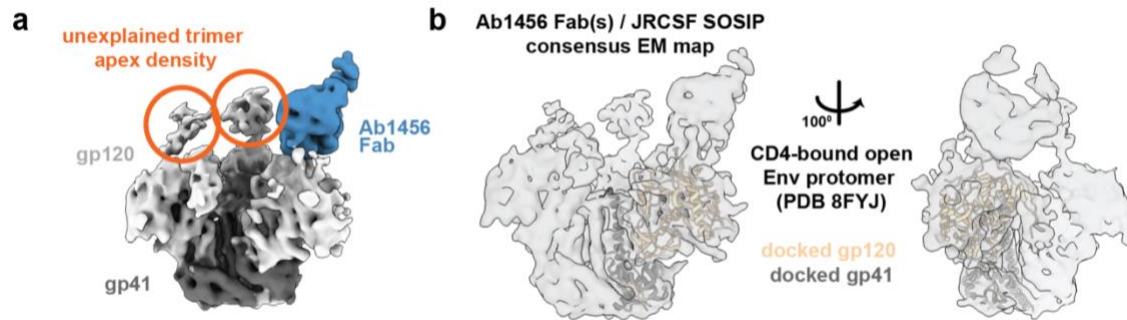


Supplementary Figures



Supplementary Figure 1: Analysis of Ab1456 Fab(s) / JRCSF SOSIP consensus map. a, Unexplained density present at the trimer apex of the consensus EM map. **b,** Consensus EM density of the Ab1456 / JRCSF structure including a cartoon representation of docked coordinates of protomer A gp120 and gp41 from an Env heterotrimer (HT2) bound by two copies of soluble CD4 (PDB 8FYJ).

JRCSF SOSIP / Ab1456 Fab dataset (page 1)

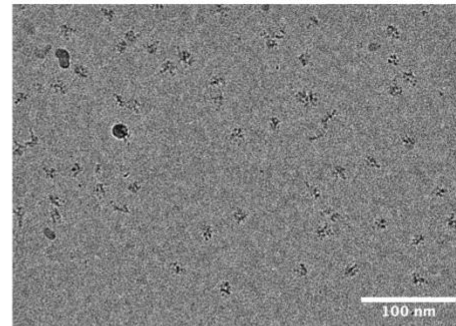
2666 Movies
Motion correction
CTF estimation
Curate micrographs (2457 accepted)

Picking and Extracting Particles

[live] Blob picker / Extract (360px)
[J137-J140] Iterative 2D classification / Select 2D classes
[J153] Manually curate particles within 100 micrographs
[J158] Topaz Train (expected number of particles = 50)
[J159] Topaz Extract (radius of extracted region = 30)
[J163] Extraction (80,319 particles; down-sampled; 360 → 180px)

[J165] Ab-Initio Reconstruction (C1; 3 classes)
[J166] NU-Refinement (45,843 particles)

Example micrograph

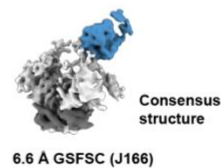


RELION processing of all extracted particles

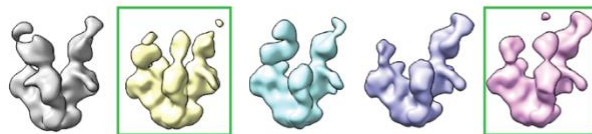
Import particles & NU-Refinement to RELION
[Job005] 3D classification (15 classes; mask = 200Å)



cryoSPARC consensus structure



[Job009] Selected 3D classes (26,720 particles)
[Job010] 3D classification (5 classes; mask = 200Å)



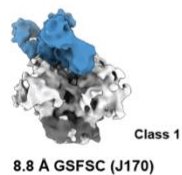
5,728 particles 5,701 particles 4,353 particles 5,638 particles 5,300 particles

[Job011] Selected 3D classes (11,001 particles)
[Job015] 3D classification (3 classes; mask = 240Å)



4,296 particles 2,565 particles 4,140 particles

CryoSPARC processing
Re-extract particles (down-sampled; 360 → 180px)
Ab-Initio (C1)
Non-uniform Refinement



CryoSPARC processing
Re-extract particles (down-sampled; 360 → 180px)
Ab-Initio (C1)
Non-uniform Refinement



Supplementary Figure 2 (page 1 of 3): Data processing of the Ab1456 Fab / JRCSF SOSIP dataset.

