

Crystal structure of the 2019-nCoV spike receptor-binding domain bound with the ACE2 receptor

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19 **Abstract**

20 A novel and highly pathogenic coronavirus (2019-nCoV) has caused an outbreak in Wuhan
21 city, Hubei province of China since December 2019, and soon spread nationwide and spilled
22 over to other countries around the world. To better understand the initial step of infection at
23 atomic-level, we determined the crystal structure of the 2019-nCoV spike receptor-binding
24 domain (RBD) bound with the cell receptor ACE2 at 2.45 Å resolution. The overall ACE2-
25 binding mode of the 2019-nCoV RBD is nearly identical to that of the SARS-CoV RBD, which
26 also utilizes ACE2 as the cell receptor. Structural analysis identified residues in 2019-nCoV
27 RBD critical for ACE2 binding, and majority of which are either highly conserved or shared
28 similar side chain properties with those in the SARS-CoV RBD. Such similarity in structure
29 and sequence strongly argue for a convergent evolution between 2019-nCoV and SARS-CoV
30 RBD for improved binding to ACE2 despite of being segregated in different genetic lineages
31 in the betacoronavirus genus. The epitopes of two SARS-CoV antibodies targeting the RBD
32 are also analyzed with the 2019-nCoV RBD, providing insights into future identification of
33 cross-reactive antibodies.

34 The emergence of a novel and highly pathogenic coronavirus (2019-nCoV) in Wuhan city,
35 Hubei province of China and its rapid international spread has posed a serious global public
36 health emergency¹⁻³. Similar to those infected by pathogenic severe acute respiratory syndrome
37 coronavirus (SARS-CoV) in 2003 and Middle East respiratory syndrome coronavirus (MERS-
38 CoV) in 2012, patients infected by 2019-nCoV manifested a range of symptoms including dry
39 cough, fever, headache, dyspnea and pneumonia with estimated mortality rate of 2.5%⁴⁻⁶. Since
40 the initial outbreak in December of 2019, 2019-nCoV has spread throughout China and to more
41 than twenty other countries worldwide. As of February 17, 2020, 70641 cases in China have
42 been confirmed with the infection while 7264 cases are suspected, and 1772 cases have died.
43 Currently, the epicenter Wuhan and the neighboring cities have been under lockdown to
44 minimize continued spread, and the WHO has announced a Public Health Emergency of
45 International Concern (PHEIC) due to the rapid and global dissemination of 2019-nCoV.

46 Phylogenetic analysis on the coronavirus genomes has revealed that 2019-nCoV is a
47 new member of the betacoronavirus genus, which includes SARS-CoV, MERS-CoV, bat
48 SARS-related coronaviruses (SARSr-CoV), as well as others identified in humans and diverse
49 animal species^{1-3,7}. Bat coronavirus RaTG13 appears to be the closest relative of the 2019-
50 nCoV sharing over 93.1% homology in the spike (S) gene. SARS-CoV and other SARSr-CoVs
51 however are rather distinct with less than 80% homology¹.

52 Coronaviruses utilize the homotrimeric spike glycoprotein (S1 subunit and S2 subunit
53 in each spike monomer) on the envelope to bind their cellular receptors. Such binding triggers
54 a cascade events leading to the fusion between cell and viral membranes for cell entry. Our
55 cryo-EM studies have shown that the binding of SARS-CoV spike to the cell receptor ACE2
56 induces the dissociation of the S1 with ACE2, prompting the S2 to transition from a metastable
57 prefusion to a more stable postfusion state that is essential for membrane fusion^{8,9}. Therefore,
58 binding to ACE2 receptor is a critical initial step for SARS-CoV to entry into the target cells.
59 Recent studies also pointed to the important role of ACE2 in mediating entry of 2019-nCoV^{1,10}.
60 HeLa cells expressing ACE2 is susceptible to 2019-nCoV infection while those without failed
61 to do so¹. In vitro SPR experiments also showed that the binding affinity of ACE2 to the spike
62 glycoprotein and to the receptor-binding domain (RBD) are equivalent, with the former of 14.7
63 nM and the latter of 15.2 nM^{11,12}. These results indicate that the RBD is the key functional
64 component within the S1 subunit responsible for binding to ACE2.

65 The cryo-EM structure of the 2019-nCoV spike trimer at 3.5 Å resolution has just been
66 reported¹². The coordinates are not yet available for detailed characterization. However,
67 inspection of the structure features presented in the uploaded manuscript on bioRxiv indicated

68 incomplete resolution of RBD in the model, particularly for the receptor-binding motif (RBM)
69 that interacts directly with ACE2. Computer modeling of interaction between 2019-CoV RBD
70 and ACE2 has identified some residues potentially involved in the actual interaction but the
71 actual interaction remained elusive¹³. Furthermore, despite of impressive cross-reactive
72 neutralizing activity from serum/plasma of SARS-CoV recovered patients¹⁴, no SARS-CoV
73 monoclonal antibodies targeted to RBD so far isolated are able to bind and neutralize 2019-
74 nCoV^{11,12}. These findings highlight some intrinsic sequence and structure differences between
75 the SARS-CoV and 2019-nCoV RBDs.

