Supporting Information

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SI Materials and Methods

Preparation of HIV-1_{NL4-3} gp120 and HIV-1_{SF162} gp120 from Cultured Cells. HIV-1_{NL4-3} gp120 was obtained from HeLa/T-env/Tat cells (1) that constitutively express HIV-1_{NL4-3} envelope protein. Cells were cultured in RPMI medium supplemented with 3% FBS and 100 μg/mL kanamycin sulfate (Meiji Seika). The culture supernatant (10 L) was concentrated using a 50-kDa cut-off ultrafiltration module (Microza UF AHP-1010; Asahi Kasei) until reaching 1/10th volume. The concentrated supernatant was loaded onto a DEAE Sephadex A-50 (Amersham Biosciences) open column equilibrated with 20 mM Tris·HCl (pH 8.0) containing 100 mM NaCl. The gp120 was recovered in the flow-through fraction and brought to 20% saturation in ammonium sulfate by addition of solid ammonium sulfate. The fraction was then filtered with a glass microfiber filter (Whatman International), and the filtrate was applied to a column (30 mm i.d. $\times$ 150 mm) packed with Phenyl-toyopearl 650M (Tosoh) that was equilibrated with 20% ammonium sulfate buffer (20 mM sodium acetate, pH 5.5/20% saturating ammonium sulfate). The column was then washed with 10% ammonium sulfate buffer and eluted with 5% ammonium sulfate buffer. The gp120-containing fractions were pooled and loaded onto a *Galanthus nivalis* lectin–agarose (Vector Laboratories) column equilibrated with 10 mM Tris, pH 8.0, in PBS. Bound gp120 was eluted with 500 mM methylmannose pyranoside (Sigma) in equilibration buffer. The fractions containing gp120 were collected, concentrated using an Amicon YM-30 membrane (Millipore), and stored at -80 °C. HIV-1_{SF162} gp120 was obtained from HeLa/M-env cells (1) that continuously express HIV-1_{SF162} envelope protein, as described above for preparation of HIV-1_{NL4-3} gp120. gp120 was detected after SDS/PAGE by Western blotting using anti-HIV-1_{IIIB}gp120 polyclonal antibody (EM) as a primary antibody and rabbit anti-goat IgG-h 1 alkaline phosphatase (Bethyl) as a secondary antibody. Both gp120 preparations gave a single band.

Crystallization of Actinohivin (AH). Crystallization conditions were surveyed by the hanging-drop vapor-diffusion method at 293 K. Protein droplets prepared by mixing 2 μL of 10 mg mL^{-1} protein in 20 mM sodium citrate buffer solution at pH 5.6 and 1 μL of reservoir solution were equilibrated to 700 μL of reservoir solution. Many kinds of reservoir solutions were prepared by Hampton Research and Emerald BioSystems. Single crystals of AH appeared when the reservoir solution contained 35% 2-ethoxyethanol in 0.1 M sodium citrate buffer (pH 5.5).

X-Ray Data Collection. Crystals with suitable size and shape were soaked in the reservoir solution containing 2 mM $K_2[Pt(CN)_4]$, and some of them were mounted in nylon loops and then flash-cooled in a nitrogen gas stream at 100 K. X-Ray diffraction patterns were taken at 100 K using a Quantum 315 CCD detector (Area Detector Systems) with synchrotron radiation (λ = 1.07 Å at BL-5A of PF in Tsukuba, Japan), and processed using the program HKL2000 (2) at 1.19-Å resolution. Statistics of data collection are summarized in Table S1. The crystal data suggests that there are two independent protein molecules in the asymmetric unit of the crystal.

Structure Determination and Refinement. The crystal structure was initially determined using the programs Il Milione (3) and ARP/wARP (4) in the CCP4 program suite (5). The structure thus constructed was then subjected to maximum-likelihood refinements with the program REFMAC (6) in CCP4, followed by manual modifications of the structure with the program XtalView (7) and Coot (8). Six sodium ions and 317 water molecules assigned using the program ARP/wARP were included in the subsequent structure refinements. The stereochemistry of the two protein structures were verified using the program PROCHECK (9). Refinement statistics are summarized in Table S1. The atomic coordinates have been deposited in the Protein Data Bank (ID code 3A07).

