Immunity, Volume 53

Supplemental Information

Cross-Neutralization of a SARS-CoV-2 Antibody to a

Functionally Conserved Site Is Mediated by Avidity

Hejun Liu, Nicholas C. Wu, Meng Yuan, Sandhya Bangaru, Jonathan L. Torres, Tom G. Caniels, Jelle van Schooten, Xueyong Zhu, Chang-Chun D. Lee, Philip J.M. Brouwer, Marit J. van Gils, Rogier W. Sanders, Andrew B. Ward, and Ian A. Wilson

А CDR H1 COVA1-16: QVQLVQSGAEVKKPGASVKVSCKASG<mark>YT</mark>FT<mark>SY</mark>YMHWVRQAPGQ IGHV1-46: OVOLVOSGAEVKKPGASVKVSCKASGYTFTSYYMHWVROAPGO CDR H2 COVA1-16: GLEWMGIINSSGGSTSYAOKFOGRVTMTRDTSTSTVYMELSSL IGHV1-46: GLEWMGIINPSGGSTSYAQKFQGRVTMTRDTSTSTVYMELSSL CDR3 H3 COVA1-16: RSEDTAVYYCARPPRNYYDRSGYYORAEYFOHWGOGTLVTVSS IGHV1-46: RSEDTAVYYCAR В CDR L1 COVA1-16: DIOLTOSPSSLSASVGDRVTITCQASQDISNYLNWYQQR IGKV1-33: DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQK CDR L2 COVA1-16: PGKAPKLLIYDASNLETGVPSRFSGSGSGTDFTFTISSL IGKV1-33: PGKAPKLLIYDASNLETGVPSRFSGSGSGTDFTFTISSL 554 552 560 CDR L3 COVA1-16: OPEDIATYYCOOYDNPPLTFGGGTKLEIK IGKV1-33: QPEDIATYYCQQYDNLP-----С C A R P P R N Y Y D R S G Y Y Q R A E Y F Q H W TGTGCGAGGCCCCCTCGAAATTACTATGATAGGAGTGGTTATTATCAGAGGGCTGAATACTTCCAGCACTGG Germline sequence: ATTACTATGATAGTAGTGGTTATTA GCTGAATACTTCCAGCACTGG IGHD3-22 IGHJ1 Total gene-derived nucleotides: 46 Total non-gene-derived nucleotides: 18

1

Figure S1, related to Figure 1. Comparison of COVA1-16 and putative germline sequences. Alignment of COVA1-16 Fab amino-acid sequence with (A) germline IGHV1-46 sequence, and (B) germline IGKV1-33 sequence. The regions that correspond to CDR H1, H2, H3, L1, L2, and L3 are indicated. Residues that differ from germline are highlighted in red. COVA1-16 Fab residues that interact with the RBD are highlighted in yellow [defined here as residues with a BSA > 0 Å² as calculated by the PISA program (Krissinel and Henrick, 2007)]. Residue positions in the CDRs are labeled according to the Kabat 9 numbering scheme. **(C)** Amino acid and nucleotide sequences of the V-D-J junction of 10 COVA1-16, with putative gene segments (blue) and N-regions from N-addition (red), are 11 indicated. The germline sequences of IGHD3-22 and IGHJ1 are also shown. The only 12 somatically mutated nucleotide in the D region is underlined that results in a V_H S100bR 13 mutation. Second binding event



