

Cryo-EM Structure of the 2019-nCoV Spike in the Prefusion Conformation

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1 **Abstract**

2 The outbreak of a novel betacoronavirus (2019-nCoV) represents a pandemic threat that has been
3 declared a public health emergency of international concern. The CoV spike (S) glycoprotein is a
4 key target for urgently needed vaccines, therapeutic antibodies, and diagnostics. To facilitate
5 medical countermeasure (MCM) development we determined a 3.5 Å-resolution cryo-EM
6 structure of the 2019-nCoV S trimer in the prefusion conformation. The predominant state of the
7 trimer has one of the three receptor-binding domains (RBDs) rotated up in a receptor-accessible
8 conformation. We also show biophysical and structural evidence that the 2019-nCoV S binds
9 ACE2 with higher affinity than SARS-CoV S. Additionally we tested several published SARS-
10 CoV RBD-specific monoclonal antibodies and found that they do not have appreciable binding to
11 nCoV-2019 S, suggesting antibody cross-reactivity may be limited between the two virus RBDs.
12 The atomic-resolution structure of 2019-nCoV S should enable rapid development and evaluation
13 of MCMs to address the ongoing public health crisis.

14 The novel coronavirus 2019-nCoV has recently emerged as a human pathogen in the city
15 of Wuhan in China's Hubei province, causing fever, severe respiratory illness and pneumonia (1,
16 2). According to the World Health Organization on February 10th, 2020, there have been over
17 40,000 confirmed cases globally, leading to at least 900 deaths. The new pathogen was rapidly
18 shown to be a novel member of the betacoronavirus genus that is closely related to several bat
19 coronaviruses as well as severe acute respiratory syndrome coronavirus (SARS-CoV) (3, 4).
20 Compared to SARS-CoV, 2019-nCoV appears to be more readily transmitted from human-to-
21 human, spreading to multiple continents and leading to the WHO declaration of a Public Health
22 Emergency of International Concern (PHEIC) (1, 5, 6).

23 2019-nCoV makes use of a densely glycosylated, homotrimeric class I fusion spike (S)
24 protein to gain entry into host cells. The S protein exists in a metastable prefusion conformation
25 that undergoes a dramatic structural rearrangement to fuse the viral membrane with the host cell
26 membrane (7, 8). This process is triggered by binding of the S1 subunit to a host-cell receptor,
27 which destabilizes the prefusion trimer, resulting in shedding of the S1 subunit and transition of
28 the S2 subunit to a highly stable postfusion conformation (9). In order to engage a host-cell
29 receptor, the receptor-binding domain (RBD) of S1 undergoes hinge-like conformational
30 movements that transiently hide or expose the determinants of receptor binding. These two states
31 are referred to as the “down” conformation and the “up” conformation, where “down”
32 corresponds to the receptor-inaccessible state and “up” corresponds to the receptor-accessible
33 state, which is thought to be less stable (10-13). Due to the indispensable function of the S
34 protein it represents a vulnerable target for antibody-mediated neutralization, and
35 characterization of the prefusion S structure would provide atomic-level information to guide
36 vaccine design and development.

37 Based on the reported genome sequence of 2019-nCoV(4), we expressed ectodomain
38 residues 1–1208 of 2019-nCoV S (**Figure 1A, Supplementary Figure 1**), adding two stabilizing
39 proline mutations in the C-terminal S2 fusion machinery based on a previous stabilization
40 strategy which proved highly effective for betacoronavirus S proteins (11, 14). We obtained
41 roughly 0.5 mg/L of the recombinant prefusion-stabilized S ectodomain from FreeStyle 293
42 cells, and the protein was purified to homogeneity by affinity chromatography and size-exclusion
43 chromatography (**Supplementary Figure 1**). Cryo-EM grids were prepared using this purified,
44 fully glycosylated S protein and preliminary screening revealed a high particle density with little
45 aggregation near the edges of the holes.

46 After collecting and processing 3,207 micrograph movies, we obtained a 3.5 Å-resolution
47 3D reconstruction of an asymmetrical trimer in which a single RBD was observed in the “up”
48 conformation. (**Figure 1B, Supplementary Figure 2**). Due to the small size of the RBD (~21
49 kDa), the asymmetry of this conformation was not readily apparent until *ab initio* 3D
50 reconstruction and 3D classification were performed (**Figure 1B, Supplementary Figure 3**). By
51 using the 3D variability feature in cryoSPARC v2 (15), we were able to observe breathing of the
52 S1 subunits as the RBD underwent a hinge-like movement, which likely contributed to the
53 relatively poor local resolution of S1 compared to the more stable S2 subunit (**Supplementary**
54 **Movies 1 and 2**). This seemingly stochastic RBD movement has been captured during structural
55 characterization of the closely related betacoronaviruses SARS-CoV and MERS-CoV, as well as
56 the more distantly related alphacoronavirus porcine epidemic diarrhea virus (PEDV)(10, 11, 13,
57 16). The observation of this phenomenon in 2019-nCoV S suggests that it shares the same
58 mechanism of triggering that is thought to be conserved among the *Coronaviridae*, wherein

59 receptor-binding to exposed RBDs leads to an unstable 3 RBD-up conformation that results in
60 shedding of S1 and refolding of S2 (11, 12).

61 Because the S2 subunit appeared to be a symmetric trimer, we performed a 3D
62 refinement imposing C3 symmetry, resulting in a 3.2 Å-resolution map, with excellent density
63 for the S2 subunit. Using both maps we built the vast majority of the 2019-nCoV S ectodomain,
64 including glycans at 44 of the 66 N-linked glycosylation sites per trimer (**Supplementary Figure**
65 **4**). Our final model spans S residues 27–1146, with several flexible loops omitted. Like all
66 previously reported coronavirus S ectodomain structures, the density for 2019-nCoV S begins to
67 fade after the connector domain (CD), reflecting the flexibility of the heptad repeat 2 (HR2)
68 domain in the prefusion conformation (**Supplementary Figure 4A**) (13, 16-18).

69 The overall structure of 2019-nCoV S resembles that of SARS-CoV S, with a root mean
70 square deviation (RMSD) of 3.8 Å over 959 C α atoms. The largest discrepancy between these
71 two structures is a conformational difference between the positions of the RBDs in their
72 respective “down” conformations (**Figure 2A**). Whereas the SARS-CoV RBD in the “down”
73 conformation packs tightly against the N-terminal domain (NTD) of the neighboring protomer,
74 the 2019-nCoV RBD in the “down” conformation is angled closer to the central cavity of the
75 homotrimer. Despite this observed conformational difference, when the individual structural
76 domains of 2019-nCoV S are aligned to their counterparts from SARS-CoV S, they reflect the
77 high degree of structural homology between the two proteins, with the NTDs, RBDs,
78 subdomains 1 and 2 (SD1 and SD2) and S2 subunits yielding RMSD values of 2.6 Å, 3.0 Å, 2.7
79 Å and 2.0 Å, respectively (**Figure 2B**).

