Cell Reports, Volume *43*

Supplemental information

Design of soluble HIV-1 envelope trimers free of covalent gp120-gp41 bonds with prevalent native-like conformation

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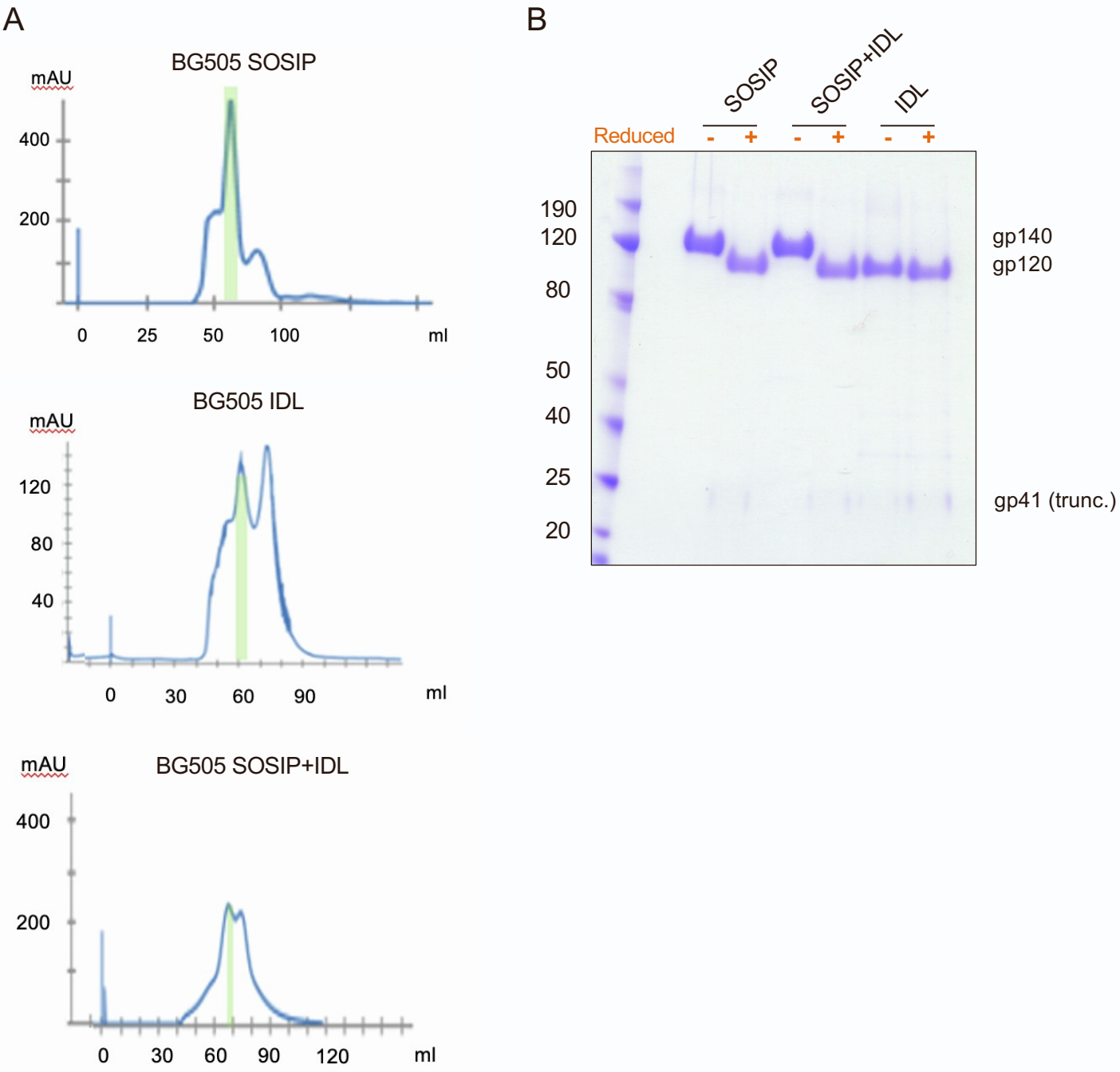

Figure S1. Characteristics of recombinant BG505 SOSIP and IDL trimers (related to Fig. 2).
(A) Analysis of BG505 SOSIP, IDL, and combined SOSIP+IDL trimers by size-exclusion chromatography (SEC). The trimer peaks are highlighted by the green shade; the peaks on the right side of the trimers correspond to the monomers, while the peaks on the left side correspond to larger aggregates.
(B) Analysis of BG505 SOSIP, IDL, and combined SOSIP+IDL trimers by SDS PAGE under reducing and non-reducing conditions.

