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3 **Structural investigation of ACE2 dependent**
4 **disassembly of the trimeric SARS-CoV-2 Spike**
5 **glycoprotein**
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22
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24
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26 post-fusion, virus entry, RBDs, receptor binding domains, soluble angiotensin-converting enzyme 2

27

28 Abstract

29

30 The human membrane protein Angiotensin-converting enzyme 2 (hACE2) acts as the main receptor for host
31 cells invasion of the new coronavirus SARS-CoV-2. The viral surface glycoprotein Spike binds to hACE2,
32 which triggers virus entry into cells. As of today, the role of hACE2 for virus fusion is not well understood.
33 Blocking the transition of Spike from its prefusion to post-fusion state might be a strategy to prevent or treat
34 COVID-19. Here we report a single particle cryo-electron microscopy analysis of SARS-CoV-2 trimeric Spike
35 in presence of the human ACE2 ectodomain. The binding of purified hACE2 ectodomain to Spike induces the
36 disassembly of the trimeric form of Spike and a structural rearrangement of its S1 domain to form a stable,
37 monomeric complex with hACE2. This observed hACE2 dependent dissociation of the Spike trimer suggests
38 a mechanism for the therapeutic role of recombinant soluble hACE2 for treatment of COVID-19.

39

40 Introduction

41 Coronavirus is a family of single-stranded RNA viruses, many of which can infect animals and humans
42 (MacLachlan and Dubovi, 2017; Monto, 1984). The symptoms of coronavirus-related diseases can be mild
43 and mainly occur in respiratory tract. For example, roughly 15%-30% cases of the common cold are caused
44 by human coronaviruses (Mesel-Lemoine *et al.*, 2012). A coronavirus infection sometimes can develop serious
45 illnesses, such as SARS (severe acute respiratory syndrome), MERS (Middle East respiratory syndrome) and
46 also the current pandemic COVID-19 (coronavirus disease 2019) (Tang *et al.*, 2020). SARS-CoV is a beta-
47 coronavirus that caused a pandemic in 2002. SARS-CoV-2 is a novel coronavirus that is genetically similar to
48 the previous SARS-CoV. SARS-CoV-2 causes the ongoing pandemic COVID-19 and has been spreading
49 globally since the first quarter of this year (Ciotti *et al.*, 2020). The symptoms of COVID-19 vary from person
50 to person. In some cases, the illness is very serious, in particular for the elderly (Pascarella *et al.*, 2020). As of
51 today, no specific antiviral drugs were approved for use against COVID-19 and vaccine development is still
52 at the phase of clinical testing.

53 Cryogenic electron microscopy (Cryo-EM) is a technique for structure determination of biomacromolecules,
54 which has been particularly successful for studying high molecular-weight proteins. Cryo-EM does not require
55 crystallization of the target protein. COVID-19 related protein structures have been widely investigated since
56 the spread of SARS-CoV-2 in this year, using cryo-EM single particle analysis (SPA). The protein nicknamed
57 Spike is with its 180kDa monomeric molecular weight the largest viral surface protein of SARS-CoV-2. It
58 consists of two domains S1 and S2 that are connected by a short linker. Spike forms stable trimers on the virus
59 surface that are attached to the virus membrane. This Spike trimer is the key molecule for host cells receptor
60 binding and invasion of the host cells. The cryo-EM structure of the entire Spike homotrimer was determined
61 recently, showing a mushroom shaped overall architecture (Walls *et al.*, 2020; Wrapp *et al.*, 2020a). As also
62 for the SARS-CoV, the viral fusion bridge from SARS-CoV-2 to the host cell is formed by Spike and the
63 ectodomain of the human Angiotensin-converting enzyme 2 (hACE2), which is the virus receptor on the host
64 cell that triggers virus entry. *In vitro* studies have shown that the Spike receptor binding domains (RBDs) from
65 SARS-CoV as well as SARS-CoV-2 can both bind to the ectodomain of hACE2 with comparable binding
66 affinities in low nanomolar levels (Lan *et al.*, 2020a). However, the new SARS-CoV-2 exhibits a more potent
67 capacity of host cells adhesion, as well as a larger virus-entry efficiency than other beta-coronaviruses (Shang
68 *et al.*, 2020).

69 The membrane-attached hACE2 is known to be the key molecule for the infection by several viruses, including
70 SARS-CoV, Human coronavirus NL63 (HCoV-NL63) and SARS-CoV-2. The infection process primarily
71 involves virus adhesion and fusion (Bao *et al.*, 2020; Fehr and Perlman, 2015; Kuba *et al.*, 2005; Sia *et al.*,
72 2020). Interestingly, hACE2 may not only serve as a drug target to prevent SARS-Co-2 infection, but hACE2
73 itself may also be considered as a potential therapeutic drug candidate for the usage against COVID-19 or
74 other beta-coronavirus related diseases. The clinical-grade soluble form of hACE2 has been reported to be a
75 potential novel therapeutic approach for reducing the infection of SARS-CoV-2 (Monteil *et al.*, 2020) by

76 preventing the viral Spike from interacting with other hACE2 present on human cells. Recently, researchers
77 have also characterized the entire architecture of the inactivated authentic virions from SARS-CoV-2 using
78 cryo-electron tomography, observing that post-fusion S2 trimers are distributed on the surface of SARS-CoV-
79 2 virions (Ke *et al.*, 2020; Turonova *et al.*, 2017). The exact role of hACE2 so far is not yet fully understood
80 in terms of its interaction with full-length Spike protein.

81 In this study, we present a cryo-electron microscopy (cryo-EM) study of the SARS-CoV-2 Spike protein in
82 complex with hACE2. Our analysis reveals a monomeric complex of Spike S1 domain with hACE2, requiring
83 a large structural rearrangement in S1 compared to its isolated structure. Our data show that hACE2 binding
84 induces a conformational change in Spike, leading to Spike trimer dissociation.

85 Results

86 Spike and hACE2 production and its complex assembly.

87 The prefusion Spike 2P ectodomain was expressed in ExpiCHO cells and affinity purified via its twin Strep-
88 tag. SDS-PAGE analysis showed the presence of pure full-length Spike protein, consisting of both, the S1 and
89 S2 domains at the expected molecular weight of 180 kDa for the Spike monomer. (**Suppl. Fig. S1a**). The
90 purified Spike sample in PBS buffer was imaged as negatively stained preparations by transmission electron
91 microscopy (TEM). This revealed the expected trimeric shape, and 2D class averages of selected particles in
92 negative stain TEM images showed the typical, mushroom-shaped particles (**Suppl. Fig. S2a,c**), in accordance
93 with the expected structure of the SARS-CoV-2 Spike in the pre-fusion state.

94 Human ACE2 ectodomain was expressed in HEK293 cells and purified via a poly-histidine immobilized metal
95 affinity chromatography (IMAC) with a Fastback Ni²⁺ column, followed by another anion exchange column
96 (**Suppl. Fig. S1b**). For details, see Methods.

97 Purified Spike protein was mixed with excess hACE2 (molar ratio Spike:hACE2 of 1:5) and incubated for 12
98 hours at 4° C. Samples were prepared by negative staining and imaged by TEM. Unexpectedly, the observed
99 particle features were largely different from the typical Spike trimers in shape. A 2D analysis of 5'854 picked
100 negatively stained particles revealed in class averages that the majority of the particles were smaller in size
101 and asymmetrical, compared to the non-incubated Spike trimeric samples (**Suppl. Fig. S2b,d**). This suggests
102 that the prolonged incubation with hACE2 led to Spike trimer dissociation. A similar observation for SARS-
103 CoV-ACE2 complex was recently reported by Song *et al* (Song *et al.*, 2018). Due to particle heterogeneity, we
104 decided to further purify the complex by size exclusion chromatography (SEC) and indeed the SEC profile
105 showed three distinct peaks, called Peak1, Peak2 and Peak3 (**Suppl. Fig. S1c,d**).

