# A highly conserved cryptic epitope in the receptor-binding domains of SARS-CoV-2 and SARS-CoV

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## ABSTRACT

The outbreak of COVID-19, which is caused by SARS-CoV-2 virus, continues to spread globally, but there is currently very little understanding of the epitopes on the virus. In this study, we have determined the crystal structure of the receptor-binding domain (RBD) of the SARS-CoV-2 spike (S) protein in complex with CR3022, a neutralizing antibody previously isolated from a convalescent SARS patient. CR3022 targets a highly conserved epitope that enables cross-reactive binding between SARS-CoV-2 and SARS-CoV. Structural modeling further demonstrates that the binding site can only be accessed when at least two RBDs on the trimeric S protein are in the “up” conformation. Overall, this study provides structural and molecular insight into the antigenicity of SARS-CoV-2.

## ONE SENTENCE SUMMARY

Structural study of a cross-reactive SARS antibody reveals a conserved epitope on the SARS-CoV-2 receptor-binding domain.

## MAIN

The ongoing outbreak of Coronavirus Disease 2019 (COVID-19) originally emerged in China during December 2019 (*1*) and has now spread over 120 countries as of March 12, 2020 and become pandemic. COVID-19 is caused by a novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (*2*). In fact, two other coronaviruses have caused global outbreak in the past two decades, namely SARS-CoV (2002-2003) and Middle East respiratory syndrome coronavirus (MERS-CoV) (2012-present). The surface spike glycoprotein (S), which is critical for virus entry through engaging the host receptor and mediating virus-host membrane fusion, is the major antigen of coronaviruses. The S proteins of SARS-CoV-2 and SARS-CoV, which are phylogenetically closely related, have an amino-acid sequence identity of around 77% (*3*). Such a high degree of sequence similarity raises the possibility that cross-reactive epitopes may exist. A recent study has shown that CR3022, which is a human neutralizing antibody that targets the receptor-binding domain (RBD) of SARS-CoV (*4*), can bind to the RBD of SARS-CoV-2 (*5*). This finding provides an opportunity to uncover a cross-reactive epitope.

CR3022 was previously isolated from a convalescent SARS patient and is encoded by germline genes IGHV5-51, IGHD3-10, IGHJ6 (heavy chain), and IGKV4-1, IGKJ2 (light chain) (*4*). Based on IgBlast analysis (*6*), the IGHV of CR3022 is 3.1% somatically mutated at the nucleotide sequence level, which results in eight amino-acid changes from the germline sequence, whereas IGKV of CR3022 is 1.3% somatically mutated resulting in three amino-acid changes from the germline sequence (fig. S1). We therefore determined the crystal structure of CR3022 with the SARS-CoV-2 RBD at 3.1 Å resolution (table S1 and fig.S2, A and B) (*7*). CR3022 uses both heavy and light chains (Fig. 1B), and all six complementarity-determining region (CDR) loops (Fig. 1C)

for interaction with the RBD. The buried surface area on the epitope is 917 $Å^2$ and SARS-CoV-2 recognition by CR3022 is largely driven by hydrophobic interactions (Fig. 1E). Five out of 11 somatic mutations are found in the paratope region (fig. S2C), implying their likely importance in the affinity maturation process.

Out of 28 residues in the epitope (defined as residues buried by CR3022), 24 (86%) are conserved between SARS-CoV-2 and SARS-CoV (Fig. 1D). This high sequence conservation explains the cross-reactivity of CR3022. Nonetheless, despite having a high conservation in the epitope residues, CR3022 Fab binds to SARS-CoV RBD ($K_d$ = 1 nM) with a much higher affinity than to SARS-CoV-2 RBD ($K_d$ = 115 nM) (Table 1 and fig. S3). We postulate that the difference in binding affinity of CR3022 between SARS-CoV-2 and SARS-CoV RBDs is due to the non-conserved residues in the epitope (fig. S4). The most dramatic difference between the CR3022 epitope in SARS-CoV-2 and SARS-CoV is an additional N-glycosylation site at N370 (N357 in SARS-CoV numbering). The N-glycan sequon (NxS/T) arises from an amino-acid difference at residue 372, where SARS-CoV has a Thr compared to Ala in SARS-CoV-2 (fig. S4B). Mass spectrometry analysis has shown that a complex glycan is indeed present at this N-glycosylation site in SARS-CoV (*8*). An N-glycan at N370 would fit into a groove formed between heavy and light chains (fig. S4C), which could increase contact and, hence, binding affinity to CR3022. We then tested whether CR3022 was able to neutralize SARS-CoV-2 and SARS-CoV in an *in vitro* microneutralization assay (*7*). While CR3022 could neutralize SARS-CoV, it did not neutralize SARS-CoV-2 at the highest concentration tested (400 µg/mL) (fig. S5). This *in vitro* neutralization result is consistent with lower affinity binding of CR3022 for SARS-CoV-2, although other explanations are possible as outlined below.

