

Supplemental Information

Mapping Neutralizing and Immunodominant Sites on the SARS-CoV-2 Spike Receptor-Binding Domain by Structure-Guided High-Resolution Serology

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	SARS-CoV-2 S/S2H13 (closed) PDB-7JV6 EMD-22494	SARS-CoV-2 S/S2H13 (local refinement) PDB-7JV2 EMD-22491	SARS-CoV-2 S/S2H13 (one RBD open) PDB-7JV4 EMD-22492	SARS-CoV-2 S/S2H14 (two RBDs open) EMD-22507	SARS-CoV-2 S/S2H14 (three RBDs open) EMD-22508	SARS-CoV-2 S/S2A4 PDB-7JVC EMD-22506	SARS-CoV-2 2 S/S2A4 (local refinement) PDB-7JVA EMD-22497	SARS-CoV-2 S/S304 PDB-7JW0 EMD-22512	SARS-CoV-2 S/S2X35 EMD-22516	SARS-CoV-2 S/ S2X35 (local refinement) EMD-22517
Data collection and processing										
Magnification	130,000	130,000	130,000	36,000	36,000	130,000	130,000	130,000	36,000	36,000
Voltage (kV)	300	300	300	200	200	300	300	300	200	200
Electron exposure (e ⁻ /Å ²)	70	70	70	60	60	70	70	70	60	60
Defocus range (µm)	0.4-3.4	0.4-3.4	0.4-3.4	1.0-2.5	1.0-2.5	0.4-2.4	0.4-2.4	1.5-2.8	0.8-2.5	0.8-2.5
Pixel size (Å)	0.525	0.525	0.525	1.16	1.16	0.525	0.525	0.525	1.16	1.16
Symmetry imposed	C3	C1	C1	C1	C1	C3	C1	C3	C3	C1
Final particle images (no.)	122,343	311,071	137,924	9,957	10,938	28,599	64,141	39,520	25,634	62,547
Map resolution (Å)	3.0	3.5	3.4	7.8	8.5	3.3	3.6	4.3	~5.0	~5.0
FSC threshold	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143
Map sharpening <i>B</i> factor (Å ²)	-68	-66	-52	-368	-458	-46	-15	-120	-77	-167
Validation										
MolProbity score	0.85	0.84	0.75			0.88	0.71	0.72		
Clashscore	0.61	0	0.43			0.95	0.64	0.32		
Poor rotamers (%)	0	1.32	0.1			0.13	0.3	0		
Ramachandran plot										
Favored (%)	97.19	96.35	97.52			97.57	98.79	97.52		
Allowed (%)	2.81	3.65	2.48			2.29	0.97	2.33		
Disallowed (%)	0	0	0			0.14	0.24	0.14		
EMRinger score	3.26	3.48	3.1			3.47	3.5	1.89		

Table S1. CryoEM data collection and refinement statistics. Related to Figures 3-6.

	Total residues in motif	Natural Variants, n>1			
		Unique positions in motif	Total variant count	Total variants prevalence [%]	Avg. count per position
S309	22	12	116	0.16	9.7
S304	25	9	96	0.13	10.7
S2A4	19	11	105	0.14	9.5
S2H14	23	15	137	0.19	9.1
S2H13	20	13	244	0.33	18.8
RBM (CoV-2)	23	14	342	0.47	24.4
RBM (CoV + CoV-2)	30	19	894	1.22	47.1
RBD	205	90	1713	2.33	19.0

Table S2. Conservation of RBD, RBM and epitope residues in ~74'000 SARS-CoV-2 sequences (GISAID, August 11th 2020). Related to Figure 3-6. Sum total of the variant prevalence and count for the RBD, RBM and the mAb epitope residues as well as the average count per position. CoV-2 RBM residues were determined from an improved 6M0J and CoV RBM residues from 2AJF, using a 5.0A distance cutoff between RBD and ACE2 residues using MOE.

	S2H14 Fab	S2A4 Fab	S2X35 Fab	RBD/S304/S309/S2H14 [#]
PDB accession code	7JXC	7JXD	7JXE	7JX3
Data collection				
X-ray source	ALS BL5.0.2	ALS BL5.0.2	ALS BL5.0.2	ALS BL4.2.2
Space group	P2 ₁ 2 ₁ 2	P2 ₁	P4 ₃ 2 ₁ 2	C2
Cell dimensions				
<i>a</i> , <i>b</i> , <i>c</i> (Å)	56.4, 70.7, 115.6	59.1, 63.3, 113.9	56.5,56.5,252.2	79.5, 127.8, 192.3
α , β , γ (°)	90, 90, 90	90, 90, 90	90,90,90	90, 96.7, 90
Resolution (Å)	50-2.5 (2.6-2.5)	46-2.5(2.54-2.5)	63.04-2.04 (2.1-2.04)	47.75-2.65 (2.73-2.65)
<i>R</i> _{sym} or <i>R</i> _{merge}	0.084 (0.34)	0.13 (0.37)	0.15 (2.36)	0.18 (2.15)
<i>CC</i> _{1/2}	0.99 (0.92)	0.98 (0.93)	1.0 (0.86)	0.996 (0.39)
<i>I</i> / σ <i>I</i>	13 (4.5)	16 (2.7)	14.2 (2.1)	8.7 (0.7)
Completeness (%)	99 (98)	88 (59)	99.5 (95.1)	99.6 (99.9)
Redundancy	5.8 (6)	8.7 (6)	20.8 (18)	7.4 (6.8)
Refinement				
Resolution (Å)	2.5	2.5	2.04	2.65
No. reflections	17070	24676	26591	52027
<i>R</i> _{work} / <i>R</i> _{free}	0.19/0.23	0.20/0.26	0.20/0.25	0.258/0.288
No. atoms				
Protein	3172	6488	3313	11482
Ligand	28			14
<i>B</i> -factors (Å ²)				
Protein	45	60	43	32.6
Ligand	53			90.0
R.m.s. deviations				
Bond lengths (Å)	0.01	0.01	0.01	0.004
Bond angles (°)	1.03	0.98	0.55	1.40

Table S3. Crystallographic data collection and refinement statistics. Related to Figure 4-6.

*Seven datasets were merged *Values in parentheses are for highest-resolution shell.