Supplementary Figure 1. Binding of HIV-1 antibodies to sCD4-Env or CD4m-Env complexes.

ELISAs evaluating binding of IgG versions of anti-Env antibodies 17b, 21c, BG1, PG16, and 10-1074 or control (no antibody added) to a, sCD4-BG505 Env, b, BMS-626529-BG505, c, BNM-III-170-BG505 Env, and d, M48U1-BG505 Env. Values are shown as mean of two individual replicates. Colors: 17b = light blue, 21c = light orange, BG1 = purple, PG16 = hot pink, 10-1074 = green, control = black. Results shown for n=2 individual replicates. Connecting line is for mean of individual replicates.
Supplementary Figure 2. Data Processing for BNM-III-170-BG505-17b complex.

**a**, Representative EM micrograph of data set. 2739 and 2048 micrographs were collected for data set 1 and 2, respectively. Scale bar is 50nm. 

**b**, Gold Standard 3D FSC chart for final reconstruction map using combined data sets.

**c**, Particle orientation distribution and sphericity for final reconstruction of combined data sets.

**d**, Schematic of processing pipeline.
Supplementary Figure 3. Data Processing for the M48U1-BG505-17b complex.

a, Representative EM micrograph of data set. 2688 total micrographs were collected. Scale bar is 50nm. 
b, Gold Standard 3D FSC chart for final reconstruction map using combined data sets. 
c, Particle orientation distribution and sphericity for final reconstruction of combined data sets. d, Schematic of Processing pipeline.
Supplementary Figure 4: 3D classification of BNM-III-170-BG505-17b shows differences in positioning of gp120 and 17b.

a, Local resolution map of BNM-III-170-BG505-17b reconstruction. b, Overlay of 3D classes of BNM-III-170-BG505-17b produced in final classification round after merging and polishing particles for classes (gray, pale cyan, pale yellow, light purple for Class 1-4, respectively). Double-headed arrow on Protomer 3 shows direction of displacement of 17b and gp120 between 3D classes. c, Cartoon models of gp120 subunits rigid body fit into BNM-III-170-BG505-17b 3D...
classification maps. The β4/β26 strands were fit separately from the rest of the gp120 and alignments were done using Cα of β4/β26 strands. d, Cartoon model overlay of Protomer 3 showing gp120 Phe43 pocket for each 3D class and BNM-III-170 (magenta) from final model.
Supplementary Figure 5: 3D classification of M48U1-BG505-17b shows differences in positioning of gp120 and 17b.

a, Local resolution map of M48U1-17b reconstruction. b, Overlay of 3D classes of M48U1-BG505-17b (gray, pale cyan, pale yellow, light purple for Class 1-4, respectively). Double-headed arrow on Protomer 3 shows direction of displacement of 17b and gp120 between 3D classes. c, Cartoon model overlay of Protomer 3 gp120 Phe43 pocket for all 3D classes with M48U1 (red) from final model. M48U1 helix removed for clarity.
**Supplementary Figure 6. CD4m density is present within gp120 Phe43 cavity.**

a. Top-down view of BNM-III-170-BG505-17b density (top left, 17b = light blue, gp120 = gray, gp41 = light orange) with regions containing BNM-III-170 circled. Surrounding panels show zoomed-in views of densities (blue) for the BNM-III-170 molecule and a cartoon/stick representation of the coordinates (BNM-III-170 = magenta, gp120 = gray) in Protomer 1 (gray), Protomer 2 (purple) and Protomer 3 (teal). Densities for protomers 1 and 2 are shown at 7σ and for protomer 3 at 5σ.

b. Top-down view of M48U1-BG505-17b density (top left, 17b = light blue, gp120 = gray, gp41 = light orange) with regions containing M48U1 (red) circled in Protomer 1 (gray), Protomer 2 (purple) and Protomer 3 (teal). Surrounding panels show zoomed-in views of densities (blue) for the M48U1 molecule and a cartoon/stick representation of the coordinates (M48U1 = red, gp120 = gray) in each protomer. Density for protomer 1 is shown at 7σ and for protomers 2 and 3 at 5σ.
Supplementary Figure 7. Overlays of CD4m-gp120 core crystal structures with gp120 core portions of structures of CD4m-BG505 Env trimer complexes.

a, Alignment of BNM-III-170-gp120 core crystal structure (PDB 5F4P [https://doi.org/10.2210/pdb5F4P/pdb], magenta) and gp120 core regions from protomers 1 (gray), 2 (purple), and 3 (teal) of the BNM-III-170-BG505-17b complex. b, Alignment of M48U1-gp120 core crystal structure (PDB 4JZZ [https://doi.org/10.2210/pdb4JZZ/pdb], red) and gp120 core regions from protomers 1 (gray), 2 (purple), and 3 (teal) of M48U1-BG505-17b complex.
## Supplementary Table 1: Cryo-EM data collection, refinement and validation statistics

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