14

15 Figure S2, related to Figures 2 and 3. Competition assay between different IgGs and

16 ACE2 and negative-stain EM analysis of COVA1-16 binding to SARS-CoV-2 S trimer. 17 Competition between COVA1-16 IgG, CR3022 IgG, and Fc-tagged ACE2 was measured 18 by biolayer interferometry (BLI). Y-axis represents the response. The biosensor was first 19 loaded with SARS-CoV-2 RBD, followed by two binding events: 1) CR3022 IgG or 20 COVA1-16 IgG, and 2) ACE2, CR3022 IgG, or COVA1-16 IgG. A period of 300 s was 21 used for each binding event. A further increase in signal during the second binding event 22 (starting at 300 s time point) indicates lack of competition with the first ligand. (B) An atomic 23 model from the crystal structure of SARS-CoV-2 RBD bound to COVA1-16 Fab was fit 24 into the negative-stain EM reconstruction of the SARS-CoV-2 spike bound to COVA1-16 25 Fab. The COVA1-16 Fab approaches the apex of the S trimer in a perpendicular 26 orientation. A secondary structure backbone representation of the prefusion spike model 27 (PDB: 6Z97, green) (Huo et al., 2020) was also fit into the EM density with RBD residues 28 (334-528) removed from one of the protomers here for clarity. The COVA1-16 heavy and 29 light chains are in magenta and pink, respectively, and COVA1-16-bound RBD in yellow. 30 (C) Conformation of RBD in an up conformation from an unliganded SARS-CoV-2 S trimer 31 (PDB: 6Z97, green) (Huo et al., 2020) is compared to that of the RBD (yellow) bound by 32 COVA1-16 Fab. The arrow indicates that the RBD further rotates and opens up when 33 bound to COVA1-16, thereby moving further away from the trimer threefold axis. (D) An 34 atomic model of the spike RBD bound to COVA1-16 Fab is fit into a negative-stain EM 35 reconstruction, where COVA1-16 Fab approaches the SARS-CoV-2 S trimer from the 36 side. COVA1-16 is modelled as an IgG to illustrate the feasibility of bivalent binding to 37 adjacent spike proteins on the virus surface. The Fab heavy and light chains are shown in 38 magenta and pink. A schematic representation of the Fc domain of the IgG is shown in 39 magenta. The RBD model and spike density for each trimer is shown in yellow and cyan. 40 (E) In the crystal structure of the RBD-bound form of COVA1-16 Fab, the CDR H3 loop is 41 completely ordered (red). (F) In the crystal structure of the apo form of COVA1-16, the 42 distal end of the CDR H3 loop is intrinsically disordered or flexible (red).





Figure S3, related to Figures 2 and 3. Sensorgrams for binding of COVA1-16 to
SARS-CoV-2 RBD and SARS-CoV RBD. (A-B) Binding kinetics of COVA1-16 Fab and

46 IgG to (A) SARS-CoV-2 RBD and (B) SARS-CoV RBD were measured by biolayer 47 interferometry (BLI) with RBD on the biosensor and antibody in solution. An anti-HIV His-48 tagged Fab (4E1) was used as a negative control. (C) The relationship between SARS-49 CoV-2 RBD loading concentration on the biosensor and the dissociation constant of 50 COVA1-16 IgG is shown. (D) Binding kinetics of COVA1-16 wild-type and V_{H} R100bS 51 mutant Fab to SARS-CoV-2 RBD were measured by biolayer interferometry (BLI) with 52 RBD on the biosensor and antibody in solution. Unlike panels A-C, which used HEK293F-53 expressed SARS-CoV-2, the experiment here used insect cell-expressed SARS-CoV-2. 54 (E) Binding kinetics of COVA1-16 IgG to SARS-CoV-2 RBD WT, A372T, and P384A were 55 measured by biolayer interferometry (BLI) with RBD on the biosensor and antibody in 56 solution. A372T and P384A are the only two mutations that differ between the SARS-CoV-57 2 and SARS-CoV sequences in COVA1-16 epitope. The affinity of COVA1-16 IgG to the 58 A372T mutant did not show any detectable difference from WT. Although the affinity (K_D) 59 of COVA1-16 IgG to the P384A mutant decreases, the binding is still 100 times tighter 60 than that measured between COVA1-16 IgG and SARS-CoV RBD (see panel B). For all 61 sensorgrams in this figure, Y-axis represents the response. Dissociation constants (K_D) 62 for IgG and Fab were obtained using a 1:2 bivalent model and 1:1 binding model, 63 respectively, which are represented by the red lines. Representative results of two 64 replicates for each experiment are shown.