76 To elucidate the 2019-nCoV RBD and ACE2 interaction at a higher resolution, we
77 chose to determine the complex structure of 2019-nCoV RBD bound with ACE2 by X-ray
78 crystallography. The atomic-level structural information would greatly improve our
79 understanding of interaction between 2019-nCoV and susceptible cells, providing precise
80 target for neutralizing antibodies, and assisting structure-based vaccine design urgently needed
81 in our ongoing combat against 2019-nCoV. Specifically, we expressed the 2019-nCoV RBD
82 (residues Arg319-Phe541) (Fig. 1a and 1b) and the N-terminal peptidase domain of ACE2
83 (residues Ser19-Asp615) in Hi5 insect cells and purified them by Ni-NTA affinity and gel-
84 filtration (Fig. S1). The complex structure was determined by molecular replacement using the
85 SARS-CoV RBD and ACE2 structures as search models, and refined at 2.45 Å resolution to
86 final R_{work} and R_{free} factors of 21.9% and 27.8%, respectively (Fig. S2 and Table S1). The final
87 model contains residues Cys336 to Glu516 of 2019-nCoV RBD and residues Ser19 to Asp615
88 of ACE2 N-terminal peptidase domain, as well as 63 solvent molecules.

89 The 2019-nCoV RBD has a twisted four-stranded antiparallel β sheet (β 1, β 2, β 3 and
90 β 6) with short connecting helices and loops forming as the core (Fig. 1b and 1c). Between the
91 β 3 and β 6 strands in the core, there is an extended insertion containing short β 4 and β 5 strands,
92 α 4 and α 5 helices and loops (Fig. 1b and 1c). This extended insertion is the receptor-binding
93 motif (RBM) containing most of the contacting residues of 2019-nCoV for ACE2 binding. A
94 total of nine cysteine residues are found in the RBD, six of which forming three pairs of
95 disulfide bonds are resolved in the final model. Among these three pairs, two are in the core
96 (Cys336-Cys361 and Cys379-Cys432) to help stabilize the β sheet structure (Fig. 1c) while the
97 remaining one (Cys480-Cys488) connects loops in the distal end of the RBM (Fig. 1c). The N-
98 terminal peptidase domain of ACE2 has two lobes, forming the peptide substrate binding site
99 between them. The extended RBM in the 2019-nCoV RBD contacts the bottom side of the
100 ACE2 small lobe, with a concave outer surface in the RBM accommodating the N-terminal
101 helix of the ACE2 (Fig. 1c). The overall structure of the 2019-nCoV RBD is similar to that of

102 the SARS-CoV RBD (Fig. 2a), with an r.m.s.d. of 1.2 Å for 174 aligned C α atoms. Even in the
103 RBM that has more sequence variations, the overall structure is also highly similar (r.m.s.d. of
104 1.3 Å) with only one obvious conformational change in the distal end (Fig. 2a). The overall
105 binding model of the 2019-nCoV RBD to the ACE2 is also nearly identical to that observed in
106 previously determined SARS-CoV RBD/ACE2 complex structure¹⁵ (Fig. 2b).

107 The cradling of the ACE2 N-terminal helix by the RBM outer surface results in a large
108 buried surface of ~ 1700 Å² between the 2019-nCoV RBD and ACE2 receptor (Fig. 1c). With
109 a distance cutoff of 4 Å, a total of 18 residues of the RBD contact 20 residues of the ACE2
110 (Fig. 3a and Table S2). Analysis of interface between SARS-CoV RBD and ACE2 revealed a
111 total of 16 residues of the SARS-CoV RBD contact 20 residues of the ACE2 (Fig. 3a and Table
112 S2). Among the 20 residues interacting with the two different RBDs, 17 are shared and most
113 of which are located at the N-terminal helix (Fig. 2a). One prominent and common feature
114 presented at both interfaces is the networks of hydrophilic interactions. There are 17 hydrogen
115 bonds and 1 salt bridge at the 2019-nCoV RBD/ACE2 interface, and 12 hydrogen bonds and 2
116 salt bridges at the SARS-CoV RBD/ACE2 interface (Table 1). Another shared feature is the
117 involvement of multiple tyrosine residues in forming hydrogen-bonding interactions with the
118 polar hydroxyl group. These include Tyr449, Tyr489, Tyr495 and Tyr505 from the 2019-nCoV
119 RBD and Tyr436, Tyr475 and Tyr491 from the SARS-CoV RBD (Table 1). To further identify
120 and compare the ACE2-interacting residues, we used structure-guided sequence alignment and
121 mapped them onto their respective RBD sequences (Fig. 3b). Among 14 shared amino acid
122 positions utilized by both RBMs for ACE2 interaction, eight have the identical residues
123 between the 2019-nCoV and SARS-CoV RBDs including Tyr449/Tyr436, Tyr453/Tyr440,
124 Asn487/Asn473, Tyr489/Tyr475, Gly496/Gly482, Thr500/Thr486, Gly502/Gly488 and
125 Tyr505/Tyr491 (Fig. 3b). Five positions have residues demonstrating similar biochemical
126 properties despite of having different side chains including Leu455/Tyr442, Phe456/Leu443,
127 486Phe/Leu472, Gln493/Asn479 and Asn501/Thr487 (Fig. 3b). Four of the five SARS-CoV
128 residues such as Tyr442, Leu472, Asn479 and Thr487 have previously been shown to be
129 critical for ACE2 binding¹³. The remaining one is at the Gln498/484Tyr position, while the
130 SARS-CoV RBD Tyr484 is not involved in hydrogen-bonding interaction, the Gln498 of the
131 2019-nCoV RBD forms hydrogen-bonding interaction with Gln42 of ACE2 (Table 1). Outside
132 RBM, there is a unique ACE2-interacting residues Lys417 in the 2019-nCoV, forming a salt-
133 bridge with ACE2 Asp30 (Fig. 3b). This position is replaced by a valine in the SARS-CoV
134 RBD that fails to participate in ACE2 binding (Fig. 3b). Consistently, comparison of the
135 surface electrostatic potential also identified a positive-charged patch on the 2019-nCoV RBD

