Structure-Guided Design of Therapeutic Antibodies Targeting SARS-CoV-2 Omicron Variants

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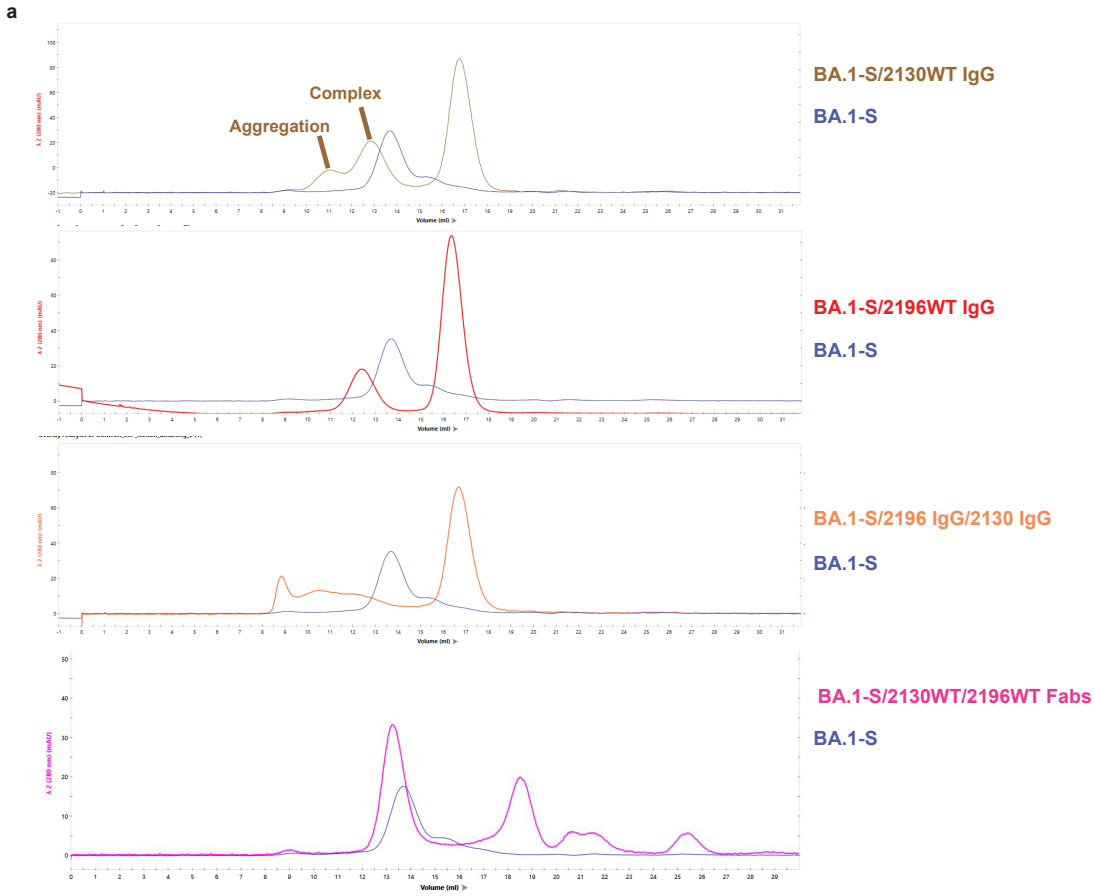
