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Molecular basis of receptor binding and antibody neutralization of Omicron

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Abstract

The SARS-CoV-2 Omicron exhibits striking immune evasion and is spreading rapidly worldwide. Understanding the structural basis of the high transmissibility and enhanced immune evasion of Omicron is of high importance. Here through cryo-EM analysis, we present both the closed and open states of the Omicron spike (S), which appear more compact than the counterparts of the G614 strain¹, potentially related to Omicron residue substitutions-induced enhanced inter-protomer and S1-S2 interactions. The closed state showing dominant population may indicate a conformational masking mechanism for Omicron's immune evasion. Moreover, we capture three states for the Omicron S-ACE2 complex, revealing that the substitutions on the Omicron RBM result in new salt bridges/H-bonds, more favorable electrostatic surface properties, and overall strengthened S-ACE2 interaction, in line with the observed higher ACE2 affinity of Omicron S relative to G614. Furthermore, we determine structures of Omicron S in complex with the Fab of S3H3, an antibody able to cross-neutralize major variants of concern including Omicron, elucidating the structural basis for S3H3-mediated broad-spectrum neutralization. Our findings shed new lights on the receptor engagement and antibody neutralization/evasion of Omicron and may also inform design of broadly effective SARS-CoV-2 vaccines.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has undergone considerable evolution since its initial discovery, leading to the emergence of a number of variants of concern (VOCs) including Alpha²⁻⁶, Beta⁵⁻¹⁰, Gamma¹¹, and Delta^{12,13}. These variants that harbor multiple mutations on their spike (S) protein show enhanced transmissibility and resistance to antibody neutralization¹³. Recently, a new variant named Omicron (B.1.1.529), first reported in South Africa in November 2021, was classified as the fifth VOC by the World Health Organization (WHO)¹⁴. Omicron bears 37 mutations in its S protein relative to the original SARS-CoV-2 strain^{15,16}. As the consequence, Omicron has been observed to extensively escape neutralization by previously developed neutralizing monoclonal antibodies (MAbs) or sera from vaccines or convalescent individuals^{15,17-22}. Among all of the Omicron S mutations, 15 are present in the receptor-binding domain (RBD) that mediates the virus binding to its host-cell receptor—angiotensin-converting enzyme 2 (ACE2) and is also a major target for neutralizing antibodies²³⁻²⁷. However, Omicron still uses ACE2 as its entry receptor²². Moreover, the Omicron S appears to have an increased binding affinity to ACE2 relative to the wildtype (WT) S^{15,16,28}.

The high transmissibility and greatly enhanced resistance to antibody neutralization observed for Omicron makes this VOC particularly threatening. Therefore, further understanding of the nature of Omicron is of significant importance and may help in developing countermeasures against this VOC. To address from a structural aspect how Omicron binds the ACE2 receptor and how it recognizes or evades neutralizing antibodies raised against the original virus, here we perform cryo-EM analyses on the Omicron S trimer and its complexes with ACE2 or with neutralizing MAbs.

Results

Closed and open states of Omicron spike

We prepared a prefusion-stabilized trimeric S protein of SARS-CoV-2 Omicron variant (Extended Data Fig. 1) and determined its cryo-EM structures. Three cryo-EM maps, including an all RBD down conformation (termed Omicron S-close), an RBD-1 up open conformation (termed Omicron S-open), and an RBD-1 up while RBD-3

disordered open conformation (termed Omicron S-open-2), were determined at 3.08, 3.40, and 3.41-Å-resolution, respectively (Fig. 1a-b, Extended Data Fig. 2a-e, Extended Data Table 1). For the Omicron S-close state, the three protomers are well resolved and they display similar conformation with their RBDs in the down position (Fig. 1c, Extended Data Fig. 2d). Strikingly, the Omicron S-close appears more twisted/compact in the RBDs relative to the G614 S-close structure¹ (Fig. 1d). Also, in the Omicron S-open state, the RBDs are slightly more twisted/compact than that of the G614 S-open¹ (Fig. 1e). There is no linoleic acid (termed LA) in the Omicron S-close, S-open and S-open-2 maps, and the LA has been suggested to lead to more compacted RBDs²⁹. Thus, the Omicron S trimer is more compact than that of G614, and this is not caused by LA binding. Moreover, in the Omicron S-open-2 map, the RBD-3 density appears mostly disordered indicating an extremely dynamic nature of RBD-3 (Fig. 1a). Our further 3D variability analysis (3DVA)³⁰ on the Omicron S trimer dataset revealed an intrinsic rising up motion of RBD-1, which could alter the original RBD-1/-3 contact and destabilize RBD-3, making it extremely dynamic and may transiently rise up (Fig. 1f, Supplementary Video).

Noteworthy, the population distribution of the Omicron closed and open state (S-open/open-2) is about 60.8% and 39.2%, respectively (Extended Data Fig. 2a), displaying a considerable population shift to the closed state than that of the Beta and Kappa S (both around 50.0%-50.0% open-transition ratio) or that of the Delta S (75.3%-24.7% open-transition ratio) from our recent studies^{10,31}, with these S structures obtained in the same sample preparation and processing schemes. Thus, the Omicron S trimer appears more prone to the closed state and potentially stabilized relative to the counterparts of the Delta/Kappa/Beta/G614 variants. The population distribution of the closed and open states of these S trimers varies among different studies, which could be potentially due to subtle difference in chemical condition used by different research groups³²⁻³⁵. Inspection of the protomer interaction interface of Omicron S-close revealed new hydrogen bond (H-bond) interactions induced by the unique Omicron substitutions in SD1 and S2 regions (Fig. 1g, Extended Data Fig. 2f, Extended Data Table 2). Specifically, the T547K from the SD1 subdomain of protomer 1 forms a new H-bond with the N978 from S2 subunit of protomer 3, potentially enhancing the S1-S2 interaction between the two protomers; the

N856K/N764K from protomer 1 can form H-bonds with T572/Q314 from protomer 2, respectively. We also observed multiple new such interactions between the N317/R319 of protomer 1 and the D737 of protomer 3 (Fig. 1g, Extended Data Fig. 2f). The SD1 and SD2 of S1 is the hinge for RBD upward rotation^{31,35-38}, and disturbing the SD1-S2 interface could destabilize the interface and increase S1 mobility³⁶. Collectively, these extra H-bonds mainly induced by Omicron substitutions contribute greatly to strengthen the inter-protomer and S1-S2 interactions, markedly stabilizing the Omicron S trimer and inhibiting its transformation towards the fusion-prone open state and subsequent shielding of S1.

Structure of enhanced S-ACE2 interaction

Compared with the WT strain, Omicron bears 15 mutations in the RBD region, 9 of which are located in the receptor-binding motif (RBM)¹⁵. We assessed whether these mutations affect the human ACE2 receptor-binding ability of the Omicron S trimer by performing biolayer interferometry (BLI) assay. We found that the ACE2-binding affinity of the Omicron S (KD = 80 nM) is comparable to that of the Delta S (KD = 88 nM) but is about 3 folds higher than that of the G614 S (KD = 237 nM, Fig. 2a), consistent with other recent reports^{15,16,28}.

Next, we carried out cryo-EM study on the Omicron S in complex with human ACE2 peptidase domain (PD) (Extended Data Fig. 3a). We obtained three cryo-EM maps of the S trimer engaged with ACE2, including a one-RBD-up state (termed Omicron S-ACE2-C1), a two RBD-up state (termed Omicron S-ACE2-C2), and an all-three-RBD-up state (termed Omicron S-ACE2-C3) at 3.69, 3.70, and 4.04-Å-resolution, respectively (Fig. 2b, Extended Data Fig. 4, Extended Data Table 1). The population distribution among Omicron S-ACE2-C1/-C2/-C3 is about 43.9%, 41.2% and 14.9%, respectively (Extended Data Fig. 3b), displaying an obvious higher one-RBD-up C1 population (43.9%) than that of the Beta/Kappa/Delta variants (C1 population ranges from 8.3% to 14.1%) observed in our recent studies^{10,31}, with these S-ACE2 structures obtained in the same sample preparation and processing schemes. Taken together, the Omicron S trimer exhibits less ability to transform to the more RBD-up C2/C3 states in the presence of ACE2, as compared to that of the Beta, Kappa, and Delta variants.

