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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  
23 **Running title:** Cryo-EM of SARS-CoV-2 S1 and hACE2

24  
25 **Keywords:** SARS-CoV-2, COVID-19, Spike, S1/S2, cleavage site, cryo-EM, single particle, pre-fusion,  
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 Ni<sup>2+</sup> 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<sup>up</sup> 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<sup>up</sup> 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% CO<sub>2</sub> 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% CO<sub>2</sub> 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 (Mastrorade, 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.

## 255 **Acknowledgements**

256 We thank Prof. Jason McLellan from University of Texas, Austin for the Plasmids. We thank Lubomir Kovacik  
257 and Mohamed Chami for assistance in cryo-electron microscopy. This work was supported by the Swiss  
258 National Science Foundation, grant NCCR TransCure. We thank Laurence Durrer and Soraya Quinche from  
259 PTPSP, EPFL for work with mammalian cell cultures.

## 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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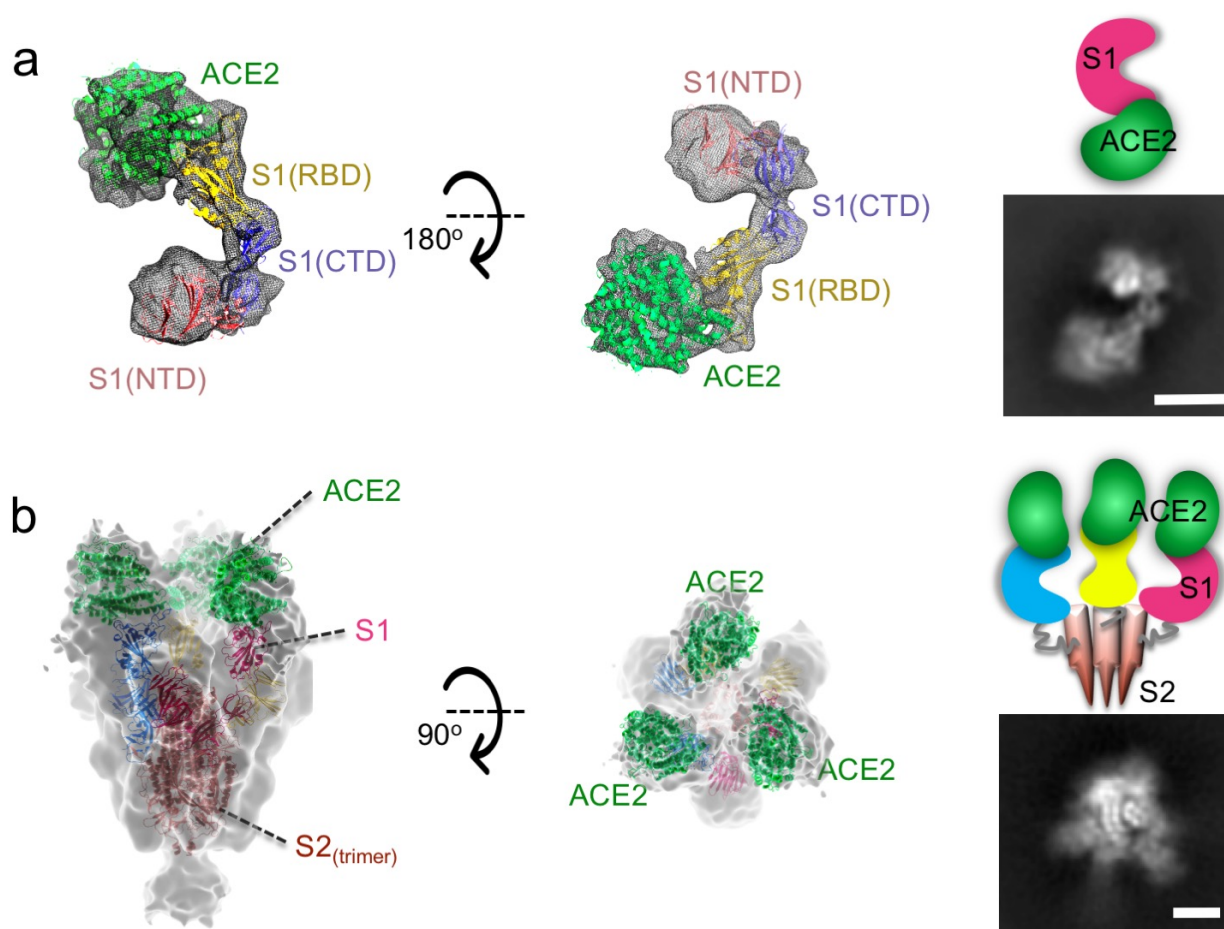
**Table 1: EM data collection statistics**

	Spike S1-hACE2 monomeric complex	Spike-hACE2 trimeric complex
<b>Cryo-electron microscopy data collection and processing</b>		
Microscope	FEI Titan Krios	FEI Titan Krios
Voltage (keV)	300	300
Camera	Gatan K2-Summit	Gatan K2-Summit
Electron exposure (e-/Å <sup>2</sup> /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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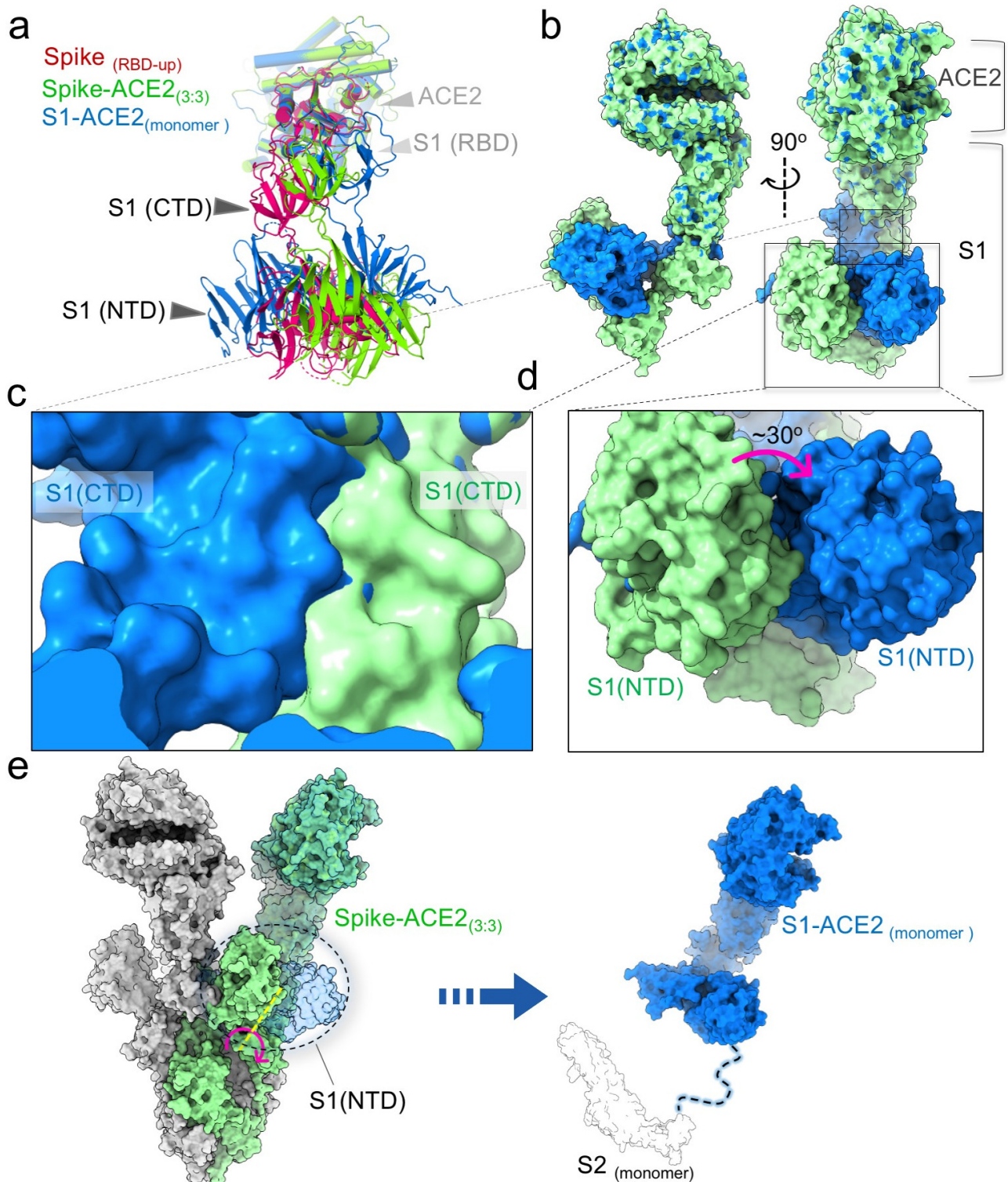