65

6

	319
SARS-CoV-2	RV0PTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSV <mark>LYNSA</mark> -S <mark>F</mark> ST <mark>FKCYGVSPT</mark> KLNDLCF
Pangolin-CoV	RVQPTESIVRFPNITNLCPFGEVFNATTFASVYAWNRKRISNCVADYSV <mark>LYNST</mark> -S <mark>F</mark> ST <mark>FKCYGVSPT</mark> KLNDLCF
RaTG13	RVQPTDSIVRFPNITNLCPFGEVFNATTFASVYAWNRKRISNCVADYSV <mark>LYNST</mark> -S <mark>F</mark> ST <mark>FKCYGVSPT</mark> KLNDLCF
WIV1	RVAPSKEVVRFPNITNLCPFGEVFNATTFPSVYAWERKRISNCVADYSVLYNST-SFSTFKCYGVSATKLNDLCF
WIV16	RVAPSKEVVRFPNITNLCPFGEVFNATTFPSVYAWERKRISNCVADYSV <mark>LYNST</mark> -S <mark>F</mark> ST <mark>FKCYGVSAT</mark> KLNDLCF
SARS-CoV	RVVPSGDVVRFPNITNLCPFGEVFNATKFPSVYAWERKKISNCVADYSVLYNST-FFSTFKCYGVSATKLNDLCF
BM48-31	RVTPTTEVVRFPNITOLCPFNEVFNITSFPSVYAWERMRITNCVADYSVLYNSSASFSTFOCYGVSPTKLNDLCF
GX2013	RVSPT0EVVRFPNITNRCPFDKVFNATRFPNVYAWERTKISDCVADYTVLYNST-SFSTFKCYGVSPSKLIDLCF
HKU3-1	RVSPTÕEVIRFPNITNRCPFDKVFNATRFPNVYAWERTKISDCVADYTV <mark>LYNST</mark> -S <mark>F</mark> ST <mark>FKCYGVSPS</mark> KLIDLCF
ZC45	RV0PT0SVVRFPNITNVCPFHKVFNATRFPSVYAWERTKISDCIADYTVFYNST-SFSTFKCYGVSPSKLIDLCF
ZXC21	RVOPTOSIVRFPNITNVCPFHKVFNATRFPSVYAWERTKISDCIADYTVFYNST-SFSTFKCYGVSPSKLIDLCF
Longguan-140	RVŠPTÕEVIRFPNITNRCPFDKVFNVTRFPNVYAWERTKISDCVADYTVLYNST-SFSTFKCYGVSPSKLIDLCF
HuB2013	RVTPTÕEVVRFPNITNRCPFDRVFNASRFPSVYAWERTKISDCVADYTVLYNST-SFSTFKCYGVSPSKLIDLCF
Rp3	RVSPTOEVIRFPNITNRCPFDKVFNATRFPNVYAWERTKISDCVADYTVLYNST-SFSTFKCYGVSPSKLIDLCF
Rs672	RVSPTHEVIRFPNITNRCPFDKVFNASRFPNVYAWERTKISDCVADYTVLYNST-SFSTFKCYGVSPSKLIDLCF
Rf1	RVSPVTEVVRFPNITNLCPFDKVFNATRFPSVYAWERTKISDCVADYTVFYNST-SFSTFNCYGVSPSKLIDLCF
SX2013	RVSPVTEVVREPNTTNLCPEDKVENATREPSVYAWERTKISDCVADYTVEVNST-SESTENCYGVSPSKLTDLCF
0/12020	
	393
SARS-CoV-2	TNVYADSFVIRGDEVROIAPGOTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERD
Panaolin-CoV	TNVYADSFVVRGDEVROIAPGOTGRIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERD
RaTG13	TNVYADSFVITGDEVROIAPGOTGKIADYNYKLPDDFTGCVIAWNSKHIDAKEGGNFNYLYRLFRKANLKPFERD
WIV1	SNVYADSFVVKGDDVROIAPGOTGVIADYNYKLPDDFTGCVLAWNTRNIDATOTGNYNYKYRSLRHGKLRPFERD
WIV16	SNVYADSFVVKGDDVR0IAPG0TGVIADYNYKLPDDFTGCVLAWNTRNIDAT0TGNYNYKYRSLRHGKLRPFERD
SARS-CoV	SNVYADSFVVKGDDVROIAPGOTGVIADYNYKLPDDFMGCVLAWNTRNIDATSTGNYNYKYRYLRHGKLRPFERD