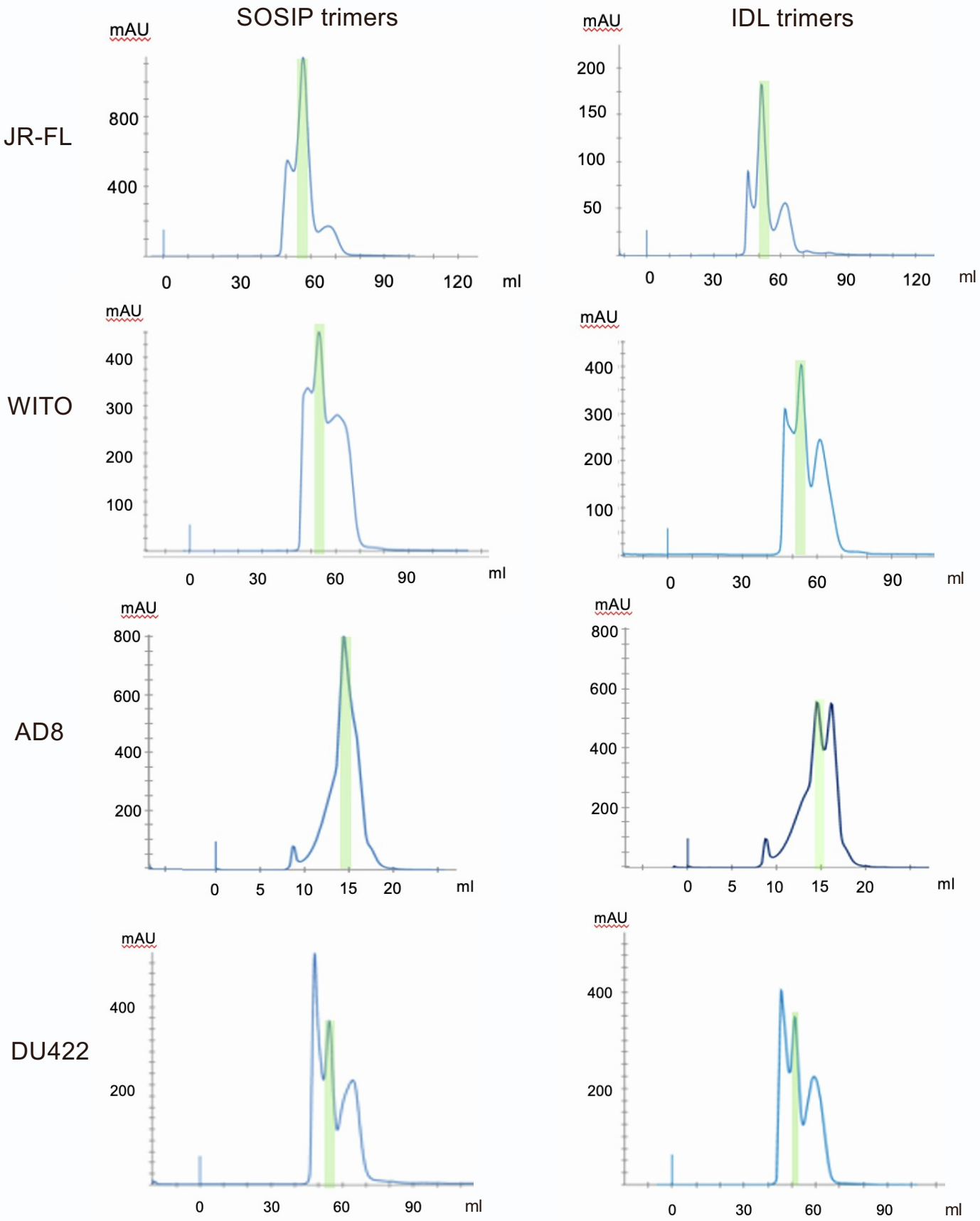

Figure S2. Analysis of SOSIP and IDL trimers from clade B (JR-FL, WITO, AD8) and clade C (DU422) by size-exclusion chromatography (SEC) (related to Fig. 3). The trimer peaks are highlighted by the green shade; the peaks on the right side of the trimers correspond to the monomers, while the peaks on the left side correspond to larger aggregates.