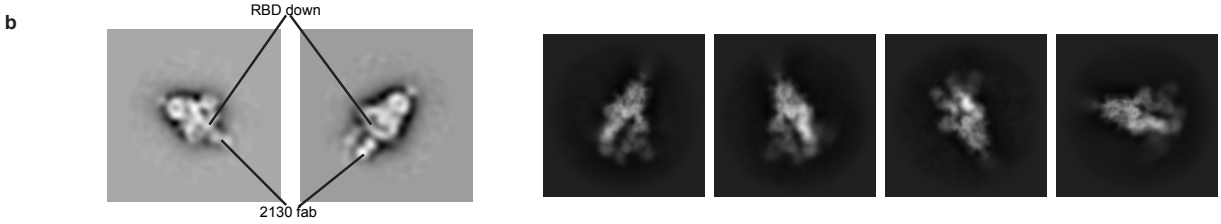
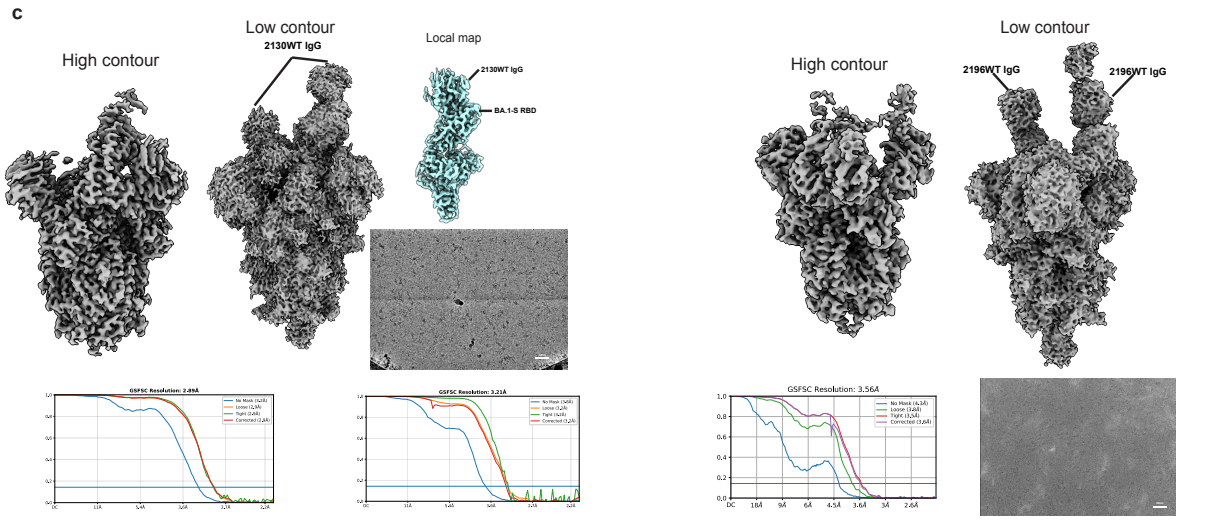
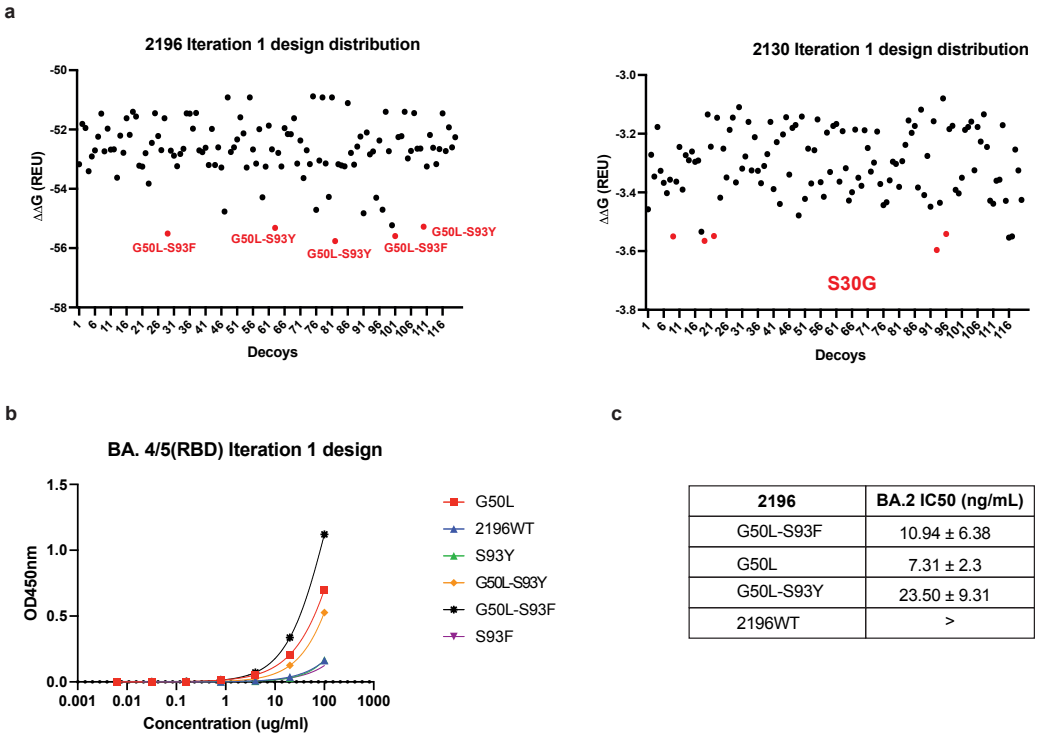
Extended Data