BM48-31	SSVYADYFVVKGDDVROIAPAOTGVIADYNYKLPDDFTGCVIAWNTNSLDSSNEFFYRRFRHGKIKPYGRD
GX2013	TSVYADTFLIRSSEVROVAPGETGVIADYNYKLPDDFTGCVIAWNTAKODTGNYYYRSHRKTKLKPFERD
HKU3-1	TSVYADTFLIRSSEVROVAPGETGVIADYNYKLPDDFTGCVIAWNTAKHDTGNYYYRSHRKTKLKPFERD
ZC45	TSVYADTFLIRFSEVROVAPGOTGVIADYNYKLPDDFTGCVIAWNTAKODVGNYFYRSHRSTKLKPFERD
ZXC21	TSVYADTFLIRFSEVROVAPGOTGVIADYNYKLPDDFTGCVIAWNTAKODTGHYFYRSHRSTKLKPFERD
Lonaauan-140	TSVYADTFLIRSSEVROVAPGETGVIADYNYKLPDDFTGCVIAWNTAKODIGNYYYRSHRKTKLKPFERD
HuB2013	TSVYADTFLIRSSEVROVAPGETGVIADYNYKLPDDFTGCVIAWNTAKODTGYYYYRSHRKTKLKPFERD
Rp3	TSVYADTFLIRSSEVROVAPGETGVIADYNYKLPDDFTGCVIAWNTAKODOGOYYYRSHRKTKLKPFERD
Rs672	TSVYADTFLIRSSEVROVAPGETGVIADYNYKLPDDFTGCVIAWNTAKODOGOYYYRSSRKTKLKPFERD
Rf1	TSVYADTFLIRFSEVROVAPGOTGVIADYNYKLPDDFTGCVIAWNTAKODYGSYFYRSHRSSKLKPFERD
SX2013	TSVYADTFLIRFSEVRÖVAPGÖTGVIADYNYKLPDDFTGCVIAWNTAKÖDVGSYFYRSHRSSKLKPFERD
0/12020	
	468
SARS-CoV-2	ISTEIYOAGSTPCNGVEGENCYFPLOSYGFOPTNGVGYOPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNF
Panaolin-CoV	ISTEIYOAGSTPCNGVEGFNCYFPLOSYGFHPTNGVGYOPYRVVVLSFELLNAPATVCGPKOSTNLVKNKCVNF
RaTG13	ISTEIYOAGSKPCNGOTGLNCYYPLYRYGFYPTDGVGHOPYRVVVLSFELLNAPATVCGPKKSTNLVKNKCVNF
WIV1	ISNVPFSPDGKPCT-PPAFNCYWPLNDYGFYITNGIGYOPYRVVVLSFELLNAPATVCGPKLSTDLIKNOCVNF
WIV16	ISNVPFSPDGKPCT-PPAFNCYWPLNDYGFYITNGIGYOPYRVVVLSFELLNAPATVCGPKLSTDLIKNOCVNF
SARS-CoV	TSNVPESPDGKPCT-PPALINCYWPLNDYGEYTTTGTGYOPYRVVVLSEELLNAPATVCGPKLSTDLTKNOCVNE
BM48-31	L SNVL ENPSGGTCS-AFGLNCYKELASYGETOSSGTGEOPYRVVVL SEELLNAPATVCGEKOSTELVKNKCVNE
GX2013	LSSDDGNGVYTLSTYDENPNPVAY0ATRVVVLSELLNAPATVCGPKLSTOLVKNOCVNE
HKU3-1	LSSDDGNGVYTLSTYDENPNVPVAY0ATRVVVLSEELINAPATVCGPKLSTELVKNOCVNE
7045	I SSDE-NGVRTI STYDENPNVPI EYOATRVVVI SEELI NAPATVCGPKI STOLVKNOCVNE
7XC21	I S SDE - NGVRTI STYDENPNYDI EYOATRVVVI SEELI NAPATVCGPKI STOLVKNOCVNE
Longguan-140	LSSDDGNGVYTLSTYDENPNVPVAY0ATRVVVU SEEL I NAPATVCGPKU STOLVKNOCVME
HuB2013	I S SDDCNGVYTI STYDENDNVPVAY0ATRVVVI SEELI NAPATVCGPKI STELVKNGCVME
Rn3	I S SDF NGVRTI STYDFYPSVPVA/0ATRVVVI SFEI I NAPATVCGPKI STOI VKNOCVME
Rs672	LT OF _NGVRTI STYDEYDNVD EFE I NADATVCGDKI STCI VKNOCVME
Rf1	I S SFE NGVRTI STYDENONVOI EVOATDVVVI SEELI NADATVCGDKI STSI VKNOCVIJE
SX2013	LSSEE
3.LU13	

