Cell

Supplemental Information

Broadly Neutralizing Antibody 8ANC195 Recognizes Closed and Open States of HIV-1 Env

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Supplemental Experimental Procedures

Protein Production and Purification. 8ANC195, 8ANC195$_{G52K5}$, 17b, PGT145, mG053, 2G12, CD4-Fc (domains 1 and 2 of human CD4 fused to human IgG1 Fc), human IgG1 Fc, and partially gl-reverted IgGs and/or Fabs were produced and purified as described in previous studies (Diskin et al., 2011; Scharf et al., 2014). Briefly, IgGs and Fabs were expressed by transient transfection in HEK293-6E cells with expression vectors containing the appropriate heavy and light chain genes. IgGs, CD4-Fc, and Fc were purified from cell supernatants using mAb Select Sure affinity chromatography (GE Healthcare). His-tagged Fabs were purified using Ni^{2+}-NTA affinity chromatography (GE Healthcare) and Superdex 200 16/60 size exclusion chromatography (SEC) (GE Healthcare).

sCD4 (domains 1 and 2; residues 1-186 of mature CD4) was produced in baculovirus-infected insect cells as described previously (Diskin et al., 2010; Scharf et al., 2014). 6x-His-tagged protein was expressed in Hi5 cells, captured using Ni^{2+}-NTA resin (GE Healthcare), and further purified using Superdex 200 16/60 SEC (GE Healthcare).

A gene encoding untagged BG505 SOSIP, a soluble gp140 trimer produced from HIV-1 clade A strain BG505, was constructed as described (Sanders et al., 2013) to include the 'SOS' substitutions (A501C$_{gp120}$, T605C$_{gp41}$), the 'IP' substitution (I559P$_{gp41}$), the *N*-linked glycan site at residue 332$_{gp120}$ (T332N$_{gp120}$); changing the gp120-gp41 cleavage site to 6R (REKR to RRRRRR), and introducing a stop codon after residue 664$_{gp41}$ (Env numbering according to HX nomenclature). HEK293-6E cells treated with 5 μM kifunensine (Sigma) were co-transfected with plasmids encoding BG505 SOSIP and soluble furin at a ratio of 4:1. BG505 SOSIP protein was harvested from cell supernatants using a 2G12 immunoaffinity column made by covalently coupling 2G12 IgG monomer to an NHS-activated Sepharose column (GE Healthcare). After elution with 3M $MgCl_2$ followed by immediate buffer exchange into Tris-buffered saline pH 8.0 (TBS), trimers were purified using Superdex 200 16/60 SEC (GE Healthcare). The trimer fractions were pooled and re-purified using Superose 6 10/300 SEC (GE Healthcare).

Crystallization

Samples for crystallography were produced by incubating BG505 SOSIP with a 3-fold molar excess of sCD4 for 16 hrs at 4°C, followed by purification using Superdex 200 10/300 SEC (GE Healthcare). The resulting complex was incubated with a 3-fold molar excess of 8ANC195$_{G52K5}$ Fab and purified using Superose 6 10/300 SEC (GE Healthcare). Fractions corresponding to ternary complex were pooled and concentrated to a final OD of 14.0. Crystals of 8ANC195$_{G52K5}$ Fab–BG505 SOSIP complex (space group $P2_1$ *a* = 117.74 Å, *b* = 195.22 Å, *c* = 119.09 Å; β = 101.6°; one molecule per asymmetric unit) were obtained by combining 0.2 μL of crystallization sample with 0.2 μL of 100 mM Tris pH 8.0, 15% PEG 3,350 and 2% 1,4-dioxane at 20°C. The crystals were cryoprotected in mother liquor solution supplemented with 25% ethylene glycol and plunge-frozen in liquid N_2.