a
Complex
Aggregation
BA.1-S/2130WT IgG
BA.1-S
BA.1-S/2196WT IgG
BA.1-S
BA.1-S/2196 IgG/2130 IgG
BA.1-S
BA.1-S/2130WT/2196WT Fabs
BA.1-S
Volume (ml)


b
RBD down
2130 fab

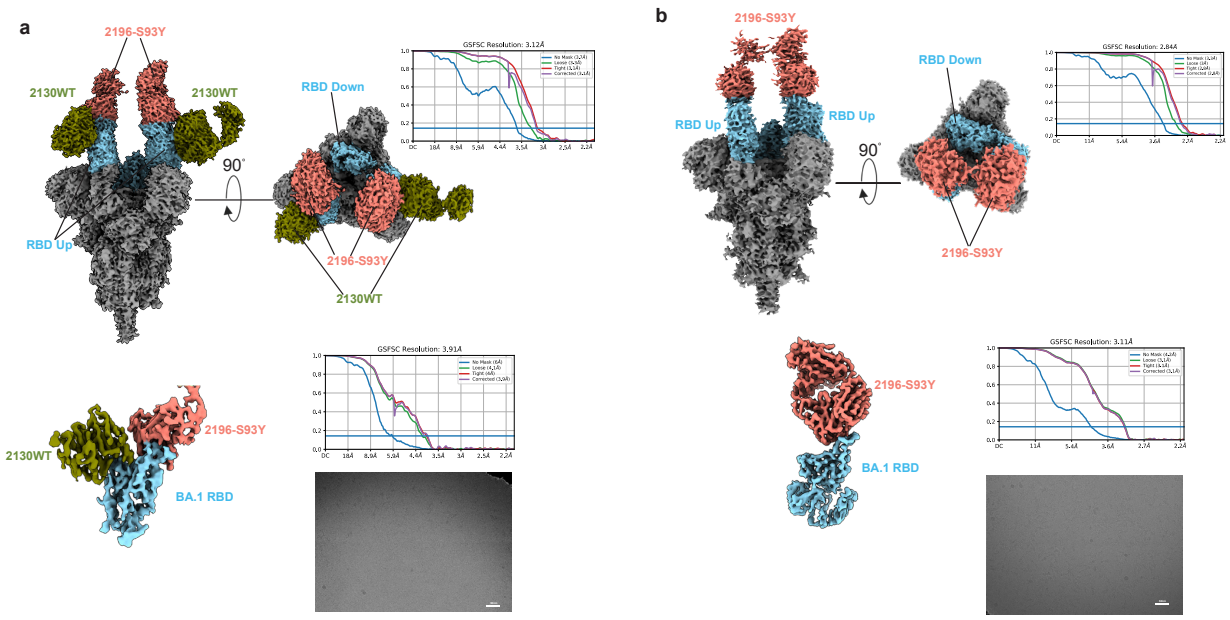

c
High contour
Low contour
2130WT IgG
Local map
2130WT IgG
BA.1-S RBD
High contour
Low contour
2196WT IgG
2196WT IgG
GSFSC Resolution: 2.49Å
GSFSC Resolution: 3.21Å
GSFSC Resolution: 3.56Å

