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

| | BG505 SOSIP.664 bound to 3-sCD4, 3-17b Fab and 3-VRC34.01 Fab | BG505 SOSIP.664 bound to 3-sCD4, 3-17b Fab and 2-VRC34.01 Fab | BG505 SOSIP.664 bound to 3-sCD4, 3-17b Fab and 1-VRC34.01 Fab | BG505 SOSIP.664 bound to 3-sCD4, 3-VRC34.01 Fab with one gp120 rotated | BG505 SOSIP.664 bound to 3-sCD4, 3-VRC34.01 Fab with two gp120 rotated |
|---|---|---|---|---|---|
| | **Population 1** | **Population 2** | **Population 3** | **Population 4** | **Population 5** |
| **PDB ID** | 9D90 | | | 9D8Y | 9D98 |
| **EMDB ID** | EMD-46655 | EMD-46671 | EMD-46672 | EMD-46653 | EMD-46670 |
| **Data Collection and processing** | | | | | |
| Microscope | FEI Titan Krios | | | | |
| Detector | Gatan K3 | | | | |
| Magnification | 81000 | | | | |
| Voltage (kV) | 300 | | | | |
| Electron exposure (e–/Å^2) | 58.7 | 58 | | 59.1 | |
| Defocus Range (μm) | 2.4 to 0.8 | | | | |
| Pixel size (Å) | 1.08 | | | | |
| Micrographs collected | 18,253 | 21,125 | | 23,157 | |
| Reconstruction software | cryoSPARC | | | | |
| Symmetry imposed | C1 | C1 | C1 | C1 | C1 |
| Initial particle images (no.) | 1,473,619 | 1,105,644 | 1,105,644 | 998,476 | |
| Final particle images (no.) | 1,028,498 | 58,240 | 25,613 | 274,345 | 138,204 |
| Map resolution (Å) | 3.9 | 4.9 | 6.4 | 4.1 | 4.2 |
| FSC threshold | 0.143 | 0.143 | 0.143 | 0.143 | 0.143 |
| **Model composition** | | | | | |
| Nonhydrogen atoms | 24524 | | | 18800 | 19100 |
| Protein residues | 3151 | | | 2384 | 2441 |
| **R.M.S. deviations** | | | | | |
| Bond lengths (Å) | 0.005 | | | 0.005 | 0.005 |
| Bond angles (°) | 0.964 | | | 0.944 | 1.04 |
| **Validation** | | | | | |
| MolProbity score | 1.64 | | | 1.62 | 1.68 |
| Clashscore | 4.78 | | | 4.25 | 4.98 |
| Favored rotamers (%) | 99.94 | | | 99.92 | 99.75 |
| **Ramachandran plot** | | | | | |
| Favored regions (%) | 94.15 | | | 93.76 | 93.74 |
| Disallowed regions (%) | 0.06 | | | 0.09 | 0.25 |

**Supplementary Table 2. Summary of structural states identified in this study.**

| | Population 4 | Population 5 | Population 1 | Population 2 | Population 3 |
|---|---|---|---|---|---|
| Ligands | CD4, VRC34.01 | CD4, VRC34.01 | CD4, 17b, VRC34.01 | CD4, 17b, VRC34.01 | CD4, 17b, VRC34.01 |
| VRC34.01 bound | ✓✓✓ | ✓✓✓ | ✓✓✓ | ✓✓X | ✓XX |
| Bridging sheet formed | ✓XX | ✓✓X | ✓✓✓ | ✓✓✓ | ✓✓✓ |
| α0 helix formed | ✓XX | ✓✓X | ✓✓✓ | ✓✓✓ | ✓✓✓ |
| Fusion peptide accessible for antibody binding | ✓✓✓ | ✓✓✓ | ✓✓✓ | ✓✓X | ✓XX |
| Extent of receptor-induced Env conformational change* | | | | | |

✓/X indicates structural state per protomer

* Shaded by extent of Env opening, light red indicating less open Env and dark red indicating more open Env.

**Supplementary Table 3. List of structures used for vector analysis in Figure 2E.**

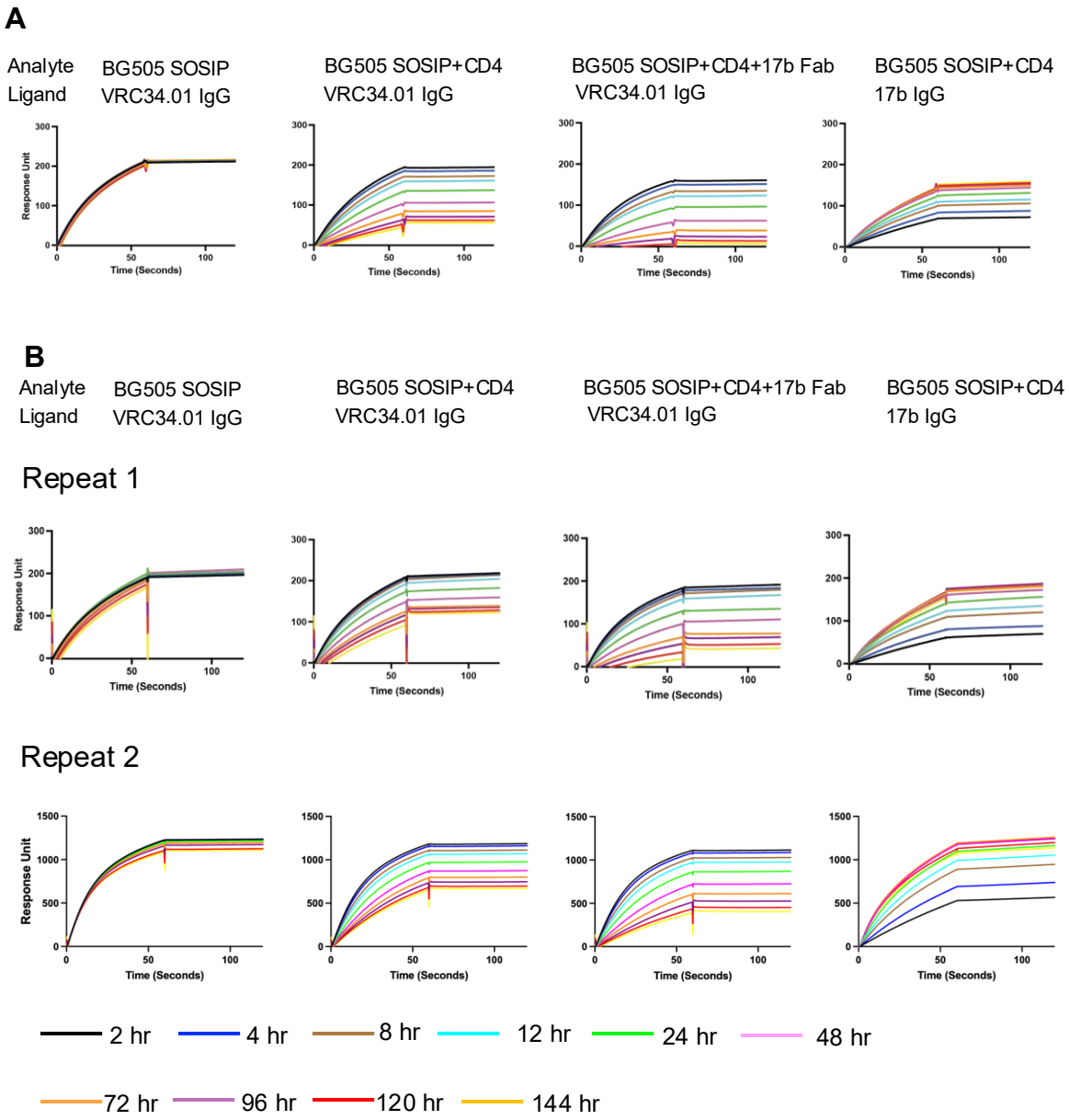
| PDB ID | Ligands | Env name |
|---|---|---|
| 9D90 (Population 1, P1) | sCD4, 17b Fab, VRC34.01 Fab | BG505 SOSIP.664 |
| 5VN3 | sCD4, 17b Fab | B41 SOSIP.664 |
| 5VN8 | b12 Fab | B41 SOSIP.664 |
| 6CM3 | sCD4, 17b Fab, 8ANC195 Fab | BG505 SOSIP.664 |
| 6EDU | sCD4, 21C Fab, 8ANC195 Fab | B41 SOSIP.664 |
| 6V8Z | VRC03 Fab, 10-1074 Fab | BG505 SOSIP.664-F14/Vt8 |
| 4TVP | PGT122 Fab, 35O22 Fab | BG505 SOSIP.664 |
| 4ZMJ | None | BG505 SOSIP.664 |
| 5ACO | PGT128 Fab | BG505 SOSIP.664 |
| 5C7K | PGT128 Fab, 8ANC195 Fab | BG505 SOSIP.664 |
| 5CEZ | Early putative precursor of the PGT121 family | BG505 SOSIP.664 |
| 5CJX | 8ANC195 Fab | BG505 SOSIP.664 |
| 5D9Q | PGT122 Fab, scFv NIH45-46 | BG505 SOSIP.664 |
| 5FUU | PGT151 Fab | JR-FL EnvdCT |
| 5FYJ | PGT122 Fab, 35O22 Fab, VRC01 Fab | HIV-1 Clade G X1193.c1 SOSIP.664 |
| 5FYK | PGT122 Fab, 35O22 Fab, VRC01 Fab | JR-FL SOSIP.664 |
| 5FYL | PGT122 Fab, 35O22 Fab | BG505 SOSIP.664 |
| 5I8H | PGT122 Fab, VRC34.01 Fab | BG505 SOSIP.664 |
| 5T3X | IOMA, 10-1074. | BG505 SOSIP.664 |
| 5T3Z | IOMA, 10-1074. | BG505 SOSIP.664 |
| 5U1F | sCD4, PGT145 Fab | BG505 DS-SOSIP |
| 5U7M | BMS-378806, PGT122 Fab, 35O22 Fab | BG505 SOSIP.664 |
| 5U7O | BMS-626529, PGT122 Fab, 35O22 Fab | BG505 SOSIP.664 |
| 5UM8 | 35O22 Fab,PGT124 Fab | 16055 NFL TD CC (T569G) |
| 5UTF | PGT122 Fab, 35O22 Fab | DS-SOSIP.6mut BG505 gp140 HIV-1 |
| 5UTY | PGT122 Fab, 35O22 Fab | DS-SOSIP.mut4 BG505 gp140 HIV-1 |
| 5V7J | 3H+109L Fab, 35O22 Fab | BG505 SOSIP.664 |
| 5V8L | 3BNC117 Fab, PGT145 Fab | BG505 SOSIP.664 |
| 5V8M | 3BNC117 Fab | BG505 SOSIP.664 |
| 5VIY | BG1 Fab, 8ANC195 Fab | BG505 SOSIP.664 |
| 5VJ6 | PG9 Fab, 8ANC195 Fab | BG505 SOSIP.664 |
| 5WDU | PGT122 Fab, 35O22 Fab, NIH45-46 scFv | BG505 SOSIP.664 H72C-H564C |
| 6B0N | PGT122 Fab, PGV19 Fab | BG505 isolate (NFL construct) |
| 6CCB | 10-1074 Fab | 253-11 SOSIP trimer |
| 6CDE | VRC03 Fab, PGT122 Fab, vFP20.01 Fab | BG505 DS-SOSIP |
| 6CDI | VRC03 Fab, PGT122 Fab, vFP16.02 Fab | BG505 DS-SOSIP |
| 6CE0 | PGT124 Fab, 35O22 Fab | H078.14 UFO-BG |
| 6CH7 | BG18 Fab, 35O22 Fab | BG505 SOSIP.664 |
| 6CH8 | BG18 Fab, 35O22 Fab | BG505 SOSIP.664 |
| 6CH9 | BG18 Fab, 35O22 Fab | B41 SOSIP.664 |
| 6CHB | BG18 Fab, IOMA Fab | BG505 SOSIP.664 |
| 6CK9 | 3H109L Fab, 35O22 Fab | HIV-1 ConC_Base0 |
| 6CUE | VRC03 Fab, PGT122 Fab, vFP7.04 Fab | BG505 DS-SOSIP |
| 6CUF | VRC03 Fab, PGT122 Fab, vFP1.01 Fab | BG505 DS-SOSIP |
| 6DE7 | PGT122 Fab, 35O22 Fab | BG505 SOSIP.664 |
| 6DID | Fabs from immunized rabbit #3417 post-boost#1 | BG505 SOSIP.664 |
| 6E5P | 2G12 Fab, VRC03 Fab | BG505 DS-SOSIP |
| 6IEQ | PGT124 Fab, 35O22 Fab | HIV-1 Env ConM SOSIP.v7 |
| 6-Mar | PGT151 Fab | BG505 delCT N332T |
| 6MCO | PGT124 Fab, 35O22 Fab | B41 SOSIP.664 |
| 6MDT | PGT124 Fab, 35O22 Fab | B41 SOSIP.664 |
| 6MN7 | BF520.1 antigen binding fragment | BG505 SOSIP.664 |
| 6MPG | PGT122 Fab, VRC03 Fab, A12V163-b.01 Fab | BG505 DS-SOSIP |
| 6MPH | PGT122 Fab, VRC03 Fab, DF1W-a.01 Fab | BG505 DS-SOSIP |
| 6MTJ | BMS-378806, 3H109L Fab, 35O22 Fab | BG505 SOSIP.664 |
| 6MTN | Compound 484, 3H109L Fab, 35O22 Fab | BG505 SOSIP.664 |
| 6MU6 | BMS-814508, 3H109L Fab, 35O22 Fab | BG505 SOSIP.664 |
| 6MU7 | BMS-818251, 3H109L Fab, 35O22 Fab | BG505 SOSIP.664 |
| 6MU8 | BMS-386150, 3H109L Fab, 35O22 Fab | BG505 SOSIP.664 |
| 6MUF | 3H109L Fab, 35O22 Fab | B41 SOSIP.664 |
| 6MUG | BMS-386150, 3H109L Fab, 35O22 Fab | B41 SOSIP.664 |
| 6N1V | A12V163-a.01 Fab, VRC03 Fab, PGT122 Fab | BG505 DS-SOSIP |
| 6N1W | DFPH-a.15 Fab, VRC03 Fab, PGT122 Fab | BG505 DS-SOSIP |
| 6NC2 | ACS202 Fab | AMC011 v4.2 SOSIP Env |
| 6NC3 | VRC34 Fab | AMC011 v4.2 SOSIP Env |
| 6NF2 | 0PV-c.01 Fab, VRC03 Fab, PGT122 Fab | BG505 DS-SOSIP |
| 6NIJ | PGT145 Fab | AMC011 HIV-1 Env |
| 6NM6 | N6 FR3-03 scFv, 3H109L Fab, 35O22 scFv | BG505 SOSIP.664 |
| 6NNF | FR3-03 scFv, 3H109L Fab, 35O22 scFv | BG505 SOSIP.664 |
| 6NNJ | CH31 scFv, 3H109L Fab, 35O22 scFv | BG505 SOSIP.664 |
| 6OHY | None | Chimpanzee SIV Env trimeric ectodomain |
| 6OKP | SF12 Fab, 10-1074 Fab | B41 SOSIP.664 |
| 6OLP | PGT151 Fab | HIV-1 Env AMC011 |
| 6ORN | 10-1074 Fab | BG505 SOSIP.664 based immunogen RC1 |
| 6ORO | Ab874NHP Fab | BG505 SOSIP.664 based immunogen RC1 |
| 6ORP | Ab897NHP Fab | BG505 SOSIP.664 based immunogen RC1 |
| 6ORQ | Ab275NHP Fab | BG505 SOSIP.664 based immunogen RC1 |
| 6OSY | VRC03 Fab, PGT122 Fab, 0PV-a.01 Fab | BG505 DS-SOSIP |
| 6OT1 | VRC03 Fab, PGT122 Fab, 0PV-b.01 Fab | BG505 DS-SOSIP |
| 6UDA | CH235UCA Fab | CH505.N279K.G458Y.SOSIP.664 |

