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Supplemental Information

Neutralization of SARS-CoV-2 by Destruction

of the Prefusion Spike

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Figure S1 Binding affinity between RBD and CR3022 Fab, related to STAR Methods, (Surface plasmon resonance and Bio-layer Interferometry).

(A-B) Surface plasmon resonance binding sensorgrams measured with a Biacore T200. Biotinylated (Bio-) RBD was immobilised as the ligand and CR3022 Fab was used as analyte at five concentrations (5.9, 11.9, 23.8, 47.5 and 95 nM). (C-D) CR3022 IgG was immobilised as the ligand and RBD-His was used as analyte at five concentrations (6.25, 12.5, 25, 50, 100 nM). Data were fitted to a 1:1 binding model using the Biacore T200 Evaluation Software 3.1. The average kinetic values from these two sets of experiment are listed in Extended Table 1. (E) Binding sensorgram of the interaction between RBD and CR3022 Fab measured with an Octet platform. CR3022 Fab was immobilized onto AR2G biosensors, and RBD was used as analyte with a serial dilution of 5,10, 20, 40 and 80 nM. The measured K_D is 19 nM using a global 1:1 fitting model. Binding competition of ACE2 and CR3022 Fab for RBD: Surface plasmon resonance binding sensorgrams measured with a Biacore T200. (F) CR3022 IgG was immobilised as the ligand, and the following samples were injected: (1) a mixture of 1 µM ACE2 and 0.1 µM RBD; (2) a mixture of 1 µM E08R (a non-binding anti-caspr2 Fab) and 0.1 µM RBD; (3)) 0.1 µM RBD; (4) 1 µM ACE2; (5) E08R Fab. (G) ACE2-hIgG1Fc was immobilised as the ligand, and the following samples were injected: (1) a mixture of 1 μM CR3022 Fab and 0.1 μM RBD; (2) a mixture of 1 μM E08R Fab and 0.1 μM RBD; (3) 0.1 µM RBD; (4) 1 µM CR3022 Fab; (5) 1 µM E08R Fab.



Figure S2 Dose Response Curve for 'leave' and 'wash' PRNT with CR3022, related to Figure 2 and STAR Methods (Neutralisation).

(A) 'Leave' plate. For CR3022 at a starting concentration of 1 mg/mL, the dilutions used were from 1:160 to 1:327,680, the virus/antibody mix was left on the plate. The probit midpoint is 1:4,666 (95% confidence intervals: 2,619-8,028) and the positive control 1:629 (95% confidence intervals: 382-958). (B) 'Wash' plate. As for (A) but the virus/antibody mix was washed. The probit mid-point is 1:6,504 (95% confidence intervals: 3,412-12,239). The positive control probit mid-point is 1:629 (95% confidence intervals: 382-958). (C) Plate photographs. Note that the plate is photographed transversed relative to the lid, so the 1:160 dilution duplicate wells are in the top right corner, and the 1:327,680 dilution duplicate wells are in the bottom left corner of the stained plate.



Figure S3 Crystal lattice of the RBD/CR3022 complex, related to Figure 3 and STAR Methods (Crystallisation, data collection and X-ray structure determination). (A) The packing of the RBD/CR3022 complex within the first crystal form. The RBD is shown as a grey surface and CR3022 Fab as cyan ribbons. (B) A closeup of the crystal lattice with the RBD of the receptor complex overlapped onto the RBD of the Fab complex showing that the receptor binding site of the RBD is not blocked in the crystal. (C-D) The ACE2 binding sites of the 2 RBDs in the second crystal form are blocked by crystal contact (indicated by red arrows).



Figure S4 Electron density maps. related to Figure 3 and STAR Methods (X-ray crystallographic refinement and electron density map generation).

(A) 4.4 Å resolution electron density map for crystal form 1, produced with Vagabond (see methods) and contoured at 1.2 σ showing the overall quality of the structure. (B) Difference electron density map (green) contoured at 3 σ showing the glycosylation site at N343 of the RBD. The glycan was not modelled into the structure used for the map calculation. (C-D), Electron density maps of the glycosylation sites N331 (C) and N343 (D) in the second, high resolution (2.4 Å), crystal form.



Figure S5 Buried solvent accessible area of the CR3022 antigen binding region due to engagement with the RBD, related to Figure 4.

Buried areas are coloured in blue for the heavy chain (Vh) and cyan for the light chain (Vl).