136 contributed by Lys417 that is absent on the SARS-CoV RBD (Fig. 3c). Taken together, these
137 results show that the 2019-nCoV RBD/ACE2 and SARS-CoV RBD/ACE2 interfaces share
138 substantial similarity in the buried surface area, the number of interacting residues, and
139 hydrophilic interaction networks although some differences in surface electrostatic potential
140 were identified (Fig. 3). Such similarity argues strongly for the convergent evolution of the
141 2019-nCoV and SARS-CoV RBD structures to improve binding affinity to the same ACE2
142 receptor despite of being in different genetic lineages in the betacoronavirus genus.

143 Consistent with structural similarity, the binding affinities between ACE2 and 2019-
144 nCoV and SARS-CoV RBDs also fall into the same range (~10-60 nM) as previously
145 reported^{11,12}. However, this is somewhat different from a recent report where an ~10-20 fold
146 increased binding between ACE2 and 2019-nCoV spike trimer was found (K_D of 14.7 nM)
147 compared with that between ACE2 and SARS-CoV RBD-SD1 (K_D of 325 nM)¹². This is
148 perhaps due to the different proteins used in the assay or some other unknown reasons.
149 Nevertheless, the binding affinity alone is unlikely to explain the unusual transmissibility of
150 2019-nCoV. Other factors such as the unique “RRAR” furin cleavage site at the S1/S2
151 boundary of the 2019-nCoV spike may play more important roles in facilitating the rapid
152 human-to-human transmission.

153 Neutralizing antibodies represent a critical component of immune system in fighting
154 against viral infection. It has been reported that the 2019-nCoV could be cross-neutralized by
155 horse anti-SARS-CoV serum and convalescent serum from SARS-infected patient^{1,14},
156 reinforcing structural similarity between 2019-nCoV and SARS-CoV RBDs. Such similarity
157 also raised the hope of rapid application of previously characterized SARS-CoV monoclonal
158 antibodies in the clinical setting. However, no antibody targeted to SARS-CoV (m396, S230,
159 80R and CR3014) has so far demonstrated any impressive cross-binding and neutralization
160 activity against 2019-nCoV spike or RBD^{11,12,16-19}. One exception is SARS-CoV antibody
161 CR3022 that binds to the 2019-nCoV RBD with a K_D of 6.2 nM, although its neutralizing
162 activity against 2019-nCoV has not been reported yet¹¹. Currently, we are uncertain where
163 exactly the epitope of CR3022 on SARS-CoV nor on 2019-nCoV RBDs. Among the three
164 antibodies incapable of binding to the 2019-nCoV RBD, two (m396 and 80R) have the epitopes
165 resolved by high resolution crystal structure determination of SARS-CoV RBD-Fab complexes.
166 Through mapping these epitope residues onto the sequence of SARS-CoV RBD aligned with
167 the sequence of 2019-nCoV RBD (Fig. 4), we found that antibody m396 has seven residue
168 changes in the 2019-nCoV RBD among 21 epitope positions (Fig. 4). There are 15 residue
169 changes in the 2019-nCoV RBD among 24 epitope positions by antibody 80R (Fig. 4). This

170 may provide the structural basis for the lack of cross-reactivity by m396 and 80R. However,
171 conserved residues between 2019-nCoV and SARS-nCoV RBD indeed exist, even in the more
172 variable RBM (Fig. 4). The cross-neutralization of 2019-nCoV by horse anti-SARS-CoV
173 serum and serum/plasm from recovered SARS patients indicates a great potential in identifying
174 antibodies with cross-reactivity between these two coronaviruses^{1,14}. Such antibody will
175 present a great promise for developing therapeutic agents toward diverse coronavirus species
176 including 2019-nCoV.
177