**Supplementary Table 4. Relative state occupancy and fitting parameters of conformational distributions of the virus Env observed from two different structural perspectives, with reference to Figure 4E.**

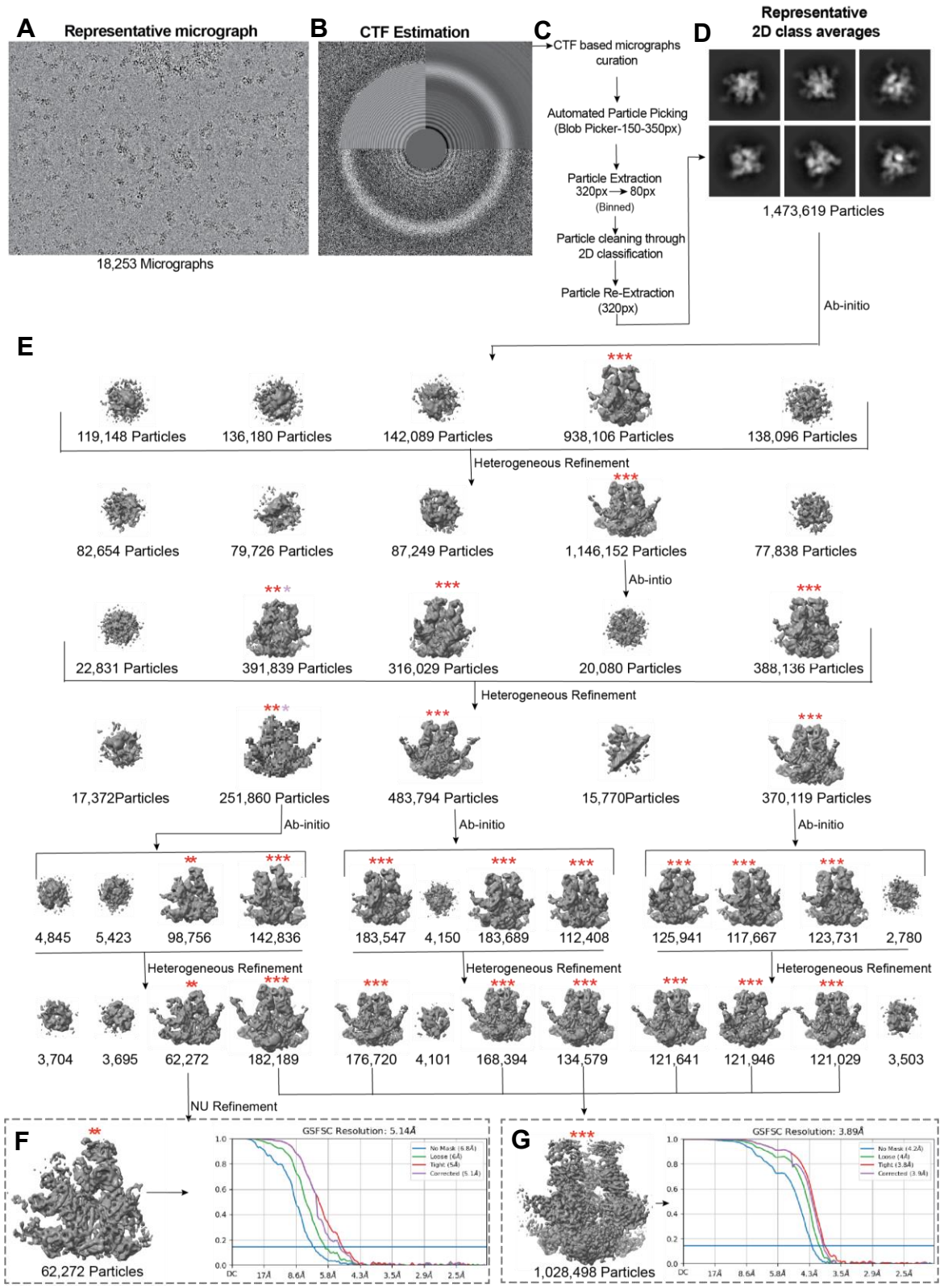
| **BG505 Virus Env** | Curve fitting $R^2$ | RMSE | Pre-triggered<br>μ: 0.1 ± 0.02<br>σ: 0.07 ± 0.01 | Pre-fusion closed<br>μ: 0.60 ± 0.03<br>σ: 0.15 ± 0.02 | Three-CD4 open<br>μ: 0.3 ± 0.03<br>σ: 0.1 ± 0.01 |
|---|---|---|---|---|---|
| **$N136_{TAG}$ $S401_{TAG}$** | | | | | |
| Ligand-free | 0.9891 | 9.4074e-04 | 52% ± 07% | 18% ± 12% | 30% ± 10% |
| + VRC34 | 0.9669 | 0.0012 | 35% ± 11% | 45% ± 14% | 20% ± 11% |
| + sCD4 + 17b + VRC34 | 0.9891 | 7.044e-04 | 28% ± 06% | 38% ± 14% | 34% ± 14% |
| + sCD4 + 17b | 0.9791 | 9.2681e-04 | 26% ± 09% | 33% ± 14% | 41% ± 13% |