80 2019-nCoV S shares roughly 96% sequence identity with the S protein from the bat
81 coronavirus RaTG13, with the most notable variation arising from an insertion in the S1/S2

82 protease cleavage site that results in an “RRAR” furin recognition site in 2019-nCoV, rather than
83 the single arginine in SARS-CoV (**Supplementary Figure 5**) (19-22). A similar phenomenon
84 has been observed for influenza viruses, where amino acid insertions that create a polybasic furin
85 site in a related position in influenza hemagglutinin proteins are often found in highly virulent
86 avian and human influenza viruses (23). In addition to this insertion of residues in the S1/S2
87 junction, 29 variant residues exist between 2019-nCoV S and RaTG13 S, with 17 of these
88 positions mapping to the RBD (**Supplementary Figures 5 and 6**). We also analyzed the 61
89 available 2019-nCoV S sequences in GISAID and found that there were only 9 amino acid
90 substitutions among all deposited sequences. Most of these substitutions are relatively
91 conservative and they are not expected to have a dramatic effect on the structure or function of
92 the 2019-nCoV S protein (**Supplementary Figure 6**).

93 Recent reports demonstrating that 2019-nCoV S and SARS-CoV S share the same
94 functional host-cell receptor—angiotensin-converting enzyme 2 (ACE2) (21, 24-26)—prompted
95 us to quantify the kinetics mediating this interaction via surface plasmon resonance (SPR).
96 Surprisingly, ACE2 bound to 2019-nCoV S ectodomain with ~15 nM affinity, which is
97 approximately 10- to 20-fold higher affinity than ACE2 binding to SARS-CoV S (**Figure 3A**,
98 **Supplementary Figure 7**) (14). We also formed a complex of ACE2 bound to the 2019-nCoV S
99 ectodomain and observed it by negative-stain EM, where it strongly resembled the complex
100 formed between SARS-CoV S and ACE2, which has been observed at high-resolution by cryo-
101 EM (**Figure 3B**) (14, 27). The high affinity of 2019-nCoV S for human ACE2 may contribute to
102 the apparent ease with which 2019-nCoV can spread from human-to-human(1), however
103 additional studies are needed to investigate this possibility.

104 The overall structural homology and shared receptor usage between SARS-CoV S and
105 2019-nCoV S prompted us to test published SARS-CoV RBD-directed monoclonal antibodies
106 (mAbs) for cross-reactivity to the 2019-nCoV RBD (**Figure 4A**). A 2019-nCoV RBD-SD1
107 fragment (S residues 319–591) was recombinantly expressed, and appropriate folding of this
108 construct was validated by measuring ACE2 binding using biolayer interferometry (BLI)
109 (**Figure 4B**). Cross-reactivity of the SARS-CoV RBD-directed mAbs S230, m396 and 80R was
110 then evaluated by BLI (12, 28-30). Despite the relatively high degree of structural homology
111 between the 2019-nCoV RBD and the SARS-CoV RBD, no binding to the 2019-nCoV RBD
112 could be detected for any of the three mAbs at the concentration tested (1 μ M) (**Figure 4C**,
113 **Supplementary Figure 8**). Although the epitopes of these three antibodies represent a relatively
114 small percentage of the surface area of the 2019-nCoV RBD, the lack of observed binding
115 suggests that SARS-directed mAbs will not necessarily be cross-reactive and that future antibody
116 isolation and therapeutic design efforts will benefit from using 2019-nCoV S proteins as probes.

117 The rapid global spread of 2019-nCoV, prompting the PHEIC declaration by WHO
118 signals the urgent need for coronavirus vaccines and therapeutics. Knowing the atomic-level
119 structure of the spike will support precision vaccine design and discovery of antivirals,
120 facilitating medical countermeasure development.

121 **Materials and Methods**

122 *Protein expression and purification*

123 To express the prefusion S ectodomain, a gene encoding residues 1–1208 of 2019-nCoV S
124 (GenBank: MN908947) with proline substitutions at residues 986 and 987, a “GSAS”
125 substitution at the furin cleavage site (residues 682–685), a C-terminal T4 fibritin trimerization
126 motif, an HRV3C protease cleavage site, a TwinStrepTag and an 8XHisTag was synthesized and
127 cloned into the mammalian expression vector p α H. To express the 2019-nCoV RBD-SD1,
128 residues 319–591 of 2019-nCoV S were cloned upstream of a C-terminal HRV3C protease
129 cleavage site, a monomeric Fc tag and an 8XHisTag. Similarly, to express the SARS-CoV RBD-
130 SD1, residues 306–577 of SARS-CoV S (Urbani strain) were cloned upstream of a C-terminal
131 HRV3C protease cleavage site, a monomeric Fc tag and an 8XHisTag. Lastly, a plasmid
132 encoding residues 1–615 of human ACE2 with a C-terminal HRV3C protease cleavage site, a
133 TwinStrepTag and an 8XHisTag was generated.

134 These expression vectors were used to transiently transfect FreeStyle293F cells (Thermo
135 Fischer) using polyethylenimine. Protein was purified from filtered cell supernatants using either
136 StrepTactin resin (IBA) or Protein A resin (Pierce) before being subjected to additional
137 purification by size-exclusion chromatography using either a Superose 6 10/300 column (GE
138 Healthcare) or a Superdex 200 10/300 Increase column (GE Healthcare) in 2 mM Tris pH 8.0,
139 200 mM NaCl and 0.02% NaN₃. ACE2 and the 2019-nCoV RBD-SD1 were incubated with 10%
140 (wt/wt) HRV3C protease for 2 hours at room temperature. Cleaved protein was then passed over
141 either NiNTA resin (ACE2) or Protein A and NiNTA resins (2019-nCoV RBD) to remove
142 cleaved tags and His-tagged protease before being run over a Superdex 200 10/300 Increase
143 column in 2 mM Tris pH 8.0, 200 mM NaCl and 0.02% NaN₃.

144 Plasmids encoding the heavy and light chains of S230, 80R and m396 IgG were transiently
145 transfected into Expi293 (Thermo Fischer) using polyethylenimine. Antibodies were purified
146 from cell supernatants using Protein A resin before being used for biolayer interferometry.

147 Cryo-EM sample preparation and data collection

148 Purified 2019-nCoV S was diluted to a concentration of 0.35 mg/mL in 2 mM Tris pH 8.0, 200
149 mM NaCl and 0.02% NaN₃. 3 uL of protein was deposited on a CF-1.2/1.3 grid that had been
150 plasma cleaned for 30 seconds in a Solarus 950 plasma cleaner (Gatan) with a 4:1 ratio of O₂/H₂.
151 Excess protein was blotted away for 6 seconds before being plunge frozen into liquid ethane
152 using a Vitrobot Mark IV (Thermo Scientific). Frozen grids were imaged in a Titan Krios
153 (Thermo Scientific) equipped with a K3 detector (Gatan). Movies were collected using Legion
154 (31) at a magnification of x22,500, corresponding to a calibrated pixel size of 1.047 Å/pixel. A
155 full description of the cryo-EM data collection parameters can be found in **Supplementary**
156 **Table 1**.