SARS-CoV-2 uses the same host receptor, angiotensin I converting enzyme 2 (ACE2) as SARS-CoV (*3, 9-11*). Interestingly, the epitope of CR3022 does not overlap with the ACE2-binding site (Fig. 2A). Structural alignment of CR3022-SARS-CoV-2 RBD complex with the ACE2-SARS-CoV-2 RBD complex (*11*) further indicates that binding of CR3022 would not clash with ACE2 (*12*). This analysis implies that the neutralization mechanism of CR3022 does not depend on direct blocking of receptor binding, which is consistent with the observation that CR3022 does not compete with ACE2 for binding to the RBD (*5*). Unlike CR3022, most known SARS RBD-targeted antibodies compete with ACE2 for binding to RBD (*4, 13-16*). The epitopes of these antibodies are very different from that of CR3022 (Fig. 2B). In fact, it has been shown that CR3022 can synergize with other RBD-targeted antibodies to neutralize SARS-CoV (*4*). Although CR3022 itself cannot neutralize SARS-CoV-2 in this *in vitro* assay, whether CR3022 can synergize with other SARS-CoV-2 RBD-targeted monoclonal antibodies for neutralization remains to be determined.

Recently, the cryo-EM structure of homotrimeric SARS-CoV-2 S protein was determined (*17, 18*) and demonstrated that the RBD, as in other coronaviruses (*19, 20*) adopts two different dispositions in the trimer. The RBD can then undergo a hinge-like movement to transition between “up” or “down” conformations (Fig. 3A). ACE2 host receptor can only interact with the RBD when it is in the “up” conformation, whereas the “down” conformation is inaccessible to ACE2. Interestingly, the epitope of CR3022 is also only accessible when the RBD is in the “up” conformation (Fig. 3, B and C). Furthermore, the ability for CR3022 to access the RBD also depends on the relative disposition of the RBD on the adjacent protomer. CR3022 can only access RBD when the targeted RBD on one protomer of the trimer and the RBD on the adjacent protomer are both in the “up” conformation. The variable region of CR3022 would clash with the RBD on the adjacent

protomer if the latter adopts a “down” conformation (Fig. 3D). As a homotrimer, the S protein could potentially adopt four possible RBD configurations, namely none-“up”, single-“up”, double-“up”, and triple-“up”. It appears that CR3022 can only bind to the S protein when it is in double-“up” or triple-“up” configuration. Specifically, one molecule of CR3022 can be accommodated in the double-“up” configuration (Fig. 3E), whereas three molecules of CR3022 could potentially be accommodated in the triple-“up” configuration (Fig. 3F). Previous cryo-EM studies have also shown that the recombinant SARS-CoV S protein is mostly found in the none-“up”, single-“up”, or double-”up” conformations (*19, 21*), but rarely in the triple-“up” conformation, even with ACE2 receptor bound (*21, 22*). Together with the fact that CR3022 was isolated from a convalescent SARS patient (*4*), these observations suggest that an antibody response can be elicited against this cryptic epitope in SARS-CoV and possibly SARS-CoV-2. Structural comparison shows that, as compared to SARS-CoV-2, the “up” conformation of RBD in SARS-CoV has a larger dihedral angle to the horizontal plane of the S protein (fig. S6), suggesting that the CR3022 epitope may be slightly more accessible in SARS-CoV than in SARS-CoV-2, in these spike ectodomain constructs. Nevertheless, the availability of this cryptic epitope on the actual virus surface still has to be quantified to fully comprehend its immunological role during natural infection.