Crystallographic Data Collection, Structure Determination, and Refinement

X-ray diffraction data were collected at the Argonne National Laboratory Advanced Photon Source (APS) beamline 23-ID-D using a Pilatus3 6M detector. The data were indexed, integrated and scaled using XDS (Kabsch, 2010). The structure was solved by molecular replacement using a trimeric model of BG505 SOSIP derived from PDB 4TVP and 3 copies of 8ANC195 Fab (PDB 4P9M). The model was refined to 3.58 Å resolution using an iterative approach involving (*i*) refinement applying NCS restraints and verification of model accuracy with simulated annealing composite omit maps using the Phenix crystallography package (Adams et al., 2010), and (*ii*) manually fitting models into electron density maps using Coot (Emsley and Cowtan, 2004). The final model (R_{work} = 24.1%; R_{free} = 28.6%) includes 21,288 protein atoms and 1,016 carbohydrate atoms (Table S1). 96%, 4% and 0% of the residues were

in the favored, allowed and disallowed regions, respectively, of the Ramachandran plot. Disordered residues that were not included in the model include residues 58-68, 134-150, 182-191, 321-332, 399-413, 459-462 and 506-517 of gp120, residues 512-518 and 547-568 of gp41, and residues 129-134 and the 6x-His tag of the 8ANC195$_{G52K5}$ HC. Solvent-exposed side chains not resolved in the electron density were stubbed.

Buried surface areas were determined using PDBePISA (Krissinel and Henrick, 2007) and a 1.4 Å probe. The following distance and geometry criteria were used for assigning potential hydrogen bonds: a distance of < 3.5 Å, and a A-D-H angle of > 90˚. The maximum distance allowed for a van der Waals interaction was 4.0 Å. Hydrogen bond assignments should be considered tentative because of the relatively low resolution of the structure. Superimposition calculations were done and molecular representations were generated using PyMOL (Schrödinger, 2011) or UCSF Chimera (Pettersen et al., 2004). PDBeFold (Krissinel and Henrick, 2004) was used to perform pairwise Cα alignments.

Surface Plasmon Resonance

Experiments were performed using a Biacore T200 (Biacore). Protein A (Pierce) was primary amine-coupled on a CM5 chip (Biacore) at a coupling density of 12,000 resonance units (RUs). Capture proteins (PGT145 IgG, PGT121 IgG, CD4-Fc, 17b IgG, 21C IgG, or mG053 IgG) were injected over the chip at 100 nM concentration, followed by injection of 1 µM human Fc to block remaining protein A binding sites. mG053 does not bind BG505 SOSIP and was used to generate a reference flow cell. 500 nM BG505 SOSIP was subsequently injected, followed by a wash step with running buffer (HBS-EP+, GE Healthcare). 8ANC195, 8ANC195$_{G52K5}$, and mutant/chimeric Fabs were injected over flow cells at increasing concentrations (1.95 to 1,000 nM), at flow rates of 50 µl/min for 180 sec and allowed to dissociate for 600 sec. Flow cells were regenerated with 1 pulse each of 10 mM glycine pH 2.5 and 1 M guanidine HCl at a flow rate of 90 µl/min. On/off rates (k_a/k_d) and binding constants (K_D (M)) were calculated by kinetic analyses after subtraction of backgrounds using a 1:1 binding model with or without a bulk reflective index (RI) correction as appropriate (Biacore T200 Evaluation software).

Neutralization Assays

Neutralization of pseudoviruses derived from primary HIV-1 isolates was determined by observing the reduction of HIV-1 Tat-induced luciferase reporter gene expression in the presence of a single round of pseudovirus infection in TZM-bl cells as described (Montefiori, 2005). Pseudoviruses were produced by cotransfection of HEK 293T cells with an Env expression plasmid and a replication-defective backbone plasmid. Pseudoviruses containing only high-mannose glycans were produced in HEK 293S (GnTI$^{-/-}$ cells). Neutralization assays were performed in-house for evaluating 8ANC195 mutants and YU2 gp41 glycosylation mutants (Table S3). Some of the data were generated with neutralization assays that were dispensed by a Freedom EVO® (Tecan) liquid handler (IC_{50} values derived from manual and robotic assays agreed to within 2-4 fold). In all cases, neutralization was monitored in the presence of a 4-fold Ab dilution series (each concentration run in duplicate or triplicate) after a single round of pseudovirus infection in the TZM-bl cell line (Montefiori, 2005). Nonlinear regression analysis was used to derive the concentrations at which half-maximal inhibition was observed (IC_{50} values).