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**a.** The 3D reconstruction of SARS-CoV-2 Spike and human ACE2 (mixed at a molar ratio 1:5) incubated for 12hrs and further purified by SEC shows a structure corresponding to monomeric Spike S1 protein in complex with hACE2. No density for S2 is observed. 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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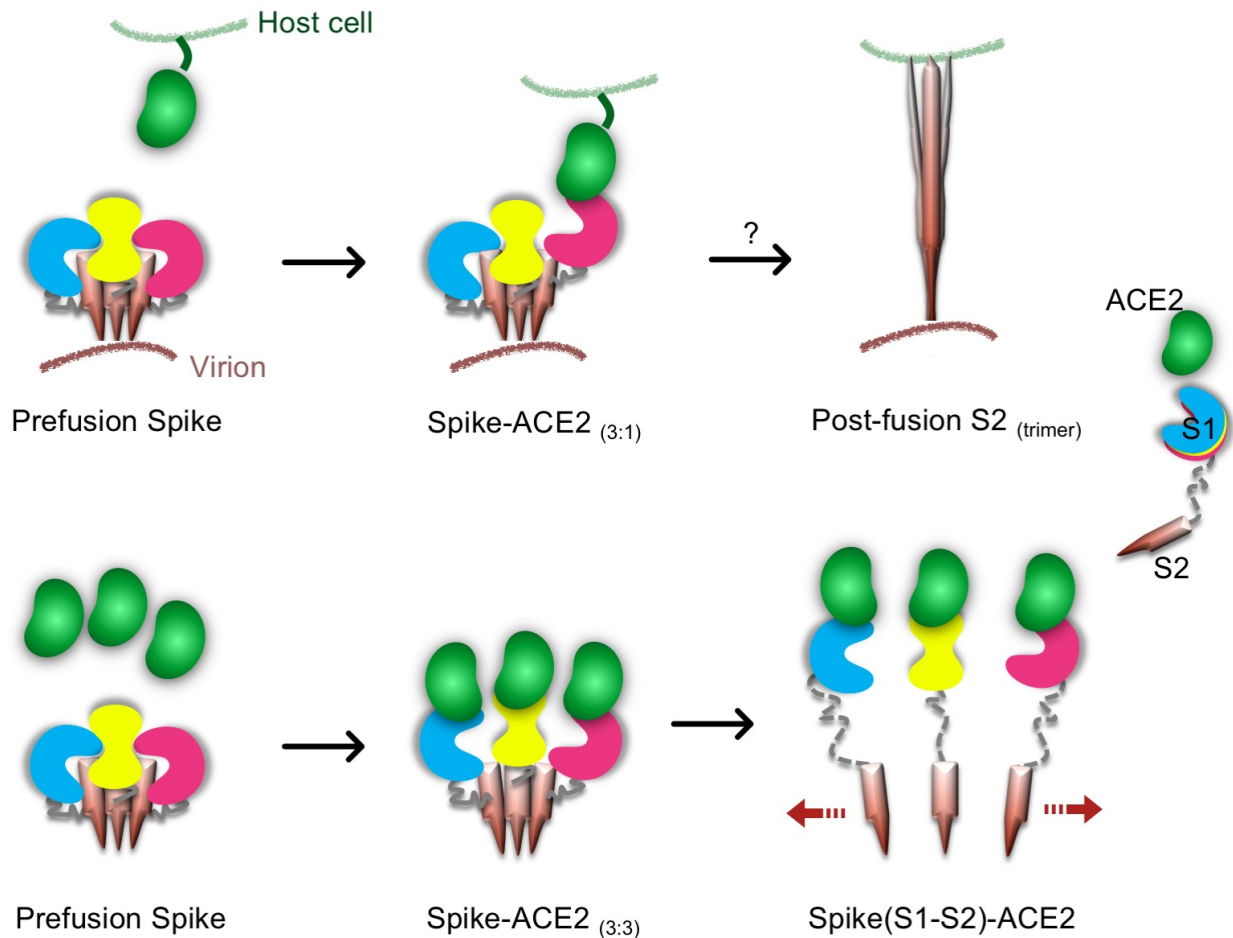
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**a.** Superposition of S1-hACE2 complex structures derived from different conformations. Structures are shown as a cartoon and colored as follows: monomeric Spike-hACE2 complex, blue; trimeric form of Spike-hACE2 complex, green; Spike one RBD-up (PDB 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.