157 Cryo-EM data processing

158 Motion correction, CTF-estimation and non-templated particle picking were performed in Warp
159 (32). Extracted particles were imported into cryoSPARC v2.12.4 (15) for 2D classification, 3D
160 classification and non-uniform 3D refinement. The C1 RBD “up” reconstruction was sharpened
161 in cryoSPARC, and the 3D reconstruction with C3 symmetry was subjected to local B-factor
162 sharpening using LocalDeBlur (33). Models were built in Coot, before being iteratively refined
163 in both Phenix and ISOLDE (34-36). Some of the data processing and refinement software was
164 curated by SBBGrid (37). The full cryo-EM data processing workflow is described in
165 **Supplementary Figure 3** and the model refinement statistics can be found in **Supplementary**
166 **Table 1**.

167 Surface plasmon resonance

168 His-tagged 2019-nCoV S was immobilized to an NiNTA sensorchip (GE Healthcare) to a level
169 of ~800 response units (RUs) using a Biacore X100 (GE Healthcare) and a running buffer
170 composed of 10 mM HEPES pH 8.0, 150 mM NaCl and 0.05% Tween 20. Serial dilutions of
171 purified and untagged ACE2 were injected ranging in concentration from 250 to 15.6 nM. The
172 resulting data were fit to a 1:1 binding model using Biacore Evaluation Software (GE
173 Healthcare). His-tagged SARS-CoV RBD-SD1 was immobilized to an NiNTA sensorchip to a
174 level of ~350 RUs using a Biacore X100 and the same running buffer listed above. Serial
175 dilutions of purified and untagged ACE2 were injected ranging in concentration from 500 to 31.3
176 nM. The resulting data were fit to a 1:1 binding model using Biacore Evaluation Software.

177 Negative stain EM

178 Purified 2019-nCoV S was diluted to a concentration of 0.032 mg/mL in 2 mM Tris pH 8.0, 200
179 mM NaCl and 0.02% NaN₃. Diluted S protein was mixed with a 1.5-fold molar excess of ACE2
180 and the mixture was incubated on ice for 1 minute before 4.8 uL of the protein mixture was
181 deposited on a CF400-Cu grid (Electron Microscopy Sciences) before being stained with
182 methylamine tungstate (Nanoprobes). This grid was imaged in an FEI Talos TEM (Thermo
183 Scientific) equipped with a Ceta 16M detector. Micrographs were collected manually using TIA
184 v4.14 software at a magnification of x92,000, corresponding to a pixel size of 1.63 Å/pixel. CTF
185 estimation, particle picking and 2D class averaging were performed in *cis*TEM (38).

186 Biolayer interferometry

187 Fc-tagged 2019-nCoV RBD-SD1 was immobilized to an anti-human capture (AHC) sensortip
188 (FortéBio) using an Octet RED96e (FortéBio). The sensortip was then dipped into 100 nM
189 ACE2 to measure association before being dipped into a well containing only running buffer

190 composed of 10 mM HEPES pH 7.5, 150 mM NaCl, 3 mM EDTA, 0.05% Tween 20 and 1
191 mg/mL bovine serum albumin to measure dissociation. Data were reference subtracted and fit to
192 a 1:1 binding model using Octet Data Analysis Software v11.1 (FortéBio).

193 S230, 80R and m396 IgGs were immobilized to AHC sensortips to a response level of
194 ~0.8 nm and dipped into wells containing 1 μ M untagged 2019-nCoV RBD-SD1 before being
195 dipped into wells containing only running buffer to measure dissociation. Data were reference-
196 subtracted and aligned to a baseline after IgG capture using Octet Data Analysis software v11.1.
197 An analogous experiment was performed under identical conditions by dipping AHC sensor tips
198 loaded with S230, 80R or m396 IgG into untagged SARS-CoV RBD-SD1. Data were reference-
199 subtracted, aligned to a baseline after IgG capture and fit to a 1:1 binding model using Octet Data
200 Analysis software v11.1.

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Figure Legends

Figure 1. Structure of 2019-nCoV S in the prefusion conformation. (A) Schematic of 2019-nCoV S primary structure, colored by domain. Domains that were excluded from the ectodomain expression construct or could not be visualized in the final map are colored white. SS= signal sequence, NTD= N-terminal domain, RBD= receptor-binding domain, SD1= subdomain 1, SD2= subdomain 2, S1/S2= S1/S2 protease cleavage site, S2'= S2' protease cleavage site, FP= fusion peptide, HR1= heptad repeat 1, CH= central helix, CD= connector domain, HR2= heptad repeat 2, TM= transmembrane domain, CT= cytoplasmic tail. Arrows denote protease cleavage sites. (B) Select 2D class averages of the particles that were used to calculate the 2019-nCoV S reconstruction (*left*). Side and top views of the prefusion structure of the 2019-nCoV S protein with a single RBD in the “up” conformation (*right*). The two RBD “down” protomers are shown as cryo-EM density in either white or gray and the RBD “up” protomer is shown in ribbons, colored corresponding to the schematic in **Fig 1A**.

Figure 2. Structural comparison between 2019-nCoV S and SARS-CoV S. (A) A single RBD “down” monomer of 2019-nCoV S is shown in ribbons, colored according to **Figure 1**. A monomer of SARS-CoV S is also shown in ribbons, colored white (PDB ID: 6CRZ). (B) The following structural domains from 2019-nCoV S have been aligned to their counterparts from SARS-CoV S; NTD (*top left*), RBD (*top right*), SD1 and SD2, (*bottom left*) and S2 (*bottom right*).

Figure 3. 2019-nCoV S binds human ACE2 with high affinity. (A) SPR sensorgram showing the binding kinetics for human ACE2 and immobilized 2019-nCoV S. Data are shown as black lines and the best fit of the data to a 1:1 binding model is shown in red. (B) Negative-stain EM 2D

class averages of 2019-nCoV S bound by ACE2. Averages have been rotated so that ACE2 is positioned above the 2019-nCoV S protein with respect to the viral membrane. A cartoon depicting the ACE2-bound 2019-nCoV S protein is shown (*right*) with ACE2 in blue and S protein monomers colored tan, pink and green.

Figure 4. Antigenicity of the 2019-nCoV RBD. (A) The SARS-CoV RBD is shown as a white molecular surface (PDB ID: 2AJF), with residues that vary in the 2019-nCoV RBD colored red. The ACE2 binding site is outlined with a black dotted line. (B) A biolayer interferometry sensorgram that shows binding to ACE2 by the 2019-nCoV RBD-SD1. Binding data are shown as a black line and the best fit of the data to a 1:1 binding model is shown in red. (C) Biolayer interferometry to measure cross-reactivity of the SARS-CoV RBD-directed antibodies S230, m396 and 80R. Sensortips with immobilized antibodies were dipped into wells containing 2019-nCoV RBD-SD1 and the resulting data are shown as a black line.