Cryoelectron tomography

Purified BG505 SOSIP-sCD4-17b-8ANC195$_{G52K5}$ complexes were diluted to 60 µg/mL in TBS immediately before plunge freezing to avoid complex dissociation at low concentration. Sample grids were prepared by adding 3 µL of sample solution to a glow-discharged Quantifoil R2/2 NH2 copper finder mesh with extra thick carbon grid (Quantifoil Micro Tools). After blotting for

2.5 sec at 24°C and 95% humidity, grids were vitrified in liquid ethane using Mark IV Vitrobot (FEI Company). Frozen grids were transferred to a FEI Tecnai G2 Polara transmission electron microscope equipped with 300 keV FEG, a Gatan energy filter and a Gatan K2 Summit direct detector. Tilt series (±60°, 1° angular increments) were digitally recorded using the UCSF tomography software package (Zheng et al., 2007). Images were acquired under low dose conditions (120 $e^{-}/Å^2$ total for the tilt series at ~8 µm underfocus) at a nominal magnification of 41,000x so that each pixel represented 2.6 Å. Tomographic reconstructions and CTF corrections were calculated using IMOD (Kremer et al., 1996). Individual tomograms were binned by 2 before alignment of particles and subtomogram averaging. Particles were picked using EMAN2.1 (Tang et al., 2007), and subtomogram averaging of 1745 subvolumes was performed using PEET (Nicastro et al., 2006) without using an external reference or applying C3 symmetry. The resolution of the resulting map was estimated to be ~23 Å using a 0.143 gold-standard Fourier shell correlation (FSC) calculated using IMOD (Kremer et al., 1996).

Negative-stain single particle EM

Purified BG505 SOSIP-sCD4-17b-8ANC195$_{G52K5}$ complexes were diluted to 10 µg/mL in TBS immediately before adding 3 µL to a glow discharged ultrathin C film on holey carbon support film, 400 mesh, Cu grids (Ted Pella, Inc.). Samples on the grids were cross-linked using glutaraldehyde vapor and then stained with 3% uranyl acetate. Data were collected using a FEI Tecnai T12 transmission electron microscope operating at 120 keV equipped with a Gatan Ultrascan 2k X 2k CCD. Images were acquired using a 0.5 sec exposure time at a nominal magnification of 42,000x at 1 µm defocus, resulting in 2.5 Å per pixel. A total of 23,951 particles were picked using manual picking in EMAN2.1 (Tang et al., 2007) and automated picking in RELION (Scheres, 2012), and the CTF correction was done using EMAN2.1 (Tang et al., 2007). Initial reference-free 2D class averaging was performed using RELION and all particles were sorted into 480 classes. 14,096 particles corresponding to good class averages were selected, and the particles were further sorted using 3D classification in RELION. Refinement was conducted using 80 Å low-pass filtered structures calculated from models of 8ANC195-sCD4-17b docked onto gp120 cores of partially-open (PDB 3DNL), open (PDB 3DNO), and closed (PDB 3DNN) trimer and 7,174 particles with C3 symmetry applied. We used the model from from the partially-open complex for the final structure, but results were similar when using the starting model for the open complex (Figure S5) or when using references calculated from model complexes without either sCD4 or 17b (data not shown). The resolution of the final reconstruction was ~17 Å calculated with RELION (Scheres, 2012) using a gold-standard FSC and a 0.143 cutoff as recommended for resolution estimations for single particle EM reconstructions (Scheres and Chen, 2012).