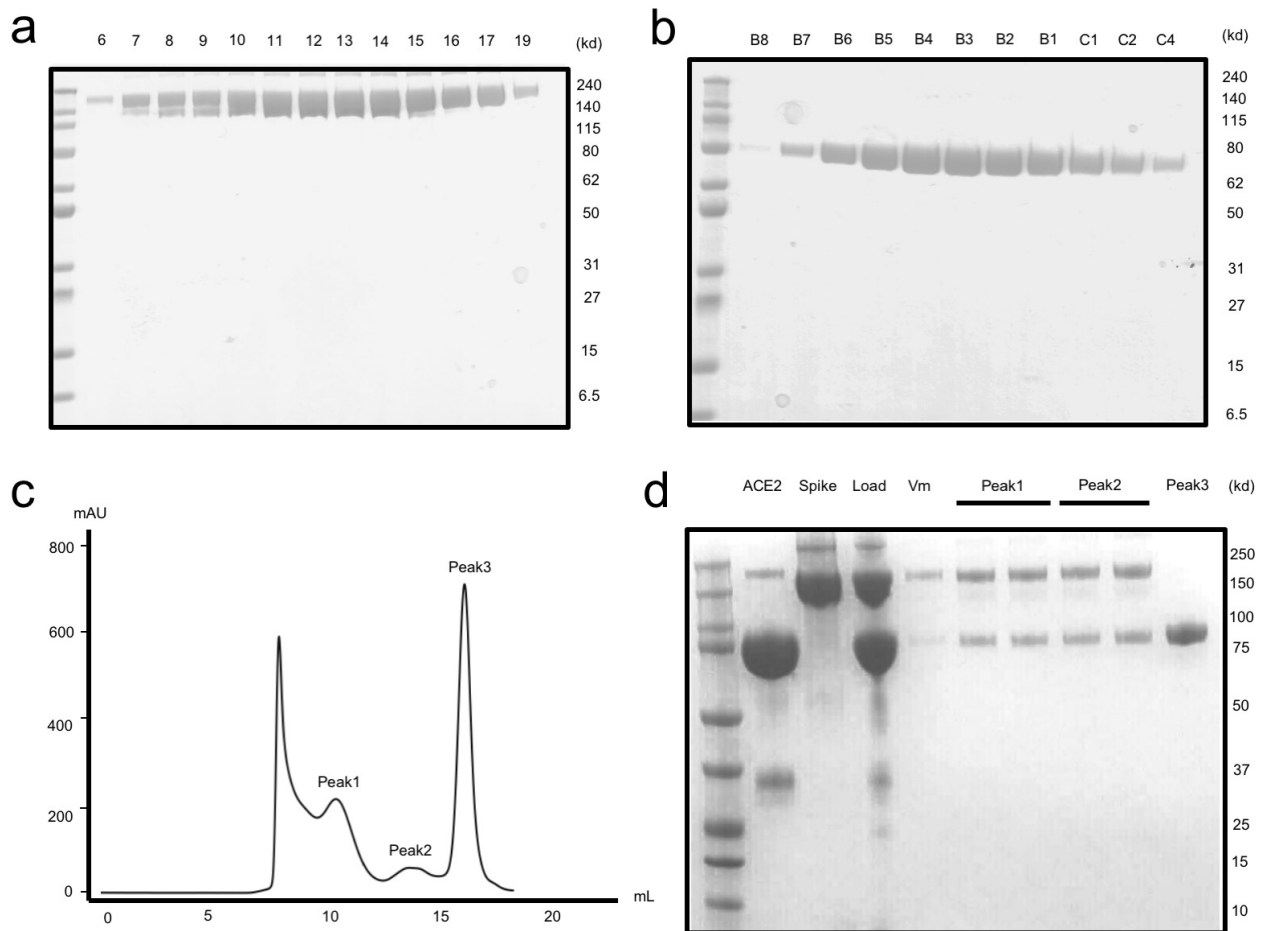




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426 **Figure 3 Proposed models for Spike-hACE2 complex formation and structural rearrangement.** The  
427 upper row shows a possible pathway leading to a conformational change of the trimeric SARS-CoV2  
428 Spike. In this model, one hACE2 molecule 