178 **Materials and Methods**

179 *Protein expression and purification*

180 The 2019-nCoV receptor-binding domain (RBD) and the N-terminal peptidase domain of
181 human ACE2 were expressed using the Bac-to-Bac baculovirus system (Invitrogen). The 2019-
182 nCoV RBD (residues Arg319-Phe541) with an N-terminal gp67 signal peptide for secretion
183 and a C-terminal 6×His tag for purification was inserted into pFastBac-Dual vector (Invitrogen).
184 The construct was transformed into bacterial DH10Bac component cells, and the extracted
185 bacmid was then transfected into Sf9 cells using Cellfectin II Reagent (Invitrogen). The low-
186 titer viruses were harvested and then amplified to generate high-titer virus stock, which was
187 used to infect Hi5 cells at a density of 2×10^6 cells/ml. The supernatant of cell culture containing
188 the secreted RBD was harvested 60 h after infection, concentrated and buffer-exchanged to
189 HBS (10 mM HEPES, pH 7.2, 150 mM NaCl). RBD was captured by Ni-NTA resin (GE
190 Healthcare) and eluted with 500 mM imidazole in HBS buffer. RBD was then purified by gel
191 filtration chromatography using the Superdex 200 column (GE Healthcare) pre-equilibrated
192 with HBS buffer. Fractions containing RBD were collected.

193 The N-terminal peptidase domain of human ACE2 (residues Ser19-Asp615) was
194 expressed and purified by essentially the same protocol used for the 2019-nCoV RBD. To
195 purify the 2019-nCoV RBD/ACE2 complex, ACE2 was incubated with RBD for 1 h on ice in
196 HBS buffer, and the mixture was then subjected to gel filtration chromatography. Fractions
197 containing the complex were pooled and concentrated to 13 mg/ml.

198 *Crystallization and data collection*

199 Crystals were successfully grown at room temperature in sitting drops, over wells containing
200 100 mM MES, pH 6.5, 10% PEG5000mme, 12% 1-propanol. The drops were made by mixing
201 200 nL RBD/ACE2 in 20 mM Tris pH 7.5, 150 mM NaCl with 200 nL well solution. Crystals
202 were harvested, soaked briefly in 100 mM MES, pH 6.5, 10% PEG5000mme, 12% 1-propanol,
203 20% glycerol, and flash-frozen in liquid nitrogen. Diffraction data were collected at the BL17U
204 beam line of the Shanghai Synchrotron Research Facility (SSRF)²⁰. Diffraction data were auto-
205 processed with aquarium pipeline and the data processing statistics are listed in Table S1²¹.

206 *Structural determination and refinement*

207 The structure was determined by the molecular replacement method with PHASER in CCP4
208 suite²². The search models are ACE2 extracellular domain and SARS-CoV RBD (PDB code
209 2AJF). Density map improvement by atoms update and refinement was performed with
210 ARP/wARP²³. Subsequent model building and refinement were performed using COOT and

211 PHENIX, respectively^{24,25}. The structural refinement statistics are listed in Table S1. All
212 structural figures were generated with PyMol²⁶.