Overall, our study provides insight into how SARS-CoV-2 can be targeted by the humoral immune response and revealed 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 also are non-neutralizing *in vitro*, but protective *in vivo* (*26*). Furthermore, there are other examples of antibodies that do not have *in vitro* neutralization activity but confer *in vivo* protection, such as reported for influenza virus (*27*), herpesvirus (*28*), cytomegalovirus (*29*), alphavirus (*30*), and dengue virus (*31*). Therefore, although CR3022 does not neutralize SARS-CoV-2 *in vitro* despite its reasonable binding affinity, it is possible that this epitope can confer *in vivo* protection. The potential existence of non-neutralizing protective antibodies to SARS-CoV-2 highlights the need for an effective SARS-CoV-2 infection mouse model, which has yet to be established.

Since there is currently great urgency in the efforts to develop a vaccine against SARS-CoV-2, characterizing the epitopes on SARS-CoV-2 S protein is extremely valuable. Much work is now ongoing in isolating human monoclonal antibodies from SARS-CoV-2 patients. We anticipate that these investigations will decipher the antigenic properties and major epitopes of SARS-CoV-2. In addition, the molecular features that antibodies use for targeting neutralizing epitopes can inspire development of therapeutics, such as antiviral peptides and small molecules (*32*). As this coronavirus outbreak continues to pose an enormous global risk (*33, 34*), the availability of conserved epitopes may allow structure-based design not only of a SARS-CoV-2 vaccine, but also for cross-protective antibody responses against future coronavirus epidemics and pandemics. While a more universal coronavirus vaccine is not the most urgent goal at present, it is certainly worthwhile for future consideration especially as cross-protective epitopes are identified so that we can be better prepared for the next novel coronavirus outbreak.

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Scheme (N_HKU737/18) (to C.K.P.M.). **Author contributions:** M.Y., N.C.W., X.Z. and I.A.W. conceived and designed the study. M.Y., N.C.W. and C.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 experiments. M.Y., N.C.W. and X.Z. collected the X-ray data, 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 at the RCSB Protein Data Bank under accession code: 6W41. All of the other data that support the conclusions of the study are available from the corresponding author upon request.