| **BG505 Virus Env** | Curve fitting $R^2$ | RMSE | μ: 0.55 ± 0.05<br>σ: 0.15 ± 0.02 | μ: 0.28 ± 0.03<br>σ: 0.1 ± 0.01 | μ: 0.10 ± 0.02<br>σ: 0.07 ± 0.01 |
|---|---|---|---|---|---|
| **$V4\text{-}A1$ $R542_{TAG}$** | | | | | |
| Ligand-free | 0.9914 | 5.0798e-04 | 53% ± 07% | 30% ± 07% | 17% ± 03% |
| + VRC34 | 0.9903 | 7.6231e-04 | 36% ± 16% | 47% ± 12% | 17% ± 13% |
| + sCD4 + 17b + VRC34 | 0.9946 | 4.9572e-04 | 33% ± 06% | 33% ± 11% | 34% ± 07% |
| + sCD4 + 17b | 0.9914 | 7.3402e-04 | 32% ± 10% | 27% ± 14% | 41% ± 10% |

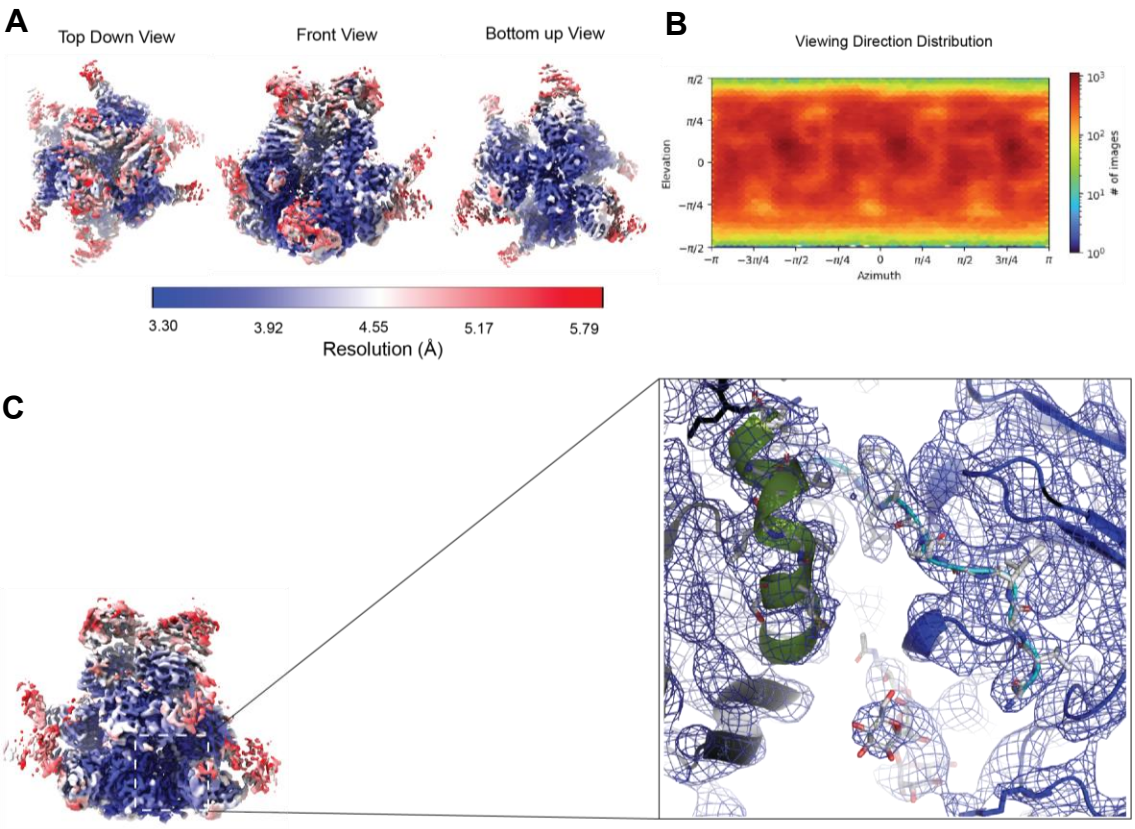
A pair of fluorophores labeled on different Env sites result in different FRET signals. In each FRET structural perspective, FRET histograms were fitted into the sum of three distinct Gaussian distributions with defined means (μ) and deviations (σ) of the individual Gaussian/normal distributions. μ and σ were determined based on the observation of unbiased raw FRET signals and were further determined using hidden Markov modeling. As a measure of uncertainty, we presented the probability of each state Env occupies as mean + s.e.m. $R^2$ and RMSE (Root Mean Square Deviation) evaluate the goodness of fitting.



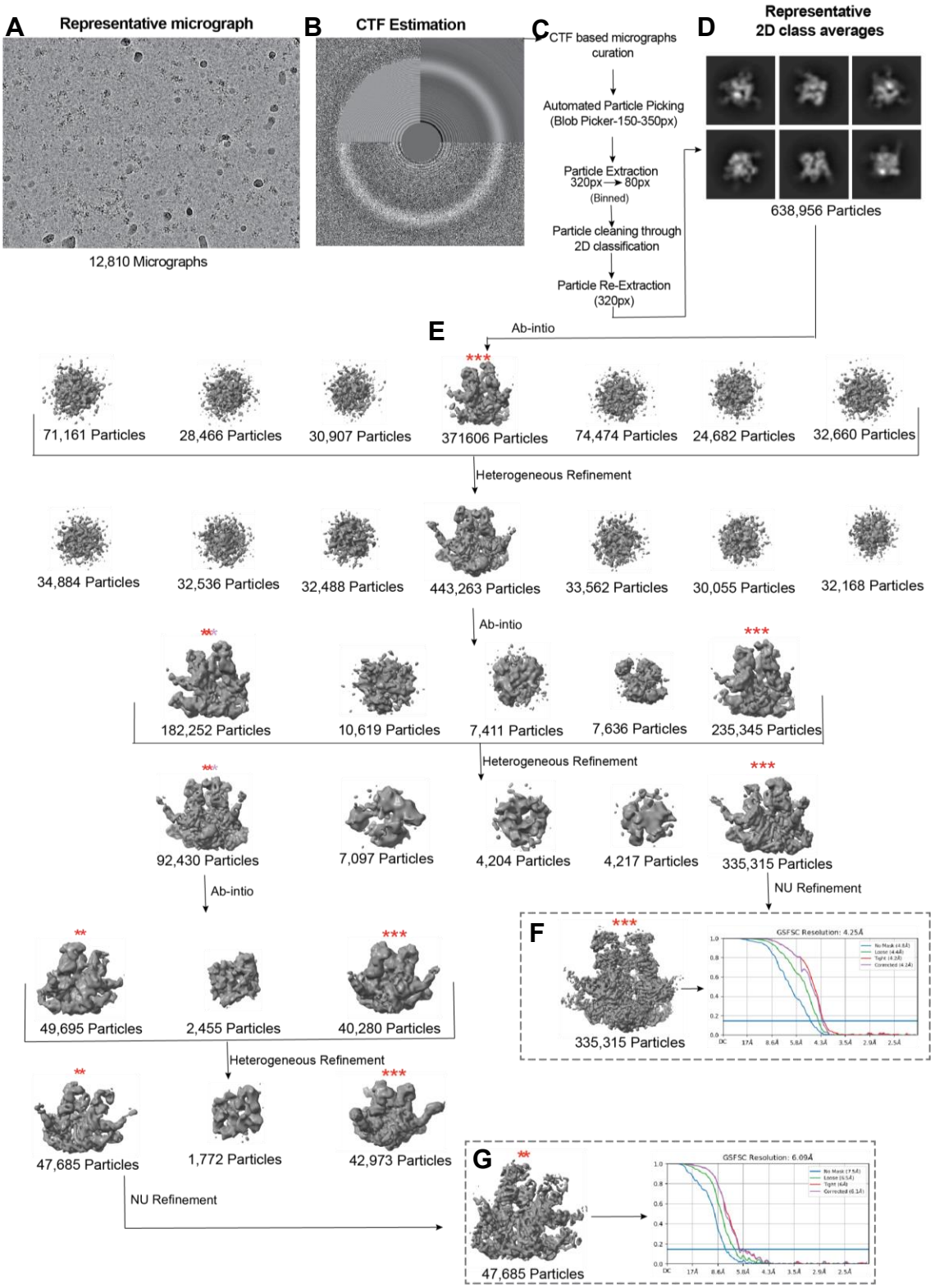

**Supplementary Figure 1. CD4-induced conformational changes in HIV-1 Env measured using SPR**. Blank subtracted, double referenced sensorgrams obtained by injecting on a ligand IgG surface the analytes at specific time-points post-incubation. **A)** Incubations and measurements performed at 25 °C. **B)** Incubations and measurements performed at 37 °C. Data shown are representative of at least two independent experiments. Two independent repeats are shown in panel B, performed at two levels of immobilization. Results were equivalent, showing progressive increase in 17b binding and reduction of VRC34.01 binding post CD4 addition to Env (an effect that was enhanced when 17b Fab was added together with CD4). Source data are provided as Source Data files.