Fitting of EM density maps

EM structures were visualized using IMOD and USCF Chimera (Kremer et al., 1996; Pettersen et al., 2004). Coordinates from crystal structures were fit into the sub-tomogram averaged or negative stain single particle EM structures using the fit in map utility within UCSF Chimera (Pettersen et al., 2004) with the following options: real-time correlation/average update, and use map simulated from atoms, resolution 15 Å. The gp120-sCD4-17b complex structure (PDB 1RZK) was first fit into EM density and then the 8ANC195 Fab (PDB 4P9M) was independently fit to remaining density. For comparisons with open (PDB 3DNO), partially-open (PDB 3DNL), and closed (PDB 3DNN) Env trimer structures, the coordinates for YU2 gp120 in the fitted complexes were replaced by coordinates for HXBc2 gp120, the strain used for those structure determinations.

Table S1. Data collection and refinement statistics, molecular replacement, Related to Figure 1.

	8ANC195$_{G52K5}$ Fab/BG505 SOSIP.664 complex
Data collection	
Resolution range (Å)	29.73 - 3.58 (3.711 - 3.58)
Space group	$P2_1$
Cell dimensions	
a, b, c (Å)	117.73, 195.22, 119.09
α, β, γ (°)	90.0, 101.6, 90.0
Total reflections	210397 (19046)
Unique reflections	61171 (5654)
Multiplicity	3.4 (3.4)
Completeness (%)	99.0 (92.0)
Mean I/σ(I)	12.96 (1.60)
Wilson B-factor	130.24
R_{merge}	0.0776 (0.823)
CC1/2	0.998 (0.525)
CC*	1 (0.83)
Refinement	
R_{work}/R_{free}	0.241/ 0.286
Number of atoms	22304
Protein	21288
Ligands	1016
Protein residues	2889
RMS (bonds)	0.007
RMS (angles)	0.87
Clashscore	13.81
Average B-factor	140.92
Protein	141.47
Ligands	129.42

Statistics for the highest-resolution shell are shown in parentheses.

Table S3. In vitro Neutralization Data, Related to Figure 3.

A.

	IC_{50} (μg/mL)	
Antibody	YU-2	BG505
8ANC195	0.421	0.100
8ANC195 LC T30A	0.446	0.059
8ANC195 LC G30aA	0.300	0.052
8ANC195 LC N31A	0.446	0.168
8ANC195 LC W32A	>50	>50
8ANC195 LC Y49A	1.614	0.414
8ANC195 LC G51A	0.455	0.122
8ANC195 LC L54A	0.589	0.143
8ANC195 LC Y91A	2.639	1.340
8ANC195 LC D92A	0.628	0.102
8ANC195 LC T93A	0.446	0.059
8ANC195 LC Y96A	0.300	0.052
8ANC195 HC D99A	0.565	0.139
8ANC195 HC K100A	0.310	0.190
8ANC195 HC W100aA	1.067	0.141
8ANC195 HC G100cA	0.407	0.154
8ANC195 HC H100eA	0.424	0.354
8ANC195 HC H100fA	0.415	0.304
8ANC195 HC D100gA	0.312	0.119

B.

	IC_{50} (μg/mL)			
Antibody	YU2 high mannose glycans	YU2 high mannose + complex glycans	PVO high mannose glycans	PVO high mannose + complex glycans
8ANC195	1.04	0.52	0.35	0.57
$8ANC195_{G52K5}$	0.59	0.33	0.23	0.28
3BNC60	0.005	0.03	0.01	0.11
PG16	0.15	0.31*	4.7	13.0*
PGT121	0.07	0.06	0.26	0.14

*maximum neutralization only reached 80%

Table S3. In vitro Neutralization Data. (**A**) Neutralization of YU2 and BG505 pseudoviruses by 8ANC195 mutants. (**B**) Neutralization of YU2 and PVO containing only high-mannose *N*-glycans or mixtures of *N*-glycans by 8ANC195-related bNAbs and control bNAbs. Consistent with previous reports that complex-type *N*-glycans partially protect the CD4 binding site (Binley et al., 2010), 3BNC60 was more potent against high-mannose-only viruses. As also previously reported, the neutralization potency of PGT121 was unaffected (Mouquet et al., 2012).