Extended Data Fig. 1 | 2130 and 2196 antibody interaction with BA.1-S. a. Size Exclusion Chromatography (SEC) analysis of BA.1-S binding to 2196WT fab or IgG with or without 2130WT fab or IgG. SEC traces of each complex are colored as indicated. **b.** Left panel: Representative ns-EM 2D class averages of BA.1-S/2130Fab/2196Fab complex. 2D classes show only 2130 fab binding to BA.1-S. Right panel: cryo-EM2D class averages of WA-S/2130/2196 fab for comparison. c. Left panel: global and local density maps of BA.1-S/2130WT IgG and corresponding FSC curves. Right panel: global density map of BA.1-S/2196WT IgG and corresponding FSC curve.

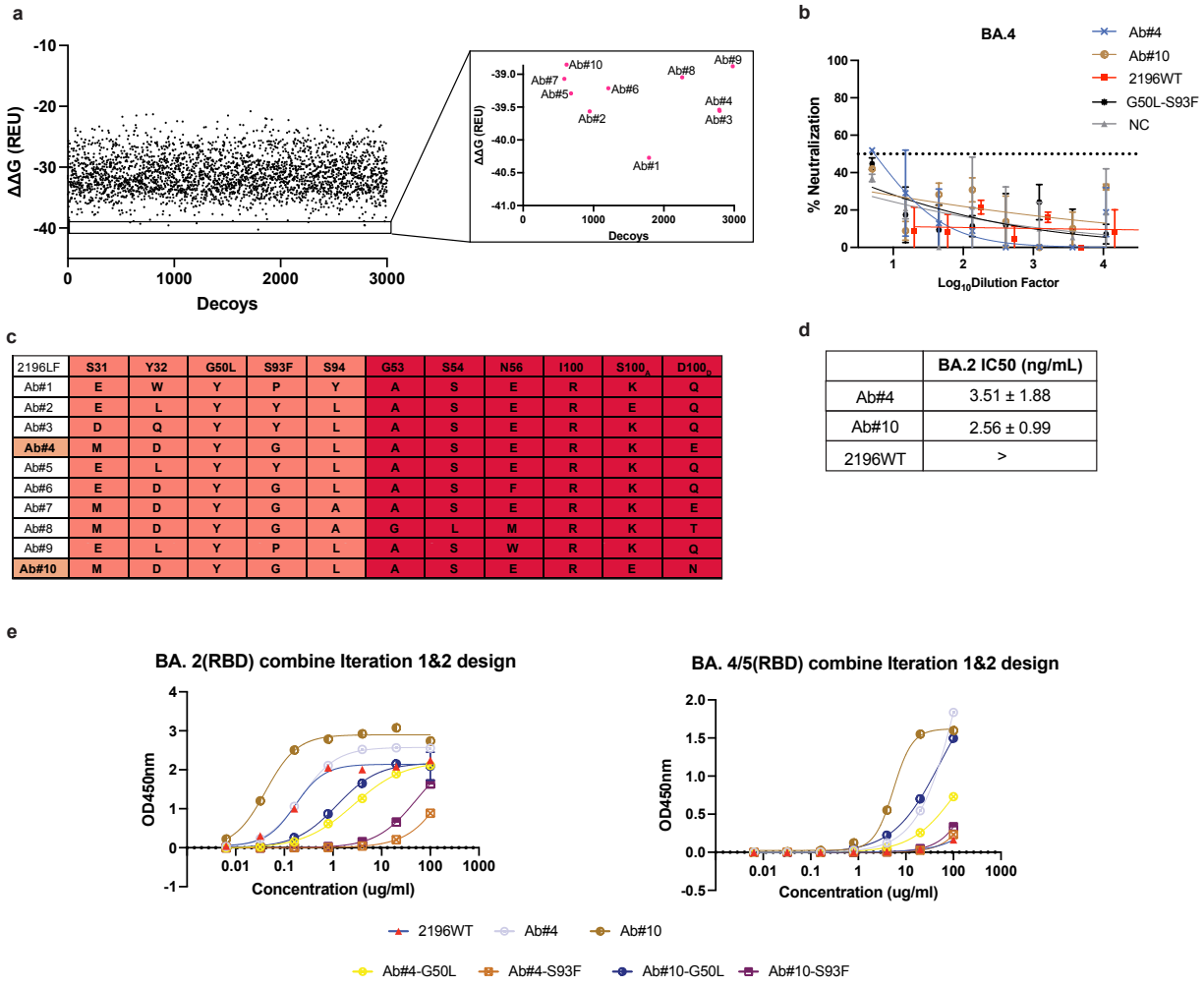


2196	BA.2 IC50 (ng/mL)
G50L-S93F	10.94 ± 6.38
G50L	7.31 ± 2.3
G50L-S93Y	23.50 ± 9.31
2196WT	>

