The outbreak of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome–coronavirus 2 (SARS-CoV-2) has now become a pandemic, but there is currently very little understanding of the antigenicity of the virus. We therefore determined the crystal structure of CR3022, a neutralizing antibody previously isolated from a convalescent SARS patient, in complex with the receptor binding domain (RBD) of the SARS-CoV-2 spike (S) protein at 3.1-angstrom resolution. CR3022 targets a highly conserved epitope, distal from the receptor binding site, that enables cross-reactive binding between SARS-CoV-2 and SARS-CoV. Structural modeling further demonstrates that the binding epitope can only be accessed by CR3022 when at least two RBDs on the trimeric S protein are in the “up” conformation and slightly rotated. These results provide molecular insights into antibody recognition of SARS-CoV-2.

**Table 1. Binding affinity of CR3022 to recombinant RBD and S protein.** Binding affinity is expressed as the nanomolar dissociation constant ($K_d$).

<table>
<thead>
<tr>
<th>Target</th>
<th>CR3022 IgG binding affinity ($K_d$)</th>
<th>CR3022 Fab binding affinity ($K_d$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SARS-CoV-2 RBD</td>
<td>&lt;0.1</td>
<td>115 ± 3</td>
</tr>
<tr>
<td>SARS-CoV RBD</td>
<td>&lt;0.1</td>
<td>1.0 ± 0.1</td>
</tr>
</tbody>
</table>

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The recent cryo-electron microscopy (cryo-EM) structures of the homotrimeric SARS-CoV-2 S protein (17, 18) demonstrated that the RBD, as in other coronaviruses (19, 20), can undergo a hinge-like movement to transition between “up” and “down” conformations (Fig. 4A). ACE2 host receptor can only interact with the RBD when it is in the up conformation—the down conformation is inaccessible to ACE2. The epitope of CR3022 is also only accessible when the RBD is in the up conformation (Fig. 4, B and C). However, even when one RBD in the SARS-CoV-2 S protein is in the up conformation, the binding of CR3022 to RBD can still be sterically hindered. Structural alignment of the CR3022–SARS-CoV-2 RBD complex with the SARS-CoV-2 S protein (17, 18) indicates that the CR3022 variable region would clash with the RBD on the adjacent protomer if the latter adopted a down conformation. In addition, the CR3022 variable domain would clash with the S2 domain underneath the RBD, and the CR3022 constant region would clash with the N-terminal domain (Fig. 4D).

**Fig. 1. Crystal structure of CR3022 in complex with SARS-CoV-2 RBD.**
(A) Overall topology of the SARS-CoV-2 spike glycoprotein. NTD, N-terminal domain; RBD, receptor binding domain; SD1, subdomain 1; SD2, subdomain 2; FP, fusion peptide; HR1, heptad repeat 1; HR2, heptad repeat 2; TM, transmembrane region; IC, intracellular domain; N, N terminus; C, C terminus. (B) Structure of CR3022 Fab in complex with SARS-CoV-2 RBD. CR3022 heavy chain is orange, CR3022 light chain is yellow, and SARS-CoV-2 RBD is light gray. (C and D) Epitope residues on SARS-CoV-2 are shown. CDR loops are labeled. Epitope residues that are conserved between SARS-CoV-2 and SARS-CoV are shown in cyan, and those that are not conserved are shown in green. (D) Epitope residues that are important for binding to CR3022 are labeled. Epitope residues are defined here as residues in SARS-CoV-2 RBD with buried surface area > 0 Å² after Fab CR3022 binding, as calculated with Proteins, Interfaces, Structures and Assemblies (PISA) (34). Single-letter abbreviations for the amino acid residues are as follows: A, Ala; D, Asp; E, Glu; F, Phe; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. (E) Several key interactions between CR3022 and SARS-CoV-2 RBD are highlighted. CR3022 heavy chain is orange, CR3022 light chain is yellow, and SARS-CoV-2 RBD is cyan. Hydrogen bonds are represented by dashed lines.