Table S4. Neutralization of YU2 $PNGS_{gp41}$ mutants by 8ANC195 mutants and chimeras, Related to Figure 3.

	IC_{50} (μg/mL) against YU2						
Antibody	wt	N611 Q	N625 Q	N637 Q	N611Q/ N637Q	N625Q/ N637Q	N611Q/ N625Q/ N637Q
8ANC195 IgG	0.4	0.42	0.76	0.57	0.37	0.79	0.46
8ANC195 mHC/glLC	7.53	17.11	ND	1.48	1.34	ND	ND
8ANC195 glCDRL1	55	>100	>100	3.42	1.80	17.40	6.64
8ANC195 glCDRL2	6.26	13.6	54	3.48	0.71	3.91	0.72
8ANC195 glCDRL3	0.79	ND	ND	ND	0.53	ND	ND
8ANC195 CDRL3Ala	38.6	ND	>100	ND	2.77	28.8	5.66

	IC_{50} fold change mutant virus/YU2 wt						
Antibody	wt	N611 Q	N625 Q	N637 Q	N611Q/ N637Q	N625Q/ N637Q	N611Q/ N625Q/ N637Q
8ANC195 IgG	1	1.0	1.9	1.4	0.9	2.0	1.2
8ANC195 mHC/glLC	19	2.3	ND	0.20	0.18	ND	ND
8ANC195 glCDRL1	138	>250	>250	0.06	0.03	0.32	0.12
8ANC195 glCDRL2	16	2.2	8.6	0.56	0.11	0.63	0.12
8ANC195 glCDRL3	2.0	ND	ND	ND	0.67	ND	ND
8ANC195 CDRL3Ala	97	ND	>250	ND	0.07	0.74	0.15

	IC_{50} fold change mutant antibody/8ANC195 wt						
Antibody	wt	N611 Q	N625 Q	N637 Q	N611Q/ N637Q	N625Q/ N637Q	N611Q/ N625Q/ N637Q
8ANC195 IgG	1	1	1	1	1	1	1
8ANC195 mHC/glLC	19	41	ND	2.6	3.6	ND	ND
8ANC195 glCDRL1	138	>1000	>1000	6.0	4.8	22	14
8ANC195 glCDRL2	16	33	71	6.0	1.9	5.0	1.6
8ANC195 glCDRL3	2.0	ND	ND	ND	1.4	ND	ND
8ANC195 CDRL3Ala	97	ND	>1000	ND	7.4	37	12

color scale (IC_{50}; μg/mL)	0.1 - 1	1.1 - 5	5.1 - 20	> 20

color scale (fold-change)	<0.05	0.051 - 0.2	0.21 - 4.9	5 - 20	> 20

Table S5: Distance comparisons in Env trimer structures, Related to Figure 6.