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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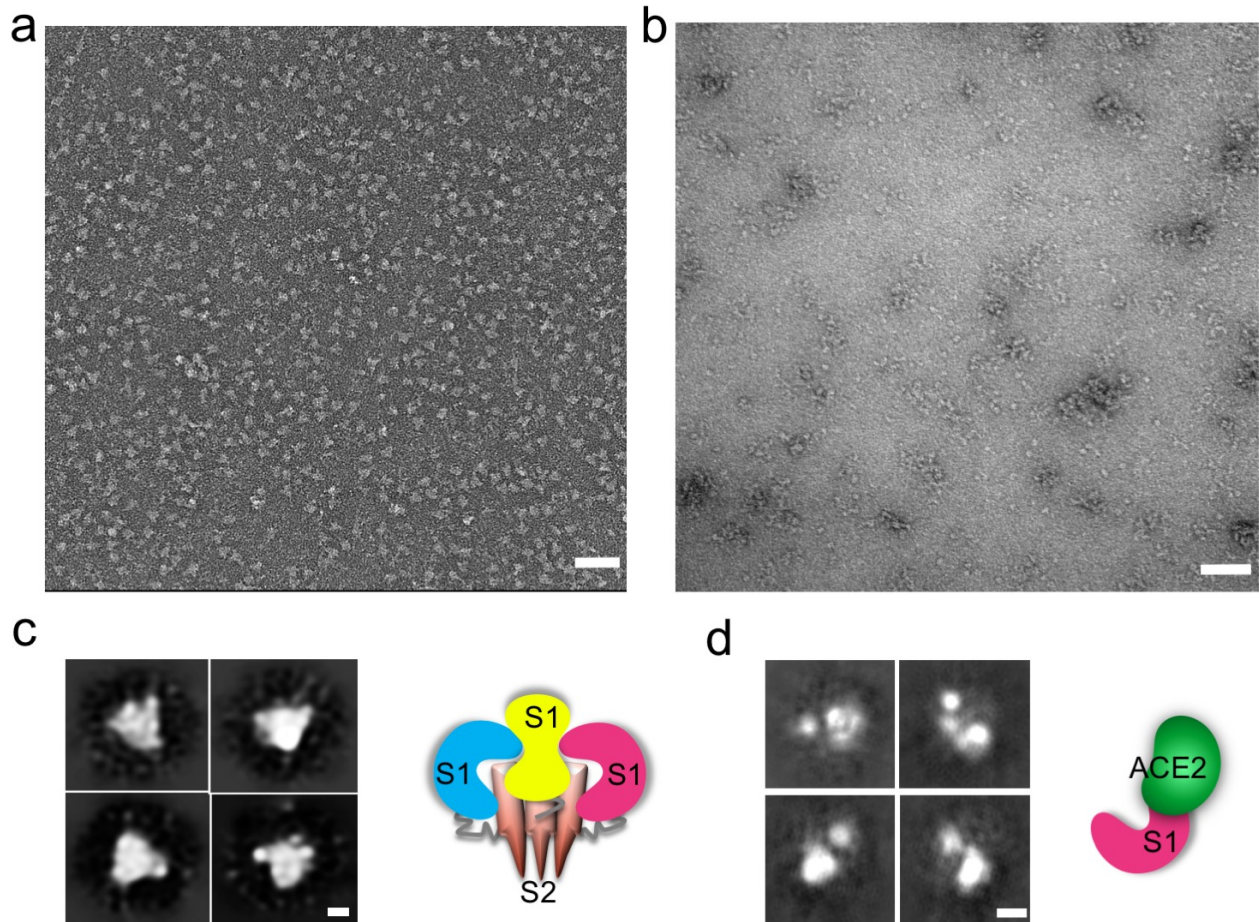


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436 **Figure S1 Protein production and purification.**

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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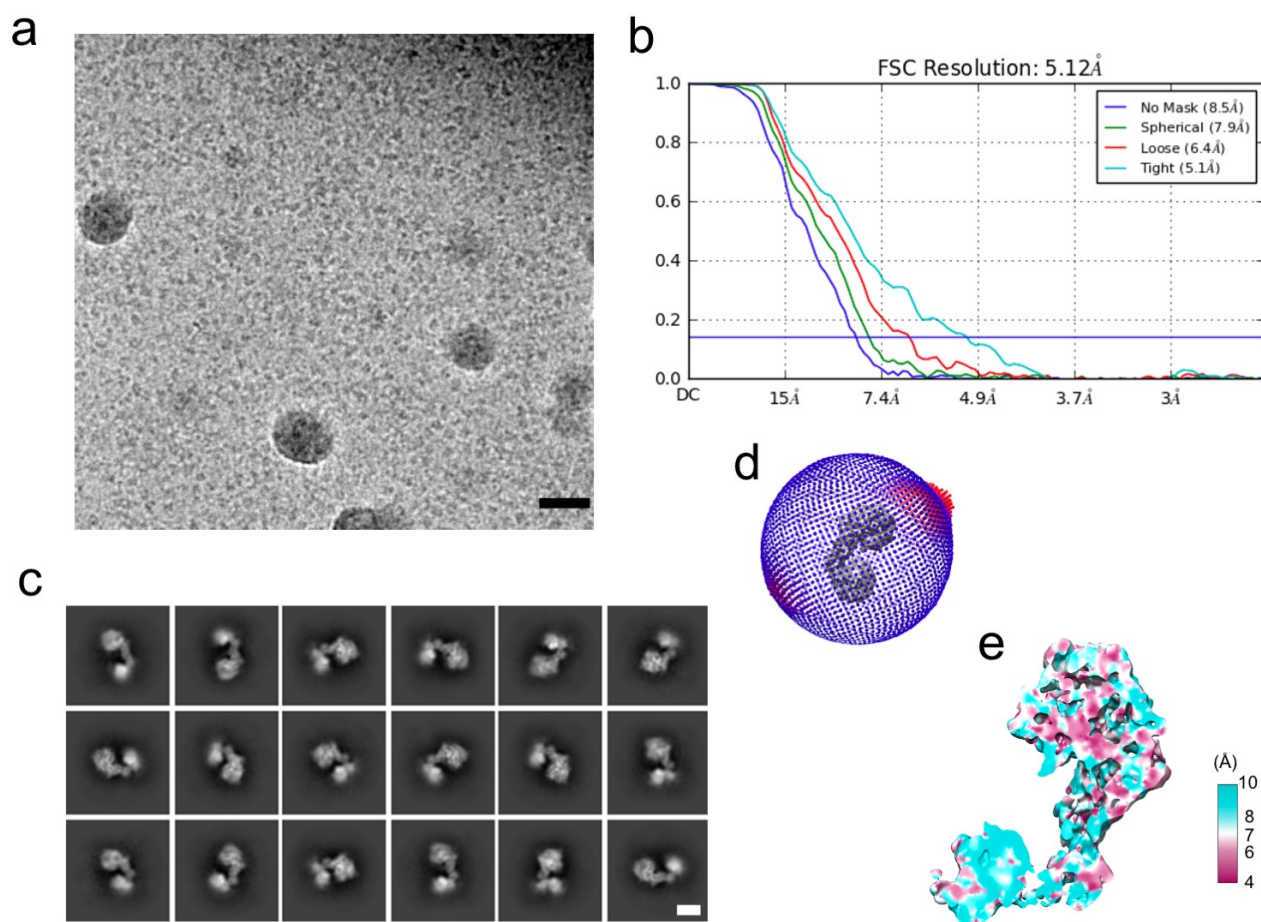
