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Supplemental information

Structure, receptor recognition, and antigenicity

of the human coronavirus

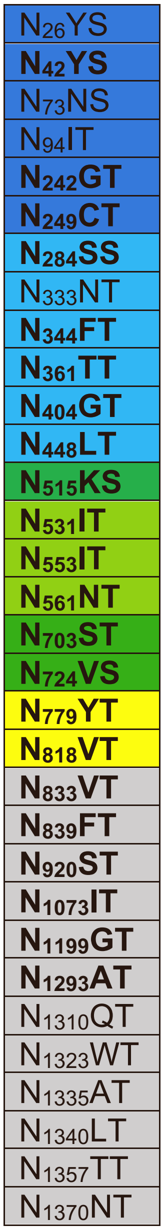
CCoV-HuPn-2018 spike glycoprotein

M. Alejandra Tortorici, Alexandra C. Walls, Anshu Joshi, Young-Jun Park, Rachel T. Eguia, Marcos C. Miranda, Elizabeth Kepl, Annie Dosey, Terry Stevens-Ayers, Michael J. Boeckh, Amalio Telenti, Antonio Lanzavecchia, Neil P. King, Davide Corti, Jesse D. Bloom, and David Veesler

Table S1. CryoEM data collection and refinement statistics for CCoV-HuPn-2018 S with domain 0 in swung-out or proximal conformations. Related to Fig 1-2.

	S trimer (swung out)	Domain 0 (swung out, local refinement)	S trimer (proximal)	Domain 0 (proximal, local refinement)
Data collection and processing				
Magnification (nominal)	130,000	130,000	130,000	130,000
Voltage (kV)	300	300	300	300
Electron exposure (e-/Å2)	84	84	84	84
Defocus range (μm)	-0.8/-2.0	-0.8/-2.0	-0.8/-2.0	-0.8/-2.0
Pixel size (Å)	0.8430	0.8430	0.8430	0.8430
Symmetry imposed	C3	C1	C3	C1
Final particle images (no.)	70,990	102,954	38,863	36,141
Map resolution (Å)	2.8	3.1	3.1	3.8
FSC threshold	0.143	0.143	0.143	0.143
Validation				
MolProbity score	1.39	1.22	1.09	1.21
Clash score	1.61	1.97	1.57	3.45
Ramachandran statistics				
Favored (%)	94.53	96.21	96.85	97.67
Allowed (%)	5.47	3.50	2.77	2.33
Outliers (%)	0	0.29	0.39	0
PDB ID	7USA	7USB	7US6	7US9
EMD ID	26730	26731	26727	26729

Table S2. N-Linked glycosylation sequons in CCoV-HuPn-2018. Glycans observed in either of the CCoV-HuPn-2018 S cryoEM maps are in bold. Glycans positions highlighted with the same color are located within the same CCoV-HuPn-2018 S domain or subunit. Color code: domain 0 (dark blue), domain A (light blue), domain B (light green), domain C (dark green), domain D (yellow) and S_2 subunit (gray). Related to Fig 1-2.



N$_{26}$YS
N$_{42}$YS
N$_{73}$NS
N$_{94}$IT
N$_{242}$GT
N$_{249}$CT
N$_{284}$SS
N$_{333}$NT
N$_{344}$FT
N$_{361}$TT
N$_{404}$GT
N$_{448}$LT
N$_{515}$KS
N$_{531}$IT
N$_{553}$IT
N$_{561}$NT
N$_{703}$ST
N$_{724}$VS
N$_{779}$YT
N$_{818}$VT
N$_{833}$VT
N$_{839}$FT
N$_{920}$ST
N$_{1073}$IT
N$_{1199}$GT
N$_{1293}$AT
N$_{1310}$QT
N$_{1323}$WT
N$_{1335}$AT
N$_{1340}$LT
N$_{1357}$TT
N$_{1370}$NT

Table S3. Biolayer interferometry binding kinetics analysis of feline, canine and porcine APNs or Fab 1AF10 to the HCoV-229E, CCoV-HuPN-2018 or TGEV B domain immobilized at the surface of SA biosensors. Related to Fig 4.

Proteins	**$K_{D,\ app}$ (nM)**	**k_{on} ($M^{-1}s^{-1}$)**	**k_{off} (s^{-1})**
HCoV-229E Domain B human-APN	9.3	6.6×10^{4}	6.1×10^{-4}
CCoV-HuPn-2018 Domain B Feline-APN	27	8×10^{4}	2.1×10^{-3}
CCoV-HuPn-2018 Domain B Canine-APN	1.0	1.7×10^{5}	1.8×10^{-4}
CCoV-HuPn-2018 Domain B Porcine-APN	41	3.5×10^{4}	1.4×10^{-3}
CCoV-HuPn-2018 Domain B 1AF10	17	1.0×10^{5}	1.7×10^{-3}
TGEV Domain B Feline-APN	47	5.7×10^{4}	2.7×10^{-3}
TGEV Domain B Canine-APN	2.1	1.3×10^{5}	2.8×10^{-4}
TGEV Domain B Pig-APN	70	3.8×10^{4}	2.6×10^{-3}
TGEV Domain B 1AF10	10	1.5×10^{5}	1.5×10^{-3}

Table S4. X-ray crystallography data collection and refinement statistics. Related to Fig 4.

	Canine APN-CCoV-HuPn-2018 B domain complex
Space group	P 21221
Cell dimensions	
a, b, c (Å)	101.43, 107.329, 239.229
α, β, γ (°)	90, 90, 90
Resolution range (Å)	48.96-3.3 (3.4-3.3)
CC1/2	0.994 (0.525)
Rmerge	0.084 (0.667)
I/σ(I)	9.5 (1.6)
Completeness (%)	99.94 (100)
Redundancy	2 (2)
Refinement	
No. reflections	40,079
Rwork/Rfree	21.55/24.01
N° of atoms	
Protein	8,876
Water	65
B factor (Wilson plot)	68.79
R.m.s. deviations	
Bond lengths (Å)	0.011
Bond angles (°)	1.49
Ramachandran favored (%)	96.16
Ramachandran allowed (%)	3.74
Ramachandran outliers (%)	0.1
PDB ID	7U0L

- Data in parentheses are for the highest resolution shell
- $R_{merge} = \sum(\sum|I_i - \langle I \rangle|/\sum|I|)$, where the first ∑ is the sum over all reflections, and the second ∑ is the sum over all measurements of a given reflection, with I_i being the ith measurement of the intensity of the reflection and $\langle I \rangle$ the average intensity of that reflection.

- $R_{\mathrm{work}}/R_{\mathrm{free}} = \sum(|F_o| - \langle|F_c|\rangle)/\sum|F_o|$, where $\langle|F_c|\rangle$ is the expectation of $|F_c|$ under the probability model used to define the likelihood function. The sum is overall reflections.