106 By analyzing the 3 peaks by SDS gel and negative stain EM, we could clearly differentiate non-structured
107 aggregates of full-length Spike and hACE2 in Peak1, that did not allow further structural analysis (data not
108 shown), to the excess of unbound hACE2 in Peak3 (**Suppl. Fig. S1c,d**). The homogenous Peak2 that contained
109 full-length Spike in complex with hACE2, was further analyzed by cryo-EM.

110 hACE2 binding can induce disassembly of Spike homotrimer

111 Peak2 (Spike:hACE2 at molar ratio 1:5 after overnight incubation at 4°C) was vitrified and frozen grids were
112 loaded into a Thermo Fisher Scientific (TFS) Titan Krios cryo-EM instrument, operated at 300kV acceleration
113 voltage, and equipped with a Gatan Quantum-LS energy filter equipped with K2 direct electron detector
114 (**Suppl. Fig. S3**). 8'927 dose-fractionated images (i.e., movies) were recorded (**Suppl. Fig. S4**), from which
115 ~1.7 million particles were extracted and subjected to image processing and 3D reconstruction. The final 3D
116 reconstruction from 72'446 particles at 5.1Å overall resolution showed a density map corresponding to a single,
117 monomeric Spike protein in complex with hACE2 (**Fig. 1a**). The map allowed docking with available
118 structures for S1 and hACE2 taken from the previously reported structures (Spike PDB ID 6VYB and Spike
119 RBD-ACE2 6M0J), revealing a structural rearrangement of the C-terminal domain (CTD) and N-terminal
120 domain (NTD) of S1 compared to a monomer from that Spike structure in the RBD^{up} conformation. The
121 interaction between the S1 RBD and hACE2 is in agreement with several other reported structures of the RBD-
122 ACE2 complex (PDB ID 6M0J, 6VW1 or 6LZG). No additional density for S2 or a fragment of S2 was
123 detected in the reconstruction.

124 We tested a shorter incubation time by mixing Spike:hACE2 (molar ratio of 1:3) and let it incubate for 3 hours
125 at 4°C, instead as overnight. No further SEC purification was performed. Subsequently, cryo-EM grids of this
126 sample were prepared and subjected to cryo-EM analysis (**Suppl. Fig. S5**). From 7'045 recorded movies,
127 615'348 particles were extracted and subjected to classification and 3D analysis. This revealed a small sub-set
128 of 47'901 particles corresponding to the prefusion Spike trimer, which allowed a 3D reconstruction at 4.2Å
129 overall resolution (no symmetry was applied), while some regions of the 3D map showed lower resolution,
130 presumably due to increased flexibility of these areas (**Suppl. Fig. S6**). A resolution-limited map at 9Å
131 resolution (**Fig. 1b**) allowed clear docking the models of Spike S1 and S2 and hACE2, which showed that the
132 complex is composed of Spike and hACE2 in a molar ratio of 3:3 (Spike:hACE2). Three hACE2 molecules
133 were observed to attach to the RBDs of Spike. All three RBDs were in the RBD^{up} conformation and slightly
134 shifted away from the central trimer axis (**Fig. 1b**). A similar arrangement was also recently observed by
135 Kirchdoerfer *et al.* (Kirchdoerfer *et al.*, 2018), see also (Zhou *et al.*, 2020).

136 Structural comparison of Spike-hACE2 complexes

137 A detailed analysis of both obtained structures shows significant structural rearrangements in different forms
138 of Spike-hACE2 complex. The resolution of our reconstructed EM maps was not sufficient for building atomic
139 models, but allowed clear placement of available atomic structures for Spike (PDB ID: 6VYB) and ACE2-
140 RBD (PDB ID: 6M0J) (Lan *et al.*, 2020b; Walls *et al.*, 2020). Comparison of the docked and fitted monomeric
141 S1-hACE2 model to that of the trimeric Spike-hACE2 (3:3) showed that a ~30° rotation of the C-terminal and
142 N-terminal sub-domains of Spike S1 were required to bring the Spike S1 protein into the monomeric
143 arrangement with hACE2. After such re-arrangement, the domains of hACE2 and the RBDs of the S1 protein
144 are in good agreement with a reported crystal structure (REF Lan *et al.*) (**Fig. 2b**).

145 When comparing the docked model of the monomeric S1-hACE2 complex with that of the trimeric Spike-
146 hACE2 complex (**Fig. 2e**), a considerable number of steric clashes at the interface between Spike S1 (CTD)
147 and its neighboring region from the S2 polypeptide chain was obvious. The docked monomeric S1-hACE2
148 complex is further structurally incompatible with the observed trimeric arrangement.

149 Discussion

150 The stoichiometric ratio of the complex of Spike:hACE2 on the host cell upon virus entry is not well
151 established. Nevertheless, one hACE2 molecule per Spike trimer is likely sufficient for binding and initializing
152 virus fusion with the host cell (Song *et al.*, 2018). Even though the structure of a post-fusion S2 trimer has
153 recently been determined (Cai *et al.*, 2020), it is not clear how membrane fusion during virus entry is
154 coordinated upon release of the S1-hACE2 caps (**Fig. 3**). We here report the cryo-EM structure of a stable
155 monomeric S1 Spike-hACE2 (1:1) complex. Even though size-wise it would have been detectable, our particle
156 classification did not reveal any particle class corresponding to an isolated S1 fragment in addition to the
157 observed S1 Spike-hACE2 (1:1) particles (**Suppl. Fig. S3c, S4**). Knowledge of the mechanism how the S1
158 fragment might be detached from the hACE2 receptors after virus entry would be relevant for understanding
159 its mechanism of infection and pathogenicity. The fact that we did not observe any free S1 fragments suggests
160 that the S1-hACE2 complex is rather stable, at least under our *in vitro* conditions.

161 Secondly, the S2 domain was not detected in the obtained structure of the Spike-hACE2 monomeric complex
162 (1:1), even though the SDS-PAGE analysis showed that S2 was present as full length in the sample (**Suppl.**
163 **Fig. S1d**). The S2 domain is expected to be connected to the S1 domain via a short loop between S1 and S2,
164 where a Furin protease cleavage site is expected (Belouzard *et al.*, 2009; Haan *et al.*, 2004; Hoffmann *et al.*,
165 2020). However, in the absence of stable trimers, the loop between S1 and S2 is likely very flexible, possibly
166 making the S2 domain undetectable by cryo-EM maps. Our cryo-EM analysis that didn't show the S2 domain
167 in the averaged 3D reconstruction therefore likely failed to align the S2 domains either due to their flexibility,
168 or due to a denaturation of S2 during sample preparation.

169 An early study presented a potential dose-dependent inhibition of SARS-CoV-2 infection by a recombinant
170 soluble form of hACE2 (Monteil *et al.*, 2020). The mechanism, how the soluble forms of hACE2 would be
171 able to neutralize the virus, is not known. One possible mechanism could be a direct competition between the
172 soluble hACE2 and the host cell hACE2 receptor, so that Spike proteins saturated with soluble hACE2 domains
173 render them unable to interact with host cell hACE2. Here, however, we report that the soluble forms of hACE2
174 induce the opening and disassembly of the trimeric Spike structure to create the stable Spike S1-hACE2
175 complex (**Fig. 3**). We propose a mechanism by which the formation of the Spike-hACE2 (3:3) complex induces
176 a high structural flexibility in the Spike trimer, allowing a conformational re-arrangement of the S1 C- and N-
177 terminal domains when interacting with hACE2. In consequence, the new S1-hACE2 complex is incompatible
178 with a trimeric arrangement, causing the dissociation of the trimeric complex (**Fig. 3**).