213

214 **Acknowledgments**

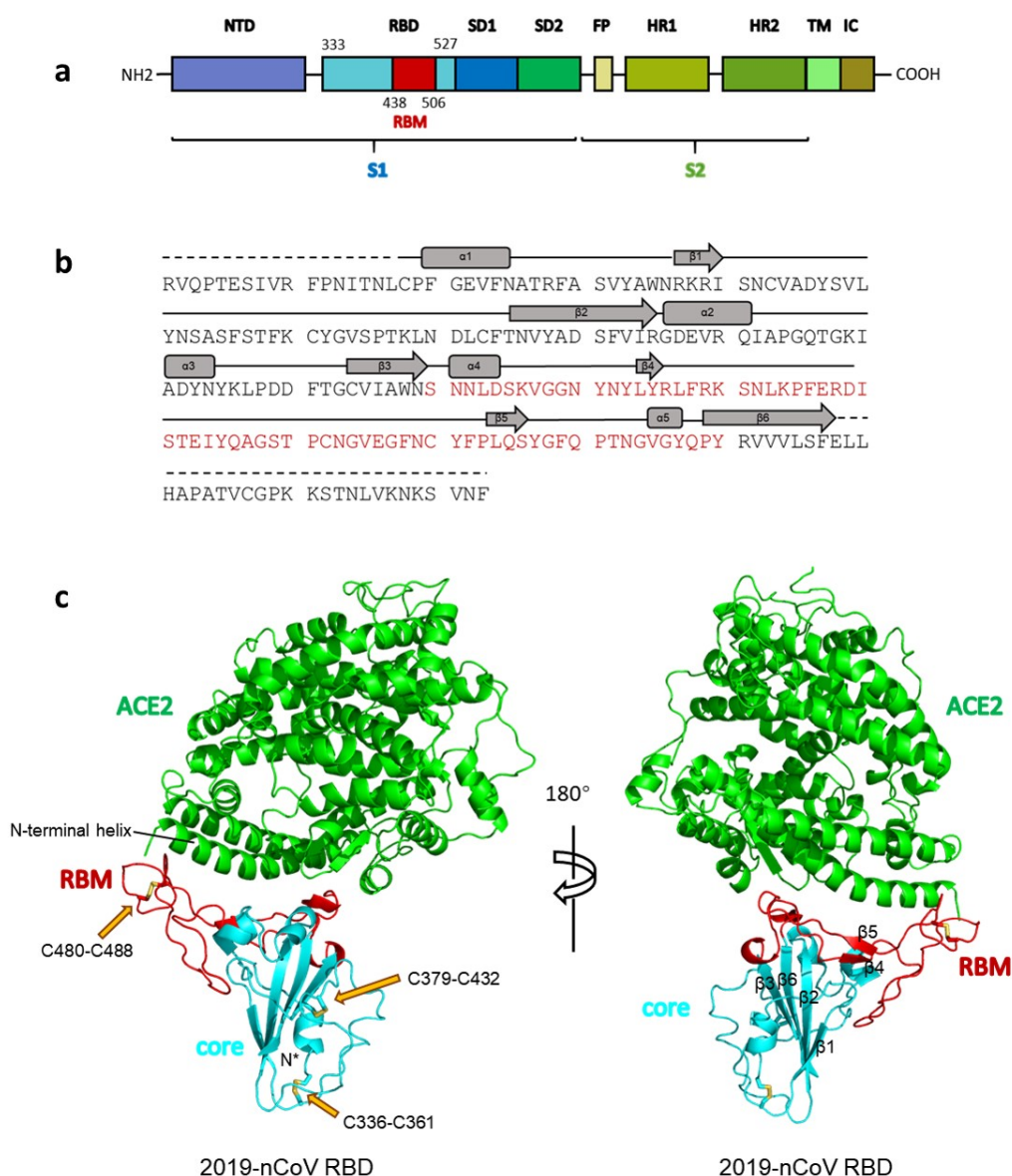
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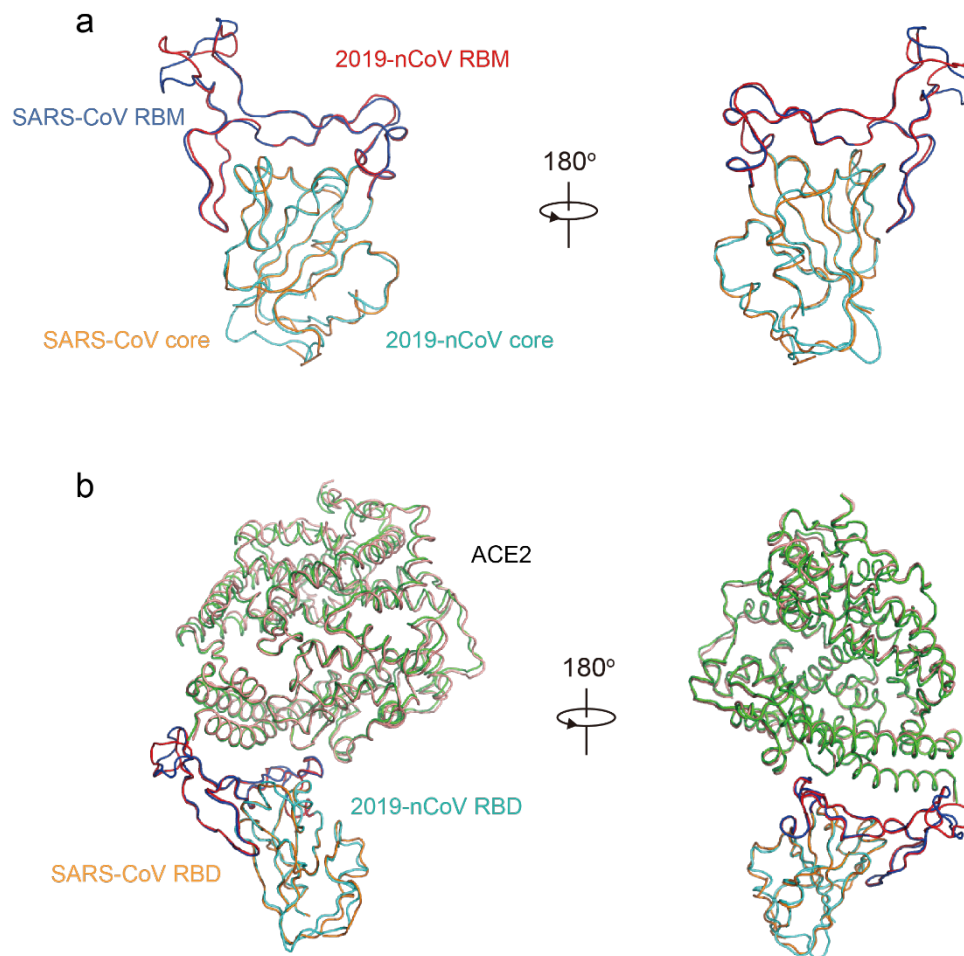
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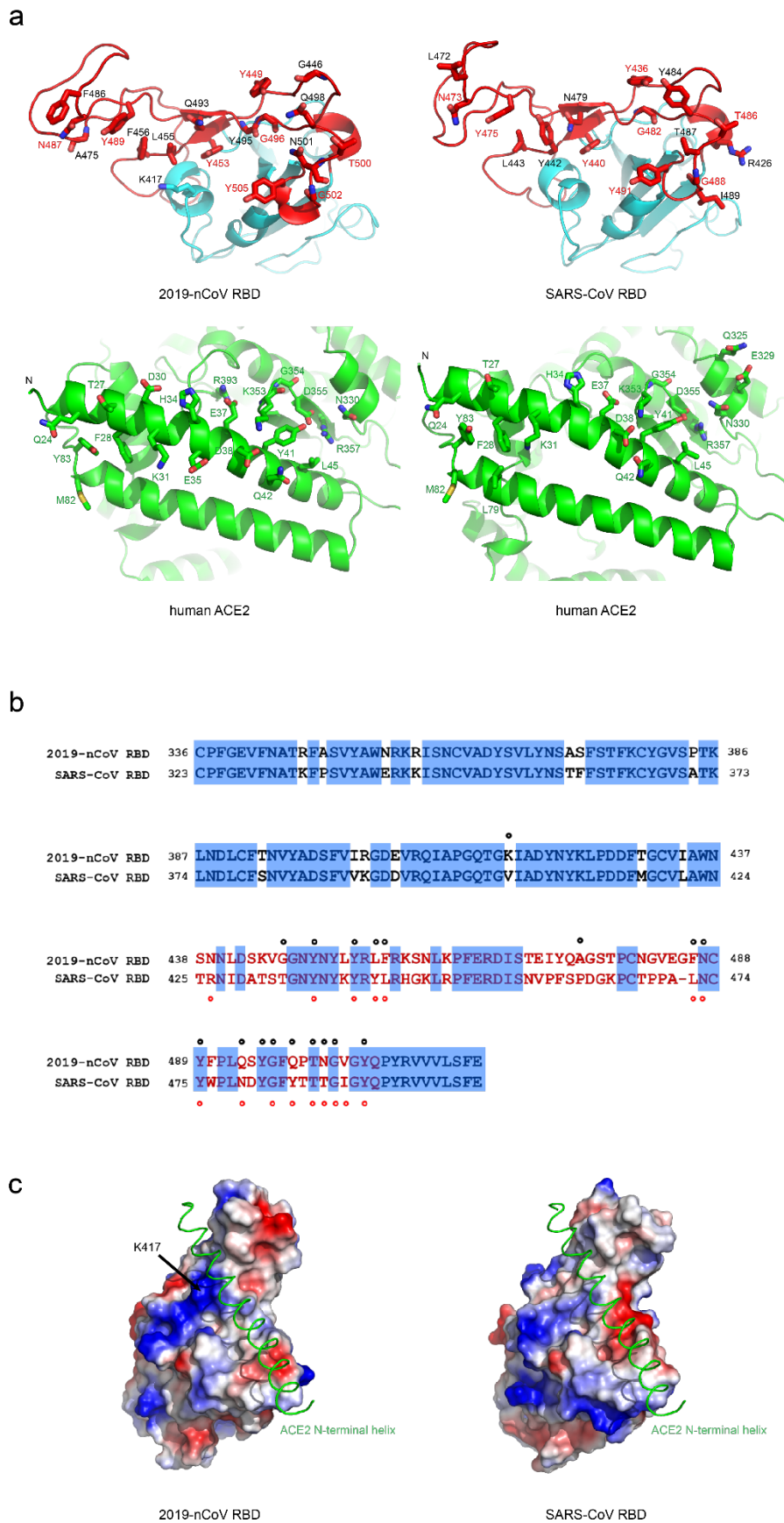


