## **Supplemental Material**

## Antibody recognition of CD4-induced open HIV-1 Env trimers

Zhi Yang<sup>a</sup>, Kim-Marie A. Dam<sup>a</sup>, Jonathan M. Gershoni<sup>b</sup>, Susan Zolla-Pazner<sup>c</sup>, Pamela J. Bjorkman<sup>a#</sup>

 <sup>a</sup>Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA 91125, USA
 <sup>b</sup>Shmunis School of Biomedicine and Cancer Research, Tel Aviv University, Israel
 <sup>c</sup>Department of Medicine, Division of Infectious Diseases, Icahn School of Medicine at Mount Sinai, New York, NY, 10029 USA

Zhi Yang and Kim-Marie A. Dam contributed equally to this work. Author order was determined by the two co-first authors.

<sup>#</sup>Address correspondence to Pamela J. Bjorkman, <u>bjorkman@caltech.edu</u>



**Figure S1. Cryo-EM data processing and validation of V2i Fab-BG505-sCD4 complexes.** Left: Example micrographs and 2D class averages of V2i Fab-BG505-sCD4 complexes. Middle: Plots of global half-map FSCs (solid red line), directional resolution values ±1σ from the mean

(left axis, green dashed lines) and distributions sampled over the 3D FSC (blue histograms, right axis). Right: Cryo-EM density maps of V2i Fab-BG505-sCD4 complexes.



Figure S2. Cryo-EM data processing and validation of CG10 Fab-B41-sCD4 complex. Left: Example micrograph and 2D class averages of the CG10-B41-sCD4 complex. Middle: Plot of global half-map FSC (solid red line), directional resolution values  $\pm 1\sigma$  from the mean (left axis, green dashed lines) and distributions sampled over the 3D FSC (blue histograms, right axis). Right: Cryo-EM map of the CG10-B41-sCD4 complex.

	1393A-BG505-	1361-BG505-	697D-BG505-	830A-BG505-
	sCD4 (EMD-27209)	sCD4 (EMD-27210)	sCD4 (EMD-27211)	sCD4 (EMD-27212)
Data collection and processing				
Magnification *	105,000x	105,000x	105,000x	105,000x
Voltage (kV)	300	300	300	300
Electron exposure $(e - / Å^2)$	60	60	60	60
Defocus range (µm)	1.8-3.0	1.8-3.0	1.8-3.0	1.8-3.0
Pixel size (Å)	0.416	0.416	0.435	0.416
Recording mode	Super resolution	Super resolution	Super resolution	Super resolution
Collected movies (no.)	3,239	2,412	2,340	1,872
Symmetry imposed	C1	C1	C1	C1
Initial particle images (no.)	943,748	843,403	367,952	436,527
Final particle images (no.)	107,234	104,002	21,655	16,575
Overall map resolution (Å)	7.5	6.1	7.0	7.3

## Supplementary Table 1. Cryo-EM data collection, refinement, and validation statistics

\* Nominal magnification

	CG10 Fab (PDB 8D54)	
Data collection		
Space group	P212121	
Cell dimensions		
<i>a, b, c</i> (Å)	75.14, 77.80, 84.03	
α, β, γ (°)	90, 90, 90	
Resolution (Å)	38.90 - 1.40 (1.42 - 1.40) *	
$R_{\text{merge}}$ (%)	6.5 (151.3)	
$R_{ m pim}$ (%)	1.8 (43.3)	
CC <sub>1/2</sub> (%)	99.9 (63.2)	
Ι/σΙ	19.6 (1.7)	
Completeness (%)	99.5 (97.1)	
Multiplicity	13.3 (12.5)	
Refinement		
Resolution (Å)	1.40	
No. reflections	97,069 (4,660)	
$R_{ m free}$ / $R_{ m work}$ (%)	18.8 / 16.9	
No. atoms		
Protein	3,330	
Ligand/ion	0	
Water	680	
R.m.s. deviations		
Bond lengths (Å)	0.008	
Bond angles (°)	0.86	
Rotamer outliers (%)	0.81	
Ramachandran plot		
Favored (%)	98.2	
Allowed (%)	1.8	
Disallowed	0	
Average <i>B</i> -factor $(Å^2)$	18.9	

Supplementary Table 2. X-ray data collection and refinement statistics

\*Values in parentheses are for highest-resolution shell.

	CG10-B41-sCD4	
	(PDB 8D5C)	
	(EMD-27208)	
Data collection and processing		
Magnification *	105,000x	
Voltage (kV)	300	
Electron exposure $(e - / Å^2)$	60	
Defocus range (µm)	1.8-3.0	
Pixel size (Å)	1.11	
Recording mode	Counting	
Collected movies (no.)	3,528	
Symmetry imposed	C1	
Initial particle images (no.)	933,854	
Final particle images (no.)	134,746	
Overall map resolution (Å) **	4.1 (4.2)	
(masked/unmasked)		
Refinement		
Initial models used (PDB code)	8D54 (this study), 5VN3 ***	
Map and model CC	0.66	
Map sharpening <i>B</i> factor ( $Å^2$ )	109.7	
Model composition		
Protein residues	2,730	
Validation		
MolProbity score	2.43	
Clashscore	31.6	
Poor rotamers (%)	0	
Ramachandran plot		
Favored (%)	93.0	
Allowed (%)	7.0	
Disallowed (%)	0	
RMS deviations		
Length (Å)	0.002	
Angles (°)	0.48	

## Supplementary Table 3. Cryo-EM data collection, refinement, and validation statistics

\* Nominal magnification; \*\* FSC threshold 0.143; \*\*\* Partial structure composed of trimeric gp120/gp41 and sCD4