179 This hypothesis is supported by the recent manuscript deposited in bioRxiv.org, which describes a similar
180 effect triggered by engineered DARPIn molecules (Walser *et al.*, 2020). Therefore, we suppose that the soluble
181 forms of hACE2 may not only block the infection and replication of SARS-CoV-2, but also destroy the trimeric
182 Spike adaptors that are responsible for viral host membrane fusion. This mechanism suggests a novel
183 therapeutic strategy for the treatment of COVID-19, by adding soluble hACE2 to dissociate the Spike trimer
184 of approaching viruses.

185 **Methods**

186 **Protein production and purification**

187 Spike protein: The gene for the prefusion ectodomain of the SARS-CoV2 Spike 2-P protein (the prefusion
188 stabilized construct (2P) that includes the putative furin cleavage site mutated; the plasmid was a generous gift
189 from Prof. Jason McLellan, University of Texas, Austin (Wrapp *et al.*, 2020b)) was transiently transfected into
190 suspension-adapted ExpiCHO cells (Thermo Fisher) with PEI MAX (Polysciences) in ProCHO5 medium
191 (Lonza). After 1 h, dimethyl sulfoxide (DMSO; AppliChem) was added to 2% (v/v). Incubation with agitation
192 was performed at 31°C and 4.5% CO₂ for 5 days. The transparent supernatant was passed over a Strep-Tactin
193 column (IBA Lifesciences) and bound protein was eluted with PBS buffer + 2.5 mM desthiobiotin. The elute
194 was dialyzed against PBS buffer. The average protein yield for Spike 2P was 15 mg/L culture.

195 hACE2 protein: The gene for the secreted expression of hACE2-Fc-His was a generous gift from Prof. Jason
196 McLellan. The construct was transiently transfected into HEK293 cells (Thermo Fisher) with PEI MAX
197 (Polysciences) in EX-CELL 293 serum-free medium (Sigma), supplemented with 3.75 mM valproic acid.
198 Incubation with agitation was performed at 37°C and 4.5% CO₂ for 8 days. The clear supernatant was purified
199 as follows via MabSelect Protein A resin (Cytiva), cleavage of the Fc-His tag by overnight treatment with 3C
200 protease, removal of the cleaved tag and 3C protease by NiNTA followed by an anion exchange column. The
201 purified protein was finally dialyzed into PBS buffer. The average yield for cleaved hACE2 was 13 mg/L
202 culture.

203 **Negative stain TEM imaging**

204 For all protein samples, 3ul of sample solution was applied to glow-discharged carbon-coated Cu-grids and
205 incubated for 1min. The free liquid was removed by blotting with filter paper and the grids were stained for
206 25s with 2% uranyl acetate solution. The negatively stained preparations were imaged with a Tecnai G2 Spirit
207 TEM, operated at 120kV at a magnification of 135kx. Image were recorded with a Veleta CCD camera (EMSIS
208 GmbH, Münster, Germany). The 2D analysis of the negative stain TEM images was performed with the
209 program cisTEM1.0 (Grant *et al.*, 2018).

210 **Cryo-EM imaging**

211 Two different preparations of cryo-EM grids were performed. Purified Spike and hACE2 proteins were mixed
212 at the molar ratio of 1:5 and incubated for 12 hours at 4°C. After that, the sample was subjected to size exclusion

213 chromatography (SEC) with a Superose 6 increase (10/300) column, and the fractions from Peak2 were pooled
214 and concentrated in 100 kDa centrifugal concentrators (Millipore).

215 Alternatively, purified Spike and hACE2 were mixed at the molar ratio of 1:3 (Spike:hACE2) and incubated
216 for 3 hours at 4°C, without further purification via SEC.

217 For both samples, the concentration was adjusted to 0.5mg/ml. Cryo-EM grids were prepared with a Vitrobot
218 Mark IV (Thermo Fisher), using a temperature of 4°C and 100% humidity. 4 µL of sample was applied onto
219 glow-discharged Quantifoil holey carbon grids (R2.0/2.0, 200 mesh, copper) and blotted for 2.0-3.0s. The grids
220 flash-frozen in a liquid ethane, cooled by liquid nitrogen.

221 For both samples, dose-fractionated images (i.e., movies) were recorded with a Titan Krios (Thermo Fisher),
222 operated at 300kV, and equipped with a Gatan Quantum-LS energy filter (20 eV zero-loss energy filtration)
223 followed by a Gatan K2 Summit direct electron detector. The data collection statistics is presented in **Table 1**.
224 Images were recorded in counting mode, at a magnification yielding a physical pixel size of 0.82Å at the
225 sample level. Images were automatically recorded with the SerialEM program (Mastronarde, 2003) at a
226 defocus range of -0.8 ~ -2.5µm. All the movies were gain-normalized, aligned, dose weighted and averaged
227 with the program of MotionCor2 (Zheng *et al.*, 2017) within FOCUS (Biyani *et al.*, 2017), which also was
228 used to sort images and reject images of insufficient quality. The pre-processed micrographs were imported
229 into cryoSPARC V2 (Punjani *et al.*, 2017).

230 Image processing

231 Data processing of the monomeric Spike-hACE2 complex was conducted in CryoSPARC V2 (Punjani *et al.*,
232 2017). The defocus and contrast transfer function (CTF) values were estimated on 8'927 micrographs and
233 7'927 good images were selected. 1'685'202 particles were auto-picked by blob picking, followed later by
234 another round of template picking. Particle clearing was performed by two rounds of 2D classifications,
235 resulting in a particles stack of 435'410 particles. 3D references were generated using ab-initio reconstruction
236 (CryoSPARC V2) and followed by two rounds of 3D hetero-refinements. The final particle set contained
237 72'446 particles, leading to a 3D map at 5.1Å overall resolution, as estimated by Fourier Shell Correlation
238 (FSC) using the 0.143 cutoff criterion.

239 For the trimeric Spike-hACE2 complex, image processing was performed similarly. The final reconstruction
240 produced a 3D map at 4.2Å overall resolution.

241 Model interpretation

242 Protein models were generated from reported structures (Spike: PDB ID 6VYB; ACE2-RBD: PDB ID 6M0J)
243 (Lan *et al.*, 2020b; Walls *et al.*, 2020). For the S1-hACE2 structure, the model was manually docked into the
244 EM density with the program Chimera (Pettersen *et al.*, 2004) and further refined using rigid-body fitting in
245 COOT (Emsley *et al.*, 2010). For the Spike-hACE2 trimer, the density corresponding to hACE2 was relatively

246 weak, so that low-pass filtration to 9Å resolution was applied to the map before proceeding with the docking
247 of hACE2 as described above.

248 **Figure preparation.**

249 Figures were created using the software PyMOL (PyMOL Molecular Graphics System, DeLano Scientific),
250 Chimera and ChimeraX (Goddard et al., 2018).

251 **Data availability**

252 The map of the SARS-CoV2 Spike-hACE2 trimer was deposited in the Electron Microscopy Data Bank under
253 EMDB ID 11832 (Spike-ACE2 monomeric complex) and the map of the S1-hACE1 complex under EMDB
254 ID 11833.

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260 **Author contributions**

261 D.N. did imaging and image processing and data analysis; D.H, K.L. and F.P. produced and purified the protein
262 samples of hACE2 and Spike; F.L. assembled the complex; A.F. performed negative stain TEM. H.S.
263 supervised the project. All the authors edited the manuscript.