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 300 **Fig. 1 Overall structure of 2019-nCoV RBD bound with ACE2.** (a) Overall topology of
 301 2019-nCoV spike monomer. NTD, N-terminal domain. RBD, receptor-binding domain. RBM,
 302 receptor-binding motif. SD1, subdomain 1. SD2, subdomain 2. FP, fusion peptide. HR1, heptad
 303 repeat 1. HR2, heptad repeat 2. TM, transmembrane region. IC, intracellular domain. (b)
 304 Sequence and secondary structures of 2019-nCoV RBD. The RBM is colored red. (c) Overall
 305 structure of 2019-nCoV RBD bound with ACE2. ACE2 is colored green. 2019-nCoV RBD
 306 core is colored cyan and RBM is colored red. Disulfide bonds in the 2019-nCoV RBD are
 307 shown as stick and indicated by yellow arrows. The N-terminal helix of ACE2 responsible for
 308 binding is labeled.
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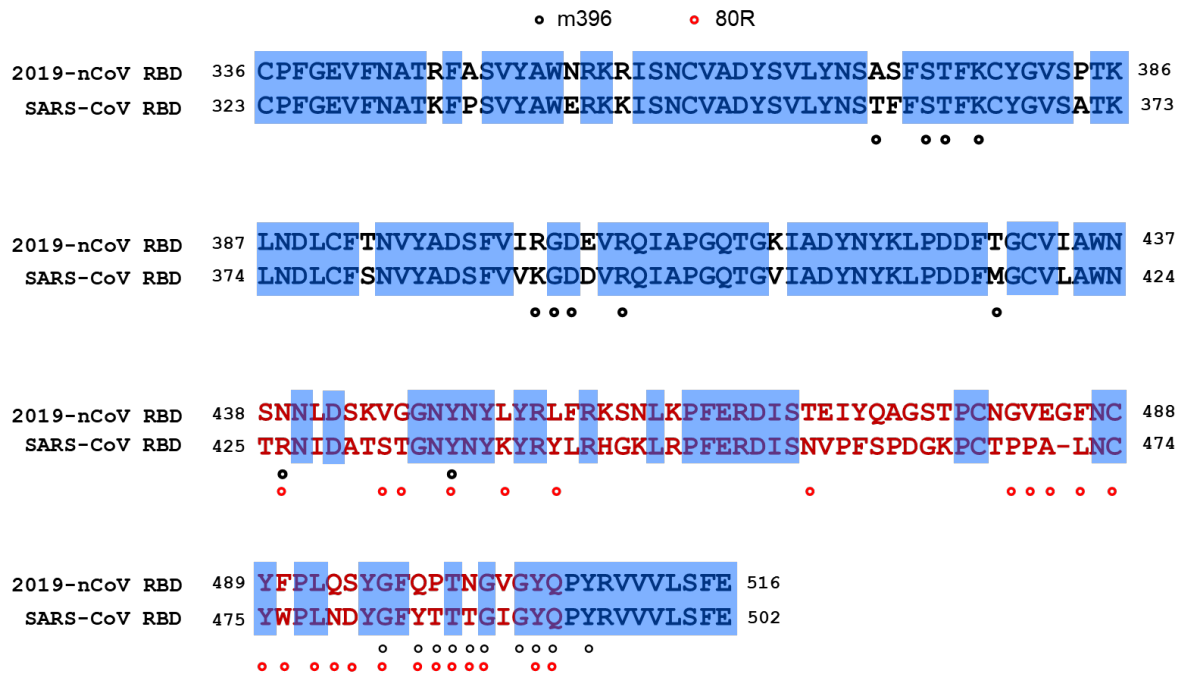