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Figure 1

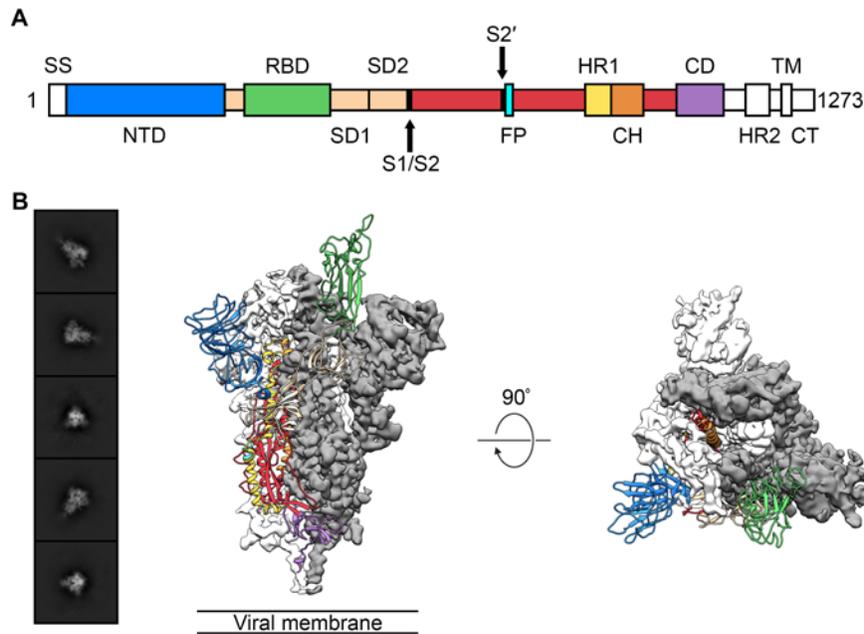


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Figure 2

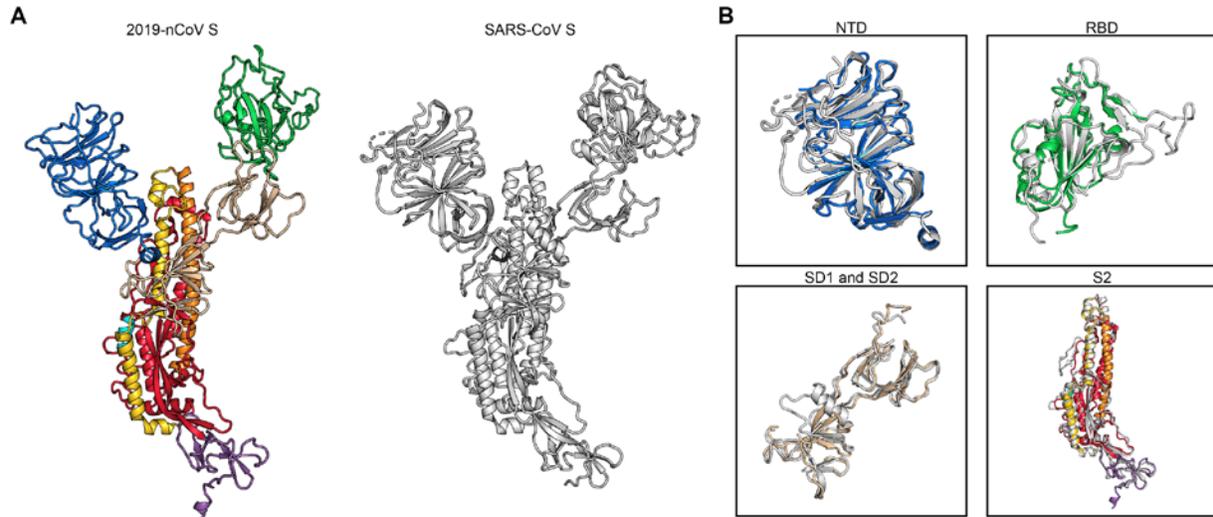


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Figure 3

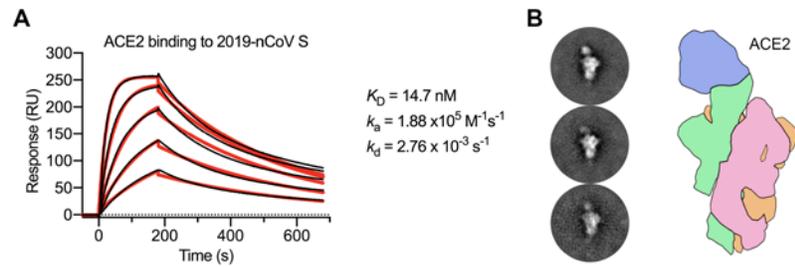


Figure 3. 2019-nCoV S binds human ACE2 with high affinity. (A) SPR sensorgram showing the binding kinetics for human ACE2 and immobilized 2019-nCoV S. Data are shown as black lines and the best fit of the data to a 1:1 binding model is shown in red. (B) Negative-stain EM 2D class averages of 2019-nCoV S bound by ACE2. Averages have been rotated so that ACE2 is positioned above the 2019-nCoV S protein with respect to the viral membrane. A cartoon depicting the ACE2-bound 2019-nCoV S protein is shown (*right*) with ACE2 in blue and S protein monomers colored tan, pink and green.