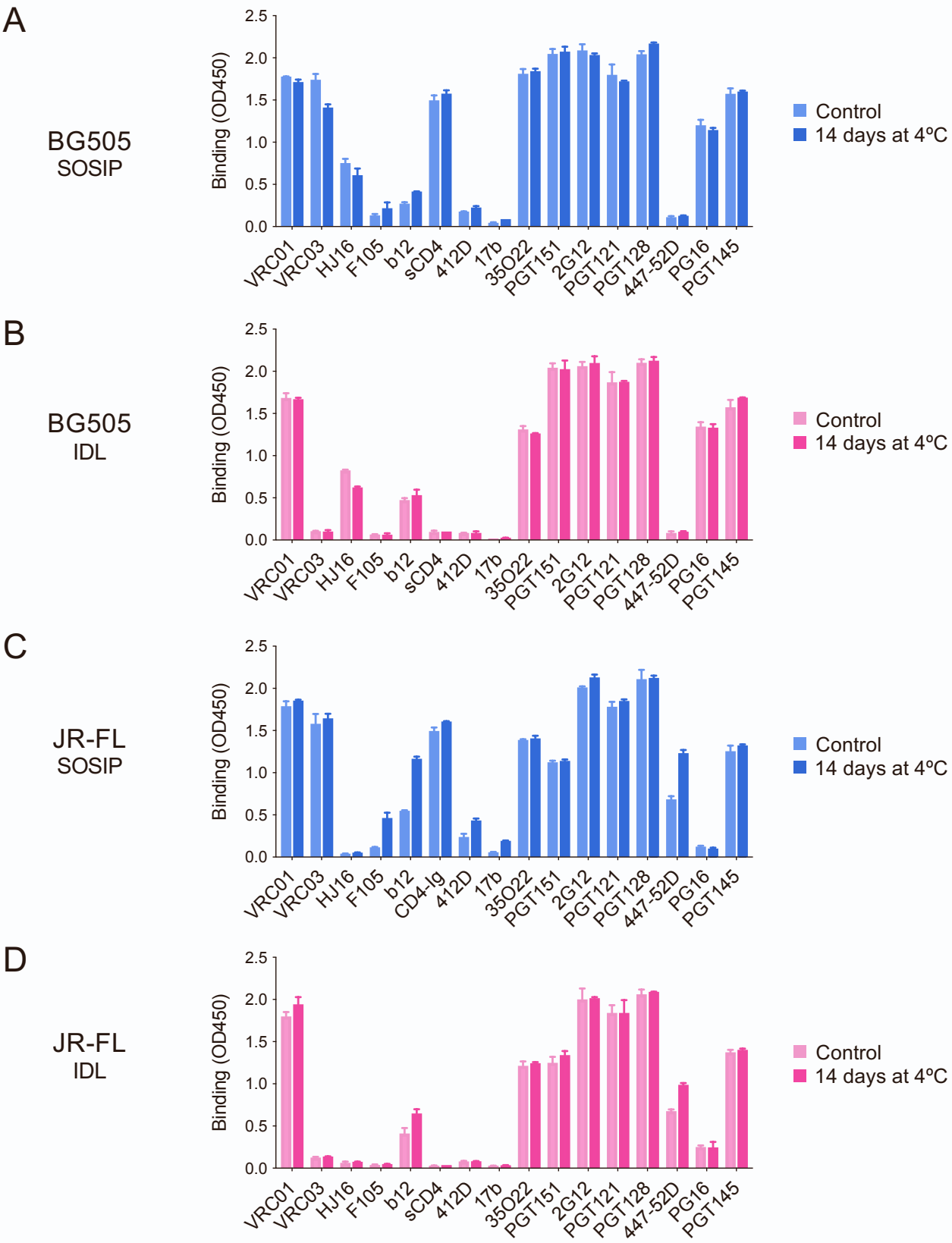

Figure S3. Long-term stability of BG505 and JR-FL SOSIP and IDL trimers after long-term incubation in the cold (related to Figs. 2 and 3). Reactivity with a panel of bNAbs and nNAbs tested by ELISA.

(A) Phenotypic profile of BG505 SOSIP trimer tested fresh or after 14 days at 4ºC.
(B) Phenotypic profile of BG505 IDL trimer tested fresh or after 14 days at 4ºC.
(C) Phenotypic profile of JR-FL SOSIP trimer tested fresh or after 14 days at 4ºC.
(D) Phenotypic profile of JR-FL SOSIP trimer tested fresh or after 14 days at 4ºC.
The data represent the mean (± standard error of the mean) of two technical replicates.