We further focus-refined the stably associated Omicron RBD-1-ACE2 region and obtained a 3.67-Å-resolution structure (Fig. 2c, Extended Data Fig. 3-4), which revealed many new interactions between the RBM substitutions and ACE2 compared with that of the WT RBD-ACE2²⁶. Specifically, the RBM Q493R and Q498R result in three new salt bridges with the ACE2 E35 and D38, respectively; the RBM G496S/Y505H with ACE2 K353, the RBM Q498R with ACE2 Q42, and the RBM S477N with ACE2 Q19 also form new H-bonds, respectively (Fig. 2d, Extended Data Table 3), generally in line with recent studies^{16,28,39-42}. Moreover, we observed an extra H-bond between RBM T500 and ACE2 D355 (Fig. 2d). Our previous study showed that Y505A obviously decreased ACE2 binding affinity³⁵, thus the Omicron Y505H substitution may maintain or even enhance ACE2 binding. Meanwhile, the K417N substitution, occurred in Omicron as well as in Beta and Delta, is known to markedly reduce ACE2 binding through abolishing multiple salt bridges/H-bonds with ACE2 D30^{26,43,44}. Together, these newly formed RBM-ACE2 interactions may compensate the loss of some original RBM-ACE2 interactions due to the residue changes introduced into the Omicron RBM.

Further inspection of the surface property showed that the substitutions in RBM, especially Q493R, G496S, Q498R, and Y505H, render the substituted site within the ACE2 interaction footprint more positively charged, which could strengthen the RBM interaction with the overall negatively charged ACE2 in the interaction interface (Fig. 2f, g). Corroborating this, the Omicron RBD-ACE2 interaction area (920.2 Å²) is enlarged compared to that of the WT (843.3 Å²), while it is comparable to that of the Delta RBD-ACE2 (928.4 Å²)³¹ (Fig. 2e). This is also in agreement with our BLI data showing that the ACE2-binding affinity of the Omicron S is similar to that of the Delta S but is higher than that of the G614 S (Fig. 2a).

Omicron sensitivity to neutralizing MAbs

We compared five previously generated MAbs, including 2H2, 3C1, 8D3, S5D2, and S3H3^{45,46}, for neutralization of the WT (Wuhan-Hu-1 strain), Delta, or Omicron pseudoviruses (Fig. 3a-b). The IC₅₀ values of MAbs 3C1, 2H2, 8D3, and S3H3 against Delta were comparable (less than 2.5-fold variation) to the corresponding ones against WT, whereas S5D2 was still neutralizing to Delta (IC₅₀ = 734.6 ng/mL) but was about 90-fold less potent. In Omicron neutralization tests, 3C1, 8D3, and S5D2 lost

neutralization activity ($IC_{50} > 10 \mu g/mL$). However, 2H2 and S3H3 remained highly effective against Omicron with IC_{50} s being 30.4 and 53.3 ng/mL, respectively, despite that a 3.3-fold increase (relative to the WT) in IC_{50} value was observed for 2H2. Thus, 2H2 and S3H3 are two potent neutralizing MAbs against Omicron.

We then compared the binding ability of the five MAbs to the WT, Delta, and Omicron S proteins by ELISA (Fig. 3c). For MAb S5D2, its binding to the Delta and to the Omicron S was nearly abolished; for MAbs 3C1 and 8D3, their reactivity profile with the Delta S closely resembled that towards the WT S but their binding to the Omicron S reduced significantly; for MAb 2H2, its binding curve to the Omicron S was similar to those towards the WT and Delta S despite the binding efficiency to the Omicron S was slightly lower; meanwhile, MAb S3H3 produced nearly identical binding curves to the three S proteins. Overall, the antigen-binding ability of the MAbs was in good agreement with their neutralization potency towards specific variant pseudovirus (Fig. 3). Collectively, the above data showed that Omicron remains sensitive to binding and neutralization by MAbs 2H2 and S3H3 whereas it displays resistance to 3C1, 8D3, and S5D2.

Structure of the Omicron S-S3H3 complex

MAb S3H3 is a unique neutralizing antibody that binds the SD1 region of the WT S⁴⁶. Our cryo-EM study revealed two states of the Omicron S in complex with S3H3 Fab. Both structures showed two engaged Fab densities on the SD1 of protomer 2 and 3, but with the RBD-1 in the up (termed Omicron S-open-S3H3) or down (termed Omicron S-close-S3H3) conformations (Fig. 4a-b). The two maps were resolved to the resolution of 3.48 Å and 3.64 Å, respectively (Extended Data Fig. 5, Extended Data Table 1). Compared with the Omicron S-open, the S trimer in the S-open-S3H3 exhibits a slight twist and the RBD-1 displays a 9.1° downward rotation (Fig. 4c), making the S trimer seemingly less “open” as a whole. Meanwhile, the SD1 shows a slight downward rotation (Fig. 4c).

We further focus-refined the SD1-S3H3 Fab region and obtained a map at 3.61-Å-resolution, with most of the sidechain densities well resolved (Fig. 4d). The heavy chain of S3H3 Fab contributes more to the interactions with SD1 than the light chain does, i.e., all the three heavy-chain CDRs of S3H3 and its CDRL1 and CDRL3

interact with T323-E324 and the three loops (loop⁵³²⁻⁵³⁷, loop⁵⁵⁴⁻⁵⁵⁶, and loop⁵⁸¹⁻⁵⁸⁴) of SD1 (Fig. 4e-f, Extended Data Table 4). Specifically, the S32 of CDRL1 forms H-bonds with the S555 and I584 of SD1, respectively, the D102 of CDRH3 forms a H-bond with the T581 from loop⁵⁸¹⁻⁵⁸⁴, and the D55 of CDRH2 forms a salt bridge with the K537 from loop⁵³²⁻⁵³⁷ (Fig. 4g, Extended Data Table 4), thus constituting an intense interaction network between S3H3 Fab and SD1. A single mutation, T547K, is present in the SD1 of Omicron, however, this mutation locates outside the footprint of S3H3 (Fig. 4f), thus will not affect the Omicron S–S3H3 interaction. Collectively, S3H3 binds the extremely conserved SD1 region, therefore retains binding and neutralizing activity towards major VOCs including Omicron.

Discussion

In this study, we performed cryo-EM study and biochemical analysis on the Omicron S trimer and its complex with ACE2 receptor. We captured both the closed and the open states of the Omicron S trimer (Fig. 1a). In contrast to the S trimer of Delta/Beta/Kappa variants^{10,31}, the Omicron S-close and S-open structures appear more twisted/compact than their counterpart of the G614 strain¹ (Fig. 1d-e), which may hinder its spike transformation towards the fusion-prone open state and shielding of S1. This could be related to enhanced inter-protomer and S1-S2 interactions induced by unique Omicron substitutions (T547K, N856K, and N764K in SD1 and S2) (Fig. 1g).

Corroborating to the enhanced inter-protomer and S1-S2 interactions of Omicron, our cryo-EM analysis revealed that for the Omicron S trimer the dominantly populated conformation is the closed state with all the RBDs buried, possibly leading to “conformational masking” that may prevent antibody binding and neutralization at sites of receptor binding, similar to that described for HIV-1 envelope^{47,48}. Such an Omicron conformational masking mechanism could potentially affect antibodies that bind to the up RBDs (such as class 1, 2, and 4 RBD antibodies⁴⁹), contributing to the observed extensive neutralization escape by Omicron. While for Delta S trimer, our recent work showed the dominant population is in the open state, indicating the conformational masking mechanism may be less effective for the Delta variant^{31,35}. This could contribute to the striking immune evasion of the Omicron variant^{15,17-22}.

We also captured three states for the Omicron S-ACE2 complex (Fig. 2b). Unlike the Delta S which tends to bind three ACE2 in majority³¹, Omicron mainly binds one or two ACE2 (Extended Data Fig. 3b). Further focus-refined RBD-1-ACE2 structure demonstrated that the substitutions on the RBM of Omicron (especially Q493R, G496S, Q498R, S477N, and Y505H) result in formation of new salt bridges/H-bonds, and more complementary electrostatic surface properties (Fig. 2d-g), which together may compensate abolished original RBM-ACE2 interactions^{26,43,44}, leading to enhanced interactions with ACE2 and potentially enhanced transmissibility of the Omicron variant.