A
Representative micrograph
18,253 Micrographs
B
CTF Estimation
C
CTF based micrographs curation
Automated Particle Picking (Blob Picker-150-350px)
Particle Extraction 320px → 80px (Binned)
Particle cleaning through 2D classification
Particle Re-Extraction (320px)
D
Representative 2D class averages
1,473,619 Particles
Ab-initio
E
119,148 Particles
136,180 Particles
142,089 Particles
938,106 Particles
138,096 Particles
Heterogeneous Refinement
82,654 Particles
79,726 Particles
87,249 Particles
1,146,152 Particles
77,838 Particles
Ab-intio
22,831 Particles
391,839 Particles
316,029 Particles
20,080 Particles
388,136 Particles
Heterogeneous Refinement
17,372Particles
251,860 Particles
483,794 Particles
15,770Particles
370,119 Particles
Ab-initio
Ab-initio
Ab-initio
4,845
5,423
98,756
142,836
183,547
4,150
183,689
112,408
125,941
117,667
123,731
2,780
Heterogeneous Refinement
Heterogeneous Refinement
Heterogeneous Refinement
3,704
3,695
62,272
182,189
176,720
4,101
168,394
134,579
121,641
121,946
121,029
3,503
NU Refinement
F
62,272 Particles
GSFSC Resolution: 5.14Å
G
1,028,498 Particles
GSFSC Resolution: 3.89Å

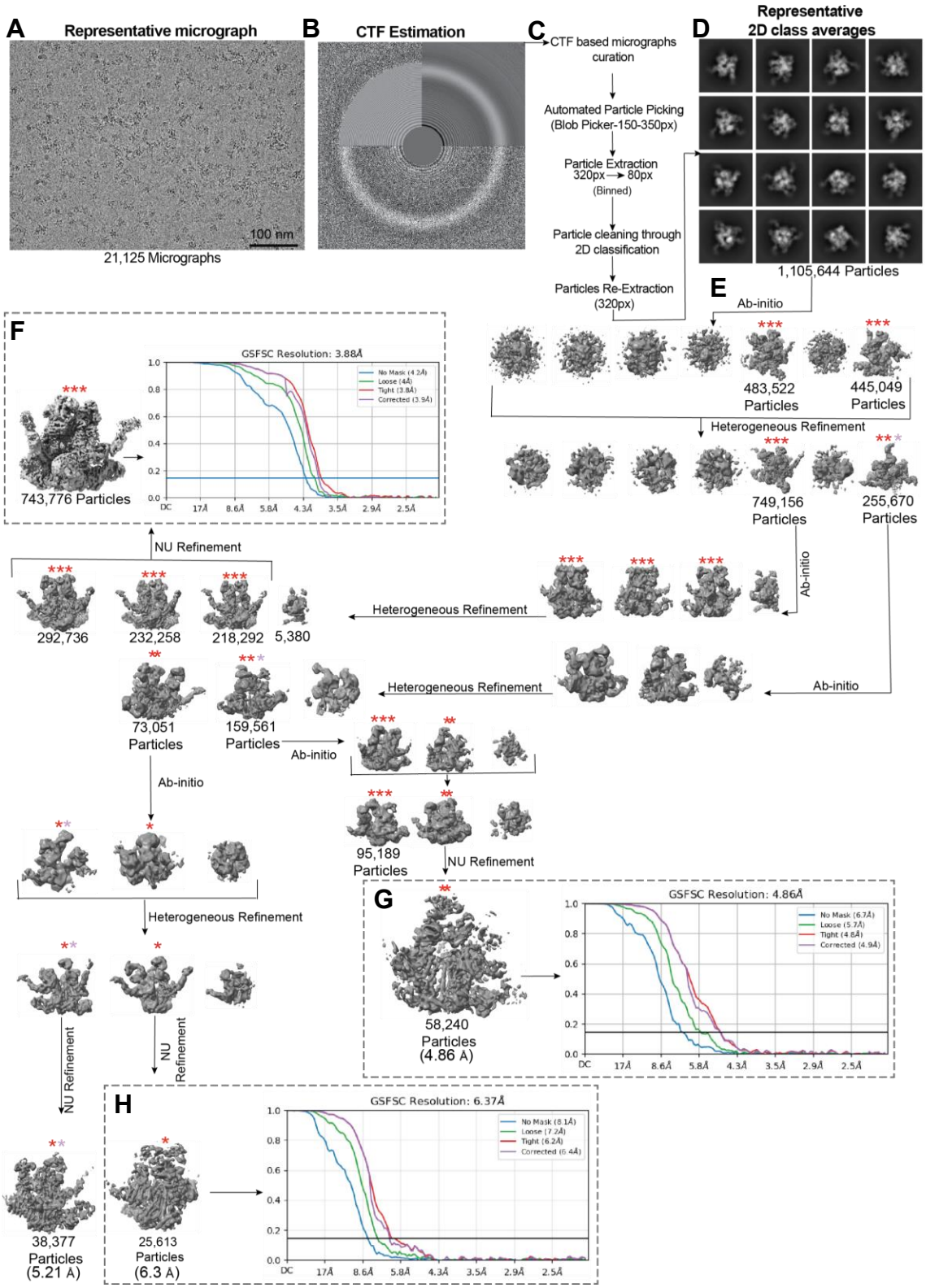
**Supplementary Figure 2. Cryo-EM data processing for BG505 SOSIP.664 incubated with CD4 and 17b for 1.3 hours at 25 °C followed by incubation with VRC34.01 Fab for 30 minutes prior to vitrification**. **A)** Representative frame aligned micrograph. **B)** Representative contrast transfer function (CTF) fit.. **C)** Data Processing workflow, leading to, **D)** Representative 2D class averages from 2D classification, followed by **, E)** 3D classifications and refinements. **F)** Refined map of Env bound to 3 molecules of CD4, 3 molecules of 17b Fabs, and 2 molecule of VRC34.01 Fabs (indicated by 2 red asterisks). **G)** Refined map of Env bound to 3 molecules of CD4, 3 molecules of 17b Fabs, and 3 molecule of VRC34.01 Fabs (indicated by 3 red asterisks). Fourier shell correlation (FSC) curves for refined 3D maps shown in panels **F** and **G** with the horizontal blue line showing FSC threshold value of 0.143.



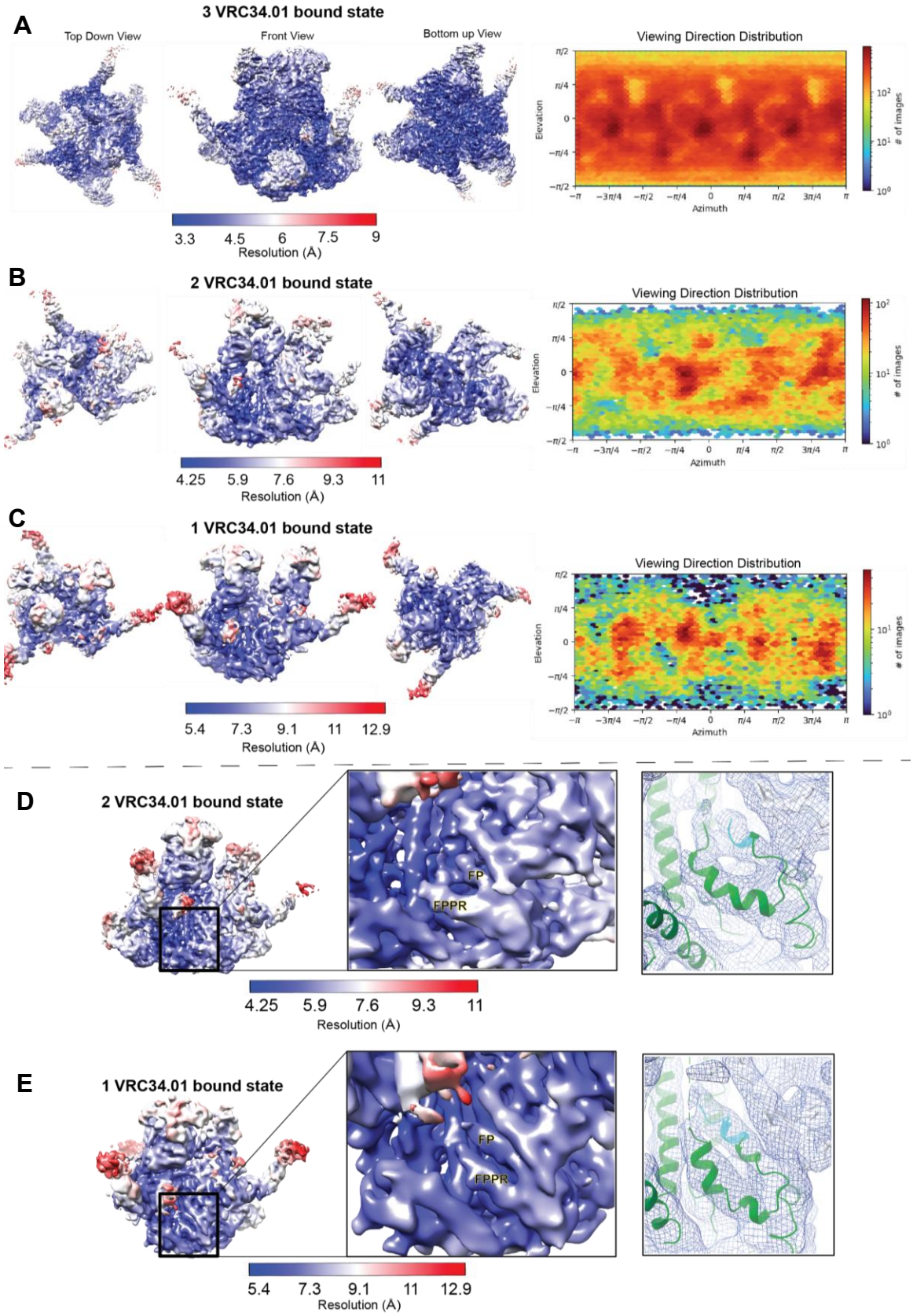

**Supplementary Figure 3. Particle distribution and local resolution estimation for cryo-EM dataset of BG505 SOSIP.664 incubated with CD4 and 17b for 1.3 hours followed by incubation with VRC34.01 Fab for 30 minutes prior to vitrification**. **A)** Refined cryo-EM map of Env complex with CD4 and 17b, bound to 3 VRC34.01 Fabs, colored by local resolution. **B)** Particle distribution. **C)** Zoomed in view of region around the FP showing the map as a blue mesh (cryo-EM map contoured at a level of 0.143 in ChimeraX) with underlying fitted model shown in cartoon representation and glycans shown as sticks. The FP is shown both in cartoon representation (colored cyan) and as light grey sticks colored by atom.