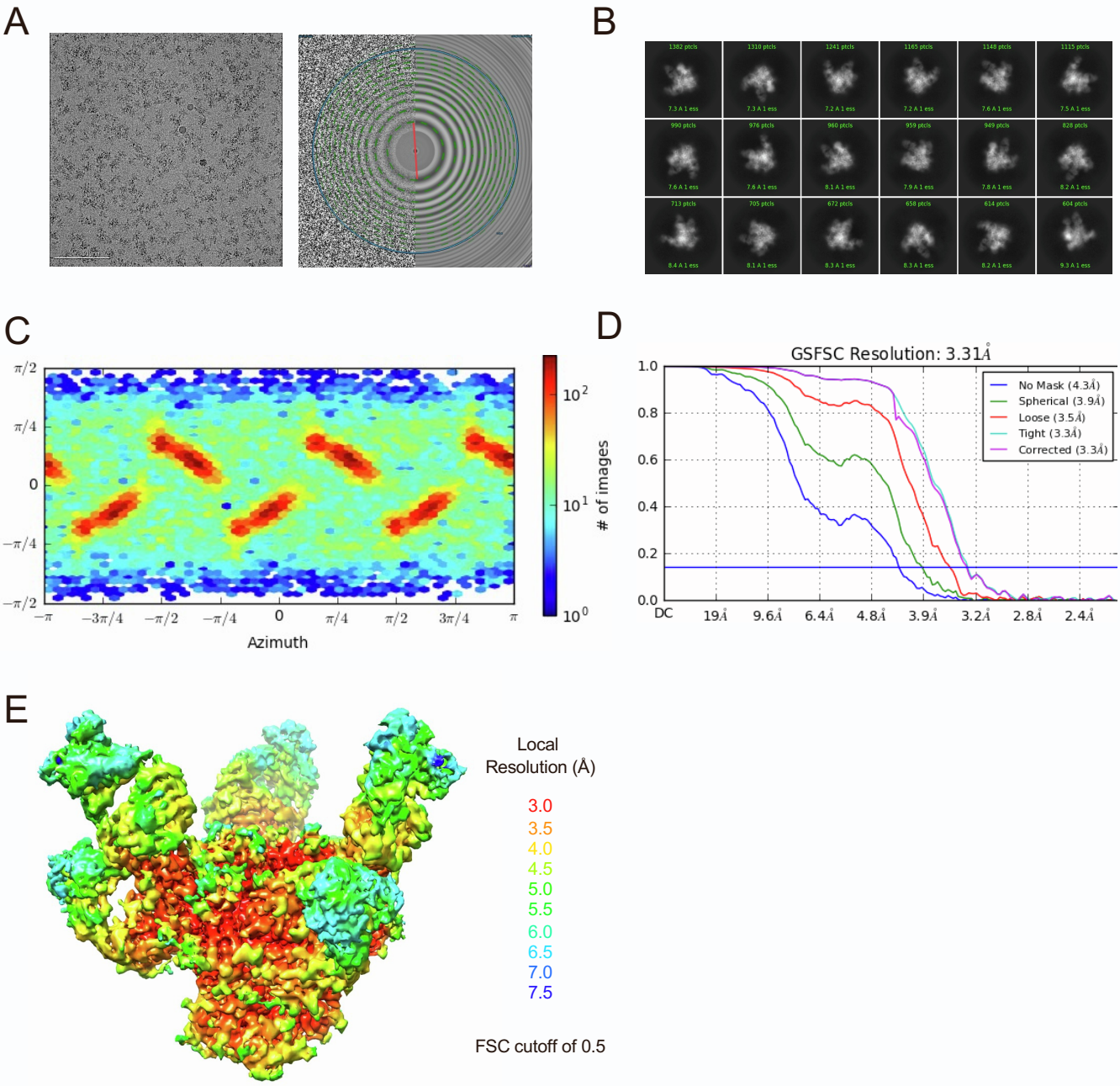

Figure S4. Cryo-EM details of HIV-1 Env BG505 IDL trimer in complex with 3BNC117 and 10-1074 Fabs statistics (related to Fig. 4).

(A) Representative micrograph and CTF of the micrograph are shown.
(B) Representative 2D class averages are shown.
(C) The orientations of all particles used in the final refinement are shown as a heatmap.
(D) The gold-standard Fourier shell correlation resulted in a resolution of 3.3 Å using non-uniform refinement with C3 symmetry.
(E) The local resolution of the full map is shown generated through cryoSPARC using an FSC cutoff of 0.5.