Figure 4

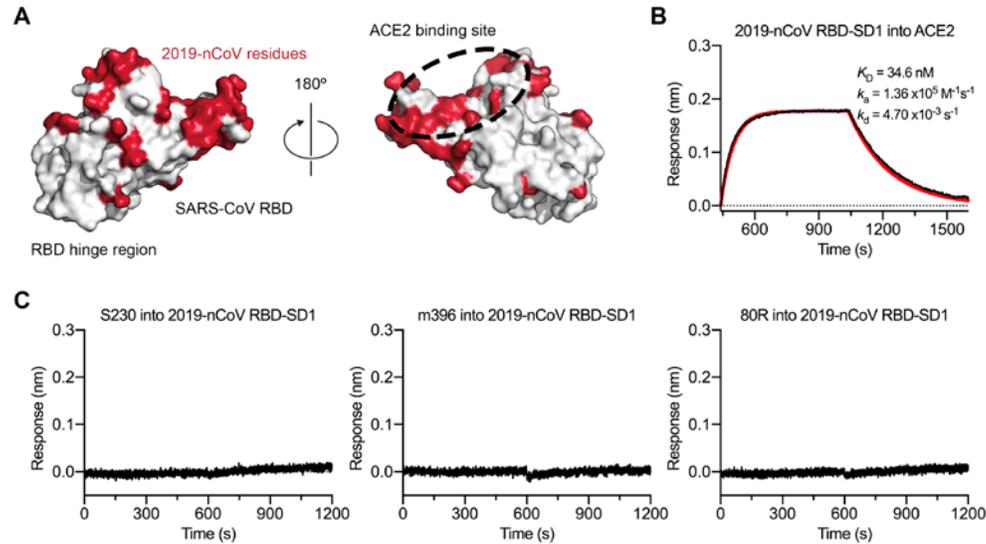
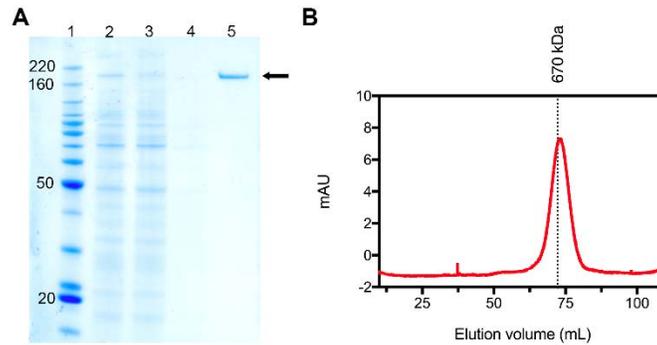
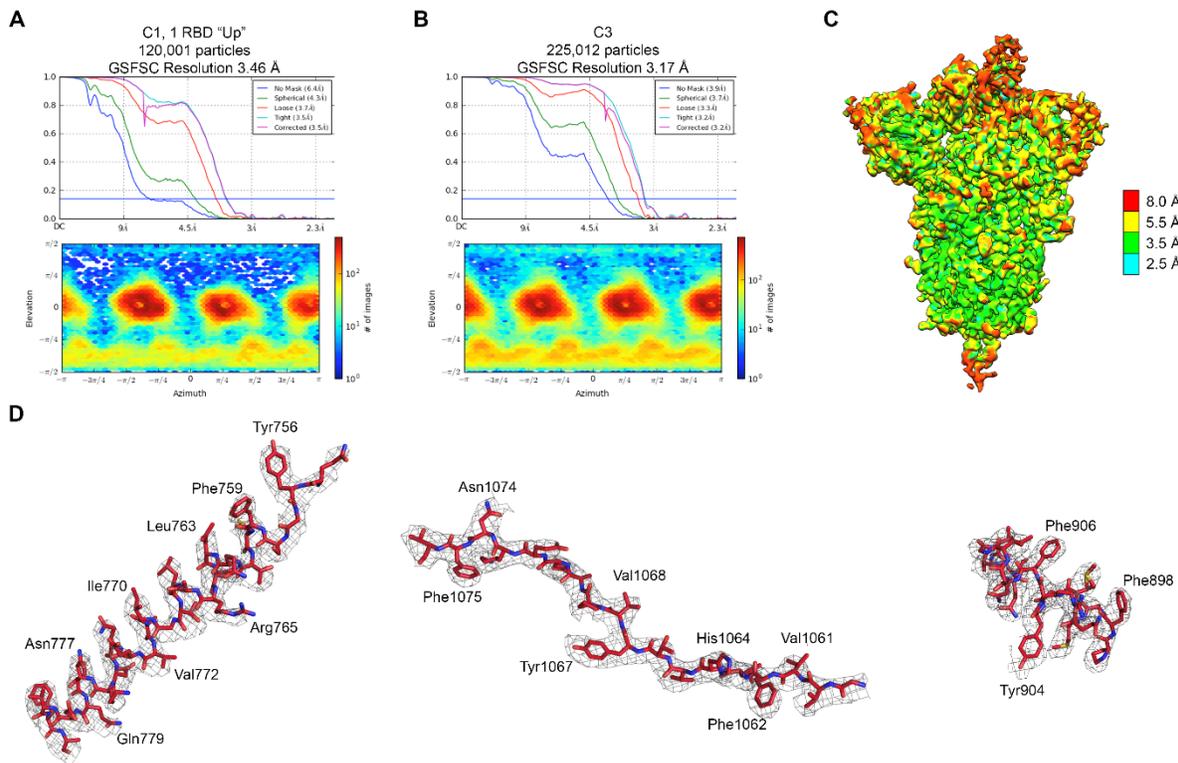


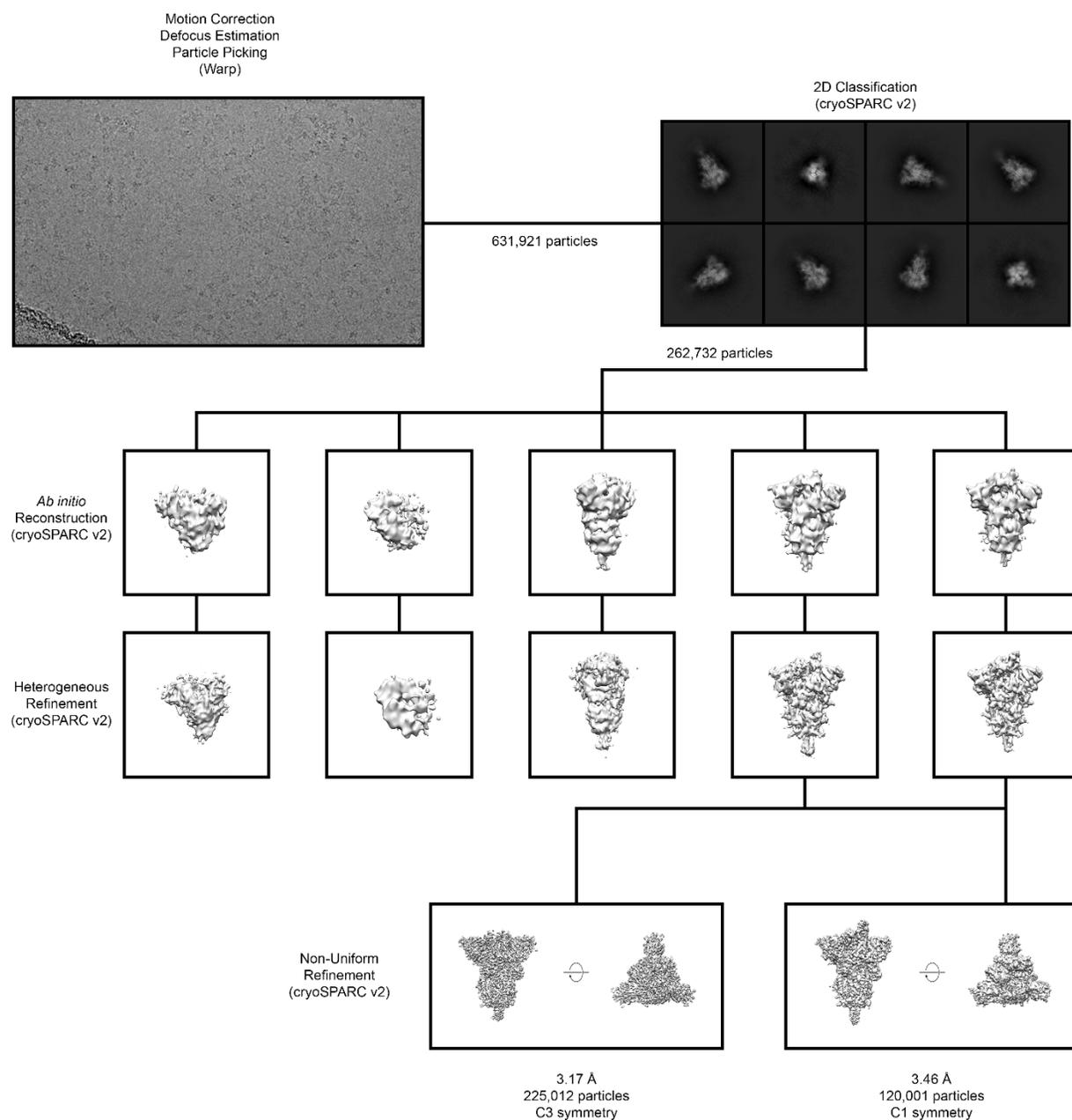
Figure 4. Antigenicity of the 2019-nCoV RBD. (A) The SARS-CoV RBD is shown as a white molecular surface (PDB ID: 2AJF), with residues that vary in the 2019-nCoV RBD colored red. The ACE2 binding site is outlined with a black dotted line. (B) A biolayer interferometry sensorgram that shows binding to ACE2 by the 2019-nCoV RBD-SD1. Binding data are shown as a black line and the best fit of the data to a 1:1 binding model is shown in red. (C) Biolayer interferometry to measure cross-reactivity of the SARS-CoV RBD-directed antibodies S230, m396 and 80R. Sensortips with immobilized antibodies were dipped into wells containing 2019-nCoV RBD-SD1 and the resulting data are shown as a black line.