A
Representative micrograph
12,810 Micrographs
B
CTF Estimation
C
CTF based micrographs curation
Automated Particle Picking (Blob Picker-150-350px)
Particle Extraction 320px → 80px (Binned)
Particle cleaning through 2D classification
Particle Re-Extraction (320px)
D
Representative 2D class averages
638,956 Particles
E
Ab-intio
71,161 Particles
28,466 Particles
30,907 Particles
371606 Particles
74,474 Particles
24,682 Particles
32,660 Particles
Heterogeneous Refinement
34,884 Particles
32,536 Particles
32,488 Particles
443,263 Particles
33,562 Particles
30,055 Particles
32,168 Particles
Ab-intio
182,252 Particles
10,619 Particles
7,411 Particles
7,636 Particles
235,345 Particles
Heterogeneous Refinement
92,430 Particles
7,097 Particles
4,204 Particles
4,217 Particles
335,315 Particles
Ab-intio
NU Refinement
49,695 Particles
2,455 Particles
40,280 Particles
Heterogeneous Refinement
47,685 Particles
1,772 Particles
42,973 Particles
NU Refinement
F
335,315 Particles
G
47,685 Particles

**Supplementary Figure 4. Cryo-EM data processing for BG505 SOSIP.664 incubated with CD4 and 17b for 20 hours at 25 °C followed by incubation with VRC34.01 Fab for 30 minutes prior to vitrification**. **A)** Representative frame aligned micrograph. **B)** Representative contrast transfer function (CTF) fit. **C)** Data Processing workflow, leading to, **D)** Representative 2D class averages from 2D classification, followed by, **E)** 3D classifications and refinements. **F)** Refined map of Env bound to 3 molecules of CD4, 3 molecules of 17b Fabs, and 3 molecule of VRC34.01 Fabs (indicated by 3 red asterisks. **G)** Refined map of Env bound to 3 molecules of CD4, 3 molecules of 17b Fabs, and 2 molecule of VRC34.01 Fabs (indicated by 2 red asterisks). Fourier shell correlation (FSC) curves for refined 3D maps shown in panels **F** and **G** with the horizontal blue line showing FSC threshold value of 0.143.

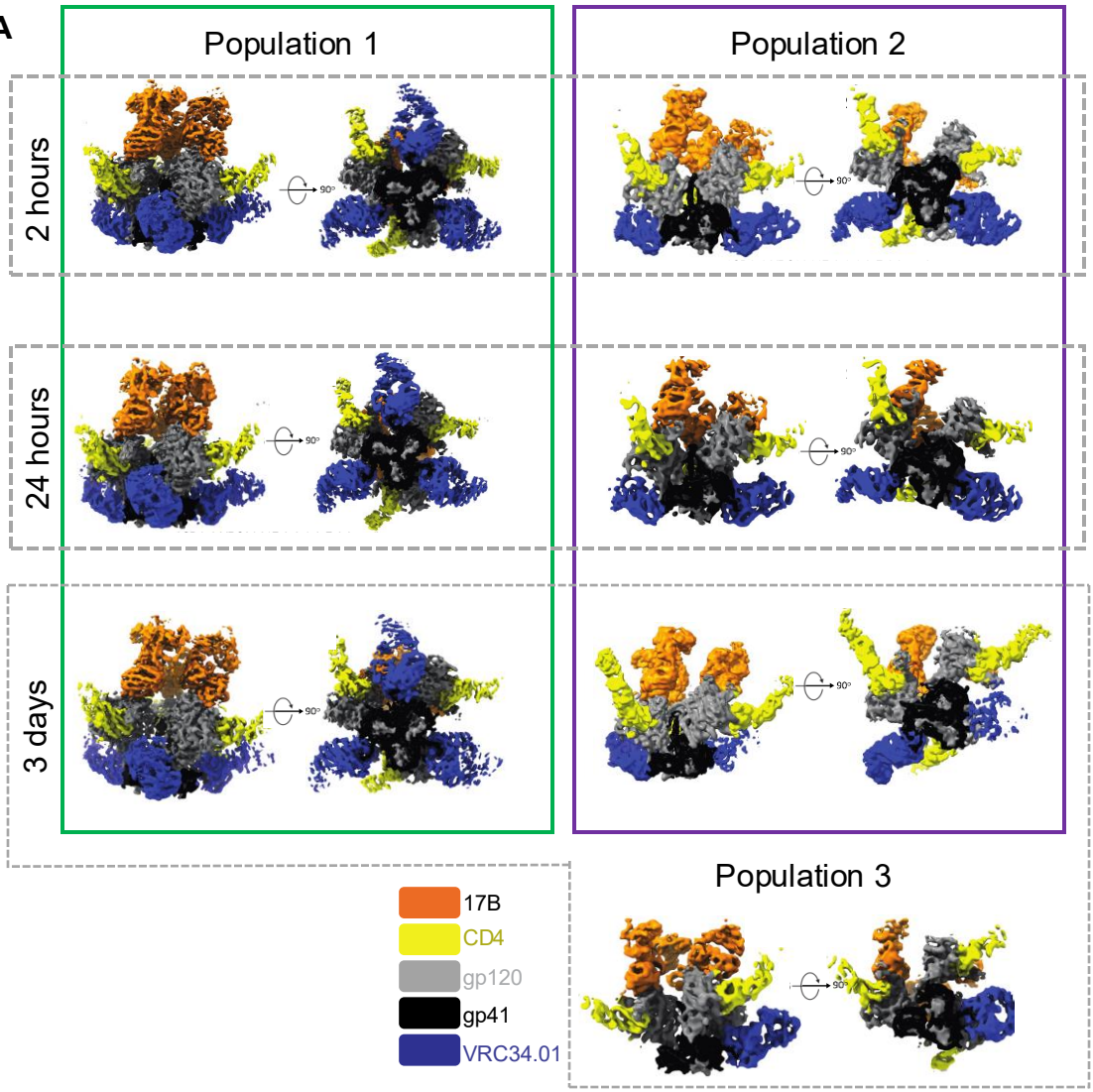

A
Representative micrograph
100 nm
21,125 Micrographs
B
CTF Estimation
C
CTF based micrographs curation
Automated Particle Picking (Blob Picker-150-350px)
Particle Extraction 320px → 80px (Binned)
Particle cleaning through 2D classification
Particles Re-Extraction (320px)
D
Representative 2D class averages
1,105,644 Particles
E
Ab-initio
483,522 Particles
445,049 Particles
Heterogeneous Refinement
749,156 Particles
255,670 Particles
Ab-initio
Heterogeneous Refinement
292,736
232,258
218,292
5,380
NU Refinement
F
GSFSC Resolution: 3.88Å
No Mask (4.2Å)
Loose (4Å)
Tight (3.8Å)
Corrected (3.9Å)
743,776 Particles
Ab-initio
Heterogeneous Refinement
73,051 Particles
159,561 Particles
Ab-initio
95,189 Particles
NU Refinement
G
GSFSC Resolution: 4.86Å
No Mask (6.7Å)
Loose (5.7Å)
Tight (4.8Å)
Corrected (4.9Å)
58,240 Particles (4.86 Å)
Heterogeneous Refinement
NU Refinement
NU Refinement
H
GSFSC Resolution: 6.37Å
No Mask (8.1Å)
Loose (7.2Å)
Tight (6.2Å)
Corrected (6.4Å)
38,377 Particles (5.21 Å)
25,613 Particles (6.3 Å)

**Supplementary Figure 5. Cryo-EM data processing for BG505 SOSIP.664 incubated with CD4 and 17b for 3 days at 25 °C followed by incubation with VRC34.01 Fab for 30 minutes prior to vitrification**. **A)** Representative frame aligned micrograph. **B)** Representative contrast transfer function (CTF) fit. **C)** Data Processing workflow, leading to, **D)** Representative 2D class averages from 2D classification, followed by , **E)** 3D classifications and refinements. **F)** Refined map of Env bound to 3 molecules of CD4, 3 molecules of 17b Fabs, and 3 molecule of VRC34.01 Fabs (indicated by 3 red asterisks). **G)** Refined map of Env bound to 3 molecules of CD4, 3 molecules of 17b Fabs, and 2 molecule of VRC34.01 Fabs (indicated by 2 red asterisks). **H)** Refined map of Env bound to 3 molecules of CD4, 3 molecules of 17b Fabs, and 1 molecule of VRC34.01 Fab. (indicated by 1 red asterisk). Fourier shell correlation (FSC) curves for refined 3D maps shown in panels **F-H** with the horizontal blue line showing FSC threshold value of 0.143. The asterisk indicate the number of VRC34.01 bound in each cryo-EM map. The red asterisks indicate well-defined density for the VRC34.01 Fab. The pink asterisks indicate partial density for the VRC34.01 Fab.