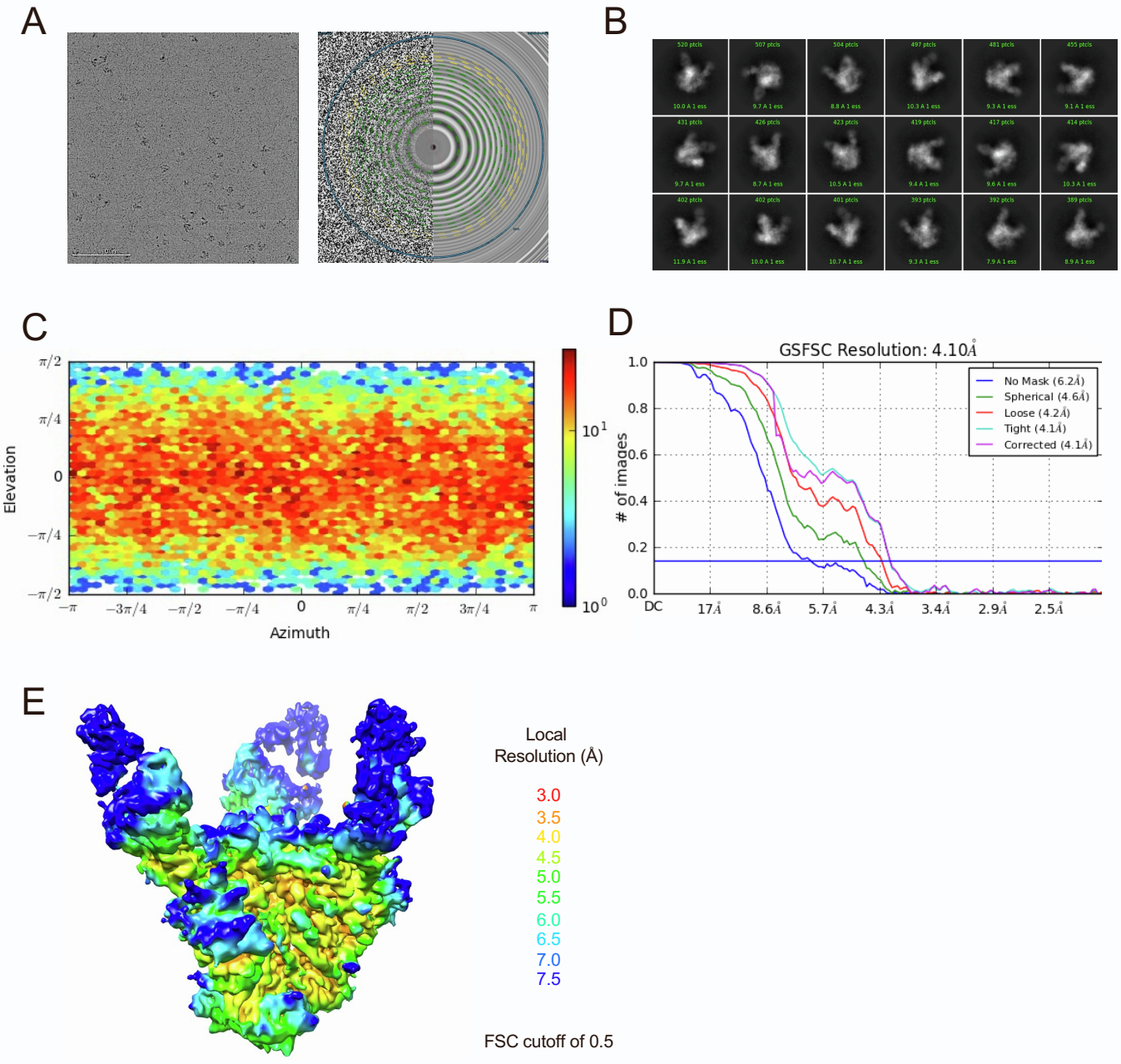

Figure S5. Cryo-EM details of HIV-1 Env JR-FL IDL trimer in complex with PGT122 Fab (related to Fig. 5).

