-fusion conformational changes^{49–51} may have effects on the

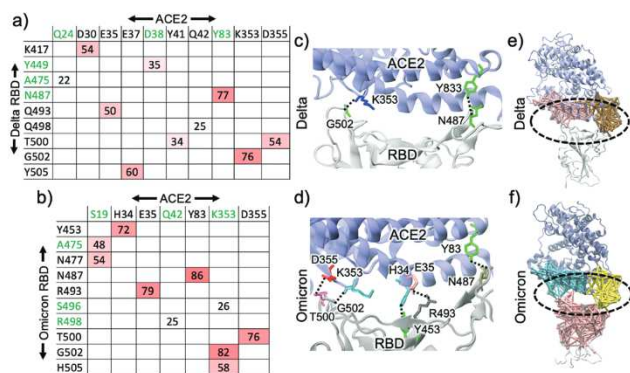


Fig. 4 Percent occupancies of the hydrogen bonds between the RBD and ACE2 for the (a) delta and (b) omicron variants. The unique interfacial hydrogen-bonds found in omicron are highlighted in green. Hydrogen bonds with >50% occupancy are shown for (c) delta and (d) omicron. The communities that span both the RBD and ACE2 are shown for the (e) delta and (f) omicron RBD–ACE2 complexes.

transmissibility and severity of infection. While the omicron RBD escapes most antibodies specific to other variants, it harbors sequences with significantly improved antigenicity compared to prior sequences. This suggests a possibility of superior neutralizing antibodies for omicron and provides insights into vaccine design as well as a perspective on the future of SARS-CoV-2 persistence.

PC designed the project. MLH performed and analyzed the simulations. PB performed the epitope analysis. All authors contributed to the analysis and writing of the manuscript.

Conflicts of interest

There are no conflicts to declare.

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