## SUPPLEMENTARY MATERIALS

Materials and Methods

Figs. S1 to S6

Tables S1 to S3

References 38-47

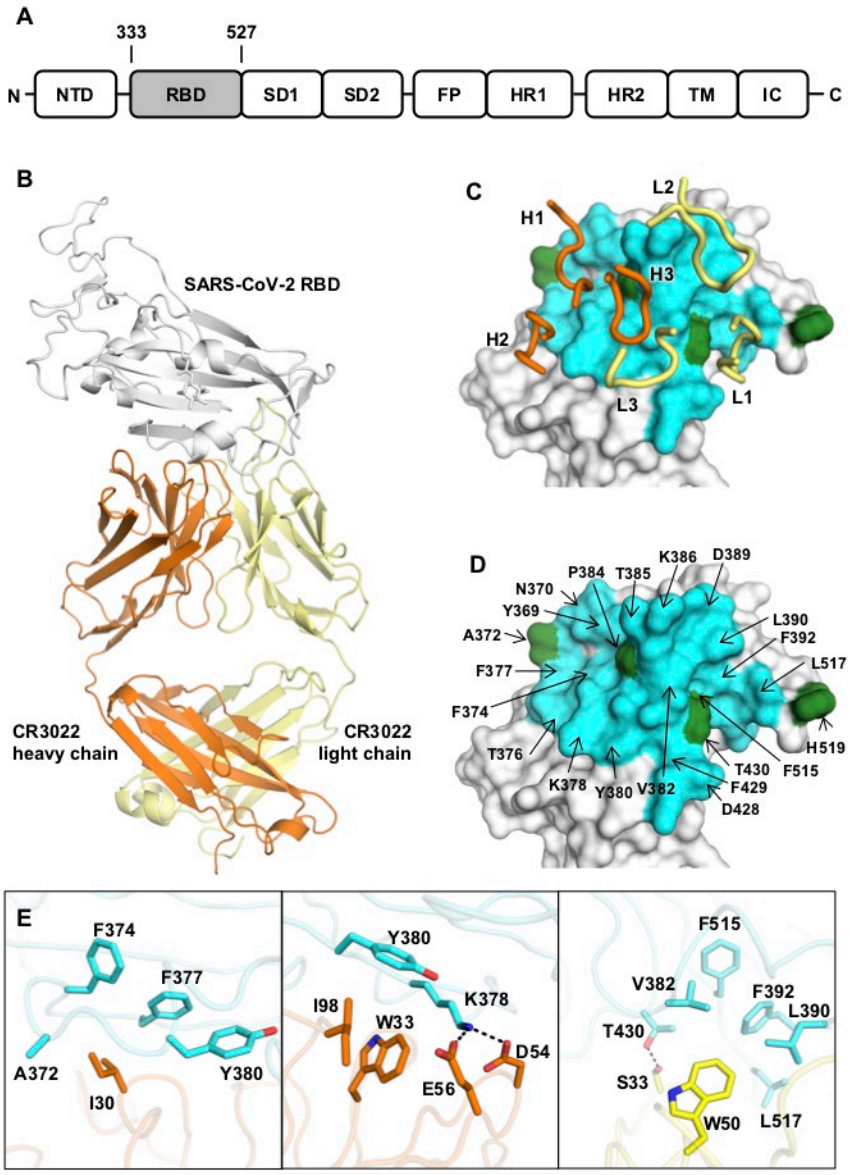



**Fig. 1. Crystal structure of CR3022 in complex with SARS-CoV-2 RBD. (A)** Overall topology of the SARS-CoV-2 spike glycoprotein. NTD: N-terminal domain, RBD: receptor-binding domain, SD1: subdomain 1, SD2: subdomain 2, FP: fusion peptide, HR1: heptad repeat 1, HR2: heptad repeat 2, TM: transmembrane region, IC: intracellular domain. **(B)** Structure of CR3022 Fab in complex with SARS-CoV-2 RBD. CR3022 heavy chain is colored in orange, CR3022 light chain in yellow, and SARS-CoV-2 RBD in light grey. **(C-D)** Epitope residues on SARS-CoV-2 are colored in cyan and

green. CDR loops are labeled. Cyan: epitope residues that are conserved between SARS-CoV-2 and SARS-CoV. Green: epitope residues that are not conserved between SARS-CoV-2 and SARS-CoV. **(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 $Å^2$ after Fab CR3022 binding as calculated with PISA (*35*). **(E)** Several key interactions between CR3022 and SARS-CoV-2 RBD are highlighted. CR3022 heavy chain is colored in orange, CR3022 light chain in yellow, and SARS-CoV-2 RBD in cyan. Hydrogen bonds are represented by dashed lines.