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272 **Competing financial interests**

273 The authors declare no competing financial interests.

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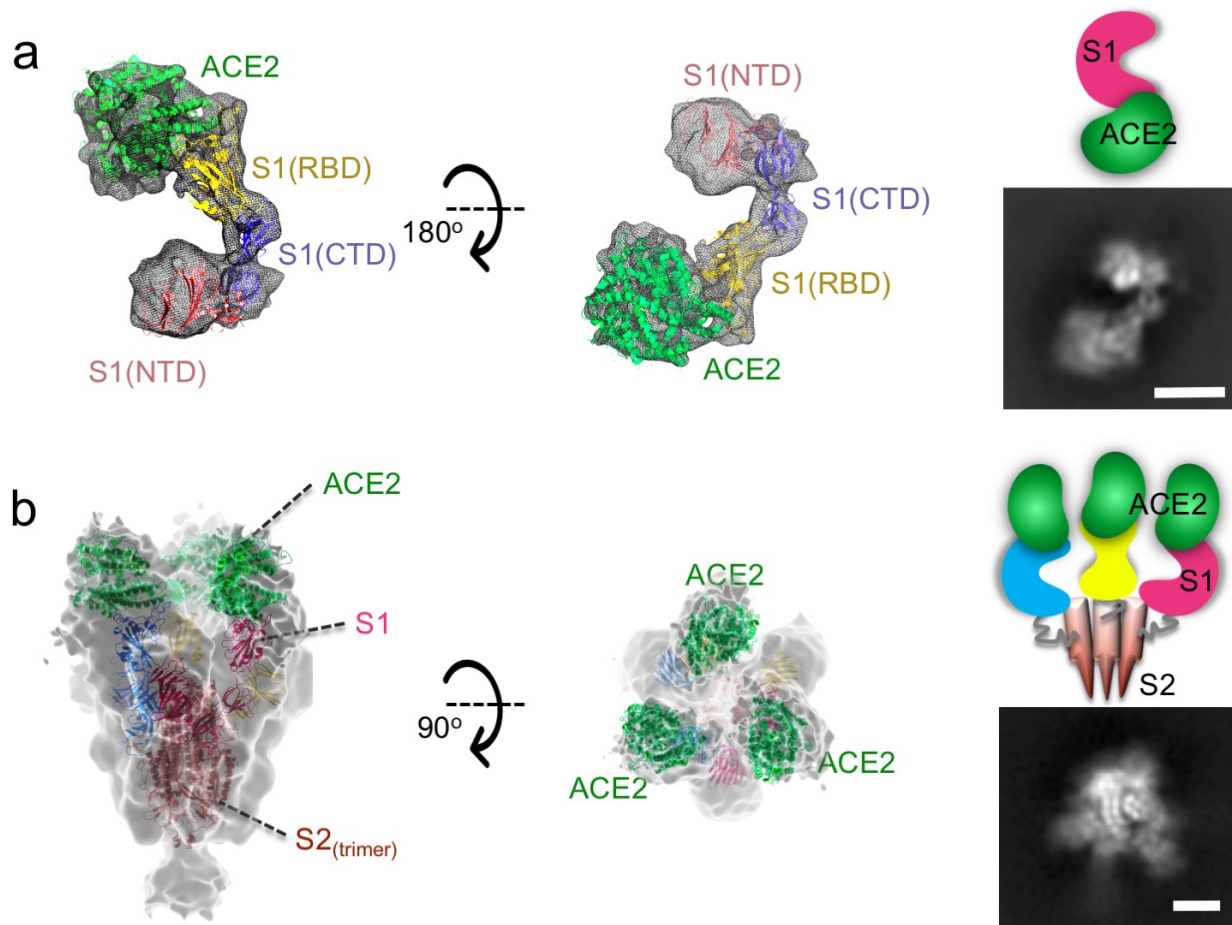
Table 1: EM data collection statistics

	Spike S1-hACE2 monomeric complex	Spike-hACE2 trimeric complex
Cryo-electron microscopy data collection and processing		
Microscope	FEI Titan Krios	FEI Titan Krios
Voltage (keV)	300	300
Camera	Gatan K2-Summit	Gatan K2-Summit
Electron exposure (e-/Å ² /frame)	1.5	1.0
Energy filter slit width (eV)	20 (Gatan Quantum-LS)	20 (Gatan Quantum-LS)
Pixel size (Å)	0.82	0.82
Defocus range (µm)	(-0.8) - (-2.5)	(-0.8) - (-2.5)
Magnification (nominal)	60'975x (165kx)	60'975x (165kx)
Number of frames per movie	40	40
Number of good micrographs	8'927	7'045
Initial particles	435'410	201'374
Final particles	72'446	47'901
Symmetry imposed	C1	C1
Map resolution (Å)	5.1	4.2
FSC threshold	0.143	0.143
Map local resolution range (Å)	20 -4.80	20 -3.60
EMDB ID	EMD-11832	EMD-11833

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Figure 1 Cryo-EM maps of SARS-CoV-2 Spike-hACE2 complexes and fitted models.

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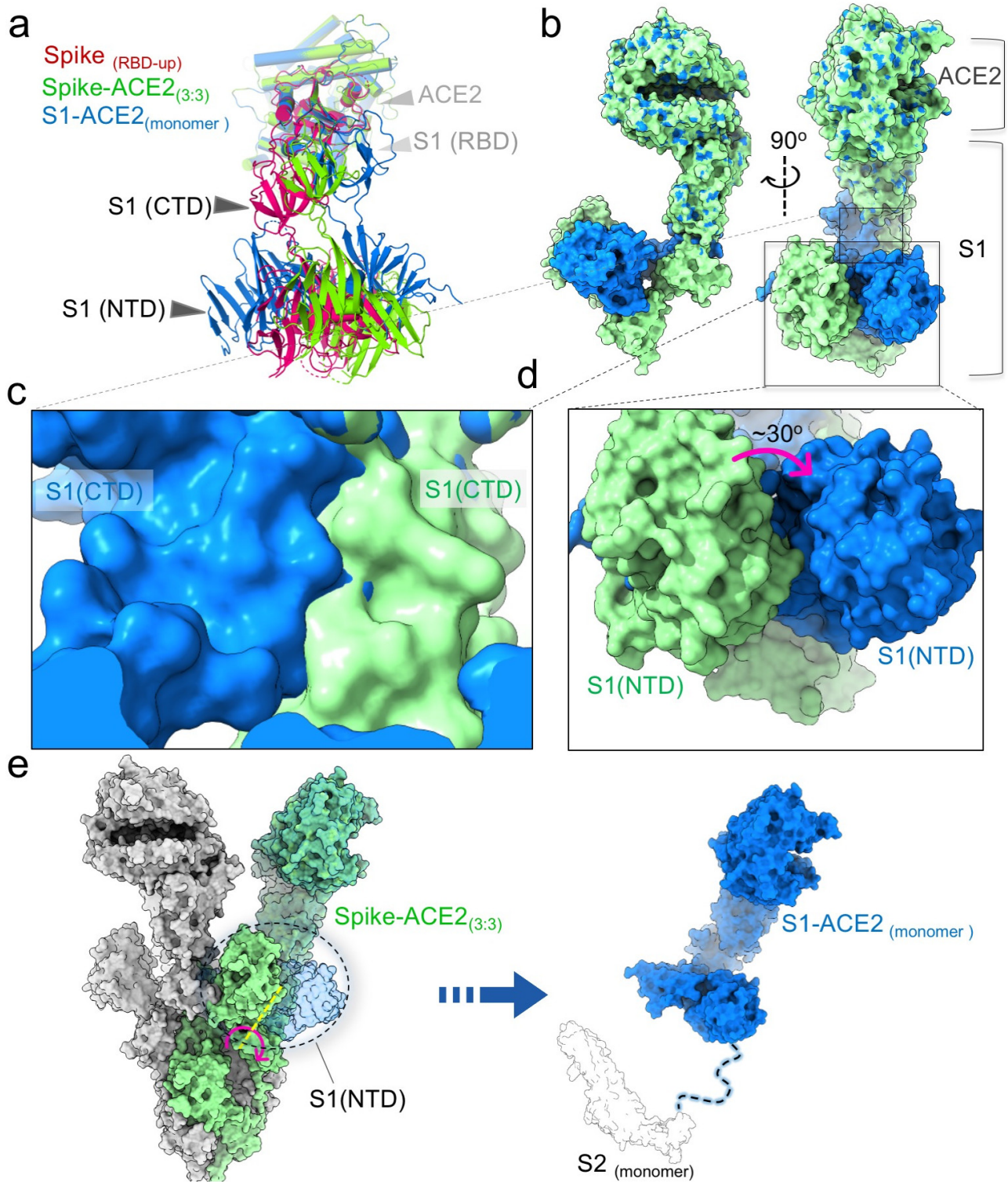
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a. The 3D reconstruction of SARS-CoV-2 Spike and human ACE2 (mixed at a molar ratio 1:5) incubated for 12hrs and further purified by SEC shows a structure corresponding to monomeric Spike S1 protein in complex with hACE2. No density for S2 is observed. The N- and C-terminal domains of S1 had to be re-arranged to fit into the map. Left: Side-view, center: 90° rotated view. The structure is colored as follows: hACE2 ectodomain green, Spike S1-RBD yellow, Spike S1-CTD slate blue, Spike S1-NTD salmon. Right: The bottom image shows a representative 2D class average of the S1-hACE2 complex. The upper cartoon is its interpretation. **b.** 3D reconstruction of Spike and hACE2 (ratio 1:3) incubated for 3 hours shows a trimeric map allowing docking of three hACE2 molecules and three S1 and three S2 molecules, all forming a trimeric complex. The map is low-pass filtered to 9Å resolution for better interpretation. The complex is arranged in a molar ratio of 3:3 (SpikeS1-S2:hACE2). The image on the left indicates the host cell membrane as a cartoon. Center: 90° rotated view. Right: The bottom image shows a representative 2D class average of Spike-hACE2 (3:3) complex, here shown from the top as in the central panel. The upper cartoon is its interpretation (here shown from the side). Scale bar in class averages: 2 nm.