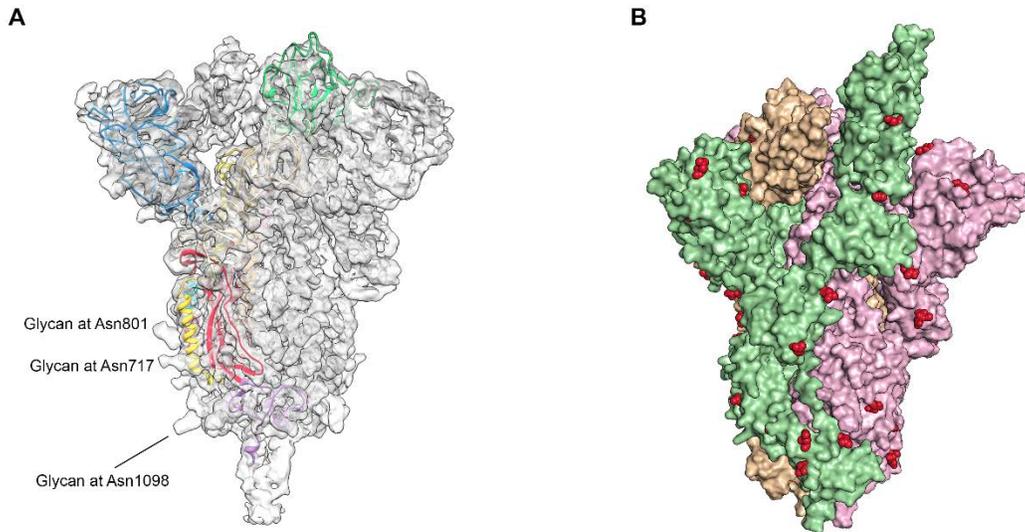
Supplementary Figure 1. 2019-nCoV S expression and purification. (A) SDS-PAGE analysis of the 2019-nCoV S protein. Lane 1: molecular weight ladder, with relevant bands labeled in kilodaltons (*left*); lane 2: filtered supernatant from transfected cells; lane 3: supernatant after passing through StrepTactin resin; lane 4: wash of StrepTactin resin; lane 5: elution from StrepTactin resin. The band corresponding to 2019-nCoV S is denoted with a black arrow. (B) Size-exclusion chromatogram of the affinity-purified 2019-nCoV S protein. Data from a Superose 6 10/300 column are shown in red. The elution volume of a 670 kilodalton molecular weight standard is shown as a black dotted line.



Supplementary Figure 2. Cryo-EM structure validation. (A) FSC curves (*top*) and the viewing direction distribution plot (*bottom*) for 2019-nCoV S with a single RBD “up”. (B) FSC curves (*top*) and the viewing direction distribution plot (*bottom*) for the 2019-nCoV S processed with C3 symmetry. (C) The cryo-EM density of the 2019-nCoV S with a single RBD “up” is shown, colored according to local resolution. (D) Density from S2 of the C3-refined 2019-nCoV S structure. Residues are shown as sticks, colored according to **Figure 1A** with oxygen atoms colored red, nitrogens colored blue and sulfurs colored yellow. The cryo-EM density map is shown as a gray mesh.



Supplementary Figure 3. Cryo-EM data processing workflow.



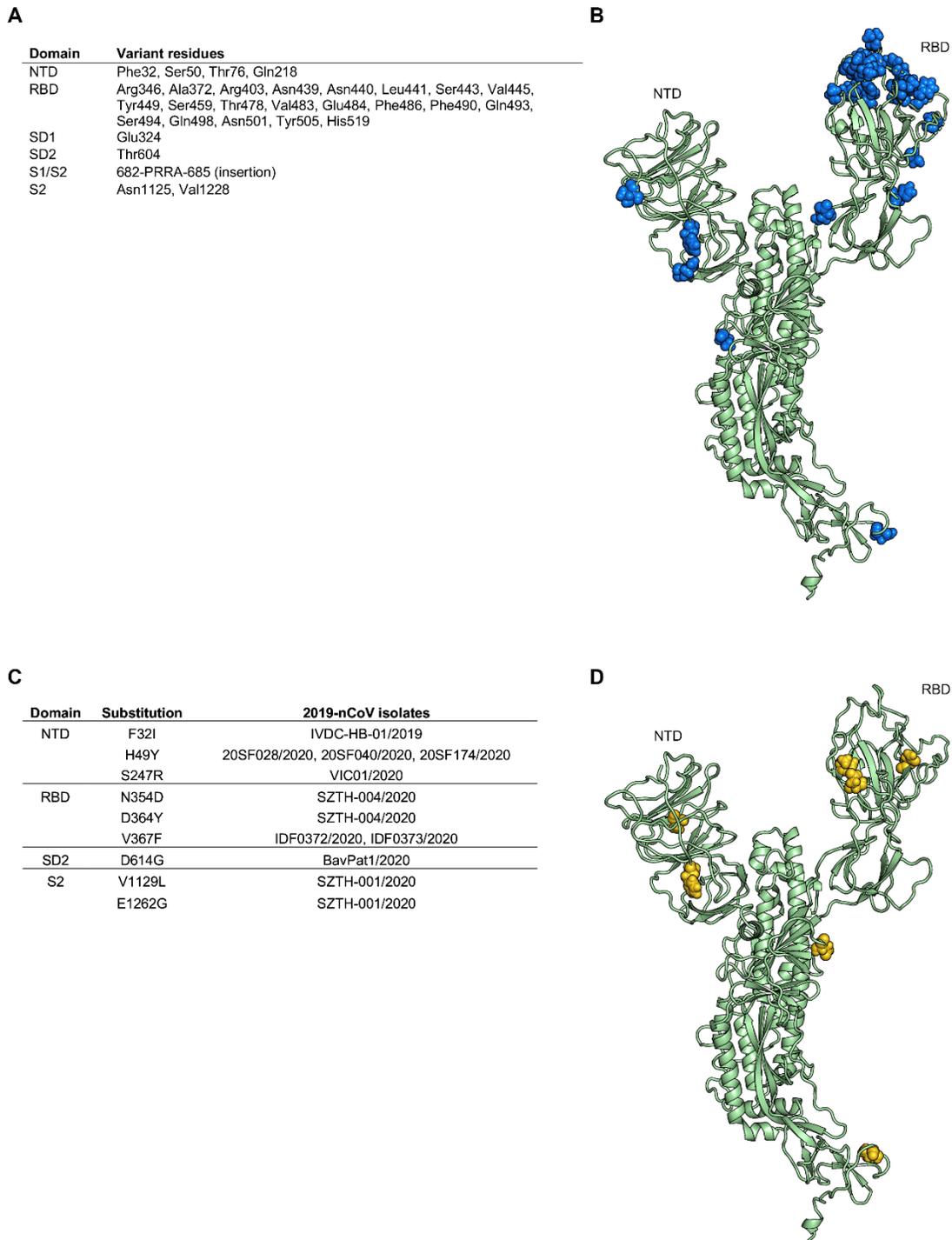
Supplementary Figure 4. Cryo-EM map and *N*-linked glycosylation sites. (A) The unsharpened cryo-EM density map for the C3-processed 2019-nCoV S is shown as a transparent molecular surface, with a single protomer fit into the map shown in ribbons and colored according to **Figure 1A**. Some S2 density that corresponds to *N*-linked glycans is labeled. (B) The 2019-nCoV S trimer is shown as a molecular surface with each protomer colored green, pink or tan. Asparagine residues that correspond to *N*-linked glycosylation sites are shown as red spheres.