The present study showed that 2H2 and S3H3 retain potent neutralization towards Omicron and Delta (Fig. 3). Further structural study revealed a unique binding epitope of S3H3 within the SD1 region (Fig. 4). S3H3 binding to S trimer may function as a “lock” to block the releasing of S1 from S2, resulting in inhibition of virus entry. The SD1 region targeted by S3H3 is extremely conserved among SARS-CoV-2 variants (Fig. 4f), thus explaining the cross-neutralization ability of S3H3 towards Omicron, Delta, and other variants⁴⁶. These findings also suggest a possibility to design SD1-based broad-spectrum SARS-CoV-2 vaccines. It is somewhat surprising that 2H2, whose epitope largely overlaps with RBM⁴⁵, remained highly neutralizing against Omicron, despite this antibody lost neutralization potency to the Kappa and Beta variants due to their E484Q/K substitution¹⁰. Docking of our previous RBD-bound 2H2 Fab structure⁴⁵ onto our current Omicron RBD-1 structure from the S-open state revealed that the 2H2 Fab could be reasonably well accommodated without major clashes with RBD (Extended Data Fig. 6a). In particular, the E484A substitution in Omicron does not appear to create steric hindrance with 2H2 due to the smaller size of the Ala side chain. In addition, the Omicron RBM substitutions such as Q493R, Q498R, and Y505H, do not introduce extra clashes between RBM and 2H2, and also make the epitope surface more positively charged compared with the WT RBD-1⁴⁵ (Extended Data Fig. 6b) potentially allowing better interaction with the 2H2 paratope which tends to be more negatively charged (Extended Data Fig. 6c).

We found that Omicron could escape three RBD antibodies, including 8D3, S5D2, and 3C1^{45,46}. Both 8D3 and S5D2 are class 1 RBD antibodies and they share similar epitopes centered around the loop⁴⁷⁷⁻⁴⁸⁹^{31,46}. Docking of the RBD-bound 8D3 or S5D2

Fab structures^{31,46} onto the Omicron RBD-1 structure revealed that several Omicron residues, especially S477N, may potentially clash with the 8D3 Fab (Extended Data Fig. 6d); S477N and T478K substitutions in Omicron may break the H-bond network between RBD and S5D2 Fabs (Extended Data Fig. 6e). 3C1, whose epitope mainly involves the relatively stable RBD core⁴⁵, belongs to the RBD antibody class 3⁴⁹. Analysis of the docked 3C1 structure on the Omicron RBD-1 showed that the S375F substitution may contribute to the Omicron's escape of 3C1 binding and neutralization through altering the interaction interface (Extended Data Fig. 6f).

In summary, the present study reveals that the Omicron spike acquires an increased RBM-ACE2 interaction network contributed by new H-bonds/salt bridges and more favorable surface properties and therefore maintains strong affinity to ACE2, providing a possible explanation to the high infectivity/transmissibility of Omicron. In addition, our study suggests that, besides individual residue substitutions in RBD antibody epitopes, a conformational masking mechanism may also contribute to the extensive antibody evasion by Omicron. Moreover, our work demonstrates that Omicron remains sensitive to S3H3, an antibody targeting the extremely conserved SD1 region. Our findings provide structural insights into how Omicron maintains high transmissibility while greatly evades immunity, and may also inform design of broadly effective vaccines against emerging variants.

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394

Figure Legends

Fig. 1 | Cryo-EM structures of the SARS-CoV-2 Omicron S trimer. **a**, Cryo-EM maps of the Omicron S-close, S-open, and S-open-2 state. Protomer 1, 2, and 3 are shown in light green, royal blue, and gold, respectively, which color scheme was followed throughout. In S-open-2, the mostly disordered RBD-3 is indicated by dotted red ellipsoid. **b**, Atomic model of the Omicron S-open, with mutations indicated by red sphere and labeled. **c**, Side view of the overlaid protomers of the Omicron S-close. **d**, Top view of the overlaid RBDs of the Omicron S-close (violet red) and the G614 S-close (PDB: 7KRQ, dark gray), indicating a twist of the Omicron S-close relative to that of G614. **e**, Top view of the overlaid RBDs of the Omicron S-open (light green) and the G614 S-open (PDB: 7KRR, purple), indicating a twist of the Omicron S-open relative to that of G614. **f**, One representative 3DVA motion of the Omicron S. The left two maps illustrate the top view of two extremes in the motion with the RBD-3 indicated by dotted black ellipsoid, and the top view of the overlaid two extreme maps is shown in the right. **g**, Newly formed H-bonds (black dashed line) in the interfaces of protomer 1/3 (blue dashed line box) and protomer 1/2 (red dashed line box) of the S-close state (see also Extended Data Fig. 2f).

Fig. 2 | Structural basis of enhanced Omicron S-ACE2 interaction. **a**, Measurement of the binding affinity between ACE2 monomer and the S trimer of the G614 (left), Delta (middle) or Omicron (right) variants using bio-layer interferometry (BLI). Biotinylated S trimers were loaded onto streptavidin sensors and then allowed to interact with different concentrations of ACE2 (shown on the right). Raw sensorgrams and fitting curves were shown in color and gray, respectively. Association and dissociation phases were divided by red dotted lines. **b**, Cryo-EM maps of the Omicron S-ACE2 complex in three distinct conformational states. In the S-ACE2-C2/C3 maps, density of RBD-2- or RBD-3-associated ACE2 appears weaker than that of the stably associated ACE2 on RBD-1 (see also Extended Data Fig. 3c). ACE2 is shown in violet red. This color scheme is followed throughout. **c**, Density map of the focus-refined Omicron RBD-1-ACE2 and the zoomed-in view of the RBD-ACE2 interaction interface, showing the side chain densities of the Q493R, G496S, Q498R, N501Y and Y505H on RBM. **d**, The substituted residues R493, S496, R498 and H505 of Omicron

RBM form new interactions with E35, D38, Q42 and K353 of ACE2 (spring represents salt bridge, and the black dashed line represents H-bond) relative to that in the WT RBD-ACE2 (PDB: 6M0J, in dark grey). A newly formed H-bond without substitution is shown in red dashed line. **e**, Interaction interface areas between ACE2 and RBD of Omicron, Delta (PDB: 7W9I), or WT (PDB: 6M0J), analyzed using PISA. **f**, The electrostatic surface properties of Omicron, Delta (PDB: 7W9I), and WT (PDB: 6M0J) RBMs, with the mutated residues indicated. Black lines depict the footprint of ACE2 on RBD. **g**, The electrostatic surface property of ACE2, with residues in proximity to RBD-1 ($< 4 \text{ \AA}$) indicated (related to Extended Data Table 3).

Fig. 3 | Neutralization and binding activities of the MAbs against Omicron and Delta variants. The MAbs were raised against WT RBD or S trimer proteins. **a**, Neutralization IC₅₀ values and fold changes in neutralization potency for Delta and Omicron variant pseudoviruses (PV) compared to WT pseudovirus. A minus sign (-) denotes decrease. Orange shade, more than 10-fold decrease; red shade, more than 1000-fold decrease. **b**, Neutralization of the MAbs towards WT, Delta, and Omicron pseudoviruses. All MAbs were 4-fold serially diluted. Data are expressed as mean $\pm$ SEM of four replicate wells. **c**, Binding activities of the MAbs to recombinant S trimers of the WT, Delta, and Omicron strains were tested by ELISA. Serially diluted S trimer proteins were coated onto the ELISA wells. Data are mean $\pm$ SD of triplicate wells. Neutralization and ELISA data are representative of two independent experiments with similar results.

Fig. 4 | Cryo-EM analyses on the Omicron S-S3H3 Fab complex. **a-b**, Side and top views of the cryo-EM map of the Omicron S-open-S3H3 (a) and S-close-S3H3 complex (b), with the heavy and light chains of S3H3 Fab in medium blue and violet red, respectively. The color scheme was followed. **c**, Conformational comparison between Omicron S-open-S-S3H3 (light green) and Omicron S-open (orange), indicating a slight twist of the RBMs of S-open-S3H3 and the downward rotations of RBD-1 (up to 9.1°) and SD1. **d**, Model-map fitting of the focus-refined Omicron SD1-S3H3 structure, and the zoomed-in view of the Omicron SD1-S3H3 interaction interface. The sidechain densities at the interface were well resolved. **e**, The S3H3 binding on SD1 of protomer 2. **f**, The interaction involved regions/residues between

460 S3H3 Fab and SD1 with T547K labeled. Residues of SD-1 in proximity to S3H3 (< 4
461 Å) indicated (related to Extended Data Table 4). **g**, The SD1-S3H3 interaction interface
462 analyzed using PISA, with major involved structural elements labeled (spring
463 represents salt bridge, and the black dashed line represents the hydrogen bond).