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**a.** Negative stain TEM image of the Spike sample alone (non-incubated sample). **b.** Negative stain TEM image of Spike protein incubated with soluble human ACE2 at a molar ratio of 1:5 for 12 hrs at 4°C, and further purified by SEC. **c.** Left: 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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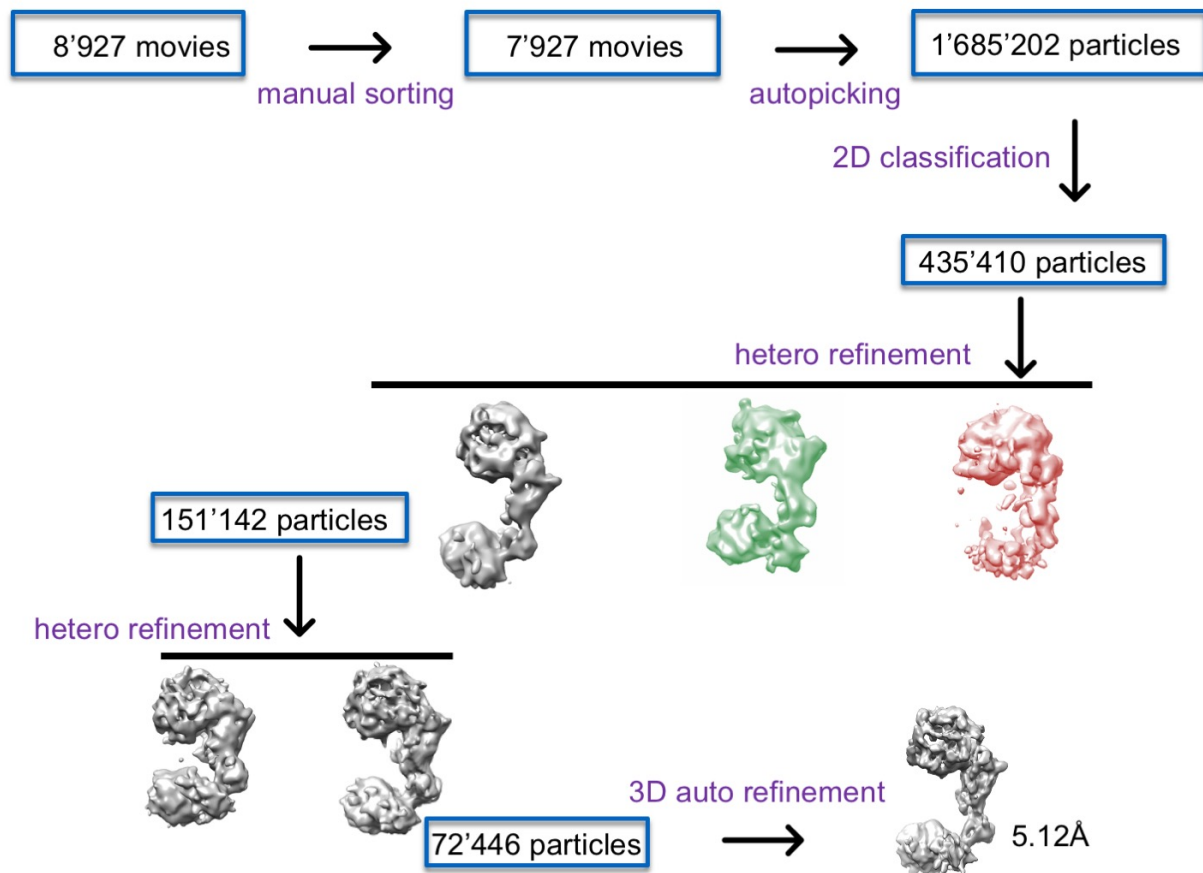