Figure S6 Cryo-EM analysis of 50 minutes incubation, related to Figure 7 and STAR Methods (Cryo-EM data processing).

2D class averages: (A) unbiased classes from blob picked particles, with particle numbers and estimated resolutions in green text (CryoSPARC). (B) Selected classes for the prefusion state reconstruction. (C) Selected classes for the dimeric association reconstruction. Prefusion Spike analysis: (D) orientation distribution, (E) Gold standard FSC analysis from CryoSPARC, with FSC = 0.143 marked with a horizontal solid blue line. Dimeric CR3022/RBD: (F) orientation distribution. (G) Gold standard FSC plot generated in CryoSPARC, with FSC = 0.143 marked with a horizontal blue line. See Methods for details.

293210 ptcls 6.8 Au ess	239795 ptcls 7.1 A 2 ets	165142 ptcs: 7.2 A 1 ess	158490 ptcb	122059 ptcls	119896 ptcls	119189 ptch	113663 ptcb	112543 ptcls	103629 ptcls
99425 ptcls	96587 ptcls	97422 ptcls 7.0 A 3 ess	96202 ptch 7.1 A 3 ess	92234 ptcls 17.0 A 3 ess	91421 ptcis 8.6 A 1 ess	88691 ptcls	87413 ptchs	87327 ptch 873 43 ess	86339 ptcls 7.4 A 3 ess
65569 ptcls 7.0 A 3 ess	85285 ptcls 7.1 A 3 ess	83100 ptcls 6.9 A 2 ess	62058 ptchs	81157 ptcls 8.5 A 3 ess	80510 ptcls 6.8 A 3 ess	80239 ptcls	78792 ptcls 7.1 A 2 ess	74222 ptchs 7.6 A 3 ess	73906 ptcls 6.8 A 3 ess
73415 ptcls 7.0 A 3 ess	73022 ptcls 6.8 A 2 ess	72938 ptcls 6.8 A 2 ess	72934 ptch 8.1 A 3 ess	72804 ptcb	72702 ptcls	69494 ptcls 9.4 A 3 ets	64972 ptch 7.4 A 4 ess	64518 ptcls 8.9 A 3 ess	63159 ptcb
62293 ptcls	62183 ptcis	61661 pachs	61261 ptcls 7.0 A 3 ess	60252 pitcls	57075 pitcls	54196 ptcls	52368 ptch	48435 ptchs	44872 pichs

В

3070 ptcls	2884 ptcls	2836 ptcls	2821 ptcls	2759 ptcls	2525 ptcls	2448 ptcls	2414 ptcls	2359 ptcls
	1	8	4	12	A	3	8	32
14.0 A 1 ess	17.3 A 1 ess	11.1 A 1 ess	16.7 A 1 ess	14.4 A 1 ess	17.3 A 1 ess	18.1 A 1 ess	13.3 A 1 ess	17.5 A 1 ess
2007 ptcls	1947 ptcls	1946 ptcls	1927 ptcls	1777 ptcls	1651 ptcls	1622 ptcls	1558 ptcls	1490 ptcls
140	10	2.	1.	3%	-	100	4	*
11.3 A 1 ess	18.9 A 1 ess	11.3 A 1 ess	18.5 A 1 ess	18.7 A 1 ess	16.6 A 1 ess	10.9 A 1 ess	10.7 A 1 ess	18.3 A 1 ess
1456 ptcls	1329 ptcls	1327 ptcls	1209 ptcls	1180 ptcls	1172 ptcls	1149 ptcls	1135 ptcls	1120 ptcls
12	8	-	-	1	112	20	5	40
19.5 A 1 ess	22.1 A 1 ess	9.8 A 1 ess	23.9 A 1 ess	19.1 A 1 ess	12.1 A 1 ess	24.9 A 1 ess	20.4 A 1 ess	12.8 A 1 ess
1025 ptcls	1008 ptcls	996 ptcls	992 ptcls	939 ptcls	867 ptcls	792 ptcls	789 ptcls	768 ptcls
1	2	1	2		1	3	32	- 34
8.7 A 2 ess	8.1 A 2 ess	12.0 A 1 ess	11.8 A 1 ess	13.6 A 1 ess	12.7 A 2 ess	13.8 A 2 ess	16.4 A 2 ess	16.0 A 2 ess
684 ptcls	629 ptcls	626 ptcls	623 ptcls	581 ptcls	547 ptcls	546 ptcls	358 ptcls	322 p/ds
10			1	-		36	1.	