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ACCELERATED ARTICLE PREVIEW

Method

Proteins expression and purification

To express SARS-CoV-2 Omicron variant S glycoprotein ectodomain, the mammalian codon-optimized gene coding SARS-CoV-2 (hCoV-19 Botswana R42B90_BHP_000842207 2021, GISAID ID: EPI_ISL_6752027) S glycoprotein ectodomain with double proline substitutions and “GSAS” substitution at the furin cleavage site was cloned into vector pcDNA 3.1+³⁵. A C-terminal T4 fibrin trimerization motif, a TEV protease cleavage site, a FLAG tag and a His tag were cloned downstream of the S glycoprotein ectodomain (Extended Data Fig. 1a). The constructs of prefusion-stabilized S proteins of SARS-CoV-2 G614 and Delta (B.1.617.2) variants were prepared as previously reported³¹. A gene encoding human ACE2 PD domain (Q18-D615) with an N-terminal interleukin-10 (IL-10) signal peptide and a C-terminal His tag was cloned into vector pcDNA 3.4³⁵. The recombinant proteins were prepared as the published protocol³⁵. Briefly, the constructs were transiently transfected into HEK293F cells (Thermo Fisher) using polyethylenimine (PEI). Three days after transfection, the supernatants were harvested by centrifugation, and then passed through 0.45 µm filter membrane. The clarified supernatants were added with 20 mM Tris-HCl pH 7.5, 200 mM NaCl, 20 mM imidazole, 4 mM MgCl₂, and incubated with Ni-NTA resin at 4°C for 1 hour. The Ni-NTA resin was recovered and washed with 20 mM Tris-HCl pH 7.5, 200 mM NaCl, 20 mM imidazole. The protein was eluted by 20 mM Tris-HCl pH 7.5, 200 mM NaCl, 250 mM imidazole.

Bio-layer interferometry (BLI) assay

Before BLI assay, Ni-NTA purified recombinant S trimer proteins of the G614, Delta and Omicron SARS-CoV-2 variants were further purified by gel filtration chromatography using a Superose 6 increase 10/300 GL column (GE Healthcare) pre-equilibrated with PBS. Then, 70 µg of purified S trimer proteins of G614, Delta and Omicron variants were separately biotinylated using the EZ-Link™ Sulfo-NHS-LC-LC-Biotin kit (Thermo Fisher) and then purified by Zeba™ spin desalting columns (Thermo Fisher).

Binding affinities of S trimers to ACE2 were determined by BLI analysis on an

Octet Red96 instrument (Pall FortéBio, USA). Briefly, biotinylated S trimer proteins (~40 µg/mL) were immobilized onto streptavidin (SA) biosensors (Pall FortéBio). After washing with kinetic buffer (0.01 M PBS with 0.02% Tween 20 and 0.1% bovine serum albumin), these sensors were incubated with 3-fold serial dilutions of ACE2 monomer protein for 500 s. Subsequently, the biosensors were allowed to dissociate in kinetic buffer for 500 s. The data were analyzed using the Octet Data Analysis 11.0 software to calculate affinity constants.

Neutralization

Luciferase (Luc)-expressing pseudoviruses bearing SARS-CoV-2 S proteins were constructed based on the HIV-1 backbone. Briefly, HEK 293T cells (ATCC) in 10-cm dish were co-transfected using PEI (polysciences) with 10 µg of pCMV-dR8.91 packaging plasmid, 10 µg of recombinant pLVX-IRES-ZsGreen1 plasmid containing luciferase reporter gene, and 2 µg of recombinant pVAX1 plasmids encoding SARS-CoV-2 S proteins. The cells were incubated with the transfection mixture for 6 h, and then 5 mL of fresh DMEM medium with 10% FBS was added to each dish. After incubation overnight, the media in the dishes was replaced with fresh DMEM medium (10% FBS). At 48 h post-transfection, the culture supernatant was harvested and frozen at -80 °C before use.

All MAbs were 4-fold serially diluted and tested by pseudovirus neutralization assay with human ACE2-overexpressing HEK 293T cells (293T-hACE2) following our previous protocol⁴⁵. Two days after pseudovirus infection, luciferase activity was measured. Data were analyzed by non-linear regression using GraphPad Prism 8 to calculate half inhibitory concentration (IC₅₀).

ELISA

To test binding activities of recombinant Omicron S protein with our previously developed anti-SARS-CoV-2 MAbs^{45,46}, recombinant S trimer proteins from WT⁴⁵, Delta, or Omicron SARS-CoV-2 strains were 2-fold serially diluted and coated onto ELISA plates at 37 °C for 2 h. The plates were blocked with 5% milk in PBS-Tween 20 (PBST) at 37 °C for 1 h. After washing with PBST, the plates were incubated with 50 ng/well of each of the anti-SARS-CoV-2 MAbs^{45,46} at 37 °C for 2 h, followed by horseradish peroxidase (HRP)-conjugated anti-mouse IgG (Sigma, 1/5,000 dilution) at

37 °C for 1 h. After washing and color development, absorbance was measured at 450 nm. ELISA data were analyzed by non-linear regression using GraphPad Prism 8.

Omicron S-S3H3 Fab complex formation

The Omicron S-S3H3 Fab complex was prepared following our previously reported protocol⁴⁶. Briefly, purified S3H3 IgG was incubated with papain (300:1 W/W) in PBS buffer (in the presence of 20 mM L-cysteine and 1 mM EDTA) for 3 h at 37 °C. The reaction was quenched by 20 mM iodoacetamide. Fab was purified by running over a HiTrap DEAE FF column (GE Healthcare) pre-equilibrated with PBS. Omicron S protein was incubated with S3H3 Fab in a 1:6 molar ratio on ice for 1 h. The Omicron S-S3H3 Fab complex was purified by size-exclusion chromatography using Superose 6 increase 10/300 GL column (GE Healthcare) in 20 mM Tris-HCl pH 7.5, 200 mM NaCl, 4% glycerol. The complex peak fractions were concentrated and assessed by SDS-PAGE.

Cryo-EM sample preparation

To prepare the cryo-EM sample of the Omicron S trimer, a 2.2 µl aliquot of the sample was applied on a plasma-cleaned holey carbon grid (R 1.2/1.3, Cu, 200 mesh; Quantifoil). The grid was blotted with Vitrobot Mark IV (Thermo Fisher Scientific) at 100% humidity and 8 °C, and then plunged into liquid ethane cooled by liquid nitrogen. To prepare the cryo-EM sample of the Omicron S-ACE2 complex, purified Omicron S trimer was incubated with ACE2 in a 1:4 molar ratio on ice for 20 min and then vitrified using the same condition. The purified Omicron S-S3H3 complex was vitrified using the same procedure as for the Omicron S sample.

Cryo-EM data collection

Cryo-EM movies of the samples were collected on a Titan Krios electron microscope (Thermo Fisher Scientific) operated at an accelerating voltage of 300 kV. For the three datasets, the movies were collected at a magnification of 64,000× and recorded on a K3 direct electron detector (Gatan) operated in the counting mode (yielding a pixel size of 1.093 Å), and under a low-dose condition in an automatic manner using EPU 2.11 software (Thermo Fisher Scientific). Each frame was exposed for 0.1 s, and the total exposure time was 3 s, leading to a total accumulated dose of 50.2 e⁻/Å² on the specimen.

Cryo-EM 3D reconstruction

For each dataset, the motion correction of image stack was performed using the embedded module of Motioncor2 in Relion 3.1^{35,50,51} and CTF parameters were determined using CTFFIND4.1.8⁵² before further data processing. Unless otherwise described, the data processing was performed in Relion 3.1.

For the Omicron S dataset (Extended Data Fig. 2), 600,845 particles remained after reference-free 2D classification in cryoSPARC v3.3.1³⁰. After 2 rounds of 3D classification and further focused 3D classification on the RBD-1 region, we obtained an Omicron S-close map from 108,509 particles and a RBD-1-up open conformation from 69,873 particles. We then performed focused 3D classification on the RBD-3 region of the open state dataset and obtained an Omicron S-open map from 30,967 particles and a S-open-2 map from 38,906 particles. After Bayesian polishing and CTF refinement, the Omicron S-close, S-open, and S-open-2 datasets were independently loaded into cryoSPARC v3.3.1³⁰ and refined to the resolution of 3.08 Å, 3.40 Å and 3.41 Å, respectively, using Non-uniform refinement. The overall resolutions for all the cryo-EM maps in this study were determined based on the gold-standard criterion using a Fourier shell correlation (FSC) of 0.143. Moreover, we performed 3D variability analysis (3DVA) on the Omicron S trimer dataset containing 178,382 particles in cryoSPARC v3.3.1 to capture its continuous conformational dynamics³⁰.

