The newly reported Omicron variant is poised to replace Delta as the most prevalent severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variant across the world. Cryo–electron microscopy (cryo-EM) structural analysis of the Omicron variant spike protein in complex with human angiotensin-converting enzyme 2 (ACE2) reveals new salt bridges and hydrogen bonds formed by mutated residues arginine-493, serine-496, and arginine-498 in the binding domain with ACE2. These interactions appear to compensate for other Omicron mutations such as the substitution of asparagine for lysine on position 417 (K417N) that are known to reduce ACE2 binding affinity, resulting in similar biochemical ACE2 binding affinities for the Delta and Omicron variants. Neutralization assays show that pseudoviruses that display the Omicron spike protein exhibit increased antibody evasion. The increase in antibody evasion and the retention of strong interactions at the ACE2 interface thus represent important molecular features that likely contribute to the rapid spread of the Omicron variant.
Fig. 1. Cryo-EM structure of the Omicron spike protein. (A) A schematic diagram illustrating the domain arrangement of the spike protein. Mutations present in the Omicron variant spike protein are labeled. RBM, receptor binding motif. (B) Cryo-EM map of the Omicron spike protein at 2.79-Å resolution. Protomers are colored in different shades of purple. (C) Cryo-EM structure of Omicron spike protein indicating the locations of modeled mutations on one protomer. (D) The Omicron spike protein RBD shown in two orthogonal orientations with Ca positions of the mutated residues shown as red spheres. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

Fig. 2. SPR analysis of the wild-type, Delta, and Omicron spike protein affinities for human ACE2. (A to C) Representative traces of single-cycle kinetic analyses of spike protein–ACE2 binding. The raw data (black) is fit (red) to a model using a 1:1 binding stoichiometry from which apparent dissociation constants were derived. The curves were obtained by injecting 6.25, 31.25, 62.5, 125, and 250 nM of each spike protein in successive cycles. RU, response units; WT, wild type. (D) Quantitation of apparent dissociation constants (K_D, app) for the wild-type, Delta, and Omicron spike protein–ACE2 interactions. The standard deviation obtained from at least three technical replicates is shown. Horizontal dotted lines are plotted for mutants carrying only K417N (top) or N501Y and E484K (Glu484 → Lys; bottom) mutations to demonstrate the range of this assay (see fig. S2 for binding data). A Tukey’s multiple comparisons test was performed on the wild-type, Delta, and Omicron binding affinities (*P ≤ 0.05; ns, not significant). A table highlighting the fold changes in K_D, app for the Delta and Omicron spike protein–ACE2 interactions relative to wild type is shown.
ACE2 residue E35 with a new salt bridge, whereas residue R498 forms a new salt bridge with ACE2 residue D38 while maintaining a hydrogen bond interaction with ACE2 residue Q42. RBD residue S496 adds a new interaction at the interface by forming a hydrogen bond with ACE2 residue K353 (Fig. 3D). Additionally, the mutated residue Y501 in the Omicron RBD makes π-stacking interactions with Y41 in ACE2, as previously seen in the Alpha (B.1.1.7), Beta (B.1.351), and Gamma (P.1) variants (8, 12), whereas mutated residue H505 (H, His) is not hydrogen-bonded to E37 in ACE2, in contrast to what we reported previously for the Y505 residue (Fig. 3E) (20).

These new interactions are offset by the loss of a key salt bridge between spike protein residue K447 and ACE2 residue D30 that is present in the Delta variant (Fig. 3F). In isolation, the K447N mutant displays reduced ACE2 binding affinity (12, 16), but our findings suggest that the new mutations in the Omicron interface have a compensatory effect on the strength of ACE2 binding, providing an explanation for the similar ACE2 binding affinities that are observed (Fig. 2).

We next investigated the effects of Omicron mutations on neutralization by (i) a selection of monoclonal antibodies, (ii) sera obtained from 30 doubly vaccinated individuals with no prior history of COVID-19 infection, and (iii) sera obtained from a set of 68 unvaccinated convalescent patients who recovered from infection with either the Alpha, Gamma, or Delta variants. (A summary of patient demographics is in table S3.) We performed neutralization experiments using pseudoviruses that incorporate the wild-type, Delta variant, or Omicron variant spike proteins and compared the ability of these pseudovirions to evade antibodies. We compare evasion relative to the Delta variant, given that the Omicron variant is rapidly supplanting the Delta variant in global prevalence, and to wild-type SARS-CoV-2, given that most SARS-CoV-2 vaccine immunogens at this time are based on this sequence (21).