A
3 VRC34.01 bound state
Top Down View
Front View
Bottom up View
3.3 4.5 6 7.5 9
Resolution (Å)
Viewing Direction Distribution
Elevation
Azimuth
# of images
B
2 VRC34.01 bound state
4.25 5.9 7.6 9.3 11
Resolution (Å)
Viewing Direction Distribution
C
1 VRC34.01 bound state
5.4 7.3 9.1 11 12.9
Resolution (Å)
Viewing Direction Distribution
D
2 VRC34.01 bound state
FP
FPPR
4.25 5.9 7.6 9.3 11
Resolution (Å)
E
1 VRC34.01 bound state
FP
FPPR
5.4 7.3 9.1 11 12.9
Resolution (Å)

**Supplementary Figure 6. Particle distribution and local resolution estimation for cryo-EM dataset of BG505 SOSIP.664 incubated with CD4 and 17b for 3 days followed by incubation with VRC34.01 Fab for 30 minutes prior to vitrification**. **A)** Left. Refined cryo-EM map of Env complex with CD4 and 17b, bound to 3 VRC34.01 Fabs, colored by local resolution. Right. Particle distribution. **B)** Same as in panel A**,** but for the 2 VRC34.01-bound population. **C)** Same as in panel A**,** but for the 1 VRC34.01-bound population. **D** and **E)** Zoomed in view of region around the FP and FPPR in the protomer that was not bound to VRC34.01 in **(D)** the 2-VRC34.01 bound structure (cryo-EM map contoured at a level of 0.111 in ChimeraX), and **(E)** one of the two protomers in the 1-VRC34.01 bound structure that was not bound to VRC34.01 (cryo-EM map contoured at a level of 0.113 in ChimeraX). Zoomed-in panels on the left shown the cryo-EM map colored by local resolution. Panels on the right show the cryo-EM map as a blue mesh with underlying fitted model in cartoon representation with fusion peptide colored cyan. All the structures shown are bound to 3 molecules of CD4 and 3 molecules of 17b Fabs.

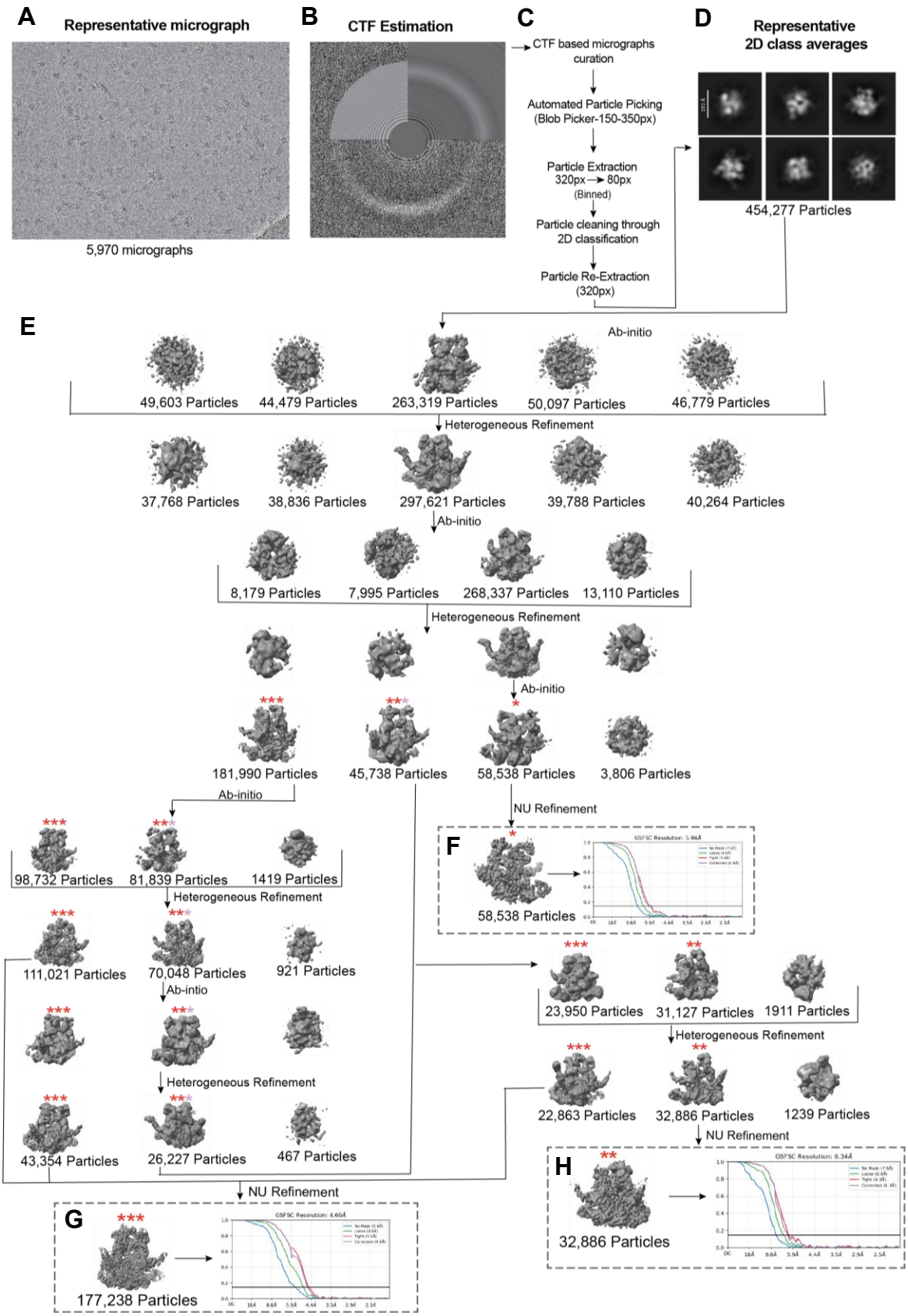
**A**




**B**

| | 3-CD4, 3-17b Fab, 3-VRC34.01 Fab bound **Population 1** | 3-CD4, 3-17b Fab, 2-VRC34.01 Fab bound **Population 2** | 3-CD4, 3-17b Fab, 2-VRC34.01 Fab bound **Population 3** |
|---|---|---|---|
| 1.3 hours | 1,028,496 (94.3 %) | 62,272 (5.7 %) | ND |
| 20 hours | 335,315 (87.5 %) | 47,685 (12.5 %) | ND |
| 3 days | 743,776 (89.86 %) | 58,240 (7.0 %) | 25,613 (3.0 %) |

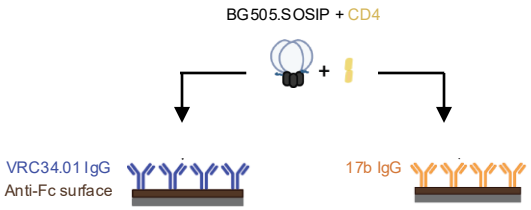
**Supplementary Figure 7. Diversity of HIV-1 Env intermediate states visualized by cryo-EM. A)** 3D reconstructions of particle populations identified in cryo-EM datasets at different time-points post CD4, 17b incubation of HIV-1 Env followed by addition of VRC34.01 Fab 30 minutes prior to sample vitrification. **B)** Summary of approximate particle numbers and relative percentages of each population identified at each timepoint.

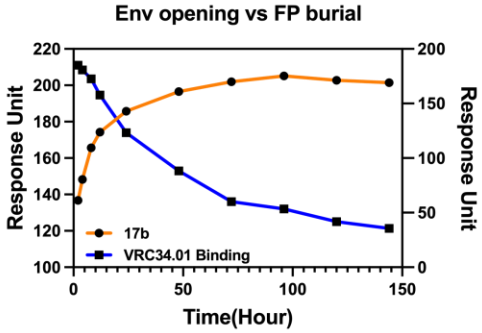

A
Representative micrograph
5,970 micrographs
B
CTF Estimation
C
CTF based micrographs curation
Automated Particle Picking (Blob Picker-150-350px)
Particle Extraction 320px → 80px (Binned)
Particle cleaning through 2D classification
Particle Re-Extraction (320px)
D
Representative 2D class averages
454,277 Particles
E
Ab-initio
49,603 Particles
44,479 Particles
263,319 Particles
50,097 Particles
46,779 Particles
Heterogeneous Refinement
37,768 Particles
38,836 Particles
297,621 Particles
39,788 Particles
40,264 Particles
Ab-initio
8,179 Particles
7,995 Particles
268,337 Particles
13,110 Particles
Heterogeneous Refinement
Ab-initio
181,990 Particles
45,738 Particles
58,538 Particles
3,806 Particles
Ab-initio
NU Refinement
98,732 Particles
81,839 Particles
1419 Particles
F
58,538 Particles
Heterogeneous Refinement
111,021 Particles
70,048 Particles
921 Particles
Ab-intio
23,950 Particles
31,127 Particles
1911 Particles
Heterogeneous Refinement
Heterogeneous Refinement
22,863 Particles
32,886 Particles
1239 Particles
NU Refinement
43,354 Particles
26,227 Particles
467 Particles
H
32,886 Particles
NU Refinement
G
177,238 Particles

**Supplementary Figure 8. Cryo-EM data processing workflow for BG505 SOSIP.664 incubated with CD4 and 17b for 2 hours at 37 °C followed by incubation with VRC34.01 Fab for 30 minutes prior to vitrification**. **A)** Representative frame aligned micrograph. **B)** Representative contrast transfer function (CTF) fit. **C)** Data Processing workflow, leading to **(D)** Representative 2D class averages from 2D classification, followed by **(E)** 3D classifications and refinements. **F)** Refined map of Env bound to 3 molecules of CD4, 3 molecules of 17b Fabs, and 1 molecule of VRC34.01 Fab (indicated by 1 red asterisks) **G)** Refined map of Env bound to 3 CD4 molecules, 3 molecules of 17b Fab, and 3 molecules of VRC34.01 Fabs. (indicated by 3 red asterisks). **H)** Refined map of Env bound to 3 CD4 molecules, 3 molecules of 17b Fabs, and 2 molecules of VRC34.01 Fabs. (indicated by 2 red asterisks). Fourier shell correlation (FSC) curves for refined 3D maps shown in panels (**F-H)** with the horizontal blue line showing FSC threshold value of 0.143. The red asterisks indicate well-defined density for the VRC34.01 Fab. The pink asterisks indicate partial density for the VRC34.01 Fab.