Trimer conformational state	Ligand	+/- sCD4	Trimer type	Method	EMDB/PBD ID	Resolution (Å)	Distance between CD4bs (D368) (Å)
closed	8ANC195$_{G52K5}$	-	BG505 SOSIP.664	crystal structure	this study	3.58	54
closed	PGT122, 35O22	-	BG505 SOSIP.664	crystal structure	4TVP	3.5	55
closed	PGT122	-	BG505 SOSIP.664	crystal structure	4NCO	4.7	56
closed	PG04	-	BG505 SOSIP.664	single particle cryo-EM	3J5M	5.8	53
closed	VRC03	-	KNH1144 SOSIP.681	single particle cryo-EM	4CC8	6	57
closed	VRC01	-	JRFL SOSIP.681	single particle NS EM	6194	20	55
closed	no ligand	-	BaL virion	cryo-ET, sub-tomogram ave	3DNN	20	63
closed	VRC01	-	BG505 SOSIP.664	single particle NS EM	6252	22	61
closed	VRC01		BaL virion	cryo-ET, sub-tomogram ave	5457	24	68
							55 ± 5
partially open	A12	-	BaL virion	cryo-ET, sub-tomogram ave	5544	N/A	75
partially open	b12	-	BaL virion	cryo-ET, sub-tomogram ave	3DNL	20	76
							75 ± 1
partially open*	8ANC195$_{G52K5}$, sCD4, 17b	+	BG505 SOSIP.664	single particle NS EM	this study	17	79
partially open*	8ANC195$_{G52K5}$, sCD4, 17b	+	BG505 SOSIP.664	cryo-ET, sub-tomogram ave	this study	23	82
							80 ± 2
open	sCD4	+	JR-FL SOSIP.681	cryo-ET, sub-tomogram ave	5322	20	94
open	sCD4	+	KNH1144 SOSIP.681	cryo-ET, sub-tomogram ave	5321	20	94
open	sCD4	+	BaL virion	cryo-ET, sub-tomogram ave	5455	24	82
open	sCD4, 17b	+	BaL virion	cryo-ET, sub-tomogram ave	3DNO	20	87
open	sCD4, 17b	+	KNH1144 SOSIP.681	cryo-ET, sub-tomogram ave	5323	20	87
open	sCD4, 17b	+	JR-FL SOSIP.681	cryo-ET, sub-tomogram ave	5324	20	90
open	sCD4, 17b	+	BG505 SOSIP.664	single particle NS EM	5723	22	85
open	17b	-	KNH1144 SOSIP.681	single particle cryo-EM	5462*	8.8	88
open	17b	-	KNH1144 SOSIP.681	cryo-ET, sub-tomogram ave	5325	20	85
open	17b	-	BaL virion	cryo-ET, sub-tomogram ave	5456	22	85
open	17b, VRC01	-	BaL virion	cryo-ET, sub-tomogram ave	5461	28	88
open	Z13e1	-	KNH1144 SOSIP.681	single particle cryo-EM	5680	18.5	83
							87 ± 4

Table S5: Distance comparisons in Env trimer structures, related to Figure 5. Structures are grouped into four conformational states: closed (unliganded and bound to Fabs), partially open (bound to A12 or b12), partially open* (bound to 8ANC195, sCD4, 17b; this study), and open (bound to sCD4 and/or 17b, or Z13e1). The EMDB or PDB identifier is given for each structure. When available, PDB coordinates for gp120 subunits within a trimer were used to measure the distances between CD4 binding site residue $Asp368_{gp120}$ on adjacent protomers (PDB coordinates for EMDB entry 5462, marked with an asterisk, were obtained from Sriram Subramaniam). When PDB coordinates were not available, gp120s were fit to density maps in the EMDB using UCSF Chimera (Pettersen et al., 2004). The mean and standard deviation for distances are listed for each category of trimer structure.

Supplemental References

Adams, P.D., Afonine, P.V., Bunkoczi, G., Chen, V.B., Davis, I.W., Echols, N., Headd, J.J., Hung, L.W., Kapral, G.J., Grosse-Kunstleve, R.W.*, et al.* (2010). PHENIX: a comprehensive Python-based system for macromolecular structure solution. Acta Crystallogr D Biol Crystallogr *66*, 213-221.

Binley, J.M., Ban, Y.E., Crooks, E.T., Eggink, D., Osawa, K., Schief, W.R., and Sanders, R.W. (2010). Role of complex carbohydrates in human immunodeficiency virus type 1 infection and resistance to antibody neutralization. Journal of virology *84*, 5637-5655.

Diskin, R., Marcovecchio, P.M., and Bjorkman, P.J. (2010). Structure of a clade C HIV-1 gp120 bound to CD4 and CD4-induced antibody reveals anti-CD4 polyreactivity. Nat Struct Mol Biol *17*, 608-613.

Diskin, R., Scheid, J.F., Marcovecchio, P.M., West, A.P., Jr., Klein, F., Gao, H., Gnanapragasam, P.N., Abadir, A., Seaman, M.S., Nussenzweig, M.C.*, et al.* (2011). Increasing the potency and breadth of an HIV antibody by using structure-based rational design. Science *334*, 1289-1293.