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Figure 2 Conformational rearrangement of Spike-hACE2.

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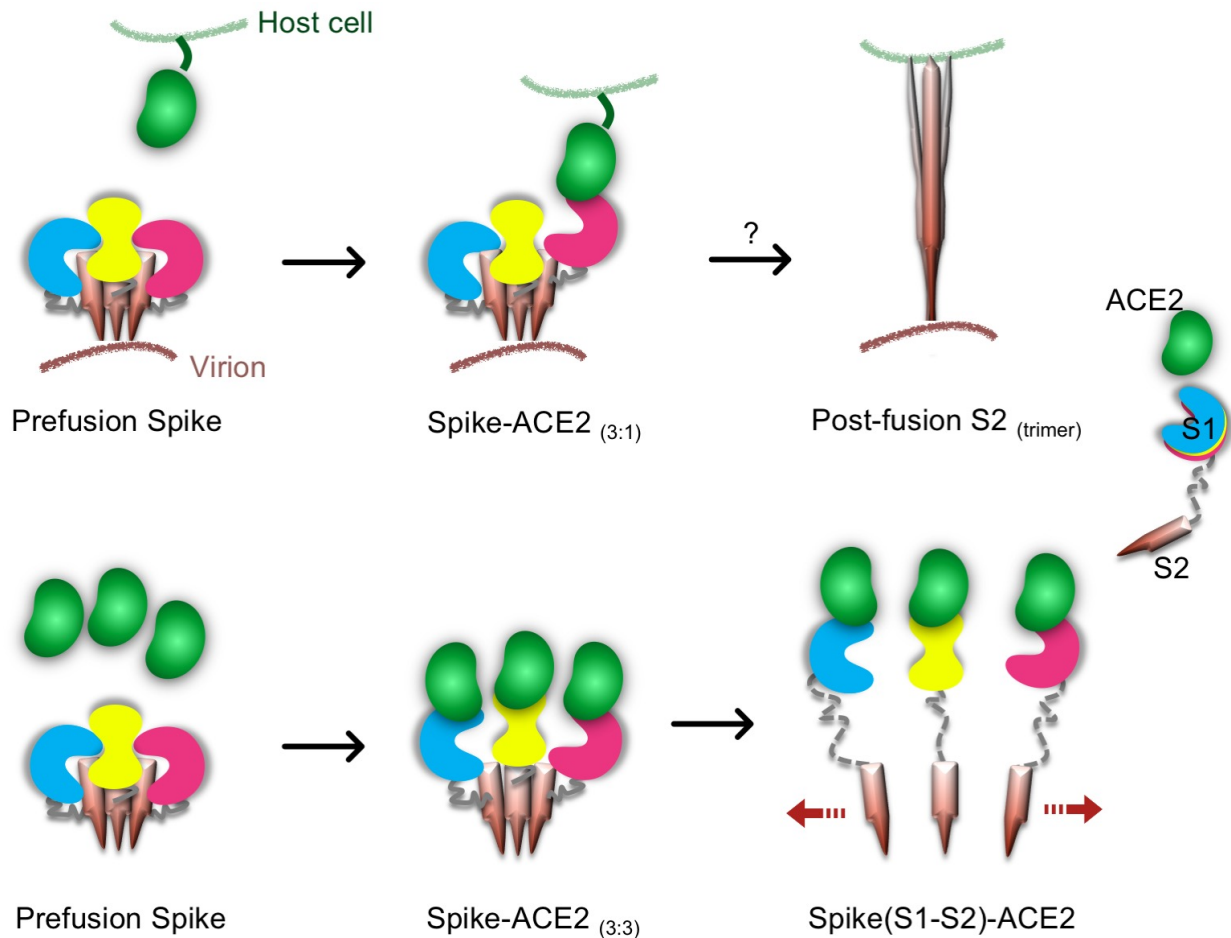
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a. Superposition of S1-hACE2 complex structures derived from different conformations. Structures are shown as a cartoon and colored as follows: monomeric Spike-hACE2 complex, blue; trimeric form of Spike-hACE2 complex, green; Spike one RBD-up (PDB ID: 6VYB), red. The RBDs have been superimposed. **b.** Structural comparison of S1-hACE2 regions. The movement is presented as two colors: SARS-CoV2 trimeric Spike-hACE2 complex (green) and S1-hACE2 (blue) **c,d.** The close-up