Figure S7 Analysis of cryo-EM data for 3 h incubation, related to Figure 7 and STAR Methods (cryo-EM data processing).

2D class averages: (A) unbiased classes i.e. following a single round of blob-picked particles in cryoSPARC, (B) selected classes showing oligomeric assemblies.

Α

Table S1 SPR kinetic results, related to Figure S1 and STAR Methods (Surface plasmon resonance).

Ligand	Biotinylated RBD	CR3022 IgG
Analyte	CR3022 Fab	His-tagged RBD
Ka $(M^{-1}s^{-1})$	6.3E+05	1.5E+06
Kd (s ⁻¹)	1.9E-02	2.3E-02
KD (nM)	30	15

Table S2 Plaque Reduction Neutralization Test results, related to Figure 2 and STARMethods (Neutralisation).

ID (batch number)	Description	PRNT ₅₀ [95% CI]
(1) Positive control	MERS convalescent serum	1:874 [663-1,220]
(1) CR3022 1.36 mg/mL	Mab (batch 2)	1:11,966 [5,297-23,038]
(2) Positive control	MERS convalescent serum	1:629 [382-958]
(2) CR3022 1.0 mg/mL	Mab (batch 1) Leave on plate	1:4666 [2,619-8,028]
(3) Positive control	MERS convalescent serum	1:629 [382-958]
(3) CR3022 1.0 mg/mL	Mab (batch 1) Wash off plate	1:6504 [3,412-12,239]

Data collection				
Data set	Crystal form 1	Crystal form 2		
Space group	$P4_{1}2_{1}2$	$P4_{1}2_{1}2$		
Cell dimensions (Å)	<i>a</i> =150.5, <i>b</i> =150.5, <i>c</i> =241.6	<i>a</i> =163.1, <i>b</i> =163.1, <i>c</i> =189.1		
Resolution (Å)	80.5-4.36 (4.44-4.36)	58.8-2.42 (2.46-2.42)		
Unique reflections	18822 (931)	97407 (4803)		
R _{merge}	0.683 ()	0.303 ()		
R_{pim}	0.097 (1.597)	0.034 (1.536)		
CC _{1/2}	0.952 (0.316)	0.997 (0.451)		
<i> /< QI></i>	4.0 (0.2)	11.6 (0.2)		
Completeness (%)	100 (100)	100 (100)		
Redundancy	51.6 (54.4)	78.7 (78.8)		
Refinement				
Resolution (Å)	35.0-4.36	55.3-2.42		
No. reflections	17940	94155		
$R_{ m work}$ / $R_{ m free}$	0.331/0.315	0.213/0.239		
No. atoms	4861	10072		
Average <i>B</i> -factors ($Å^2$)	151	89		
Parameters				
Positional	4298	N/A		
Flexibility	2391	N/A		
Total	6689	N/A		
R.m.s. deviations				
Bond lengths (Å)	N/A	0.002		
Bond angles (°)	N/A	0.5		

Table S3 X-ray data collection and refinement statistics, related to Figure 3 and STAR Methods (Crystallisation, data collection and X-ray structure determination and X-ray crystallographic refinement and electron density map generation).

Numbers in brackets refer to the highest resolution shell of data

	3h incubation	50 min incubation trimer	50 min incubation 'dimer'				
Data collection and reconstruction							
Voltage (kV) 300							
Frames	40	40					
Dose rate (e^{-1} Å ² /s)	20.2	20.7					
Total dose (e ⁻ /Å ²)	42	42.0					
Pixel size (Å) (super- resolution)	0.415	0.4	415				
Defocus (µm)	0.8-2.6	0.8	-2.6				
Symmetry	C1	0	21				
Movies	7,032	13,307					
Particles	24,303	327,945	100,295				
Resolution FSC = 0.143 (Å)		3.3	3.9/7.0				
Map sharpening B- factor $(Å^2)$		-111.1	-92.3				
	Model re	finement					
Model-to-map fit, CC_mask		0.84	0.47				
R.m.s.d., bonds (Å)		0.006	0.002				
R.m.s.d., angles (°)		0.9	0.5				
All-atom Clash score		7.6	1.8				
Rotamer outliers (%)		3.8	2.0				
Ramachandran plot							
Favored (%)		95.4	95.9				
Allowed (%)		3.8	3.9				
Outliers (%)		0.2	0.2				

Table S4 Cryo-EM data collection parameters, related to Figure 7 and STAR Methods (cryo-EM data processing).