(A) Representative micrograph and CTF of the micrograph are shown.
(B) Representative 2D class averages are shown.
(C) The orientations of all particles used in the final refinement are shown as a heatmap.
(D) The gold-standard Fourier shell correlation resulted in a resolution of 4.1 Å using non-uniform refinement with C3 symmetry.
(E) The local resolution of the full map is shown generated through cryoSPARC using an FSC cutoff of 0.5.

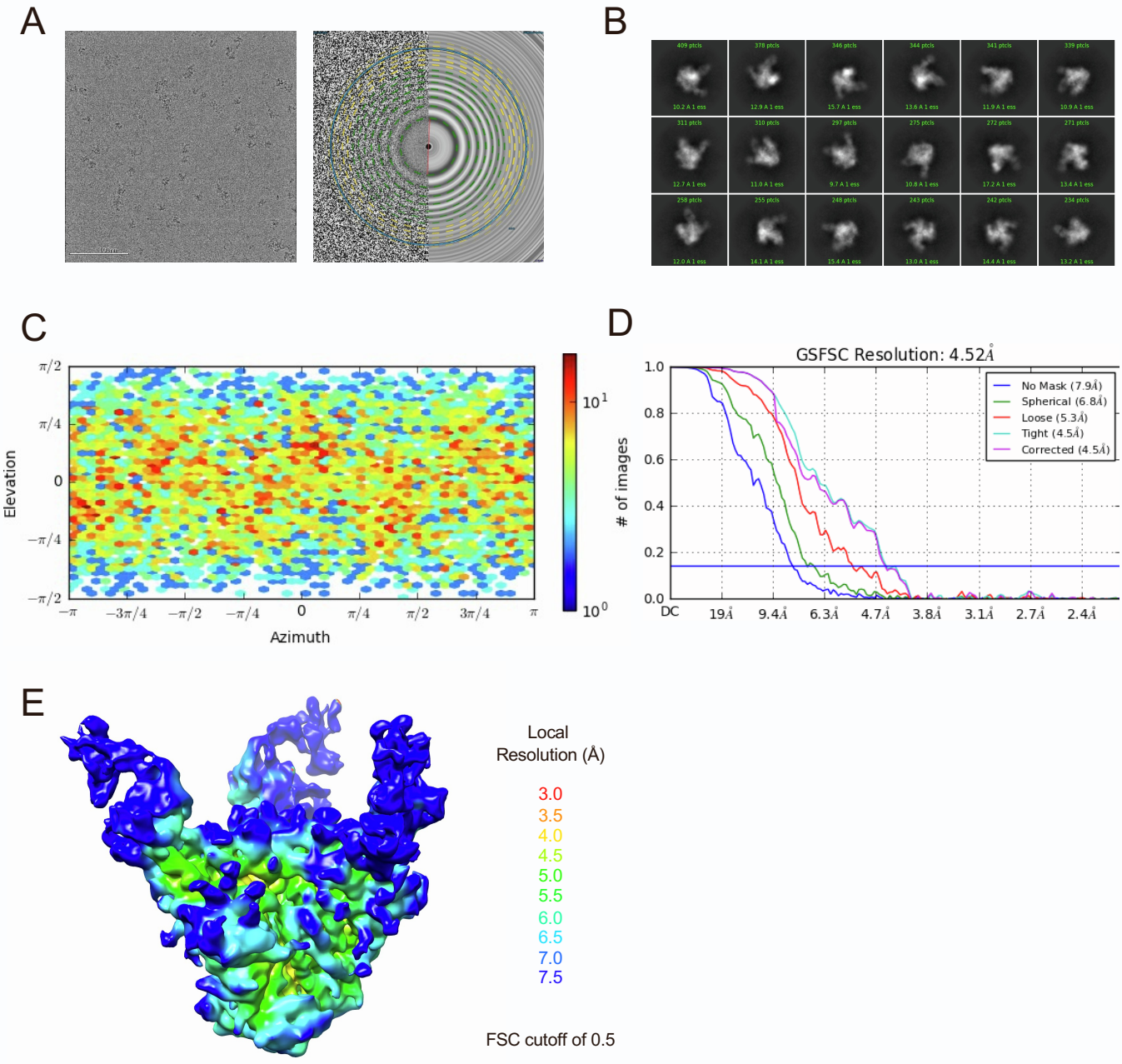

Figure S6. Cryo-EM details of HIV-1 Env WITO IDL trimer in complex with PGT122 Fab (related to Fig. 6).