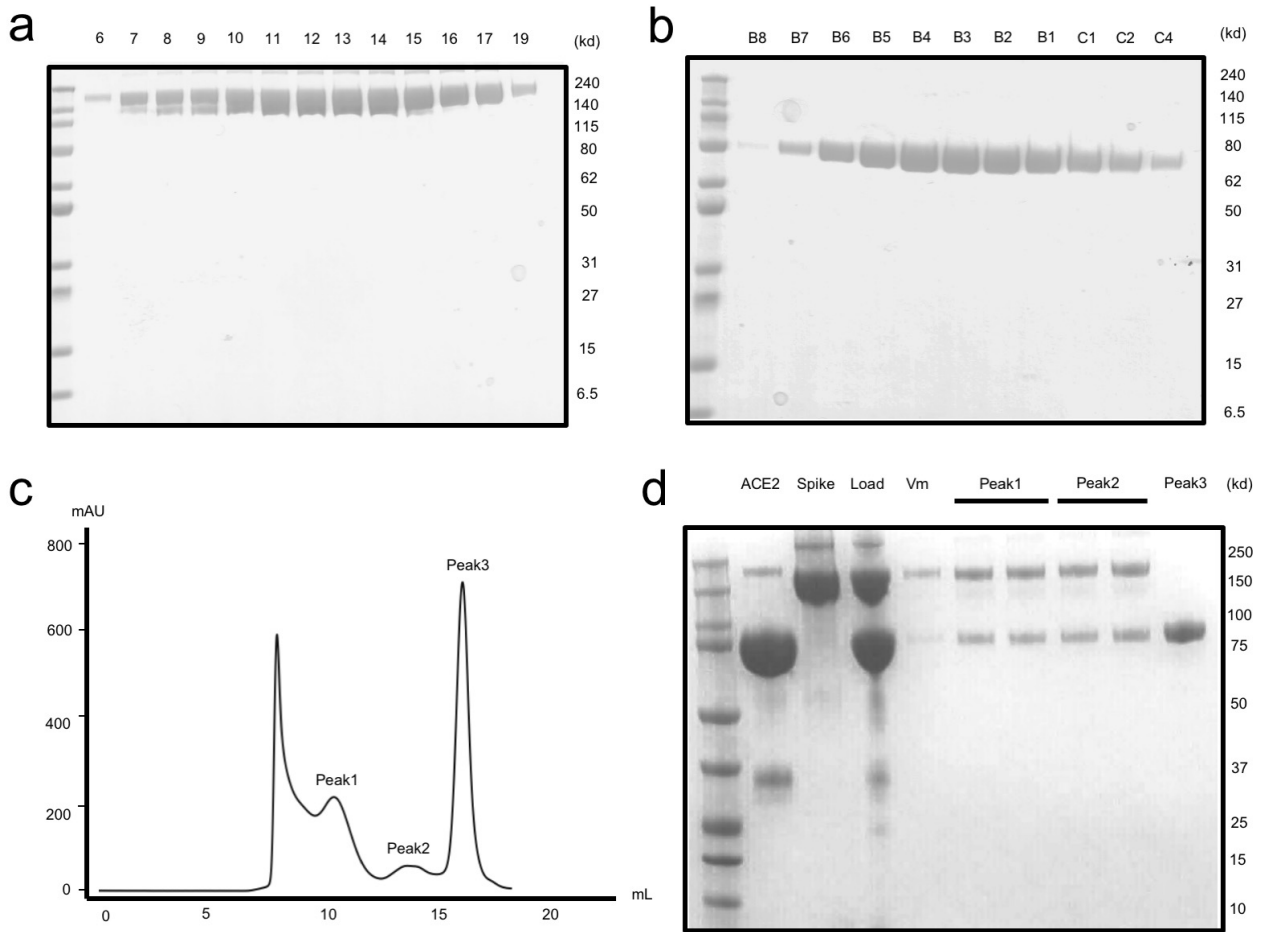
420 views show the proposed local structural rearrangements. **e.** Superposition of S1-hACE2(1:1) with
421 the trimeric form of Spike-hACE2 complex. The RBDs have been superimposed. The rearranged
422 structure of Spike-hACE2 is no longer compatible with formation of trimers so that it dissociates.
423 The right panel is a predicted structure of the dissociated Spike-hACE2 monomer.



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426 **Figure 3 Proposed models for Spike-hACE2 complex formation and structural rearrangement.** The
427 upper row shows a possible pathway leading to a conformational change of the trimeric SARS-CoV2
428 Spike. In this model, one hACE2 molecule binds to one Spike S1 monomer and induces the
429 conformational changes in the trimeric Spike. Subsequently, a post-fusion S2 trimer is formed. The
430 lower row shows a novel proposed pathway leading to Spike trimer disassembly by hACE2. In
431 presence of a high concentration of hACE2 molecules, a Spike-hACE2 (3:3) complex is formed.
432 Structural clashes between the three Spike-hACE2 elements lead to their dissociation. This induces
433 the formation of monomeric Spike-hACE2 complexes.

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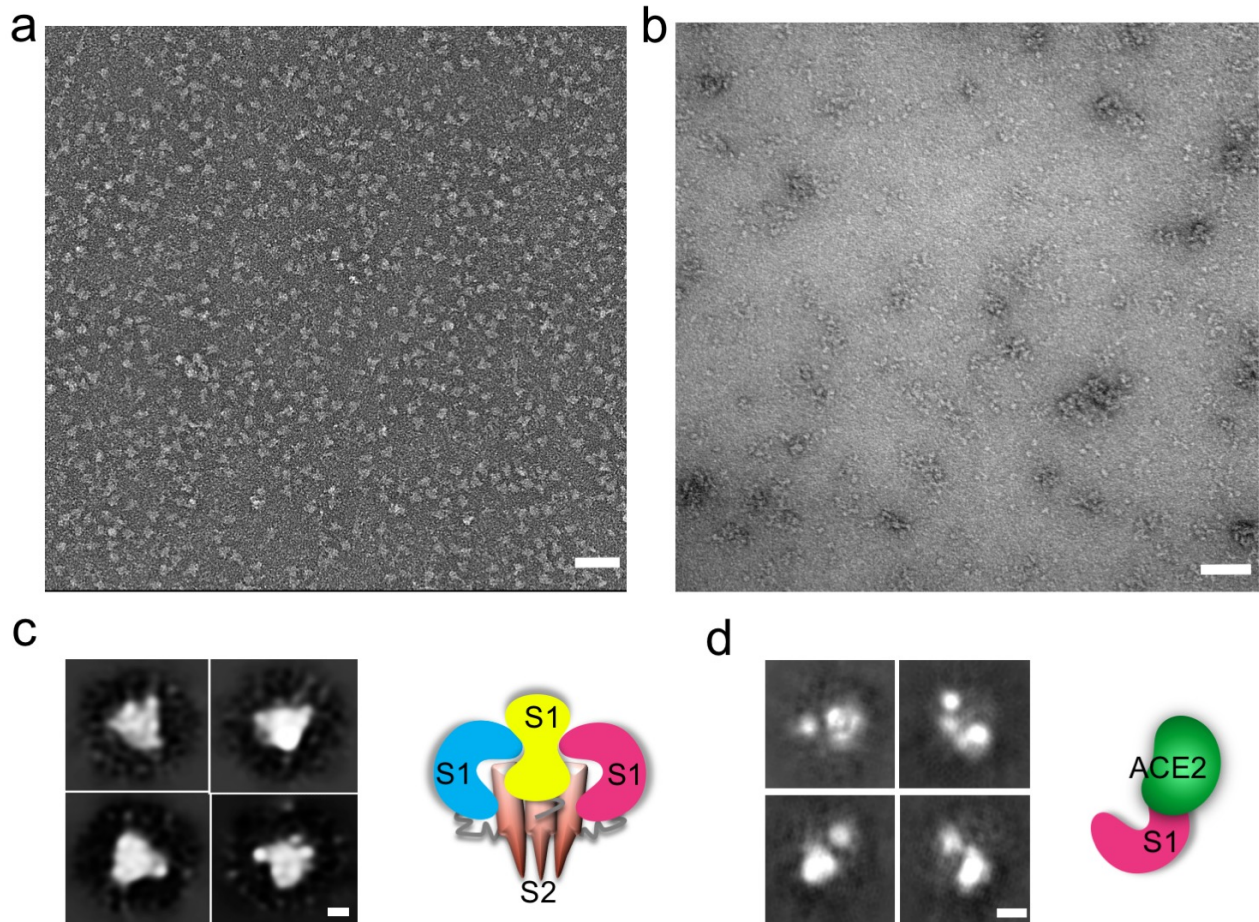


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436 **Figure S1 Protein production and purification.**

437 **a.** SDS-PAGE of the size exclusion chromatography (SEC) fractions of SARS-CoV-2 Spike (Superose 6
438 Increase 10/300GL). **b.** SDS-PAGE of the anion exchange chromatography fractions from the purified
439 sample of hACE2 ectodomain (HiTrap Q Sepharose HP). **c.** A representative size exclusion
440 chromatography curve of Spike-hACE2 complex assembly. 280nm UV absorption is shown. **d.** The
441 representative SDS-PAGE of the complex assembly. Lanes hACE2 to Load were after pre-
442 concentration in 100 kDa centrifugal concentrators (Millipore).

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Figure S2 Dissociation of SARS-CoV2 Spike trimer.