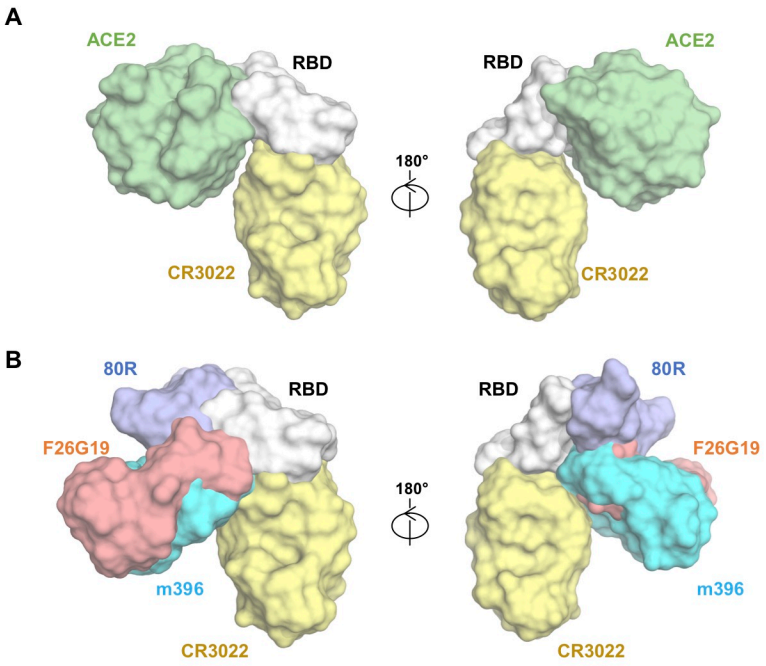
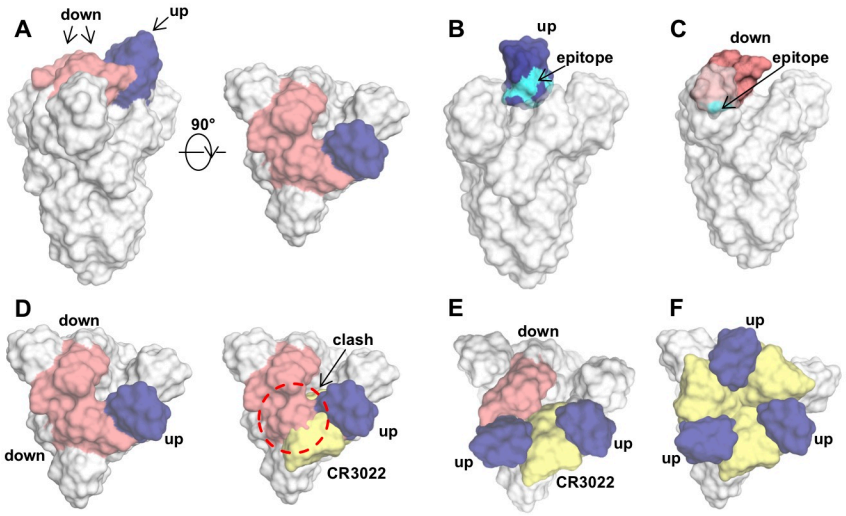



**Fig. 2. The relative binding position of CR3022 with respect to receptor ACE2 and other SARS-CoV RBD monoclonal antibodies. (A)** Structures of CR3022-SARS-CoV-2 RBD complex and ACE2-SARS-CoV-2 RBD complex (*11*) are aligned based on the SARS-CoV-2 RBD. ACE2 is colored in green, RBD in light grey, and CR3022 in yellow. **(B)** Structural superposition of CR3022-SARS-CoV-2 RBD complex, F26G19-SARS-CoV RBD complex (PDB 3BGF) (*36*), 80R-SARS-CoV RBD complex (PDB 2GHW) (*37*), and m396-SARS-CoV RBD complex (PDB 2DD8) (*16*).

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317 **Fig. 3. Binding of CR3022 depends on the RBD configurations on the S protein. (A)**
318 RBD in the S proteins of SARS-CoV-2 and SARS-CoV can adopt either an "up"
319 conformation (blue) or a "down" conformation (red). PDB 6VSB (cryo-EM structure of
320 SARS-CoV-2 S protein) (*17*) is shown. **(B-C)** CR3022 epitope (cyan) on the RBD is
321 exposed in **(B)** the "up" but not **(C)** the "down" conformation. **(D)** Binding of CR3022 to
322 single-"up" configuration would clash (indicated by the red circle) with the neighboring
323 RBD. CR3022 is colored yellow. **(E-F)** The double-"up" and triple-"up" RBD
324 configurations on the SARS-CoV-2 S protein are modeled based on PDB 6VSB (*17*). **(E)**
325 One CR3022 molecule can be accommodated per S protein in the double-"up"
326 configuration, and **(F)** three CR3022 molecules could potentially be accommodated per
327 S protein in the triple-"up" configuration.

328 **Table 1. Binding affinity of CR3022 to recombinant RBD and S protein**

| Affinity ($K_d$ in nM) | CR3022 IgG | CR3022 Fab |
|---|---|---|
| SARS-CoV-2 RBD | < 0.1 | 115 ± 3 |
| SARS-CoV RBD | < 0.1 | 1.0 ± 0.1 |

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