(A) Representative micrograph and CTF of the micrograph are shown.
(B) Representative 2D class averages are shown.
(C) The orientations of all particles used in the final refinement are shown as a heatmap.
(D) The gold-standard Fourier shell correlation resulted in a resolution of 4.5 Å using non-uniform refinement with C3 symmetry.
(E) The local resolution of the full map is shown generated through cryoSPARC using an FSC cutoff of 0.5.

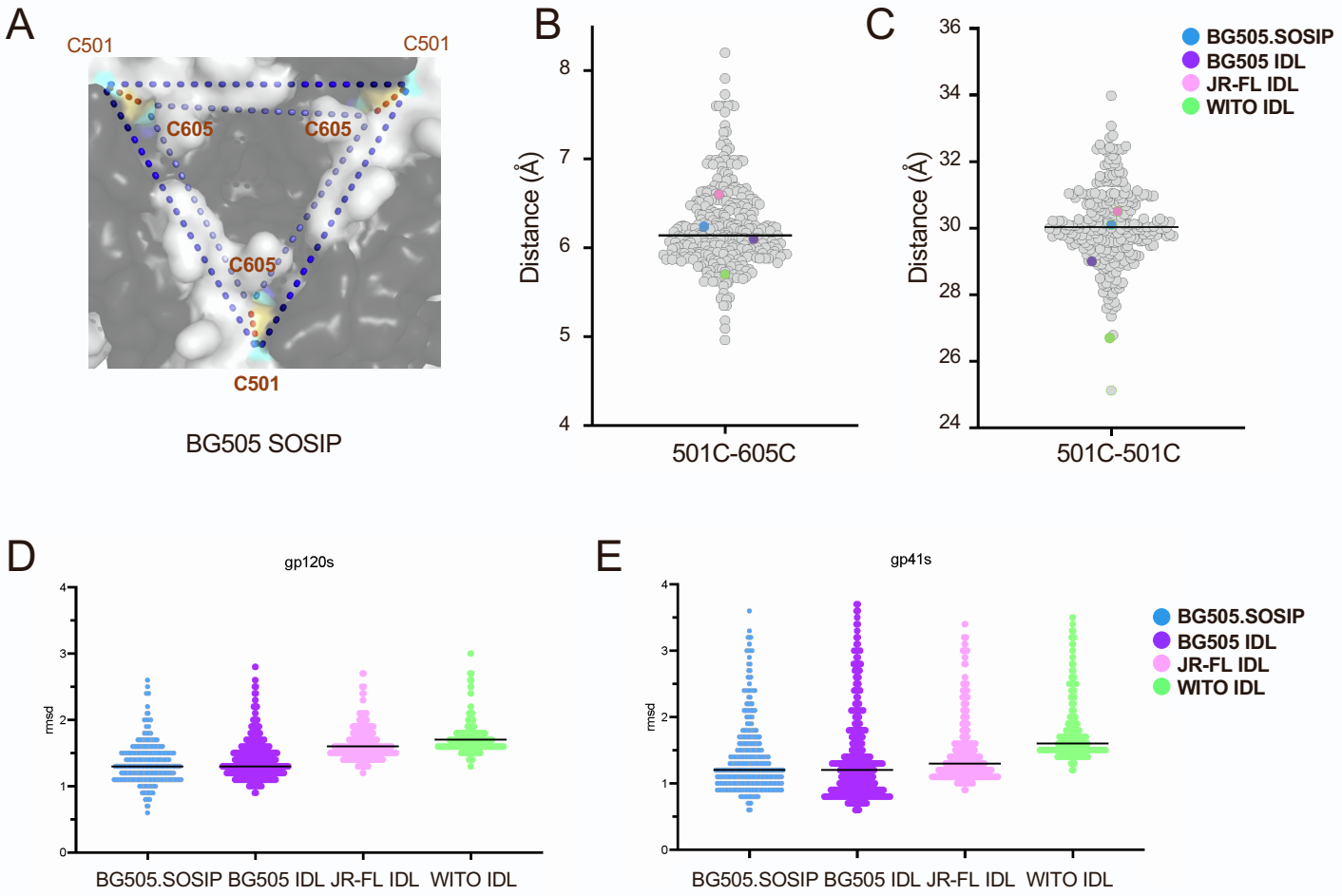

Figure S7. IDL trimers show similar conformational and interprotomer distances as SOSIP trimers (related to Figs. 4, 5 and 6).

(A) Schematic diagram showing the distance between residue 501 and residue 605 alpha carbons from different protomers. The BG505 SOSIP trimer (PDB Code: 5V8M) was used as a model.