For the Omicron S-ACE2 dataset (Extended Data Fig. 3), 1,268,072 particles remained after reference-free 2D classification. After two rounds of 3D classification and further focused 3D classification on the RBD-1-ACE2 region, we obtained an Omicron S-ACE2 map from 141,538 particles. After Bayesian polishing and CTF refinement, the map was reconstructed to 3.53-Å-resolution. We then focused on RBD-2 region for further classification and obtained two conformations, with RBD-2 in the “down” position (43.9%), termed S-ACE2-C1, or “up” position. The RBD-2 “up” dataset was further focused 3D classified on RBD-3 region. We then obtained two conformations with RBD-3 in the “down” or “up” position, termed as S-ACE2-C2 and -C3, respectively. The three datasets were independently loaded into cryoSPARC v3.3.1 and refined using Non-uniform refinement to 3.69-, 3.70-, and 4.04-Å-resolution, respectively. Here, after obtaining the 3.53-Å-resolution map of Omicron S-ACE2, we performed further local refinement on the RBD-1-ACE2 region (indicated by dotted

black ellipsoid in Extended Data Fig. 3a) in cryoSPARC v3.3.1 to acquire a 3.67-Å-resolution map of this region.

For the Omicron S-S3H3 dataset (Extended Data Fig. 5), similar data processing procedure was adapted as described for the Omicron S dataset to obtain a 3.56-Å-resolution S-S3H3 map from 238,121 particles. We then carried out focused 3D classification on the RBD-1 region, followed by Non-uniform refinement in cryoSPARC v3.3.1, and obtained a 3.48-Å-resolution S-open-S3H3 map from 162,221 particles and a 3.64-Å-resolution S-close-S3H3 map from 75,900 particles. In addition, after obtaining the 3.56-Å-resolution map, we performed focused 3D classification on the S3H3-SD1 region of protomer 2 (highlighted by dotted orange ellipsoid in Extended Data Fig. 5), leading to a dataset of 101,192 particles, which was further local refined on the S3H3-SD1 region in cryoSPARC v3.3.1, deducing a 3.61-Å-resolution map of this region. All of the obtained maps were post-processed through deepEMhancer⁵³.

Atomic model building

To build an atomic model for the Omicron S-open structure, we used the atomic model of Delta S-open (PDB: 7W92) from our prior study as the initial model³¹. We first fit the model into our Omicron S-open map in Chimera by rigid body fitting, then manually substituted the mutations of the Omicron variant in COOT⁵⁴. Subsequently, we flexibly refined the model against our Omicron S-open map using ROSETTA⁵⁵. Finally, we used the phenix.real_space_refine module in Phenix 1.19.2-4158 for the S trimer model refinement against the map⁵⁶. For the S-close model, we utilized the down protomer from our recent Delta S-transition (PDB: 7W94)³¹ structure as initial template, and followed similar procedure described above for model refinement. For the Omicron S-ACE2 and the local refined RBD-1-ACE2 structures, we used the Delta S-ACE2 model (PDB: 7W98, 7W9I)³¹ as initial template, and followed similar procedure described above for model refinement. For better fitting in the dynamic ACE2 region of the Omicron S-ACE2 C1/C2/C3 maps, we merged the better resolved Omicron RBD-1-ACE2 model with the other portion of the original model to make a complete model, and refined it against the corresponding unsharpened map. For the Omicron S-S3H3 and the local refined RBD-1-S3H3 structures, we utilized our recent Beta S-S3H3 model (PDB: 7WDF, 7WD8)⁴⁶ as template, and followed similar

procedure described above for model refinement. The atomic models were validated using Phenix.molprobrity command in Phenix. Interaction interface analyses were conducted through PISA server⁵⁷.

UCSF Chimera and ChimeraX were applied for figure generation, rotation measurement, and Coulombic potential surface analysis^{58,59}.

Data availability

The cryo-EM maps determined for the Omicron S trimer have been deposited at the Electron Microscopy Data Bank with accession codes [EMD-32556](#), [EMD-32854](#) and [EMD-32855](#), and the associated atomic models have been deposited in the Protein Data Bank with accession codes [7WK2](#), [7WVN](#) and [7WVQ](#) for S-close, S-open and S-open-2, respectively. For the S-ACE2 dataset, related cryo-EM maps have been deposited in the Electron Microscopy Data Bank with accession codes [EMD-32558](#), [EMD-32856](#), [EMD-32857](#) and [EMD-32560](#), and the associated models have been deposited in the Protein Data Bank with accession codes [7WK4](#), [7WVP](#), [7WVQ](#) and [7WK6](#) for S-ACE2-C1, S-ACE2-C2, S-ACE-C3 and RBD-1-ACE2, respectively. For the S-S3H3 Fab dataset, related cryo-EM maps have been deposited in the Electron Microscopy Data Bank with accession codes [EMD-32562](#), [EMD-32563](#), and [EMD-32564](#), and the associated models have been deposited in the Protein Data Bank with accession codes [7WK8](#), [7WK9](#) and [7WKA](#) for SD1-S3H3, S-open-S3H3 and S-close-S3H3, respectively. The structures used for initial templates or structural analysis in this work include PDB IDs: [7W92](#), [7W94](#), [7W98](#), [7W9I](#), [7WDF](#), [7WD8](#), [7KRQ](#), [7KRR](#), [6M0J](#), [7DK4](#), [7WCR](#), [7W9F](#), and [7DCC](#). For gel source images, see the Supplementary Figure 1. Source data are provided with this paper.

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Author contributions

Y.C. and Z.H. designed the experiments; Y-X. Wang expressed and purified the proteins with assistants of Z.L. and S.X.; Q.H. and W.H. performed cryo-EM data acquisitions; Q.H., W.H., J.L., and Y-F. Wang performed cryo-EM reconstructions, model buildings; C.Z. and S.X. performed biochemical analyses; J.L. Q.H., W.H., Y-F. Wang, C.X. and C.Z. analyzed the data; Y.C. and Z.H. together with Q.H., J.L. W.H., Y-X. Wang and C.Z. wrote the manuscript.

Competing interests

Z.H., S.X., and C.Z. are listed as inventors on a pending patent application for MAb S3H3. The other authors declare that they have no competing interests.

Additional information

Supplementary Information is available for this paper.

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Extended Data Figure Legends

Extended Data Fig. 1 | Purification of Omicron variant S and S-S3H3 Fab

complex. **a**, Schematic diagram of the Omicron variant S organization in this study (left, positions of all mutations are indicated), and the model of a SARS-CoV-2 S protomer (right) with mutation sites of the Omicron variant shown as red sphere. **b**, SDS-PAGE analysis of the purified Omicron variant S protein. Representative images of two independent experiments are shown. For gel source data, related to Supplementary Figure 1. **c**, Size-exclusion chromatogram and SDS-PAGE analysis of the formed Omicron S-S3H3 Fab complex. Representative images of two independent experiments are shown. For gel source data, related to Supplementary Figure 1.

Extended Data Fig. 2 | Cryo-EM analysis on the Omicron S trimer. **a**, Data processing workflow for structure determination of the Omicron S trimer. A representative original micrograph, reference-free 2D class averages, and the population distributions of the Omicron S-close, S-open, and S-open-2 states are also presented. **b**, Resolution assessment of the Omicron S-close, S-open, and S-open-2 maps by FSC at 0.143 criterion. **c-d**, Angular distribution (c) and local resolution evaluation (d) of the S-close, S-open, and S-open-2 maps. **e**, Model-map fitting for each of the three structures. **f**, Model and map fitting in the inter-protomer interaction area of Omicron S-close. Protomer 1/2/3 are colored in light green, royal blue and gold, respectively.

Extended Data Fig. 3 | Cryo-EM data processing procedure for the Omicron

S-ACE2 complex. **a**, Data processing procedure for the Omicron S-ACE2 dataset. A representative original micrograph and the reference-free 2D class averages are also presented. The masks for the focused 3D classification on RBD-2 and RBD-3 regions are shown as pink and orange transparent surfaces, respectively. **b**, Population distribution of the Omicron S-ACE2-C1/-C2/-C3 states. **c**, Lower threshold rendering of the Omicron S-ACE2-C1/-C2/-C3 maps. ACE2 density (violet red) also associates with RBD-2 or RBD-3 in the C2 and C3 states, although appearing weaker than that of the stably associated ACE2 on RBD-1, indicating a dynamic ACE2 association with RBD-2/-3.