66

67 Figure S4, related to Figure 4. Sequence alignment of the RBD from SARS-related

68 coronaviruses. Amino-acid sequences of RBDs from SARS-CoV-2, SARS-CoV, and

69 other SARS-related coronavirus (SARSr-CoV) strains are aligned. COVA1-16 epitope

70 residues are highlighted in cyan. ACE2-binding residues are highlighted in purple.

71 Conserved residues are indicated by small black dots on the top of the alignment.



72

73 Figure S5, related to Figures 4 and 5. Sequence conservation of S309 epitope and

74 additional structural analyses on COVA1-16 epitope. (A) Sequence conservation of

75 the RBD is highlighted on the structure for S309 epitope (Pinto et al., 2020). This view 76 corresponds to the opposite side (rotated 180 degrees along the vertical axis) from that 77 shown in Figure 4A-B. (B) The epitope of COVA1-16 is outlined and is mainly polar in 78 character. (C) The RBD of one of the three protomers is shown as a gray cartoon with the 79 side chains of five residues of interest shown in yellow stick representation. RBD residues 80 K378, R408, Q414, and D427 are within the COVA1-16 epitope, whereas K386 is not a 81 COVA1-16 epitope residue. The other two protomers (protomers 2 and 3) are shown in a 82 surface electrostatic representation. (D-G) Zoomed-in views for the regions surrounding 83 residues (D) R408 and Q414, (E) D427, (F) K378, and (G) K386. A hydrogen bond in (D) 84 is represented by a dashed line. Due to charge difference or similarity between the side 85 chain and the proximal region of the neighboring protomer, either repulsive (same charge) 86 or attractive (opposite charge) environments are found and visualized here. PDB 6VXX is 87 used to represent the spike protein (Walls et al., 2020). Of note, the shape 88 complementarity values (Sc) (Lawrence and Colman, 1993) of the COVA1-16 89 epitope/RBD interface, COVA1-16 epitope/S2 interface, and COVA1-16 epitope/COVA1-90 16 interface are 0.53, 0.75, and 0.74, respectively, indicating good complementarity and 91 tight fit of the COVA1-16 epitope surface with the rest of the trimer in the RBD down 92 conformation. Sc values can range from 0 to 1, with a larger Sc value represents higher 93 shape complementarity. (H) The antibody-bound RBD is shown in the up conformation on 94 the S protein (PDB 6VSB) (Wrapp et al., 2020). N-glycans on N165 (NTD), N234, N331, 95 and N343 (RBD) are modelled according to the main glycoform observed at these sites in 96 (Watanabe et al., 2020) and shown in stick representation. Antibody Fabs from published 97 crystal and cryo-EM structures are represented as globular outlines in different colors. 98 B38, CB6, C105, CC12.1, CC12.3, COVA2-04, COVA2-39, BD23, P2B-2F6 all bind at or 99 around the receptor binding site. S309 binds to the elongated accessible face of the RBD

- 100 in both up and down conformations, and CR3022 binds to the opposite face that is
- 101 exposed in the RBD up conformation, but buried in the RBD down conformation.