**Fig. 2. Conservation of epitope residues.** (A) Sequence alignment of SARS-CoV-2 RBD and SARS-CoV RBD. CR3022 epitope residues are highlighted in cyan. ACE2-binding residues are highlighted in magenta. Nonconserved epitope residues are marked with asterisks. (B to E) Interactions between the nonconserved epitope residues and CR3022 are shown. Amino acid variants observed in SARS-CoV are in parentheses. SARS-CoV-2 RBD is cyan, CR3022 heavy chain is orange, and CR3022 light chain is yellow. Residues are numbered according to their positions on the SARS-CoV-2 S protein sequence. (B) Whereas SARS-CoV-2 has an Ala at residue 372, SARS-CoV has Thr, which introduces an N-glycosylation site at residue N370. (C) The potential location of N370 glycan in SARS-CoV RBD is indicated by the dotted box. CR3022 is shown as an electrostatic potential surface presentation with units of kT/e, where e is the charge of an electron, k is the Boltzmann constant, and T is temperature in kelvin. (D) P384 interacts with T31, S96, and T100 of CR3022 heavy chain. Ala at this position in SARS-CoV would allow the backbone to adopt a different conformation when binding to CR3022. (E) T430 forms a hydrogen bond (dashed line) with S27f of CR3022 light chain. Met at this position in SARS-CoV would instead likely insert its side chain into the hydrophobic pocket formed by Y27d, I28, Y32, and W50 of CR3022 light chain.
as compared with SARS-CoV-2, the up conformation of the RBD in SARS-CoV has a larger dihedral angle to the horizontal plane of the S protein (fig. S5), the clashes described above would also exist in the SARS-CoV S protein (fig. S6).

For CR3022 to bind to the S protein, the previously described clashes need to be resolved. The clash with the CR3022 variable domain can be partially relieved when the targeted RBD on one protomer of the trimer and the RBD on the adjacent protomer are both in the up conformation (Fig. 4E). SARS-CoV S protein with two RBDs in the up conformation has been observed in cryo-EM studies (19, 21, 22). Nevertheless, clashes with the N-terminal domain (NTD) and S2 domain would still exist in the “two-up” conformation. Further structural modeling shows that all clashes can be avoided with a slight rotation of the targeted RBD in the “double-up” conformation (Fig. 4F). This conformational change is likely to be physiologically relevant because CR3022 can neutralize SARS-CoV. In addition, our enzyme-linked immunosorbent assay (ELISA)
experiment demonstrated that CR3022 is able to interact with the SARS-CoV-2 virus. Although the binding signals of CR3022 and m396, which is a SARS-CoV-specific antibody (6, 17), to SARS-CoV were comparable in ELISA ($P > 0.05$, two-tailed $t$ test) (Fig. 4G, left panel), CR3022 had a significantly higher binding signal to SARS-CoV-2 than did m396 ($P = 0.003$, two-tailed $t$ test) (Fig. 4G, left panel), but not higher than its own binding signal to SARS-CoV, which is consistent with their relative binding to the RBD (Table 1 and fig. S3).

Our study provides insight into how SARS-CoV-2 can be targeted by the humoral immune response, and it reveals a conserved, but cryptic, epitope shared between SARS-CoV-2 and SARS-CoV. Recently, our group and others have identified a conserved epitope on influenza A virus hemagglutinin (HA) that is located in the trimeric interface and is only exposed through protein “breathing” (23–25), which is somewhat analogous to the epitope of CR3022. Antibodies to this influenza HA trimeric interface epitope do not exhibit in vitro neutralization activity but can confer in vivo protection. Similarly, antibodies to another conserved epitope that partially overlaps with the influenza HA trimeric interface are also non-neutralizing in vitro but protective in vivo (26). Examples of antibodies that do not have in vitro neutralization activity but confer in vivo protection have also been reported for influenza virus (27), herpesvirus (28), cytomegalovirus (29), alpha-virus (30), and dengue virus (31). Therefore, although CR3022 does not neutralize SARS-CoV-2 in vitro, it is possible that this epitope can confer in vivo protection. Further study will require suitable animal models, which have yet to be established.

This coronavirus outbreak continues to pose an enormous global risk (32, 33), and the availability of conserved epitopes may allow structure-based design not only of a SARS-CoV-2 vaccine but also of cross-protective antibody responses against future coronavirus epidemics and pandemics. Although a more universal coronavirus vaccine is not the most urgent goal at present, it is certainly worth future consideration, especially as cross-protective epitopes are identified, so that we can be better prepared for the next novel coronavirus outbreak.