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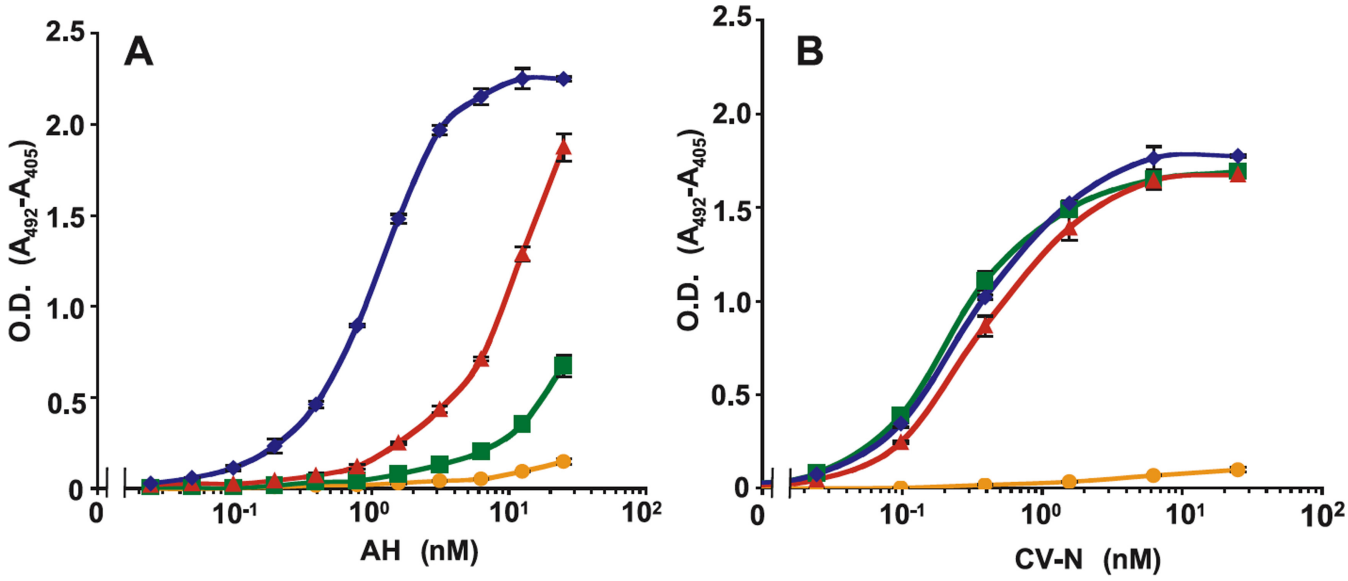

Coated protein	Number of sugar chains	Number of high-mannose type glycans
$gp120_{NL4-3}$ (100 ng/well)	24	11 (including hybrid type)
Thyroglobulin (500 ng/well)	14	4
RNase B (500 ng/well)	1	1
BSA (500 ng/well)	0	0

Fig. S1. Comparison between AH and cyanovirin (CV)-N in binding to various glycoproteins by ELISA.

Table S1. Statistics of data collection and structure refinement

Data collection		Structure refinement	
X-ray source	PF BL-5A	Resolution, Å	27.3–1.2
Wavelength, Å	1.07	Used reflections	67,142
Resolution, Å	27.30–1.19	*R*-factor, %‡	14.9
Observed reflections	580,437	*R*-free, %§	16.3
Unique reflections	69,476	No. of protein atoms	1,823
Completeness, %	97.2 (84.3)	No. of ions	$6Na^{+}$
R_{merge}, %*	8.9 (29.4)	No. of water molecules	317
$I/\sigma I$	33.6 (2.7)	rmsd	
Space group	$C222_1$	Bond lengths, Å	0.029
Unit cell *a*, Å	47.3	Bond angles, °	2.2
b, Å	51.6	Between proteins, Å	0.50
c, Å	175.3	Ramachandran plot, %	
Z†	2	Most favored regions	95.3
V_M	2.14	Add. allowed regions	4.7

Values in parentheses are for the highest-resolution shell between 1.23 and 1.19 Å.

*$R_{merge} = 100 \times \Sigma_{hj}|I_{hj} - \langle I_h \rangle| / \Sigma_{j'} \langle I_{hj} \rangle$, where I_{hj} is the jth measurement of the intensity of reflection h and $\langle I_h \rangle$ is its mean value.

†Number of subunits in the asymmetric unit.

‡R factor $= 100 \times \Sigma||F_o| - |F_c|| / \Sigma |F_o|$, where $|F_o|$ and $|F_c|$ are the observed and calculated structure factor amplitudes, respectively.

§Calculated using a random set containing 5% of the observations that were not included throughout refinement (10).