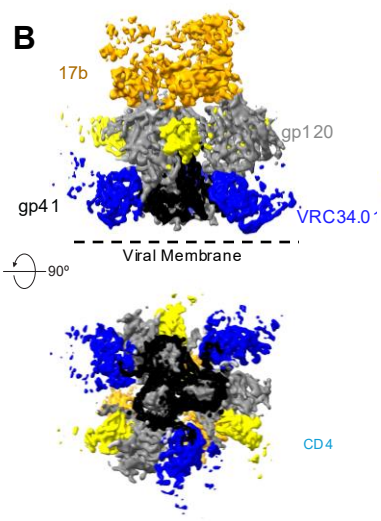
**A**

CD4-induced Env opening vs FP burial at 37℃



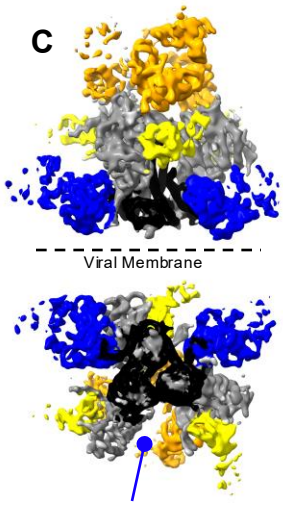




**B**



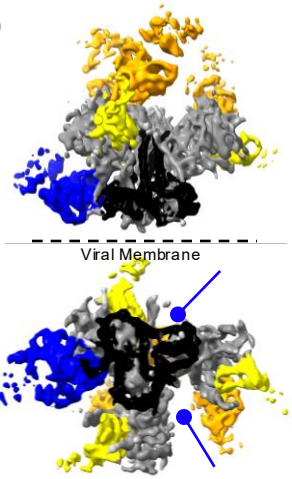

3-VRC34.01 bound state
4.6 Å, 177,238 particles

**C**



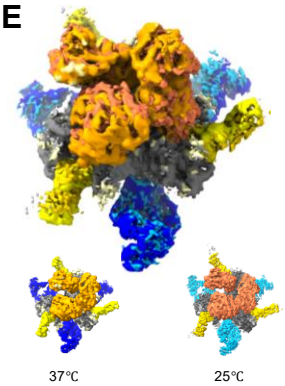

2-VRC34.01 bound state
6.34 Å, 32,866 particles

**D**



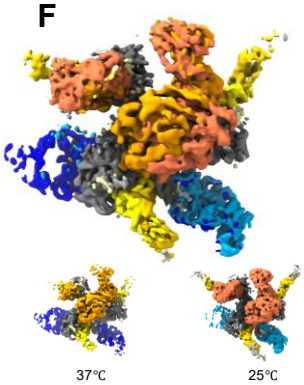

1-VRC34.01 bound state
5.94 Å 53,275 particles

**E**



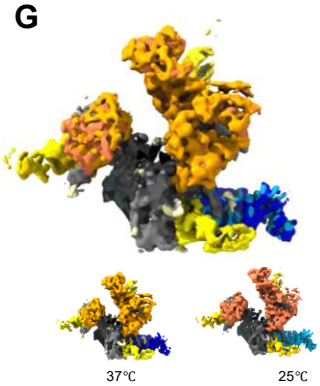

Alignment between 3-VRC34.01 bound states obtained at 25 ℃ and 37 ℃

**F**




Alignment between 2-VRC34.01 bound states obtained at 25 ℃ and 37 ℃

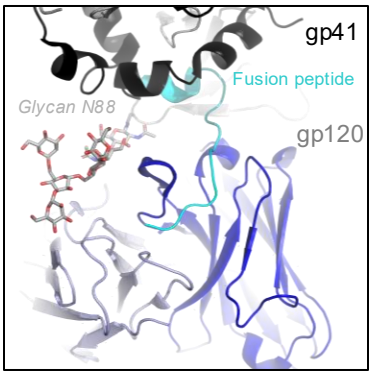
**G**



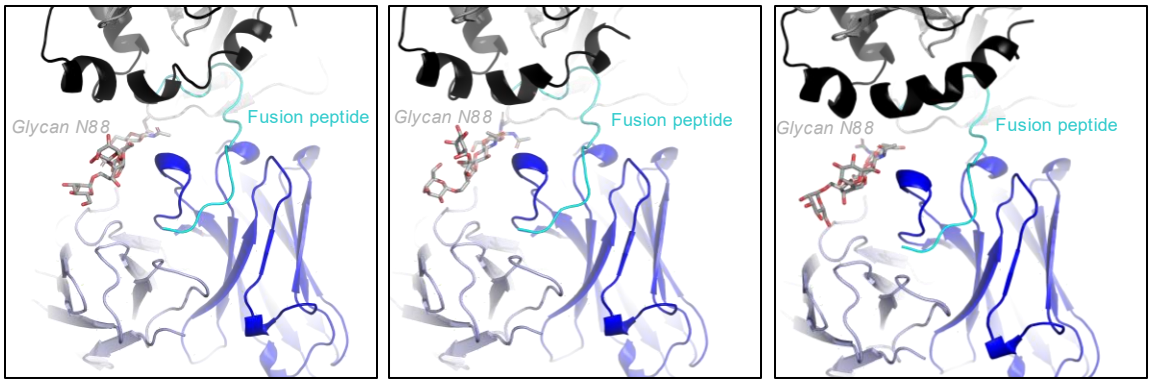

Alignment between 1-VRC34.01 bound states obtained at 25 ℃ and 37 ℃

**Supplementary Figure 9. Time-dependent conformational changes in HIV-1 BG505 SOSIP Env upon incubation with CD4 at 37 ℃. A)** Surface plasmon based binding (SPR) analysis monitoring simultaneous Env opening and fusion peptide burial were measured by incubating Env with CD4 at 37 ℃ and at different time-points injecting over a VRC34.01 IgG or a 17b IgG surface. The SPR measurements were carried out at at 37 ℃. The dataset labeled Repeat 1 in Supplementary Figure 1, panel B was used for plotting the graph. **B-D)** Cryo-EM reconstructions of CD4/17b-bound, partially open Env bound to **B)** 3 VRC34.01 Fabs, **C)** 2 VRC34.01 Fabs and **D)** 1 VRC34.01 Fab. The blue arrows indicate sites unoccupied by VRC34.01. **E)** Alignment of partially open Env states bound to 3-VRC34.01 obtained at 25 ℃ and 37 ℃. **F)** Alignment of partially open Env states bound to 2-VRC34.01 obtained at 25 ℃ and 37 ℃. **G)** Alignment of partially open Env states bound to 1-VRC34.01 obtained at 25 ℃ and 37 ℃. Source data are provided as a Source Data file.

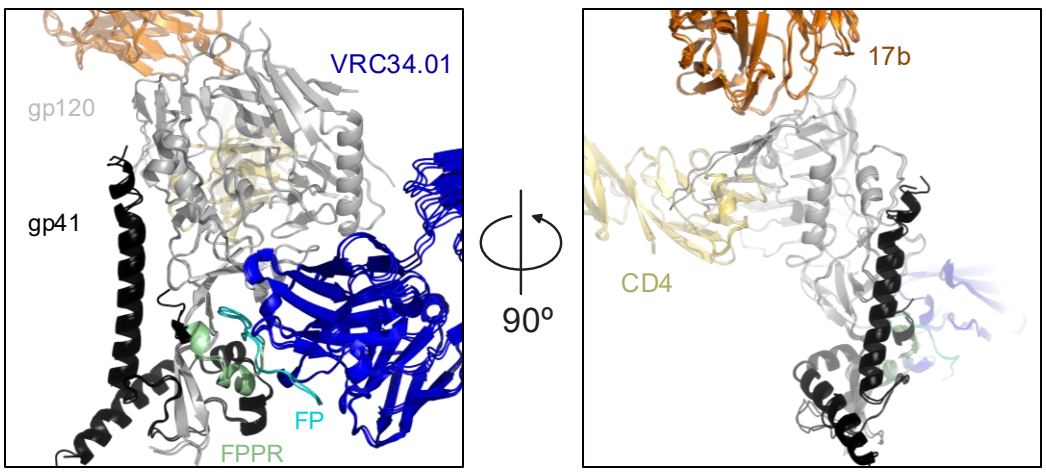
**A** BG505 SOSIP-PGT122-VRC34.01 (Closed conformation)
PDB: 5I8H