2019-nCoV	-----MEVFLVLLPLVSSQ-----C	67	
SARS-CoV	-----MFIFLLFLITLTSCLDLDRCITTFDDVQAPNYTQHTSSMRGVYYPDRIFRSDTLYLTQDIFLFFYSNVTPGIT	71	
RaTG13	MFLLTTRKRMVFLVLLPLVSSQ-----C	76	
2019-nCoV	LHVSQTNGIKRFDNVLEFNDGVYFASTEKSNLIRGWIPGTTLDSKTSGLLVNNAINVVIRKVEFQFCNDPFLGVYVHK	147	
SARS-CoV	LN-----HIFGNVPIPKDGIYFAAIEKSNVVRGWFVGSIMNKNQSVLILNNSINVIRACNFELCDNPFPAVSKPM	144	
RaTG13	LHVSQTNGIKRFDNVLEFNDGVYFASTEKSNLIRGWIPGTTLDSKTSGLLVNNAINVVIRKVEFQFCNDPFLGVYVHK	156	
2019-nCoV	NNKSMWSEFRVYSSANNCTFEYVSQPFMLDLGKQGNFKNLRVFNKIDGYFKIYSKHTPINLVRDLFQCFSALEPLV	227	
SARS-CoV	GTQ---THTMIFDNAENCTFEYISDAFSLDVEKSGNFKHLREVFVKNKDGFLVYVYQYQPIDVVRDLPSGENTLKPFE	220	
RaTG13	NNKSMWSEFRVYSSANNCTFEYVSQPFMLDLGKQGNFKNLRVFNKIDGYFKIYSKHTPINLVRDLFQCFSALEPLV	236	
2019-nCoV	DLPIGINITRFQTLALHRSYLTTPGDSSSGWTAGAAAYVGYLQPRITFLKLYNENGTITDAVDCALDPLSEKTKLKSFT	307	
SARS-CoV	KLPLGINITNFRALTAES-----PAQDIWGTSAAYFVGYLKPITFMLKYDENGITDAVDCSONPLAEKCSVKSFE	294	
RaTG13	DLPIGINITRFQTLALHRSYLTTPGDSSSGWTAGAAAYVGYLQPRITFLKLYNENGTITDAVDCALDPLSEKTKLKSFT	316	
2019-nCoV	VEKGIYQTSNFRVQPTESIVRF	387	
SARS-CoV	IDKGIYQTSNFRVQPTESIVRF	374	
RaTG13	VEKGIYQTSNFRVQPTESIVRF	396	
2019-nCoV	NDLCFITNVYADS	467	
SARS-CoV	NDFCFITNVYADS	454	
RaTG13	NDLCFITNVYADS	476	
2019-nCoV	ISTEYIQAQSTPCNGVEGFCYFPLQSYGFPQTNVGVYQPYRNVVLSPELLHAIATVCGPKKSTNLVKNKCVNENFNGLT	547	
SARS-CoV	ISNVFSPDGKPCIP-PALNICYWPLNDYGYTTTIGYQPYRNVVLSPELLHAIATVCGPKLSTDLKKNQCVNENFNGLT	533	
RaTG13	ISTEYIQAQSTPCNGVEGFCYFPLQSYGFPQTNVGVYQPYRNVVLSPELLHAIATVCGPKKSTNLVKNKCVNENFNGLT	556	
2019-nCoV	GTGVLTESNKKFLPFQCFGRDITADTTDAVRDPQTLREILDITPCSPGGVSVITPFGTNTSNQVAVLYQDVNCTEVPVAIHAD	627	
SARS-CoV	GTGVLTPSSKRFQFPQCFGRDVSDFDTSVRDPKTSSEILDISPCEAGGVSVITPFGTNTSNQVAVLYQDVNCTEVPVAIHAD	613	
RaTG13	GTGVLTESNKKFLPFQCFGRDITADTTDAVRDPQTLREILDITPCSPGGVSVITPFGTNTSNQVAVLYQDVNCTEVPVAIHAD	636	
2019-nCoV	QLTPWRVYSTGSNVQFTRAGCLIGAEHVNNSEYECDIPIGAGICASYQTQNS	707	
SARS-CoV	QLTPAWRIYSTGNVQFTRAGCLIGAEHVNTSVECDIPIGAGICASYHTVSL---	689	
RaTG13	QLTPWRVYSTGSNVQFTRAGCLIGAEHVNNSEYECDIPIGAGICASYQTQNS---	712	
2019-nCoV	SNNSIAIPNFTISVTEILPVSMTKTSVDCDITMYICGDSSTECNSLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQ	787	
SARS-CoV	SNNTIAIPNFTISITTEVMPVSMARKTSVDCNMYICGDSSTECANLLQYGSFCTQLNRALSGIAEQDRNTRVFAQVKQ	769	
RaTG13	SNNSIAIPNFTISVTEILPVSMTKTSVDCDITMYICGDSSTECNSLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQ	792	
2019-nCoV	IYKTPPIKDFGGFNFSQILPDPSPKSKRS	867	
SARS-CoV	MYKTPTLKYFPGFNFSQILPDPPLKPKRS	849	
RaTG13	IYKTPPIKDFGGFNFSQILPDPSPKSKRS	872	
2019-nCoV	EMIAQYTSALLAGTITSGWTFGAGAALQIPFAMQAYRFNG	947	
SARS-CoV	DMIAAYTAALVSGTATAGWTFGAGAALQIPFAMQAYRFNG	929	
RaTG13	EMIAQYTSALLACTITSGWTFGAGAALQIPFAMQAYRFNG	952	
2019-nCoV	LQDVVNQNAQAINTLVKQLSSNFGAISSVNLNLSRLDKVEAEVQIDRLITGRQLSQTQYVYVQQLIRAAEIRASANLAAT	1027	
SARS-CoV	LQDVVNQNAQAINTLVKQLSSNFGAISSVNLNLSRLDKVEAEVQIDRLITGRQLSQTQYVYVQQLIRAAEIRASANLAAT	1009	
RaTG13	LQDVVNQNAQAINTLVKQLSSNFGAISSVNLNLSRLDKVEAEVQIDRLITGRQLSQTQYVYVQQLIRAAEIRASANLAAT	1032	
2019-nCoV	KMSECVLQSKRVDFCGKGYHLMSFPQAPHGTVFLHVTYVPAQEKNS	1107	
SARS-CoV	KMSECVLQSKRVDFCGKGYHLMSFPQAPHGTVFLHVTYVPAQERNNT	1089	
RaTG13	KMSECVLQSKRVDFCGKGYHLMSFPQAPHGTVFLHVTYVPAQEKNS	1112	
2019-nCoV	NFYEPQIITIDNTEVSGNCDVVIIVNNTVYDPLQPELDSFKEELDKEYFNHTSPDVLGDISGINASVNNIQKEIDRLN	1187	
SARS-CoV	NFESPQIITIDNTEVSGNCDVVIIVNNTVYDPLQPELDSFKEELDKEYFNHTSPDVLGDISGINASVNNIQKEIDRLN	1169	
RaTG13	NFYEPQIITIDNTEVSGNCDVVIIVNNTVYDPLQPELDSFKEELDKEYFNHTSPDVLGDISGINASVNNIQKEIDRLN	1192	
2019-nCoV	EVAKNLNESSLIDQLGKYEYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCCLKGCSCGSCCKFDEDDSEPVKLG	1267	
SARS-CoV	EVAKNLNESSLIDQLGKYEYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCCLKGCSCGSCCKFDEDDSEPVKLG	1249	
RaTG13	EVAKNLNESSLIDQLGKYEYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCCLKGCSCGSCCKFDEDDSEPVKLG	1272	
2019-nCoV	VKLHYT	1273	
SARS-CoV	VKLHYT	1255	
RaTG13	VKLHYT	1278	