Selected all particles not in Class 1 or Class 2 (71,883 particles)

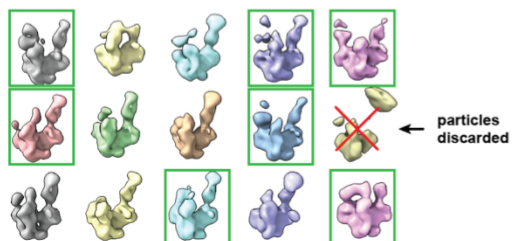
RELION processing

[Job038] 3D classification (15 classes; mask = 240Å)



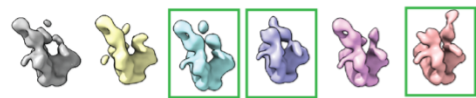
Selected classes which appear to contain Fabs bound, but trimer not fully open
- 42,180 particles

[Job047] 3D classification (15 classes; mask = 240Å)



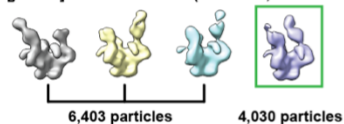
Selected classes which appear to contain particles with 2 Fabs bound
- 19,615 particles

[Job049] 3D classification (6 classes; mask = 240Å)



Selected classes which appear to contain 2 Fabs bound to "open" protomers
- 10,433 particles

[Job053] 3D classification (4 classes; mask = 240Å)

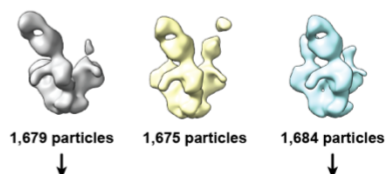


CryoSPARC processing
Re-extract particles (down-sampled; 360 → 180px)
Ab-Initio Reconstruction (C1)
Non-uniform Refinement



Selected class in which trimer appeared fully open
- 5,038 particles

[Job041] 3D classification (3 classes; mask = 240Å)

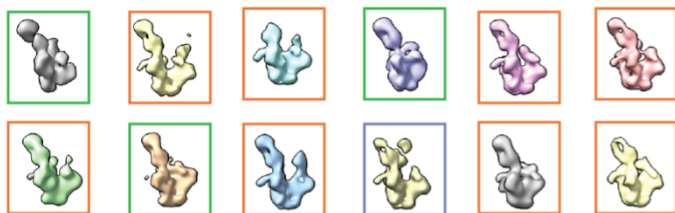


CryoSPARC processing of selected classes
Re-extract particles (down-sampled; 360 → 180px)
Ab-Initio Reconstructions (C1)
Non-uniform Refinements

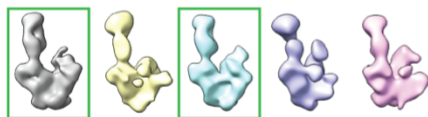


Supplementary Figure 2 (page 2 of 3): Data processing of the Ab1456 Fab / JRCSF SOSIP dataset.

[job065] selected classes particles without a **green box** or **red X** from job047, job049, and job053 (37,309 particles)
 [job066] 3D classification (12 classes; mask = 240Å)



Selected job066 **classes boxed in green** (9,905 particles)
 [job070] 3D classification (5 classes; mask = 240Å)

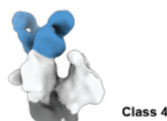


Selected classes (5,306 particles)
 Re-extract particles in cryoSPARC (down-sampled; 360 → 180px)
 Ab-Initio (C1) → Non-uniform refinement



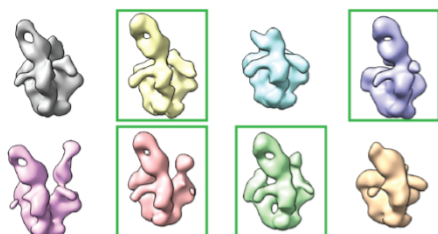
14 Å GSFSC (J288)

Selected job066 **class boxed in purple** (3,382 particles)
 Re-extract particles in cryoSPARC (down-sampled; 360 → 180px)
 Ab-Initio (C1) → Non-uniform refinement

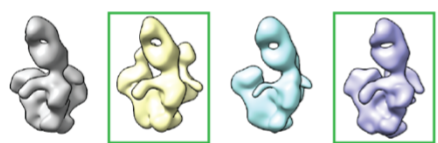


13 Å GSFSC (J279)

Selected job066 **classes boxed in orange** (24,022 particles)
 [job078] 3D classification (8 classes; mask = 240Å; 45 iterations)



Selected classes (11,930 particles)
 [job081] 3D classification (4 classes; mask = 240Å)