We used a panel of neutralizing monoclonal antibodies that include four RBD-directed antibodies [ab1, ab8, S309, and S2M11; (22–25)] and two NTD-directed antibodies [4-8 and 4A8; (26, 27)] to investigate the impact of Omicron RBD and NTD mutations on monoclonal antibody escape. In contrast to wild-type SARS-CoV-2 and the Alpha (B.1.1.7), Gamma (P.1), Kappa (B.1.617.1), and Delta (B.1.617.2) variants, the Omicron variant could not be completely neutralized at maximum concentrations of five of the six antibodies tested (Fig. 4A and fig. S4) (20, 28). The loss of neutralizing activity for both the NTD-directed antibodies (4-8 and 4A8) against Omicron is likely due to the A144–145 deletion, which falls within the footprint of both of these antibodies (Fig. 4B). The escape from RBD-directed antibodies S2M11, ab8, and ab1 is likely due to the numerous Omicron mutations that lie within their respective footprints (Fig. 4B). By contrast, S309 (an antibody undergoing evaluation in clinical trials for treating patients with COVID-19) was able to fully neutralize the Omicron variant, consistent with previous reports that show retained neutralization capacity of S309 despite a mild decrease in potency (19, 29–31). The unusually high number of mutations in the Omicron variant spike
protein thus appear to confer broad antibody escape relative to previously emerged variants of SARS-CoV-2, consistent with emerging reports (19).

Sera obtained from patients not exposed to SARS-CoV-2 (prepandemic) showed negligible neutralization activity against wild-type SARS-CoV-2 and both the Delta and Omicron variants (fig. S5). Sera from either vaccinated or convalescent patients exhibited potent neutralization of wild-type pseudoviruses (figs. S6 to S9); sera from convalescent patients displayed, on average, a 6.3× decrease in ability to neutralize the Omicron variant relative to wild type (Fig. 4C, top). Sera from the vaccinated cohort also displayed reduced neutralization ability (4.4× decrease on average) with a wider variation driven by some individuals that showed greater loss of neutralization ability against Omicron. The comparison of change in neutralization potential between the Delta and Omicron variants is perhaps more relevant given the previous worldwide dominance of the Delta variant. Sera from convalescent patients shows an even greater drop in neutralization potency relative to the Delta variant (8.2× decrease), whereas the vaccinated group also shows reduction in potency, although to a lesser extent (3.4× decrease) (Fig. 4C, bottom).

A finer analysis of the unvaccinated convalescent cohort stratified into those who recovered from infection with either the Delta, Alpha, or Gamma variants (Fig. 4D) highlights the reduction in neutralization potency against the Omicron variant relative to the Delta variant in all populations, with especially notable drops for patients who recovered from infection with the earlier Alpha and Delta variants. The findings we report here are consistent with several other recent reports (19, 32–34) that support the finding that the Omicron variant is more resistant to neutralization dependent on prior infection with an earlier variant or vaccination than any other variant of concern that has emerged over the course of the COVID-19 pandemic.

The large number of mutations on the surface of the spike protein, including the immunodominant RBD (Fig. 1), would be expected to help the virus escape antibodies elicited by vaccination or prior infection. It is interesting that the Omicron variant evolved to retain its ability to bind ACE2 efficiently despite these extensive mutations. The cryo-EM structure of the spike protein–ACE2 complex provides a structural rationale for how this is achieved: Interactions involving the new mutations in the Omicron variant at residues 493, 496, 498, and 501 appear to restore ACE2 binding efficiency that would be lost as a result of other mutations such as K417N. The Omicron variant thus appears to have evolved to selectively balance an increase in escape from neutralization with its ability to interact efficiently with ACE2. The increase in antibody evasion and the retention of strong interactions at the ACE2 interface are thus factors that likely contribute to the increase in transmissibility of the Omicron variant.

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Data and materials availability: All newly created materials described in this manuscript will be available from the corresponding author upon reasonable request. Cryo-EM reconstructions and atomic models generated during this study are available at the Protein Data Bank (PDB) and Electron Microscopy Data Bank (EMDB) databases under the following accession codes: unbound Omicron spike protein trimer (PDB ID 7T9J, EMD-25759), global ACE2-bound Omicron spike protein trimer (PDB ID 7T9K, EMD-25760), and focus-refinement of the ACE2-RBD interface for the ACE2-bound Omicron spike protein trimer (PDB ID 7T9L, EMD-25761). This work is licensed under a Creative Commons Attribution 4.0 International (CC BY 4.0) license, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. To view a copy of this license, visit https://creativecommons.org/licenses/by/4.0/.

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SUPPLEMENTARY MATERIALS

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Materials and Methods

Tables S1 to S3

References (35–39)

MDAR Reproducibility Checklist

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