(B) Measured distances from all closed Env trimers in the PDB are shown for the distance from residue 501-605.

(C) Average measured distances from all closed Env trimers in the PDB are shown for the distances from residue 501 of one protomer to 501 of the adjacent protomers.

(D) RMSD values for alignments of the gp120 of all closed Env trimers in the PDB are shown for BG505 SOSIP (5V8M) and the three IDL trimers. Single protomers were used for C3 symmetric structures while all three protomers were used for C1 structures. Structures that were rigid-body docked with a trimer already included were removed to reduce redundancy.

(E) RMSD values for alignments of the gp41 of all closed Env trimers in the PDB are shown for BG505 SOSIP (5V8M) and the three IDL trimers.

Table S1. Cryo-EM data collection and refinement statistics (related to Figs. 4, 5 and 6).

	BG505 IDL with 3BNC117 and 10-1074	JR-FL IDL with PGT122 Fab	WITO IDL with PGT122 Fab
EMDB ID	44484	44482	44491
PDB ID	9BEW	9BER	9BF6
Data Collection			
Microscope	FEI Titan Krios	FEI Titan Krios	FEI Titan Krios
Voltage (kV)	300	300	300
Electron dose ($e^-/Å^2$)	63.90	70.73	70.77
Detector	Gatan K2 Summit	Gatan K2 Summit	Gatan K2 Summit
Pixel Size (Å)	1.096	1.073	1.073
Defocus Range (μm)	-0.16 to -3.4	-0.4 to -4.0	-0.1 to -4.7
Magnification	105000	22500	22500
Reconstruction			
Software	cryoSparcV2.14	cryoSparcV2.14	cryoSparcV2.14
Particles	50,380	31,576	12,615
Symmetry	C3	C1	C3
Box size (pix)	352	320	352
Resolution (Å) ($FSC_{0.143}$)	3.3	4.1	4.5
Refinement			
Software	Phenix 1.21	Phenix 1.21	Phenix 1.21
Protein residues	3087	2416	2367
Chimera CC	0.89	0.86	0.82
EMRinger Score	3.32	1.39	0.47
R.m.s. deviations:			
Bond lengths (Å)	0.003	0.003	0.003
Bond angles (°)	0.532	0.716	0.670
Validation			
Molprobity score	1.31	1.78	1.68
Clash score	2.73	5.81	3.80
Favored rotamers (%)	1.01	0.24	0.10
Ramachandran:			
Favored regions (%)	96.33	92.53	91.4
Disallowed regions (%)	0.00	0.08	0.17

Table S2. Parameters and statistics of three-state Gaussian model FRET histograms (related to Fig. 7).

Env trimer	Curve fitting (R^2)	RMSE	State 1	State 2	State 3
			μ: 0.1 ± 0.03	μ: 0.65 ± 0.03	μ: 0.3 ± 0.04
			σ: 0.08 ± 0.01	σ: 0.17 ± 0.02	σ: 0.1 ± 0.01
BG505 IDL	0.9758	9.3454^{-04}	37% ± 13%	33% ± 12%	30% ± 14%
BG505 SOSIP+IDL	0.9562	9.3820^{-04}	20% ± 7%	49% ± 9%	31% ± 11%
BG505 SOSIP[33]	0.9737	n.a.	13% ± 6%	62% ± 4%	25% ± 7%
BG505 virus Env[33]	0.9949	n.a.	45% ± 6%	24% ± 5%	31% ± 8%

Three-state Gaussian model-fitting of smFRET histograms of soluble BG505 IDL and BG505 SOSIP+IDL trimers. More than 100 smFRET traces for each trimer (130 for BG505 IDL and 165 for BG505 SOSIP+IDL) were combined and compiled into a FRET histogram, presented as mean ± SEM determined from 3 randomized groups of smFRET traces. The FRET histograms were further fitted into a sum of a previously well-defined 3-state Gaussian model: State 1 (μ ~0.1-FRET Gaussian), State 2 (μ ~0.65-FRET Gaussian), and State 3 (μ 0.3-FRET Gaussian). Each Gaussian distribution was presented as μ and σ (mean ± SD). The probability (percentage, %) of each conformational state was evaluated by the relative area under each Gaussian curve and presented as mean ± SEM. Curve fitting R^2 and RMSE (Root Mean Square Deviation) evaluate the level of fitting. Statistics of referenced BG505 SOSIP and BG505 virus Env are from ref. 33. n.a. = not available.