102

Table S1, related to Figure 1. X-ray data collection and refinement statistics

1	02	
1	03	

Data collection					
	COVA1-16 Fab + SARS-CoV-2 RBD	COVA1-16 Fab			
Beamline	SSRL 12-1	SSRL 12-1			
Wavelength (Å)	0.97946	0.97946			
Space group	<i>P</i> 1 2 ₁ 1	P 41 3 2			
Unit cell parameters					
<i>a, b, c</i> (Å)	57.4, 124.9, 57.6	156.3, 156.3, 156.3			
α, β, γ (°)	90, 96.1, 90	90, 90, 90			
Resolution (Å) ^a	50.0-2.89 (2.95-2.89)	50.0-2.53 (2.58-2.53)			
Unique reflections ^a	17,656 (845)	22,357 (1,084)			
Redundancy ^a	3.7 (3.2)	37.0 (14.1)			
Completeness (%) ^a	97.9 (93.9)	100.0 (100.0)			
< I /\sigma _I > ^a	7.4 (1.2)	21.5 (1.3)			
R _{sym} ^b (%) ^a	15.3 (69.1)	23.6 (>100)			
R _{pim} ^b (%) ^a	9.0 (42.9)	3.8 (54.3)			
CC _{1/2} ^c (%) ^a	96.3 (66.8)	99.6 (52.1)			
Refinement statistics					
Resolution (Å)	42.8-2.89	34.1-2.53			
Reflections (work)	17,632	21,872			
Reflections (test)	948	1,069			
R _{cryst} ^d / R _{free} ^e (%)	23.7/29.4	21.2/24.4			
No. of atoms	4,873	3,284			
Macromolecules	4,845	3,223			
Glycans	28	-			
Average <i>B</i> -values (Å ²)	49	43			
Macromolecules	49	43			
Fab	45	43			
RBD	56	-			
Glycans	89	-			
Wilson <i>B</i> -value (Å ²)	43	40			
RMSD from ideal geometry					
Bond length (Å)	0.004	0.007			
Bond angle (°)	0.74	1.02			
Ramachandran statistics (%) ^f					
Favored	95.9	96.7			
Outliers	0.16	0.0			
PDB code	7JMW	7JMX			

^a Numbers in parentheses refer to the highest resolution shell.

 $104 \\ 105 \\ 106 \\ 107 \\ 108 \\ 109 \\ 110 \\ 111$ $^{b}R_{sym} = \Sigma_{hkl} \Sigma_{i} \mid I_{hkl,i} - \langle I_{hkl} \rangle \mid / \Sigma_{hkl} \Sigma_{i} \mid I_{hkl,i} \text{ and } R_{pim} = \Sigma_{hkl} (1/(n-1))^{1/2} \Sigma_{i} \mid I_{hkl,i} - \langle I_{nkl} \rangle \mid / \Sigma_{hkl} \Sigma_{i} \mid I_{hkl,i}, \text{ where } I_{hkl,i} \text{ is the scaled}$ intensity of the ith measurement of reflection h, k, I, <I_{nkl}> is the average intensity for that reflection, and n is the redundancy.

^c CC_{1/2} = Pearson correlation coefficient between two random half datasets.

^d R_{cryst} = Σ_{hkl} | $F_o - F_c$ | $/ \Sigma_{hkl}$ | F_o | x 100, where F_o and F_c are the observed and calculated structure factors, respectively.

^e R_{free} was calculated as for R_{cryst}, but on a test set comprising 5% of the data excluded from refinement.

^{*f*} From MolProbity (Chen et al., 2010).

112

114 115 116 Table S2, related to Figure 1. Hydrogen bonds identified in the antibody-RBD interface using the PISA program

COVA1-16 Fab	Distance [Å]	SARS-CoV-2 RBD
H:ARG100b[NH2]	3.3	A:TYR369[O]
H:ARG100b[NE]	3.9	A:SER371[O]
H:ARG100b[N]	3.8	A:PHE377[O]
H:TYR100[N]	2.6	A:CYS379[O]
H:GLN101[NE2]	3.1	A:GLN414[OE1]
H:ARG97[NH1]	2.5	A:ASP427[O]
H:TYR32[OH]	3.1	A:ASP427[OD1]
H:THR28[N]	3.2	A:ASP427[OD2]
H:ARG97[NH1]	3.0	A:PHE429[O]
H:TYR100[O]	2.9	A:CYS379[N]
H:SER100c[O]	3.3	A:THR385[OG1]
H:GLN101[OE1]	3.8	A:GLN414[NE2]
L:ASN53[OD1]	3.2	A:ARG408[NH2]
L:LEU54[O]	3.7	A:ARG408[NE]