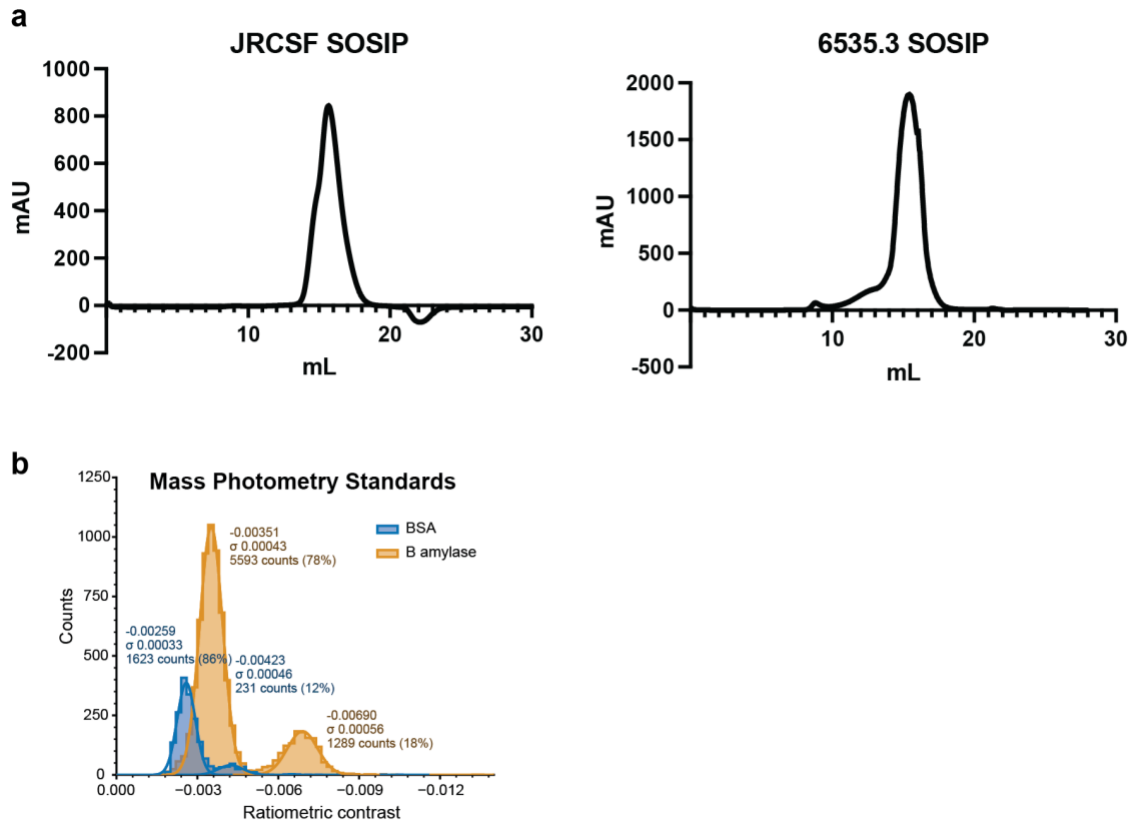


Selected classes (6,204 particles)
 Re-extract particles in cryoSPARC (down-sampled; 360 → 180px)
 Ab-Initio (C1) → Non-uniform refinement



12 Å GSFSC (J305)

Supplementary Figure 2 (page 3 of 3): Data processing of the Ab1456 Fab / JRCSF SOSIP dataset.



Supplementary Figure 3: Characterization of JRCSF and 6535.3 SOSIP Envs. a, SEC profiles for the purification of JRCSF SOSIP (left) and 6535.3 SOSIP (right). **b,** Experimental histograms for the mass standards used in mass photometry experiments of SOSIP Envs and Fab-SOSIP complexes.

6535.3 SOSIP / Ab1271 Fab dataset

Picking and Extracting Particles

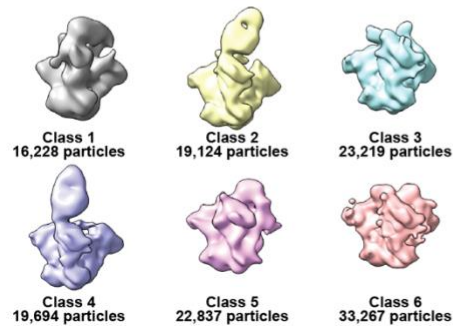
[J50] Manually picked particles within 25 micrographs
 [J53] Topaz Train (expected number of particles = 75)
 [J58] Topaz Extract (from 201 micrographs)
 [J61] Manually curate particles within 201 micrographs
 [J63] Topaz Train (expected number of particles = 100)
 [J66] Topaz Extract
 [J69] Extraction (down-sampled; 300 → 150 px)
 [J70/J71] 2D classification / Select 2D classes
 [J112] Extract from Micrographs (360 px) → 134,369 particles