Extended Data Fig. 4 | Cryo-EM analysis on the Omicron S-ACE2 complex. **a**, Resolution assessment of the cryo-EM maps by FSC at 0.143 criterion. **b**, Angular distribution of the Omicron S-ACE2-C1/-C2/-C3 and RBD-1-ACE2 maps. **c-d**, Local resolution evaluation (c) and model-map fitting (d) for the Omicron S-ACE2 maps and the RBD-1-ACE2 map.

Extended Data Fig. 5 | Cryo-EM analysis on the Omicron S-S3H3 Fab complex. **a**, Data processing workflow for the Omicron S-S3H3 Fab complex. A representative original micrograph and the reference-free 2D class averages are also presented. **b**, Resolution assessment of the cryo-EM maps by FSC at 0.143 criterion. **c**, Local resolution evaluation of the Omicron S-S3H3 and SD1-S3H3 maps. **d**, Angular distribution for the three Omicron S-S3H3 complexes. **e**, Model-map fitting for the Omicron S-S3H3 complexes.

Extended Data Fig. 6 | Structural basis of 2H2 antibody retaining neutralization potency and 8D3/S5D2/3C1 antibodies losing their neutralization potency against Omicron. **a**, Model of the Omicron RBD-2H2 Fab interaction interface, obtained by docking of the RBD-bound 2H2 (PDB ID: 7DK4) onto the RBD-1 structure from our current Omicron S-open. **b**, The electrostatic surface properties of Omicron and WT RBDs, with the black line depicting the footprint of 2H2 on RBD-1. The residues with significant electrical charge changes are indicated. **c**, The electrostatic surface property of 2H2 Fab, with the black line depicting the footprint of RBD-1 on 2H2. **d-f**, Docking of RBD-bound 8D3/S5D2/3C1 (PDB ID: 7W9F/7WCR/7DCC, respectively) onto the Omicron RBD-1 structure. Residue S477N that may potentially clash with the 8D3 Fab is labeled (d). Residues S477N and T478K that may break the H-bond network between RBD and S5D2 Fab are labeled (e). Residue S375F that may contribute to the Omicron's escape of 3C1 binding and neutralization is labeled (f). All substituted residues in Omicron RBD-1 are colored in red.

761 **Extended Data Table Legends**

762 **Extended Data Table 1 | Cryo-EM data collection and refinement statistics for**
763 **Omicron S, Omicron S-ACE2, and Omicron S-S3H3**

764 **Extended Data Table 2 | Omicron S-close protomer 1/2 and protomer 1/3**
765 **interactions**

766 **Extended Data Table 3 | Interaction interface analysis (upper table) and footprint**
767 **distance measurement (lower table) for Omicron RBD-1-ACE2 structure**

768 **Extended Data Table 4 | Interaction interface analysis (upper table) and footprint**
769 **distance measurement (lower table) for Omicron SD1-S3H3 structure**

e

Structure	Interaction interface ($\text{\AA}^2$)
Omicron S-ACE2	920.2
Delta S-ACE2	928.4
WT S-ACE2	843.3

a

MAb	IC50 value (ng/mL)			IC50 value (nM)			IC50 fold change in variant PV neutralization (relative to WT)	
	WT	Delta	Omicron	WT	Delta	Omicron	Delta	Omicron
3C1	286.7	231.1	>10000	1.911	1.541	>66.667	1.2	<-34.9
2H2	9.1	6.3	30.4	0.061	0.042	0.203	1.4	-3.3
8D3	6.5	6.4	>10000	0.043	0.043	>66.667	1.0	<-1538
S5D2	8.2	734.6	>10000	0.055	4.897	>66.667	-89.6	<-1220
S3H3	82.2	39.3	53.3	0.548	0.262	0.355	2.1	1.5

b **c**

1

NTD

RBD

SD1

SD2

FP

HR1

CH

1208

9XHis tag

FLAG tag

TEV cleavage site

T4 fibrin trimerization motif

2P Mutation

Mutated RRAR to GSAS

H69-V70 DEL

A67V

T95I

G142D

N214 DEL

ins 214 EPE

G339D

S371L

S375F

K417N

K446S

G447N

G493R

G498R

N501Y

Y505H

D614G

H655Y

P681H

N764K

D796Y

N856K

Q954H

N969K

L961P

Extended Data Fig. 2 | Cryo-EM analysis on the Omicron S trimer. **a**, Data processing workflow for structure determination of the Omicron S trimer. A representative original micrograph, reference-free 2D class averages, and the population distributions of the Omicron S-close, S-open, and S-open-2 states are also presented. **b**, Resolution assessment of the Omicron S-close, S-open, and S-open-2 maps by FSC at 0.143 criterion. **c-d**, Angular distribution (c) and local resolution evaluation (d) of the S-close, S-open, and S-open-2 maps. **e**, Model-map fitting for each of the three structures. **f**, Model and map fitting in the inter-protomer interaction area of Omicron S-close. Protomer 1/2/3 are colored in light green, royal blue and gold, respectively.

Extended Data Fig. 3 | Cryo-EM data processing procedure for the Omicron S-ACE2 complex. **a**, Data processing procedure for the Omicron S-ACE2 dataset. A representative original micrograph and the reference-free 2D class averages are also presented. The masks for the focused 3D classification on RBD-2 and RBD-3 regions are shown as pink and orange transparent surfaces, respectively. **b**, Population distribution of the Omicron S-ACE2-C1/-C2/-C3 states. **c**, Lower threshold rendering of the Omicron S-ACE2-C1/-C2/-C3 maps. ACE2 density (violet red) also associates with RBD-2 or RBD-3 in the C2 and C3 states, although appearing weaker than that of the stably associated ACE2 on RBD-1, indicating a dynamic ACE2 association with RBD-2/-3.

Extended Data Fig. 4 | Cryo-EM analysis on the Omicron S-ACE2 complex. a, Resolution assessment of the cryo-EM maps by FSC at 0.143 criterion. **b**, Angular distribution of the Omicron S-ACE2-C1/-C2/-C3 and RBD-1-ACE2 maps. **c-d**, Local resolution evaluation (c) and model-map fitting (d) for the Omicron S-ACE2 maps and the RBD-1-ACE2 map.

a

b

c

e

d

Extended Data Fig. 5 | Cryo-EM analysis on the Omicron S-S3H3 Fab complex. **a**, Data processing workflow for the Omicron S-S3H3 Fab complex. A representative original micrograph and the reference-free 2D class averages are also presented. **b**, Resolution assessment of the cryo-EM maps by FSC at 0.143 criterion. **c**, Local resolution evaluation of the Omicron S-S3H3 and SD1-S3H3 maps. **d**, Angular distribution for the three Omicron S-S3H3 complexes. **e**, Model-map fitting for the Omicron S-S3H3 complexes.

Extended Data Fig. 6 | Structural basis of 2H2 antibody retaining neutralization potency and 8D3/S5D2/3C1 antibodies losing their neutralization potency against Omicron. **a**, Model of the Omicron RBD-2H2 Fab interaction interface, obtained by docking of the RBD-bound 2H2 (PDB ID: 7DK4) onto the RBD-1 structure from our current Omicron S-open. **b**, The electrostatic surface properties of Omicron and WT RBDs, with the black line depicting the footprint of 2H2 on RBD-1. The residues with significant electrical charge changes are indicated. **c**, The electrostatic surface property of 2H2 Fab, with the black line depicting the footprint of RBD-1 on 2H2. **d-f**, Docking of RBD-bound 8D3/S5D2/3C1 (PDB ID: 7W9F/7WCR/7DCC, respectively) onto the Omicron RBD-1 structure. Residue S477N that may potentially clash with the 8D3 Fab is labeled (d). Residues S477N and T478K that may break the H-bond network between RBD and S5D2 Fab are labeled (e). Residue S375F that may contribute to the Omicron's escape of 3C1 binding and neutralization is labeled (f). All substituted residues in Omicron RBD-1 are colored in red.