Extended Data Fig. 2 | Iteration 1 design supplemental data. a. The ΔΔG distribution of decoys from Iteration 1 design on 2196 and 2130. Selected designs are colored in red and labeled. b. ELISA binding of Iteration 1 designed antibodies against BA.4/5 RBD. Each curve and data point are colored as shown in legend. Curves represent a four-parameter logistic regression model fitted in GraphPad Prism with two replicates. c. Half-maximal inhibitory concentration (IC50) of neutralization of Iteration 1 designed antibody against BA.2 pseudovirus.



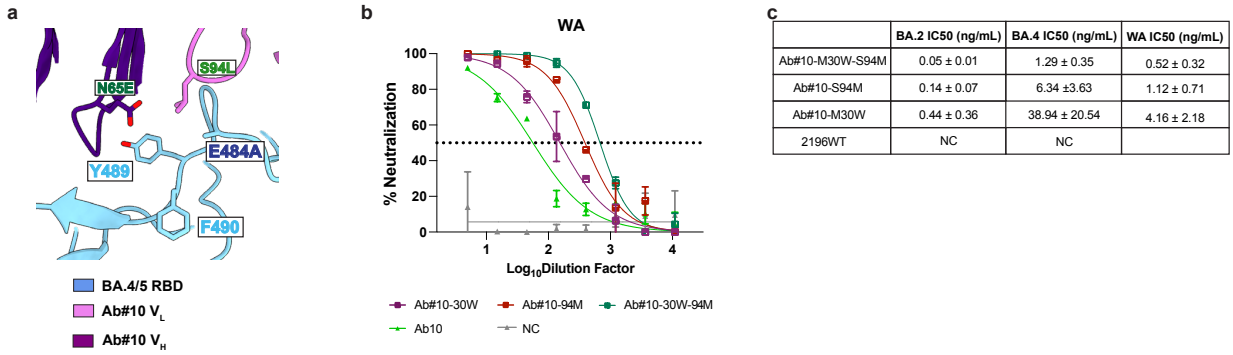
Extended Data Fig. 3 | Cryo-EM density maps of other two antibodies from Iteration 1 design in complex with BA.1-S. a. Global density map of BA.1-S/2196-S93Y/2130WT and local density map of BA.1-RBD/2196-S93Y/2130WT. **b.** Global density map of BA.1-S/2196-S93Y and local density map of BA.1-RBD/2196-S93Y. Corresponding FSC curves are next to the density maps.