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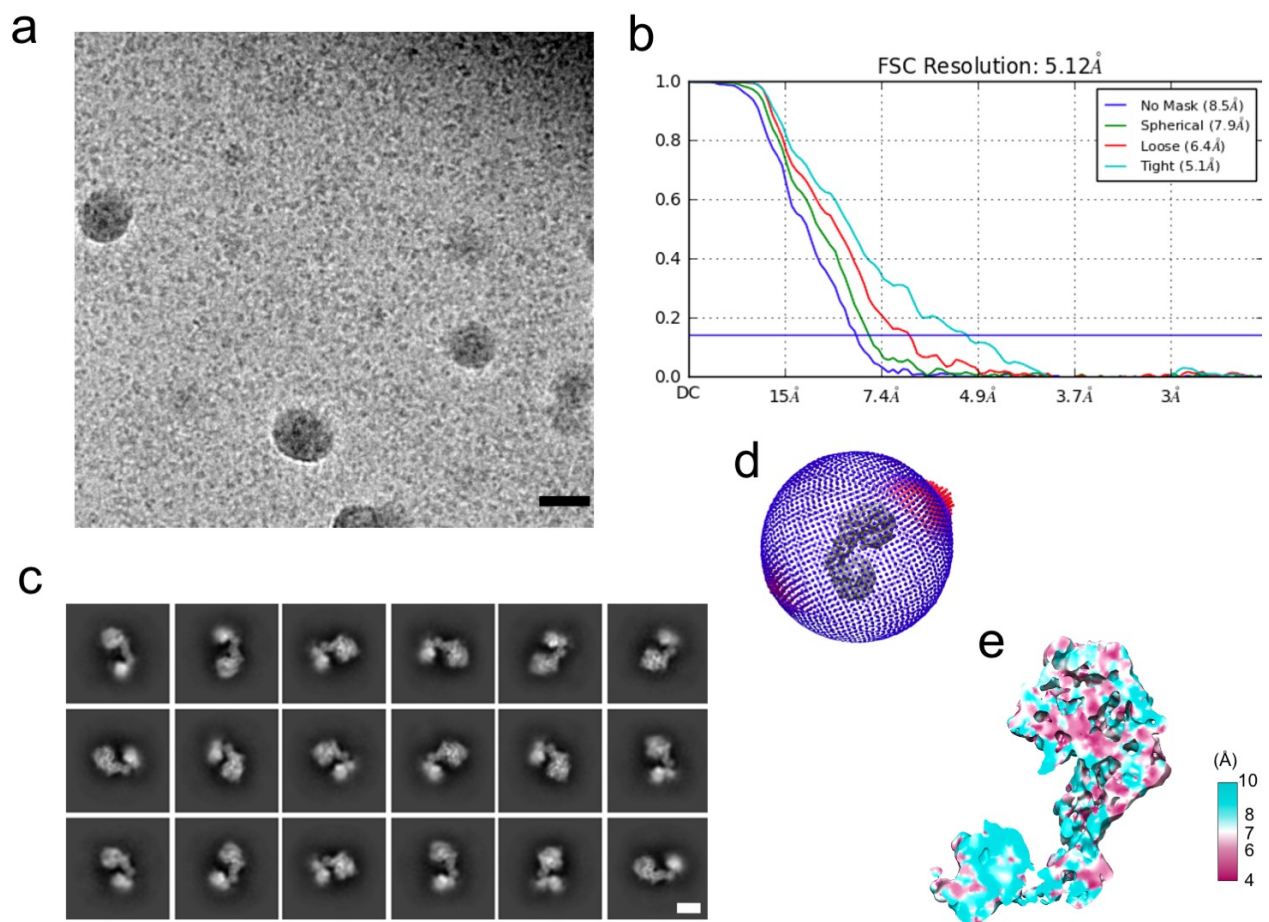
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a. Negative stain TEM image of the Spike sample alone (non-incubated sample). **b.** Negative stain TEM image of Spike protein incubated with soluble human ACE2 at a molar ratio of 1:5 for 12 hrs at 4°C, and further purified by SEC. **c.** Left: Representative class averages from particles picked from images as in a. Right: A cartoon interpretation of the structure. **d.** Left: Representative class averages from particles picked from images as in b. Right: A cartoon interpretation of the structure. Scale bars in a and b are 50nm, in c and d are 3 nm.

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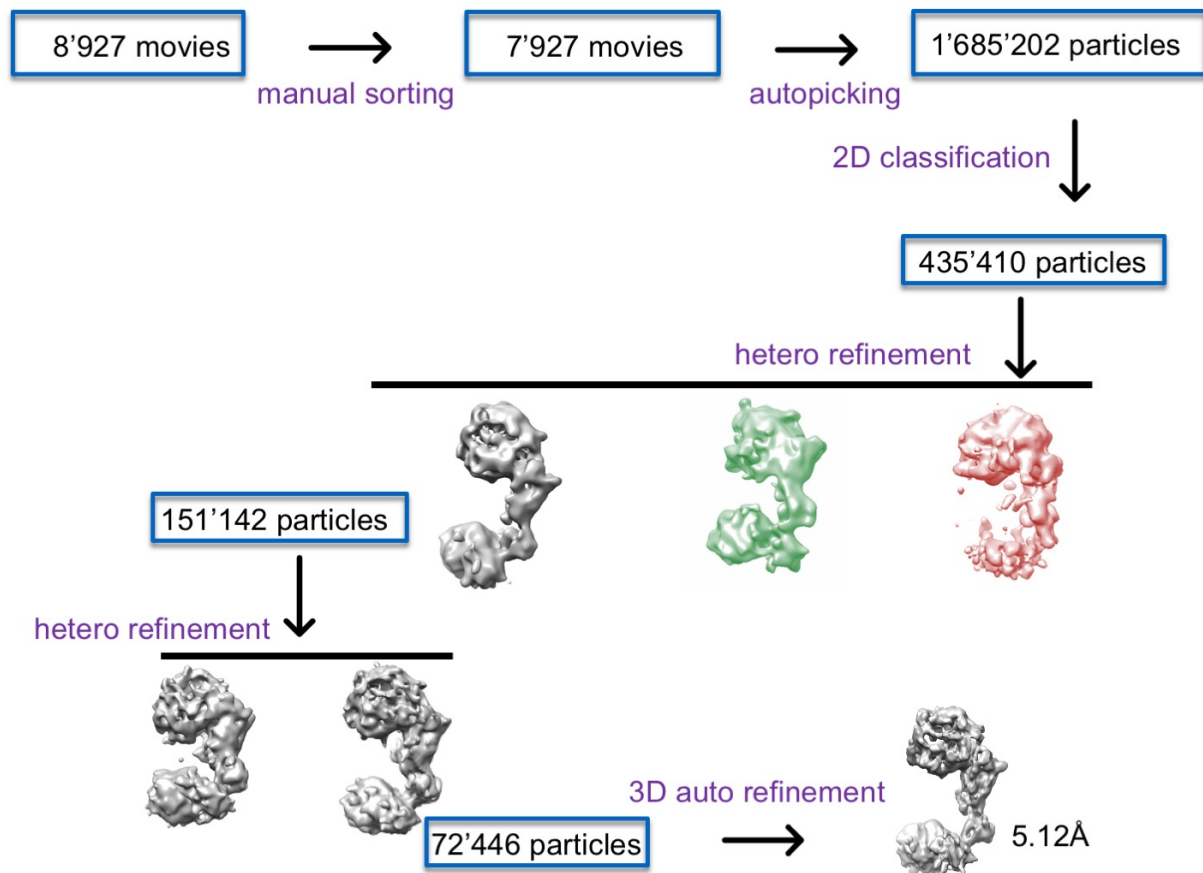
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457 **Figure S3 Data quality of Spike-ACE2 monomeric complex (S1-ACE2) sample.**

458 **a.** A representative micrograph. **b.** Overall Resolution estimation (FSC, 0.143). **c.** Representative 2D
459 average classes. **d.** Direction distributions. **e.** Local Resolution estimation (MonoRes). Scale bar in **a**
460 is 50nm and in **c** is 3nm.