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457 **Figure S3 Data quality of Spike-ACE2 monomeric complex (S1-ACE2) sample.**

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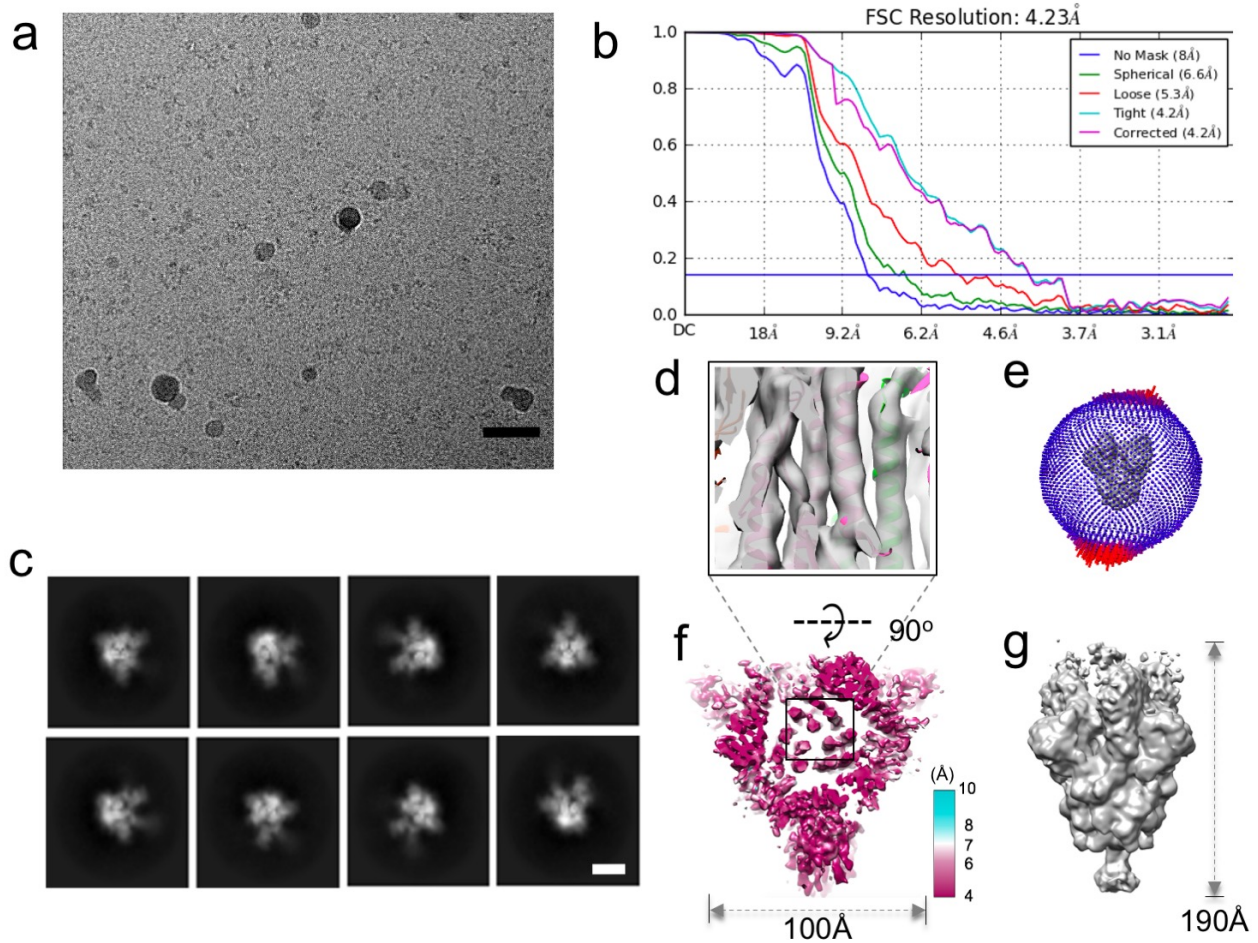
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Figure S4 Processing workflow for the Spike-hACE2 monomeric complex (S1-hACE2).