2196LF	S31	Y32	G50L	S93F	S94	G53	S54	N56	I100	$S100_A$	$D100_D$
Ab#1	E	W	Y	P	Y	A	S	E	R	K	Q
Ab#2	E	L	Y	Y	L	A	S	E	R	E	Q
Ab#3	D	Q	Y	Y	L	A	S	E	R	K	Q
Ab#4	M	D	Y	G	L	A	S	E	R	K	E
Ab#5	E	L	Y	Y	L	A	S	E	R	K	Q
Ab#6	E	D	Y	G	L	A	S	F	R	K	Q
Ab#7	M	D	Y	G	A	A	S	E	R	K	E
Ab#8	M	D	Y	G	A	G	L	M	R	K	T
Ab#9	E	L	Y	P	L	A	S	W	R	K	Q
Ab#10	M	D	Y	G	L	A	S	E	R	E	N

	BA.2 IC50 (ng/mL)
Ab#4	3.51 ± 1.88
Ab#10	2.56 ± 0.99
2196WT	>

Extended Data Fig. 4 | Iteration 2 design supplementary data. a. ΔΔG values of Iteration 2 decoys. Zoom-in view shows scoring of our selected top 10 candidates. The selected candidates are indicated in red. **b.** Pseudoviral neutralization ofAb#4 and Ab#10 against BA.2 or BA.4 pseudoviruses. Curves represent a four-parameter logistic regression model fitted in GraphPad Prism with two replicates. Neutralization data has been repeated in 3 assays. **c.** Chart shows mutated residues of designed positions of the 10 candidates. Light chain positions are shown in salmon and heavy chain positions are shown in red. Ab#4 and Ab#10 are highlighted in light brown. **d.** IC50 values of Ab#4 and Ab#10 neutralizing of BA.2 pseudovirus. **e.** ELISA binding of antibodies from Iterations 1 and 2 combined designs against BA.1/2 RBD. Each curve and data point are labeled as described in legend.



c

	BA.2 IC50 (ng/mL)	BA.4 IC50 (ng/mL)	WA IC50 (ng/mL)
Ab#10-M30W-S94M	0.05 ± 0.01	1.29 ± 0.35	0.52 ± 0.32
Ab#10-S94M	0.14 ± 0.07	6.34 ±3.63	1.12 ± 0.71
Ab#10-M30W	0.44 ± 0.36	38.94 ± 20.54	4.16 ± 2.18
2196WT	NC	NC	

Extended Data Fig. 5 | Iteration 3 design supplementary data. a. We identified the S94L position on the BA.4-RBD/Ab#10 model as an additional design opportunity. **b.** Pseudoviral neutralization of selected Iteration 3 designed antibodies against WA pseudovirus. Curves represent a four-parameter logistic regression model fitted in GraphPad Prism with two replicates. Neutralization data has been repeated in 3 assays. **c.** IC50 values of Iteration 3 designed antibodies against BA.2, BA.4 and WA pseudoviruses.

a

General Data Processing Scheme

Raw Movies

↓

MotionCorr2
Ctffind4

↓

Extraction bin4
2D Classification

↓

Ab initio (CryoSPARC)
3D Classification (Relion)

↓

Re-extraction bin2
3D Autorefinement

↓

Re-extraction in original pixel size
3D Autorefinement
Bayesian Polishing
Ctf Refinement

↓

Transfer particles to CryoSPARC
Non-Uniform Refinement (Consensus)

↓

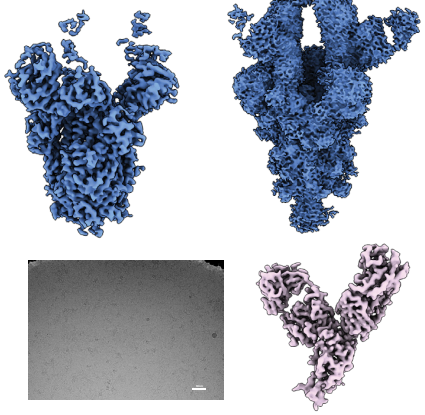
Particle subtraction
Local Refinment
EMready