Extended Data Table 1 | Cryo-EM data collection and refinement statistics for Omicron S, Omicron S-ACE2, and Omicron S-S3H3

	S-close (EMDB-3 2556) (PDB 7WK2)	S-open (EMDB-3 2854) (PDB 7WVN)	S-open-2 (EMDB- 32855) (PDB 7WVO)	S-ACE2- C1 (EMDB-3 2558) (PDB 7WK4)	S-ACE2- C2 (EMDB-3 2856) (PDB 7WVP)	S-ACE2- C3 (EMDB-3 2857) (PDB 7WVQ)	RBD-1-A CE2 (EMDB-3 2560) (PDB 7WK6)	S-open-S 3H3 (EMDB-3 2563) (PDB 7WK9)	S-close-S 3H3 (EMDB-3 2564) (PDB 7WKA)	SD1-S3H3 (EMDB-325 62) (PDB 7WK8)
Data collection and processing										
Magnification						64,000				
Voltage (kV)						300				
Electron exposure (e ⁻ /Å ²)						50.2				
Defocus range (μm)						-0.8 to -2.5				
Pixel size (Å)						1.093				
Symmetry imposed						C1				
Initial particle images (no.)	761,519	761,519	761,519	2,726,095	2,726,095	2,726,095	2,726,095	2,237,264	2,237,264	2,237,264
Final particle images (no.)	108,509	30,967	38,906	62,082	58,331	21,125	141,538	162,221	75,900	101,192
Map resolution (Å)	3.08	3.40	3.41	3.69	3.70	4.04	3.67	3.48	3.64	3.61
FSC threshold						0.143				
Map resolution range (Å)	2.5-5.0	3.0-6.5	3.0-6.5	3.0-7.0	3.0-7.0	3.0-7.0	3.0-4.6	3.0-7.0	3.0-7.0	3.1-3.9
Refinement										
Initial model used (PDB code)	7W94	7W92	7W92	7W98	7W98	7W98	7W9I	7WDF	7WDF	7WD8
Model resolution (Å)	3.8	4.0	4.0	4.5	4.4	7.8	3.9	4.1	4.2	3.6
FSC threshold						0.5				
Model resolution range (Å)	3.8	4.0	4.0	4.5	4.4	7.8	3.9	4.1	4.2	3.6
Map sharpening B factor (Å ²)	-77.8	-59.8	-60.4	-83.8	-90.1	-156.88	-129.7	-108.1	-93.9	-133.3
Model composition										
Non-hydrogen atoms	24,882	24,808	23,191	29,545	29,605	29,531	6,430	29,745	31,353	3,982
Protein residues	3,162	3,162	2,961	3,743	3,750	3,741	791	3,805	4,017	516
Ligands	0	0	0	0	0	0	0	0	0	0
B factors (Å²)										
Protein	106.21	145.32	129.50	174.20	246.11	251.45	99.00	179.47	183.22	67.06
Ligand	0	0	0	0	0	0	0	0	0	0
R.m.s. deviations										
Bond lengths (Å)	0.006	0.006	0.006	0.005	0.005	0.008	0.008	0.005	0.005	0.005
Bond angles (°)	0.814	0.793	0.794	0.857	0.833	1.027	1.301	1.130	1.145	1.117
Validation										
MolProbity score	1.24	1.20	1.20	1.41	1.41	1.53	1.66	1.38	1.37	1.59
Clashscore	2.15	1.85	1.85	3.87	3.37	3.82	5.57	3.08	3.13	5.60
Poor rotamers (%)	0.14	0.07	0.04	0.49	0.27	0.46	0.57	0.36	0.23	0.22
Ramachandran plot										
Favored (%)	96.22	96.24	96.29	96.40	95.97	94.71	94.92	95.95	96.14	95.87
Allowed (%)	3.78	3.73	3.68	3.44	3.95	5.13	4.70	3.89	3.74	3.94
Disallowed (%)	0.00	0.03	0.03	0.16	0.08	0.16	0.38	0.16	0.13	0.20

Extended Data Table 2 | Omicron S-close protomer 1/2 and protomer 1/3 interactions

S-close Protomer 1		S-close Protomer 2		Interaction	Distance (Å)
Residue	Atom	Residue	Atom		
PHE 43	[O]	ARG 567	[N]	H-bond	3.29
PRO 230	[O]	ARG 357	[OH]	H-bond	3.68
TYR 369	[OH]	ASN 460	[NH1]	H-bond	3.03
ASN 370	[OD1]	TYR 421	[ND2]	H-bond	2.39
ALA 372	[O]	ASN 417	[OH]	H-bond	3.02
PHE 375	[O]	ARG 408	[NH1]	H-bond	3.01
ASP 737	[OD1]	ASN 317	[NH2]	H-bond	2.86
THR 739	[OG1]	ARG 319	[NH2]	H-bond	3.01
GLN 755	[O]	LYS 969	[N]	H-bond	3.20
GLN 755	[O]	SER 968	[OG]	H-bond	2.40
GLN 755	[O]	PHE 970	[N]	H-bond	3.59
SER 758	[OG]	GLN 965	[NE2]	H-bond	2.47
ILE 788	[O]	ASN 703	[N]	H-bond	3.20
PRO 863	[O]	ALA 668	[N]	H-bond	2.87
LEU 864	[O]	GLY 669	[N]	H-bond	2.93
SER 884	[OG]	TYR 707	[N]	H-bond	2.40
GLN 895	[O]	ALA 713	[OH]	H-bond	3.51
PHE 981	[O]	LYS 386	[OH]	H-bond	2.47
ARG 983	[O]	SER 383	[N]	H-bond	2.94
LEU 984	[O]	LYS 386	[NZ]	H-bond	3.47
GLU 1031	[OE2]	VAL 1040	[N]	H-bond	3.50
GLU 1111	[OE2]	SER 1123	[N]	H-bond	3.31
TYR 200	[OH]	GLU 516	[O]	H-bond	2.57
TYR 369	[OH]	ASN 460	[OE2]	H-bond	3.09
ALA 372	[N]	ASN 417	[OD1]	H-bond	3.62
GLN 762	[NE2]	THR 961	[OG]	H-bond	3.07
LYS 764	[NZ]	GLN 314	[OE1]	H-bond	3.00
THR 768	[OG1]	GLN 314	[OE1]	H-bond	3.49
GLN 787	[NE2]	ALA 701	[O]	H-bond	2.76
ILE 788	[N]	ALA 701	[O]	H-bond	3.41
LYS 790	[N]	ASN 703	[OE2]	H-bond	3.87
LYS 790	[NZ]	GLU 702	[OG1]	H-bond	2.70
LYS 856	[NZ]	THR 572	[O]	H-bond	3.88
GLN 895	[NE2]	ALA 706	[OD2]	H-bond	2.77
TYR 904	[OH]	GLY 1093	[O]	H-bond	2.41
SER 975	[OG]	ASP 571	[OE2]	H-bond	3.72
ASN 978	[ND2]	LYS 547	[OE1]	H-bond	3.23
LYS 790	[NZ]	GLU 702	[OE1]	Salt bridge	3.91
LYS 790	[NZ]	GLU 702	[OE2]	Salt bridge	2.70

S-close Protomer 1		S-close Protomer 3		Interaction	Distance (Å)
Residue	Atom	Residue	Atom		
GLU 516	[OE1]	TYR 200	[OH]	H-bond	2.99
ASN 417	[OD1]	ALA 372	[N]	H-bond	2.60
GLN 314	[O]	LYS 764	[NZ]	H-bond	3.84
ALA 701	[O]	GLN 787	[NE2]	H-bond	3.00
ALA 701	[O]	ILE 788	[N]	H-bond	3.25
ASN 703	[O]	LYS 790	[N]	H-bond	3.84
GLU 702	[OE2]	LYS 790	[NZ]	H-bond	2.47
SER 698	[O]	TYR 873	[OH]	H-bond	3.53
TYR 707	[OH]	THR 883	[OG1]	H-bond	3.08
TYR 707	[OH]	SER 884	[OG]	H-bond	3.50
ALA 706	[O]	GLN 895	[NE2]	H-bond	3.00
LYS 547	[O]	ASN 978	[ND2]	H-bond	3.20
ARG 567	[N]	PHE 43	[O]	H-bond	3.83
LYS 557	[NZ]	SER 45	[OG]	H-bond	3.42
ASN 417	[ND2]	ALA 372	[O]	H-bond	2.66
ARG 408	[NH2]	PHE 375	[O]	H-bond	3.30
ARG 408	[NH2]	THR 376	[OG1]	H-bond	2.44
ASN 317	[ND2]	ASP 737	[OD2]	H-bond	2.33
ARG 319	[NH2]	ASP 737	[OD2]	H-bond	3.79
ARG 319	[NH1]	ASP 745	[OD1]	H-bond	3.68
LYS 969	[N]	GLN 755	[O]	H-bond	2.86
PHE 970	[N]	GLN 755	[O]	H-bond	3.12
GLN 965	[NE2]	SER 758	[OG]	H-bond	2.46
ALA 701	[N]	LYS 786	[O]	H-bond	3.67
ASN 703	[N]	ILE 788	[O]	H-bond	3.23
ALA 668	[N]	PRO 863	[O]	H-bond	2.96
GLY 669	[N]	LEU 864	[O]	H-bond	3.54
ALA 713	[N]	GLN 895	[O]	H-bond	3.38
LYS 386	[NZ]	PHE 981	[O]	H-bond	2.50
SER 383	[N]	ARG 983	[O]	H-bond	3.20
LYS 386	[NZ]	LEU 984	[O]	H-bond	2.68
VAL1040	[N]	GLU1031	[OE2]	H-bond	3.82
SER1123	[OG]	GLU1111	[OE2]	H-bond	2.98
GLU 702	[OE2]	LYS 790	[NZ]	Salt bridge	2.47
ARG 319	[NH2]	ASP 737	[OD2]	Salt bridge	3.79
ARG 319	[NH1]	ASP 745	[OD1]	Salt bridge	3.68