[J117] Ab-Initio (C1)

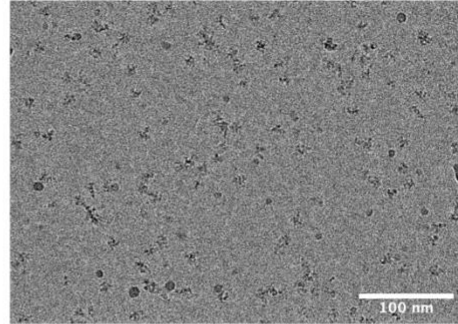
[J118] Homogeneous refinement (C1)

RELION processing

Import particles & consensus refinement
 3D classification (6 classes)

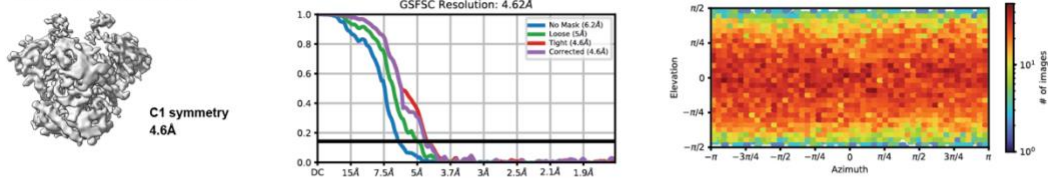


Example micrograph



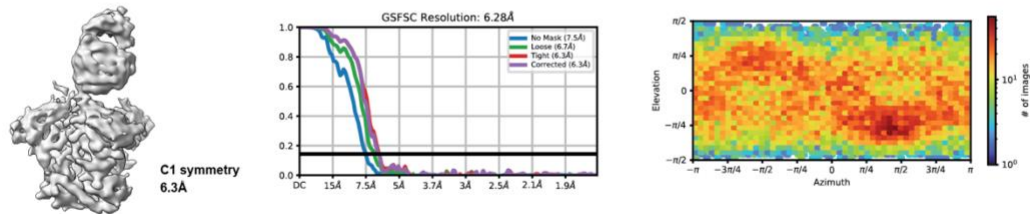
CryoSPARC processing of unbound trimer
 Re-extract particles from RELION Class 3 + Class 6
 Ab-Initio (C1)