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Fig. 2 Structural comparisons of 2019-nCoV and SARS-CoV RBDs and their binding modes to ACE2 receptor. (a) Alignment of the 2019-nCoV RBD (core in cyan and RBM in red) and SARS-CoV RBD (core in orange and RBM in blue) structures. **(b)** Structural alignment of 2019-nCoV RBD/ACE2 and SARS-CoV RBD/ACE2 complexes. 2019-nCoV RBD is colored cyan and red, its interacting ACE2 is colored green. SARS-CoV RBD is colored orange and blue, its interacting ACE2 is colored salmon. The PDB code for SARS-CoV RBD/ACE2 complex: 2AJF.



320 **Fig. 3 The 2019-nCoV RBD/ACE2 binding interface compared with that of SARS-CoV**
321 **RBD/ACE2. (a)** Contacting residues shown as stick at the 2019-nCoV RBD/ACE2 and SARS-
322 CoV RBD/ACE2 interfaces. Positions in both RBDs involved in ACE2 binding are indicated
323 by red labels. **(b)** Sequence alignment of 2019-nCoV RBD and SARS-CoV RBD. Contacting
324 residues in the 2019-nCoV RBD are indicated by black dots; contacting residues in the SARS-
325 CoV RBD are indicated by red dots. **(c)** Electrostatic potential map of 2019-nCoV RBD (left
326 panel), and SARS-CoV RBD (right panel). The position of K417 in the 2019-nCoV RBD is
327 indicated by black arrow. The N-terminal helix of ACE2 is shown as green ribbon. The PDB
328 code for SARS-CoV RBD/ACE2 complex: 2AJF.
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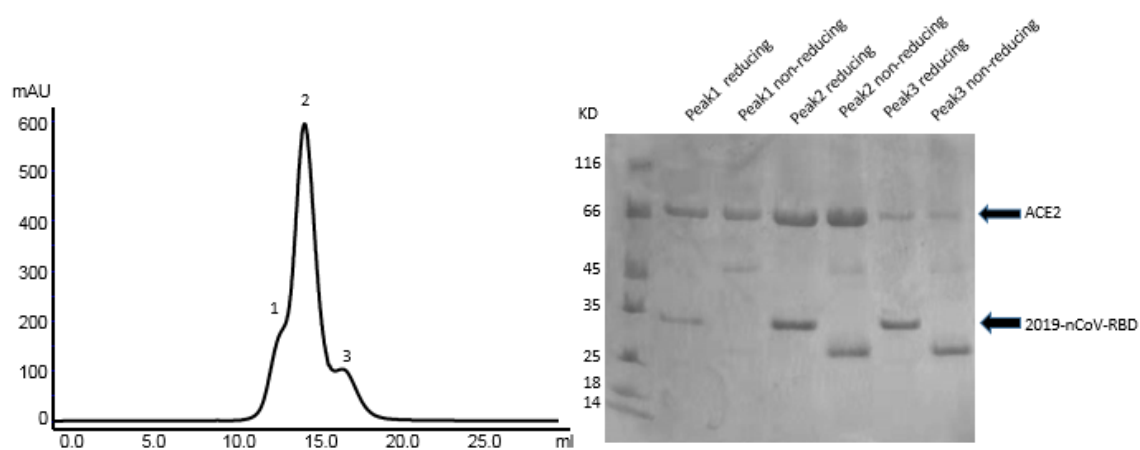
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Fig. 4 Mapping of SARS-CoV neutralizing antibody epitopes. The epitopes of SARS-CoV neutralizing antibodies m396 and 80R, which target the RBD, are labeled in the SARS-CoV sequence aligned with the sequence of 2019-nCoV RBD. Epitope residues of m396 are indicated by black dots; epitope residues of 80R are indicated by red dots.