Emsley, P., and Cowtan, K. (2004). Coot: model-building tools for molecular graphics. Acta Crystallogr D Biol Crystallogr *60*, 2126-2132.

Kabsch, W. (2010). Integration, scaling, space-group assignment and post-refinement. Acta Crystallogr D Biol Crystallogr *66*, 133-144.

Kremer, J.R., Mastronarde, D.N., and McIntosh, J.R. (1996). Computer visualization of three-dimensional image data using IMOD. J Struct Biol *116*, 71-76.

Krissinel, E., and Henrick, K. (2004). Secondary-structure matching (SSM), a new tool for fast protein structure alignment in three dimensions. Acta Crystallogr D Biol Crystallogr *60*, 2256-2268.

Krissinel, E., and Henrick, K. (2007). Inference of macromolecular assemblies from crystalline state. J Mol Biol *372*, 774-797.

Montefiori, D.C. (2005). Evaluating neutralizing antibodies against HIV, SIV, and SHIV in luciferase reporter gene assays. Current protocols in immunology / edited by John E Coligan [et al *Chapter 12*, Unit 12 11.

Mouquet, H., Scharf, L., Euler, Z., Liu, Y., Eden, C., Scheid, J.F., Halper-Stromberg, A., Gnanapragasam, P.N., Spencer, D.I., Seaman, M.S.*, et al.* (2012). Complex-type N-glycan recognition by potent broadly neutralizing HIV antibodies. Proc Natl Acad Sci U S A *109*, E3268-3277.

Nicastro, D., Schwartz, C., Pierson, J., Gaudette, R., Porter, M.E., and McIntosh, J.R. (2006). The molecular architecture of axonemes revealed by cryoelectron tomography. Science *313*, 944-948.

Pettersen, E.F., Goddard, T.D., Huang, C.C., Couch, G.S., Greenblatt, D.M., Meng, E.C., and Ferrin, T.E. (2004). UCSF Chimera--a visualization system for exploratory research and analysis. J Comput Chem *25*, 1605-1612.

Sanders, R.W., Derking, R., Cupo, A., Julien, J.P., Yasmeen, A., de Val, N., Kim, H.J., Blattner, C., de la Pena, A.T., Korzun, J.*, et al.* (2013). A next-generation cleaved, soluble HIV-1 Env Trimer, BG505 SOSIP.664 gp140, expresses multiple epitopes for broadly neutralizing but not non-neutralizing antibodies. PLoS Pathog *9*, e1003618.

Scharf, L., Scheid, J.F., Lee, J.H., West, A.P., Jr., Chen, C., Gao, H., Gnanapragasam, P.N., Mares, R., Seaman, M.S., Ward, A.B.*, et al.* (2014). Antibody 8ANC195 reveals a site of broad vulnerability on the HIV-1 envelope spike. Cell reports *7*, 785-795.

Scheres, S.H. (2012). RELION: implementation of a Bayesian approach to cryo-EM structure determination. J Struct Biol *180*, 519-530.

Scheres, S.H., and Chen, S. (2012). Prevention of overfitting in cryo-EM structure determination. Nat Methods *9*, 853-854.

Schrödinger, L. (2011). The PyMOL Molecular Graphics System (The PyMOL Molecular Graphics System).
Tang, G., Peng, L., Baldwin, P.R., Mann, D.S., Jiang, W., Rees, I., and Ludtke, S.J. (2007). EMAN2: an extensible image processing suite for electron microscopy. J Struct Biol *157*, 38-46.
Zheng, S.Q., Keszthelyi, B., Branlund, E., Lyle, J.M., Braunfeld, M.B., Sedat, J.W., and Agard, D.A. (2007). UCSF tomography: an integrated software suite for real-time electron microscopic tomographic data collection, alignment, and reconstruction. J Struct Biol *157*, 138-147.