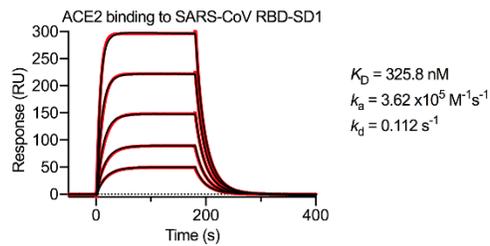
Supplementary Figure 5. Sequence alignment of 2019-nCoV S, SARS-CoV S and RaTG13 S.

Identical residues are denoted by an “*” beneath the consensus position. Structural domains are colored according to **Figure 1A**.

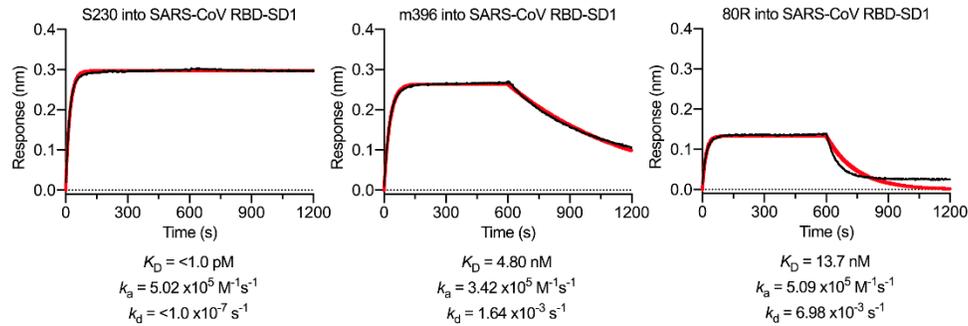


Supplementary Figure 6. Sequence variability between RaTG13 S and 2019-nCoV S clinical isolates. (A)

Table shows residues in the 2019-nCoV S protein that vary in RaTG13, grouped by structural domain. **(B)** A single monomer of the 2019-nCoV S protein is shown in ribbons, colored green. RaTG13 variant residues are shown as blue spheres. **(C)** Table shows variations in the 2019-nCoV S sequence based on 61 clinical isolates and the domains wherein these variations occur. **(D)** A single monomer of the 2019-nCoV S protein is shown in ribbons, colored green. Variant residues are shown as gold spheres.



Supplementary Figure 7. SARS-CoV RBD-SD1 binding to human ACE2. An SPR sensorgram is shown, displaying the binding between soluble human ACE2 and immobilized SARS-CoV RBD-SD1. The data are shown as black lines and the best fit of the data to a 1:1 binding model is shown in red.



Supplementary Figure 8. SARS-CoV RBD-directed antibody validation. The monoclonal antibodies that were tested for cross-reactivity to the 2019-nCoV RBD-SD1 were also tested for binding to the SARS-CoV S RBD-SD1 as a positive control. Binding data are shown as a black line and the best fit of the data to a 1:1 binding model is shown in red.

Supplementary Table 1. Cryo-EM data collection and refinement statistics.

EM data collection and reconstruction statistics

Protein	2019-nCoV S 1 RBD "up"	2019-nCoV S C3 symmetry
EMDB	EMD-21375	EMD-21374
Microscope	FEI Titan Krios	FEI Titan Krios
Voltage (kV)	300	300
Detector	Gatan K3	Gatan K3
Exposure (e ⁻ /Å ²)	36	36
Defocus range (μm)	0.8–2.8	0.8–2.8
Final particles	120,001	225,012
Symmetry imposed	n/a (C1)	C3
Resolution (Å)	3.46	3.17

Model refinement and validation statistics

PDB	6VSB
Composition	
Amino acids	2,905
Glycans	61
RMSD bonds (Å)	0.004
RMSD angles (°)	0.88
Ramachandran	
Favored (%)	94.6
Allowed (%)	5.2
Outliers (%)	0.2
Rotamer outliers (%)	0.64
Clash score	12.8
MolProbity score	1.99

Supplementary Movie 1. CryoSPARC 3D variability analysis. 2019-nCoV S trimer viewed from the side, along the viral membrane.

Supplementary Movie 2. CryoSPARC 3D variability analysis. 2019-nCoV S trimer viewed from the top, toward the viral membrane.