336 **Table 1.** The hydrogen bonds and salt bridges identified using PISA program at the 2019-
 337 nCoV RBD/ACE2 and SARS-CoV RBD/ACE2 interfaces.
 338

	2019-nCoV RBD	Distance(Å)	ACE2	Distance(Å)	SARS-CoV RBD
Hydrogen bonds	N487(ND2)	2.4	Q24(OE1)	2.9	N473(ND2)
	Q493(NE2)	3.2	E35(OE1)		
	Y505(OH)	3.5	E37(OE1)	3.4	Y491(OH)
	Y505(OH)	3.6	E37(OE2)		
	Y449(OH)	3.0	D38(OD1)	3.0	Y436(OH)
	Y449(OH)	2.8	D38(OD2)	3.0	Y436(OH)
	T500(OG1)	2.7	Y41(OH)	2.8	T486(OG1)
	N501(N)	3.7	Y41(OH)	3.3	T487(N)
	Q498(OE1)	3.1	Q42(NE2)		
	G446(O)	3.6	Q42(NE2)		
	Y449(OH)	3.2	Q42(NE2)		
			Q42(OE1)	2.7	Y436(OH)
	N487(OD1)	2.5	Y83(OH)	2.8	N473(ND2)
	Y489(OH)	3.4	Y83(OH)	3.3	Y475(OH)
			Q325(OE1)	3.8	R426(NH2)
			N330(ND2)	2.8	T486(O)
	Y495(O)	3.5	K353(NZ)		
	G496(O)	3.1	K353(NZ)		
	G502(N)	2.7	K353(O)	2.5	G488(N)
	Y505(OH)	3.8	R393(NH2)		
Salt bridges	K417(NZ)	3.1	D30(OD2)		
			E329(OE2)	3.7	R426(NH1)
			E329(OE2)	2.9	R426(NH2)

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Fig. S1 Purification of 2019-nCoV RBD/ACE2 complex



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Fig. S2 Crystal of 2019-nCoV RBD/ACE2 complex.

351 **Table S1 Data collection and refinement statistics.**
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Data Collection	
Beamline	SSRF BL17U
Wavelength	0.9796 Å
Space group	P4 ₁ 2 ₁ 2
Cell dimensions	
a, b, c (Å)	104.67, 104.67, 228.72
α, β, γ (°)	90, 90, 90
Resolution (Å)	53.1- 2.45 (2.54 - 2.45)
^a R _{merge}	0.118 (2.70)
^b R _{pim}	0.033 (0.738)
^c CC _{1/2} of the highest resolution shell	0.661
I / σI	24.2 (1.7)
Completeness (%)	99.90 (99.98)
Redundancy	26.1 (27.3)
Refinement	
Resolution (Å)	53.1- 2.45
No. Reflections	47545
^d R _{work} / R _{free} (%)	21.1/23.7
No. atoms	
Protein	6369
Water	16
B-factors (Å ²)	
Protein	68.2
Water	58.8
r.m.s. deviations	
Bond lengths (Å)	0.014
Bond angles (°)	1.90
Ramachandran plot (%)	
Most favored	95.01
Allowed	4.61
Disallowed	0.38

353 ^aR_{merge} = $\sum_{hkl} \sum_j |I_j(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_j I_j(hkl)$, where *I* is the intensity of reflection.

354 ^bR_{pim} = $\sum_{hkl} [I/(N-I)]^{1/2} \sum_j |I_j(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_j I_j(hkl)$, where *N* is the redundancy of the
 355 dataset.

356 ^cCC_{1/2} is the correlation coefficient of the half datasets.

357 ^dR_{work} = $\sum_{hkl} | |F_{obs}| - |F_{calc}| | / \sum_{hkl} |F_{obs}|$, where *F_{obs}* and *F_{calc}* is the observed and the
 358 calculated structure factor, respectively. *R_{free}* is the cross-validation *R* factor for the test set of
 359 reflections (5% of the total) omitted in model refinement.

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361 **Table S2 Contact residues at the 2019-nCoV RBD/ACE2 and SARS-CoV RBD-ACE2**
362 **interfaces**
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2019-nCoV RBD-ACE2 interface		SARS-CoV-ACE2 interface	
ACE2	2019-nCoV RBD	ACE2	SARS-CoV RBD
24Q	417K	24Q	426R
27T	446G	27T	436Y
28F	449Y	28F	440Y
30D	453Y	31K	442Y
31K	455L	34H	443L
34H	456F	37E	472L
35E	475A	38D	473N
37E	486F	41Y	475Y
38D	487N	42Q	479N
41Y	489Y	45L	482G
42Q	493Q	79L	484Y
45L	495Y	82M	486T
82M	496G	83Y	487T
83Y	498Q	325Q	488G
330N	500T	329E	489I
353K	501N	330N	491Y
354G	502G	353K	
355D	505Y	354G	
357R		355D	
393R		357R	

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