REFERENCES AND NOTES
7. See supplementary materials.
8. Y. Watanabe et al., bioRxiv 2020.02.20.957472 [Preprint].
12. ACE2 also forms a dimer when it associates with the amino acid transporter B0AT1 (W34). We modeled a CR3022 IgG onto this dimer structure and found no clashes of CR3022 with ACE2 in its dimeric form, where the RBDs would likely come from adjacent trimers on the virus (J).
22. Yuan et al. (19) observed 56% of the wild-type recombinant SARS-CoV S protein particle in “none-up” conformation and 44% in “single-up” conformation, whereas Kirchdoerfer et al. (21) found that recombinant SARS-CoV S protein, with Lys369→Pro and Val370→Pro mutations in the S2 domain to stabilize the prefusion conformation, has 58% in single-up, 39% in double-up, and 3% in triple-up conformations. However, it is not known whether the distribution of different conformations of S proteins on virus surface is the same as that of recombinant S protein.
23. S. Bangaru et al., Cell 177, 1136–1152.e18 (2019).
24. A. Watanabe et al., Cell 177, 1124–1135.e16 (2019).

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We thank H. Tian for technical support with the crystallization robot. J. Matteson for contribution to mammalian cell culture. W. Yu for contribution to insect cell culture. R. Stanfield for assistance with data collection, and A. Ward for discussion. Funding: This work was supported by National Institutes of Health grant K99 AI139445 (N.C.W.), a Calmette and Yersin scholarship from the Pasteur International Network Association (H.L.); Bill and Melinda Gates Foundation grant OPP1170236 (I.A.W.); Guangzhou Medical University High-level University Innovation Team Training Program (Guangzhou Medical University released [2017] no. 159); (C.K.P.M.); and National Natural Science Foundation of China (NSFC)/Research Grants Council (RGC) Joint Research Scheme (N_HKU737/18) (C.K.P.M.). General Medical Sciences and Cancer Institutes Structural Biology Facility at the Advanced Photon Source (APS) has been funded by federal funds from the National Cancer Institute (ACB-12002) and the National Institute of General Medical Sciences (AGM-12006). This research used resources of the APS, a U.S. Department of Energy (DOE) Office of Science User Facility operated for the DOE Office of Science by Argonne National Laboratory under contract DE-AC02-06CH11357. Author contributions: M.Y., N.C.W., X.Z., C.K.P.M., and I.A.W. conceived of and designed the study. M.Y., N.C.W., and C.-D.L. expressed and purified the proteins. M.Y. and N.C.W. performed biolayer interferometry binding assays. R.T.Y.S., H.L., and C.K.P.M. performed the neutralization and virus-binding experiments. M.Y., N.C.W., and X.Z. collected the x-ray data and determined and refined the x-ray structures. M.Y., N.C.W., and C.K.P.M. analyzed the data. M.Y., N.C.W., and I.A.W. wrote the paper, and all authors reviewed and edited the paper. Competing Interests: The authors declare no competing interests. Data and materials availability: X-ray coordinates and structure factors are deposited in the RCSB Protein Data Bank under ID 6W41. 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/. This license does not apply to figures/photos/artwork or other content included in the article that is credited to a third party; obtain authorization from the rights holder before using such material.

SUPPLEMENTARY MATERIALS
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A highly conserved cryptic epitope in the receptor binding domains of SARS-CoV-2 and SARS-CoV
Meng Yuan, Nicholas C. Wu, Xueyong Zhu, Chang-Chun D. Lee, Ray T. Y. So, Huibin Lv, Chris K. P. Mok and Ian A. Wilson

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Targeting the SARS-CoV-2 spike
The surface of severe acute respiratory syndrome–coronavirus 2 (SARS-CoV-2) is decorated with trimeric spikes that bind to host cell receptors. These spikes also elicit an antibody response, so understanding antibody recognition may aid in vaccine design. Yuan et al. determined the structure of CR3022, a neutralizing antibody obtained from a convalescent SARS-CoV–infected patient, in complex with the receptor-binding domain of the SARS-CoV-2 spike. The antibody binds to an epitope conserved between SARS-CoV-2 and SARS-CoV that is distinct from the receptor-binding site. CR3022 likely binds more tightly to SARS-CoV because its epitope contains a glycan not present in SARS-CoV-2. Structural modeling showed that the epitope is only revealed when at least two of the three spike proteins are in a conformation competent to bind the receptor.

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