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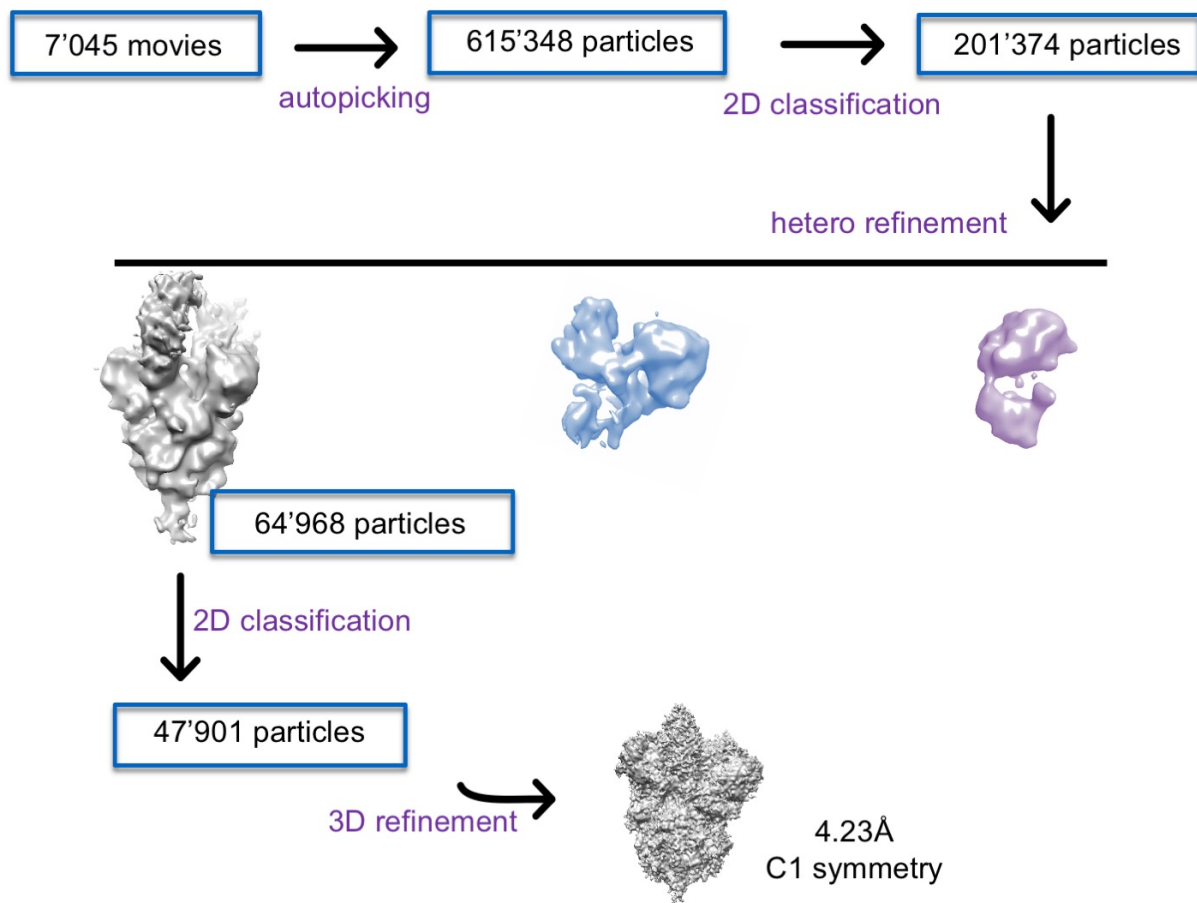
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**Figure S5 Data quality of the sample of Spike-hACE2 trimeric complex.**

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.