Non-uniform Refinement

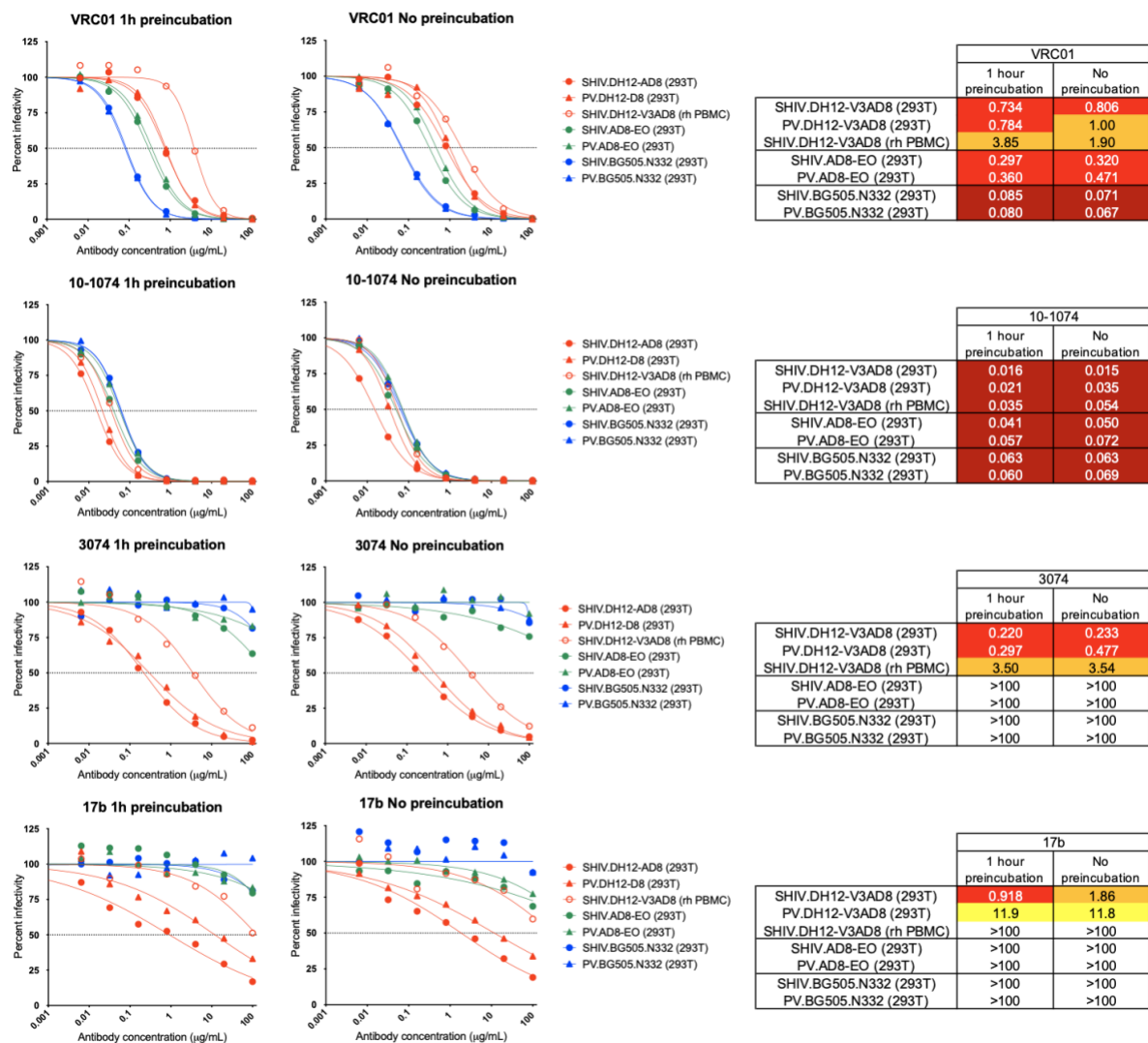


CryoSPARC processing of Ab1271 Fab-bound trimer
 Re-extract particles from RELION Class 2 + Class 4
 Ab-Initio (C1)

Non-uniform Refinement



Supplementary Figure 4: Data processing of the Ab1271 Fab / 6535.3 SOSIP dataset.



Supplementary Figure 5: Omission of the antibody-virus preincubation step does not affect the potency of bNAbs or antibodies recognizing non-closed Env trimers. The sensitivity of viruses expressing the DH12-V3AD8 (red), AD8-EO (green) and BG505.N332 (blue) Envs to neutralization by VRC01, 10-1074, 3074, and 17b in a standard TZMbl assay including a 1 hour antibody and virus preincubation step^{41,42} and a modified assay with no preincubation are shown. Neutralization curves for the indicated mAbs are shown on the left (dashed lines indicate 50% reduction in virus infectivity), with the corresponding 50% inhibitory concentrations (IC₅₀) in μg/mL shown on the right (coloring indicates relative neutralization potency). Pseudoviruses (PV) as well as replication-competent SHIVs derived either by HEK293T transfection (293T) or following propagation in rhesus macaque PBMC (rh PBMC) were tested. Note that similar to Ab1456 (Fig. 4a), the slopes of the neutralization curves for the linear V3 mAb 3074 and the CD4-induced mAb 17b are more shallow slopes than slopes of the CD4bs bNAb VRC01⁴⁶ and the V3 glycan bNAb 10-1074.⁴⁵

Ab1456 Fab / JRC5F SOSIP										Ab1271 Fab / 6535.3 SOSIP	
Data collection conditions											
Microscope	Titan Krios									Titan Krios	
Voltage (kV)	300									300	
Camera	Gatan K3 6k x 4k									Gatan K3 6k x 4k	
Energy filter slit width (eV)	10									10	
Magnification	105,000x									105,000x	
Frames per movie	40									40	
Recording mode	counting									counting	
Dose rate (e ⁻ /pixel/s)	25									26	
Total electron dose (e ⁻ /Å ²)	60									60	
Defocus range (µm)	-1 to -3									-1 to -3	
Pixel size (Å)	0.416 (super resolution)									0.416 (super resolution)	
Micrographs collected	2664									2898	
Micrographs used	2456									2756	
Total extracted particles	80,319									134,369	
	Consensus	Class 1	Class 2	Class 3	Class 4	Class 5	Class 6	Class 7	Class 8	Unbound 6535.3 SOSIP	Ab1271 Fab-bound 6535.3 SOSIP
EMD	45944	45945	45946	45947	45948	45949	45950	45951	45952	45942	45943
Particles in class	45,843	4296	4140	4030	3382	1684	5306	6204	1679	56,486	38,818
Symmetry	C1	C1	C1	C1	C1	C1	C1	C1	C1	C1	C1
Map resolution (Å)	6.6	8.8	10	13	13	10	14	12	13	4.6	6.3
FSC Threshold	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143	0.143

Supplementary Table 1: EM data collection and processing statistics.