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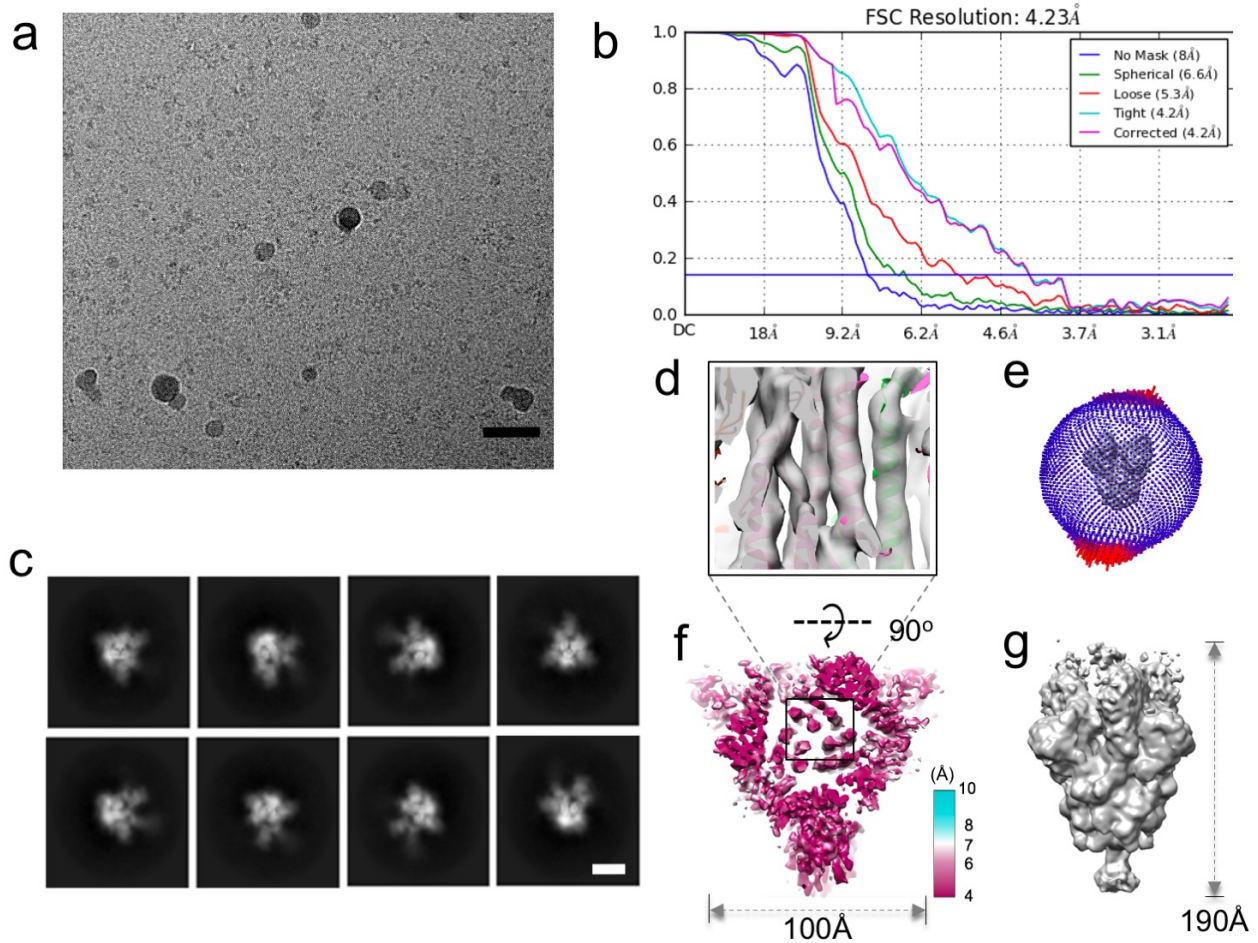
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Figure S4 Processing workflow for the Spike-hACE2 monomeric complex (S1-hACE2).

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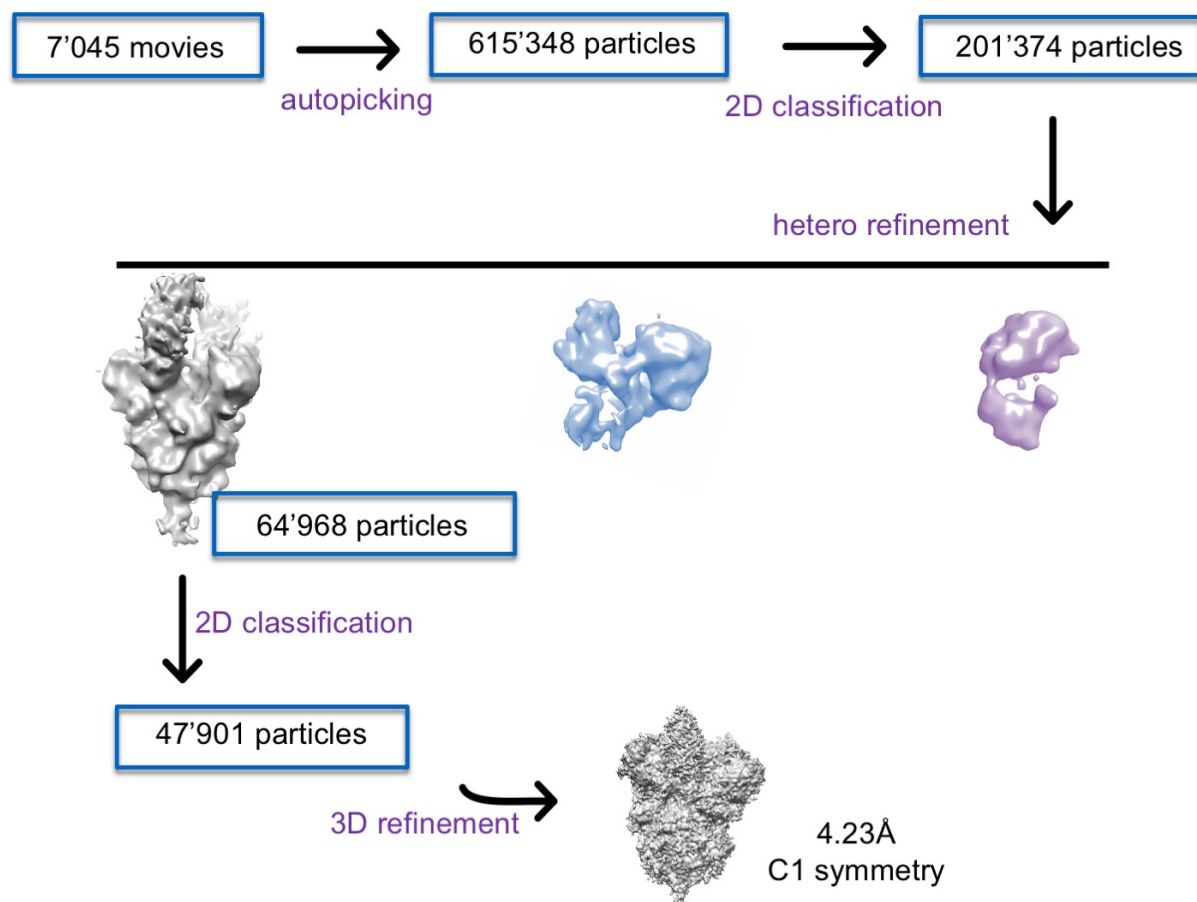
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Figure S5 Data quality of the sample of Spike-hACE2 trimeric complex.

a. A representative micrograph. b. Overall Resolution estimation (FSC, 0.143). c. Representative 2D average classes. d. Model fitted into the S2 trimeric core (The map was low-pass filtered). e. Distribution of particle orientations. f. Local resolution level at the best resolved regions of the trimeric form of Spike-hACE2 complex (bottom view, MonoRes). g. The low-pass filtered EM map at 9 Å resolution (For model generation). Scale bar in a is 50nm and in c is 3nm.

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Figure S6 Processing workflows for the trimeric Spike-hACE2 complex.