b

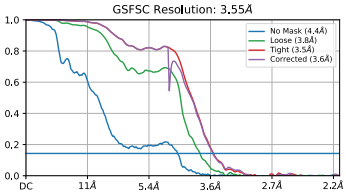
BA.1-S/2130G/2196-G50L-S93F

High contour

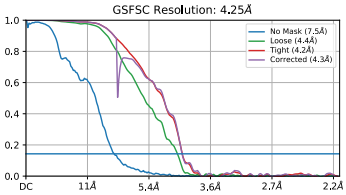
Low contour



Global Non-Uniform Refinment
BA.1-S/2130G/2196-G50L-S93F
GSFSC Resolution: 3.55Å



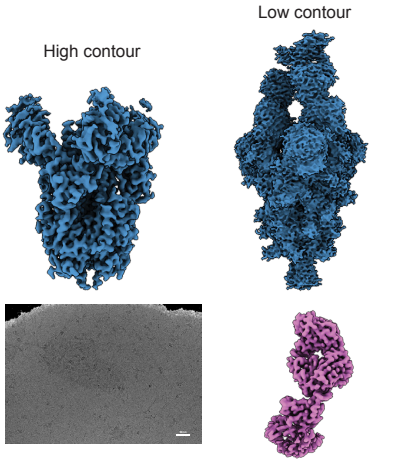
Local Refinment
BA.1-RBD/2130G/2196-G50L-S93F
GSFSC Resolution: 4.25Å



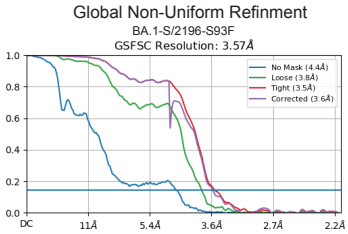
BA.1-S/2196-G50L-S93F

High contour

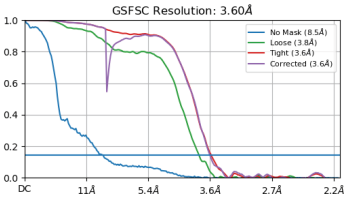
Low contour



Global Non-Uniform Refinment
BA.1-S/2196-S93F
GSFSC Resolution: 3.57Å



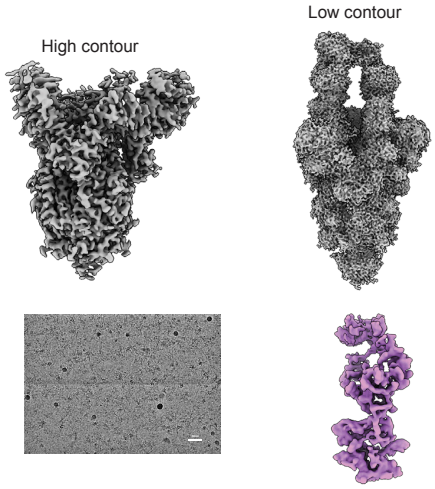
Local Refinment
BA.1-RBD/2196-S93F
GSFSC Resolution: 3.60Å



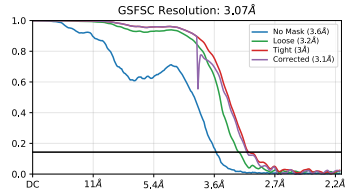
BA.4-S/Ab#10-M30W-S94M

High contour

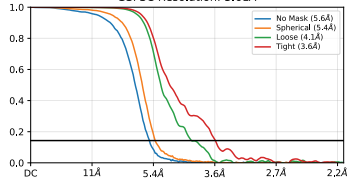
Low contour



Global Non-Uniform Refinment
BA.4-S/Ab#10-30W-94M
GSFSC Resolution: 3.07Å



Local Refinment
BA.4-S/Ab#10-30W-94M
GSFSC Resolution: 3.61Å



Extended Data Fig. 6 | Cryo-EM data processing scheme and additional density maps. a. General data processing scheme. **b.** BA.1-S/2196-G50L-S93F/2130-S30$_{B}$G, BA.1-S/2106-S93F and BA.4-S/Ab#10-M30W-L94M global density maps, local density maps and corresponding GSFSC curve plots.