Extended Data Table 3 | Interaction interface analysis (upper table) and footprint distance measurement (lower table) for Omicron RBD-1-ACE2 structure

Omicron S RBD-1		ACE2		Interaction	Distance(Å)
Residue	Atom	Residue	Atom		
ASN 487	[OD1]	TYR 83	[OH]	H-bond	2.45
TYR 489	[OH]	TYR 83	[OH]	H-bond	3.00
SER 494	[O]	HIS 34	[NE2]	H-bond	3.09
SER 496	[O]	LYS 353	[NZ]	H-bond	3.65
SER 496	[OG]	LYS 353	[NZ]	H-bond	2.82
ASN 477	[ND2]	SER 19	[O]	H-bond	3.86
ASN 477	[ND2]	SER 19	[OG]	H-bond	3.38
ASN 487	[ND2]	GLN 24	[OE1]	H-bond	2.74
ARG 493	[NH2]	GLU 35	[OE1]	H-bond	3.58
TYR 449	[OH]	ASP 38	[OD1]	H-bond	2.79
ARG 498	[NH1]	ASP 38	[OD1]	H-bond	3.37
TYR 449	[OH]	ASP 38	[OD2]	H-bond	2.62
TYR 449	[OH]	GLN 42	[OE1]	H-bond	3.27
ARG 498	[NH1]	GLN 42	[OE1]	H-bond	3.04
HIS 505	[ND1]	LYS 353	[O]	H-bond	2.85
THR 500	[OG1]	ASP 355	[OD2]	H-bond	3.40
ARG 493	[NH2]	GLU 35	[OE1]	Salt bridge	3.58
ARG 493	[NH2]	GLU 35	[OE2]	Salt bridge	3.93
ARG 498	[NH1]	ASP 38	[OD1]	Salt bridge	3.37

Omicron S RBD-1	ACE2
Y449	D38, Q42
Y453	H34
L455	D30
F456	T27, D30, K31
A475	Q24, T27
N477	S19
F486	M82, Y83
N487	Q24, Y83
Y489	T27, F28
R493	H34, E35
S494	H34
S496	D38, K353
R498	D38, Y41, Q42
T500	Y41, D355, R357
Y501	Y41, K353, G354, D355
G502	G354
H505	K353, G354

Extended Data Table 4 | Interaction interface analysis (upper table) and footprint distance measurement (lower table) for Omicron SD1-S3H3 structure

Omicron S		S3H3		Interaction	Distance(Å)
Residue	Atom	Residue	Atom		
LYS 537	[NZ]	ASP 55	[OD2]	H-bond	3.35
THR 581	[OG1]	ASP 102	[OD1]	H-bond	3.25
SER 555	[O]	SER 32	[OG]	H-bond	3.05
ILE 584	[O]	SER 32	[OG]	H-bond	3.80
LYS 537	[NZ]	ASP 55	[OD2]	Salt bridge	3.35

Omicron S	S3H3
T323	S54
E324	R31
N532	F32
L533	Y101
V534	R31, W33
K535	Y101
N536	W33, R59, L98
K537	H52, D55
E554	Y36, S95, R96
S555	A31, S32
N556	A31, R96
T581	D102
L582	Y34
E583	Y103
I584	S32

Heavy chain
Light chain

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n/a Confirmed

- ☒ ☐ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- ☒ ☐ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- ☒ ☐ The statistical test(s) used AND whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- ☒ ☐ A description of all covariates tested
- ☒ ☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- ☐ ☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- ☒ ☐ For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
- ☒ ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- ☒ ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- ☒ ☐ Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection FEI TEM user interface, EPU 2.11

Data analysis Octet Data Analysis 11.0, GraphPad Prism (version 8), MotionCor2, RELION 3.1, CTFFIND 4.1.8, Phenix 1.19.2-4158, Rosetta 2017, COOT 0.9.6.2-pre, UCSF Chimera 1.15, UCSF ChimeraX 1.3, PDBePISA, cryoSPARC v3.3.1, deepEMhancer

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- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

All data presented in this study are available within the figures and in the Supplementary Information. For the SARS-CoV-2 Omicron variant, related cryo-EM maps have been deposited at the Electron Microscopy Data Bank with accession codes EMD-32556 [<https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-32556>], EMD-32557 [<https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-32557>], EMD-32558 [<https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-32558>], EMD-32559 [<https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-32559>], EMD-32560 [<https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-32560>], EMD-32562 [<https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-32562>], EMD-32563 [<https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-32563>], and EMD-32564 [<https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-32564>], and associated atomic models have been deposited in the Protein Data Bank with accession codes 7WK2 [<https://doi.org/10.2210/pdb7WK2/pdb>], 7WK3 [<https://doi.org/10.2210/pdb7WK3/pdb>], and 7WK4 [<https://doi.org/10.2210/pdb7WK4/pdb>].

doi.org/10.2210/pdb7WK3/pdb], 7WK4 [https://doi.org/10.2210/pdb7WK4/pdb], 7WK5 [https://doi.org/10.2210/pdb7WK5/pdb], 7WK6 [https://doi.org/10.2210/pdb7WK6/pdb], 7WK8 [https://doi.org/10.2210/pdb7WK8/pdb], 7WK9 [https://doi.org/10.2210/pdb7WK9/pdb], and 7WKA [https://doi.org/10.2210/pdb7WKA/pdb] for S-close, S-open, S-ACE2-C1, S-ACE2-C2, RBD-1-ACE2, SD1-S3H3, S-open-S3H3 and S-close-S3H3, respectively.

Field-specific reporting

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No statistical methods were used to determine sample size for EM data. Sample sizes were determined based on the ability to get sufficient particles for 3D reconstruction and particles with good features are selected.
Data exclusions	The cryo-EM images of poor quality were deleted based on the defocus, astigmatism and reslution for better resolution.
Replication	Experimental findings were reliably reproduced. Most of the experiments were replicated two or three times.
Randomization	Randomization was not relevant for this study, as data were collected automatically and there were no samples allocated into control and experimental groups.
Blinding	Blinding was not relevant for this study, as data were collected automatically, and the data under investigation need to be known.

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Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	HRP-conjugated anti-mouse IgG (Sigma, A0168-1ML); anti-WT-Spike-protein MAbs 3C1, 2H2, 8D3, S5D2, and S3H3 (50 ng/well [1 µg/mL] for each MAb in ELISA) were prepared in Zhong Huang's lab.
Validation	The specifications of commercially available HRP-conjugated anti-mouse IgG can be found on the manufacture's website (https://www.sigmaaldrich.com/HK/zh/product/sigma/a0168). Anti-WT-Spike-protein MAbs 2H2, 3C1, and 8D3 (Zhang et al. Nature communications. 2021, 12, 264; https://doi.org/10.1038/s41467-020-20465-w), and S5D2 and S3H3 (Xu et al., 2021, Emerging Microbes & Infections. https://doi.org/10.1080/22221751.2021.2024455) have been validated previously.

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)	Human ACE2-expressing HEK 293T cells (293T-hACE2) were generated in Zhong Huang's lab; HEK293F suspension cells, Thermo fisher.
Authentication	293T-hACE2 cells have been validated previously (Zhang et al. Nature communications. 2021, 12, 264; https://doi.org/10.1038/s41467-020-20465-w). HEK293F cells were not authenticated further after purchase.

Mycoplasma contamination

Cell lines have not recently been tested for Mycoplasma contamination.

Commonly misidentified lines
(See [ICLAC](#) register)

No commonly misidentified lines were used.