**B** BG505 SOSIP-CD4-17b-VRC34.01 (Partially open conformation)
PDB: 9D90 (this study)



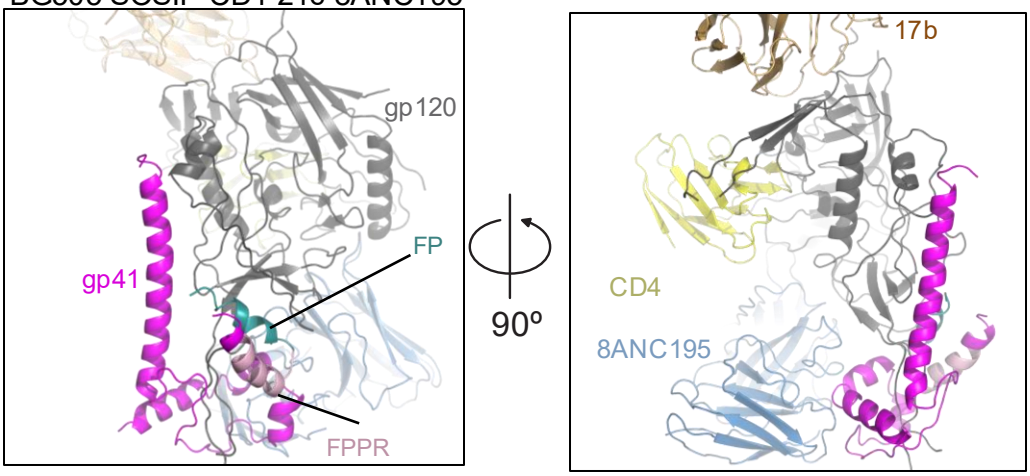

**C** BG505 SOSIP-CD4-17b-VRC34.01 (Partially open conformation)
PDB: 9D90 (this study)



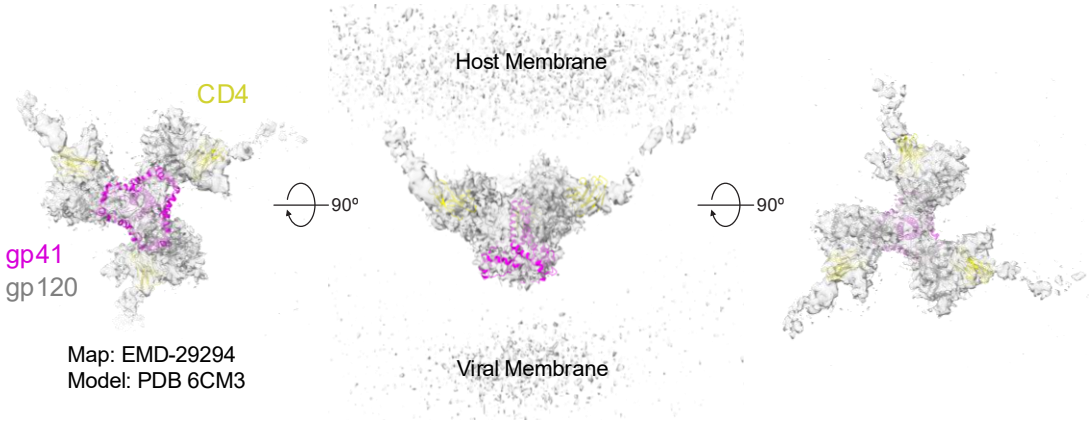
**Supplementary Figure 10. Antibody recognition of FP in the context of diverse Env conformations. A)** VRC34.01 bound to HIV-1 Env in closed conformation. VRC34.01 heavy and light chains are shown as dark blue and light blue cartoon, respectively; gp120 is colored grey with the glycan at position N88 shown in stock representation; gp41 is colored black; FP s colored cyan. **B)** VRC34.01 bound to HIV-1 Env in partially open conformation. Color scheme is same as in panel **A**. **C)** The three protomers of the Population 1 structure (PDB: 9D90; this study) overlayed by their gp120 subunits. VRC34.01 bound to HIV-1 Env in partially open conformation. Color scheme is same as in panel **A,** except both VRC34.01 heavy and light chains are colored dark blue. FPPR is colored pale green, CD4 yellow and 17b orange.

A

PDB:6CM3
BG505 SOSIP-CD4-21c-8ANC195

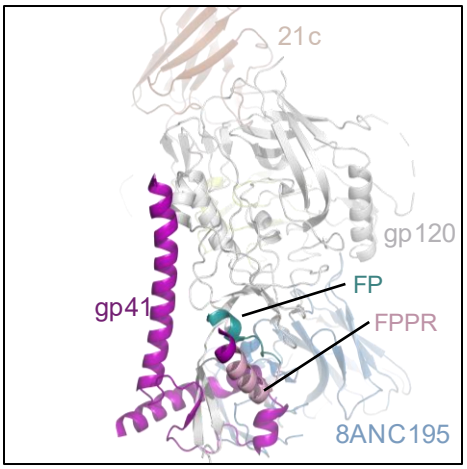



B



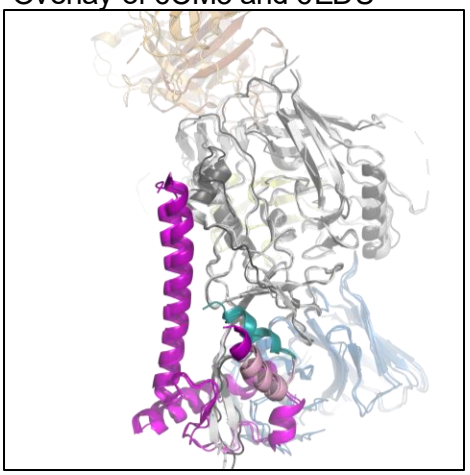

C

PDB:6EDU
B41 SOSIP-CD4-21c-8ANC195

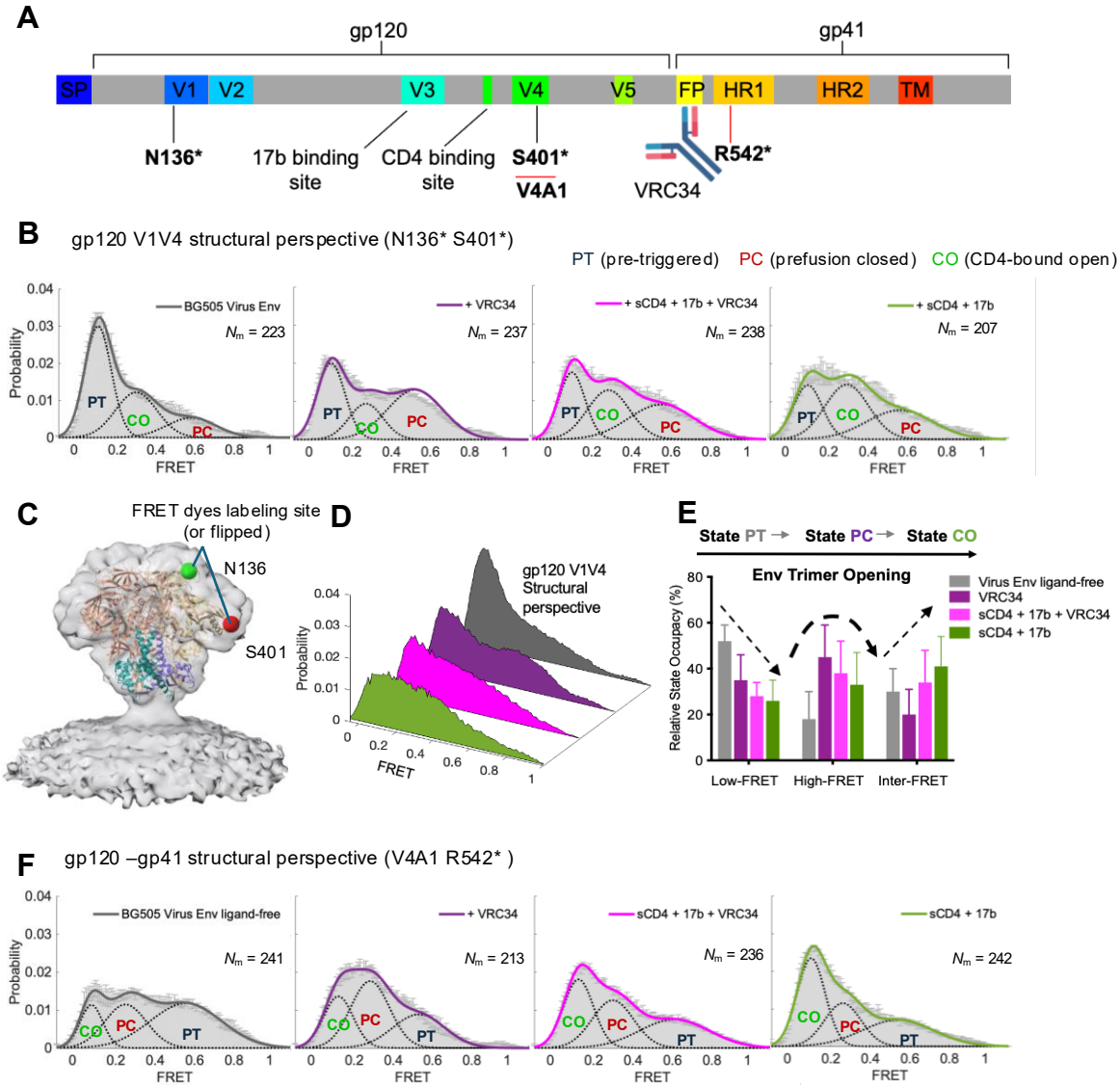



D

Overlay of 6CM3 and 6EDU



**Supplementary Figure 11. Cryo-EM structure of BG505 SOSIP Env bound to CD4, 17b and 8ANC195 (PDB: 6CM3)**. **A)** One protomer of the partially open Env bound to CD4, 17b Fab and 8ANC195 Fab (PDB ID: 6CM3) shown in cartoon representation zoomed-in at the gp120/gp41 interface. The gp120 subunit is colored dark grey, gp41 magenta, FP dark teal and FPPR light pink. The bound CD4 is colored yellow, 8ANC195 Fab marine blue, 17b Fab brown. **B)** Coordinates of PDB: 6CM3 including Env (gp120 in light gray, gp41 in magenta) and CD4 (yellow) fitted into the *in situ* cryo-ET reconstruction of a partially open CD4-bound Env (EMD-29294). **C)** One protomer of the partially open Env bound to CD4, 21c Fab and 8ANC195 Fab (PDB ID: 6EDU) shown in cartoon representation zoomed-in at the gp120/gp41 interface. The gp120 subunit is colored light grey, gp41 dark purple, FP dark teal and FPPR light pink. The bound CD4 is colored yellow, 8ANC195 Fab marine blue, 21c Fab chocolate. **D)** Structures of BG505 SOSIP bound to CD4, 17b Fab and 8ANC195 Fab, and of B41 SOSIP bound to CD4, 21c Fab and 8ANC195 Fab, overlaid by their gp120 subunits.


A
gp120
gp41
SP
V1
V2
V3
V4
V5
FP
HR1
HR2
TM
N136*
17b binding site
CD4 binding site
S401*
V4A1
VRC34
R542*
B
gp120 V1V4 structural perspective (N136* S401*)
PT (pre-triggered) PC (prefusion closed) CO (CD4-bound open)
BG505 Virus Env
Nm = 223
+ VRC34
Nm = 237
+ sCD4 + 17b + VRC34
Nm = 238
+ sCD4 + 17b
Nm = 207
Probability
FRET
C
FRET dyes labeling site (or flipped)
N136
S401
D
gp120 V1V4 Structural perspective
E
State PT → State PC → State CO
Env Trimer Opening
Virus Env ligand-free
VRC34
sCD4 + 17b + VRC34
sCD4 + 17b
Relative State Occupancy (%)
Low-FRET
High-FRET
Inter-FRET
F
gp120 –gp41 structural perspective (V4A1 R542* )
BG505 Virus Env ligand-free
Nm = 241
+ VRC34
Nm = 213
sCD4 + 17b + VRC34
Nm = 236
sCD4 + 17b
Nm = 242

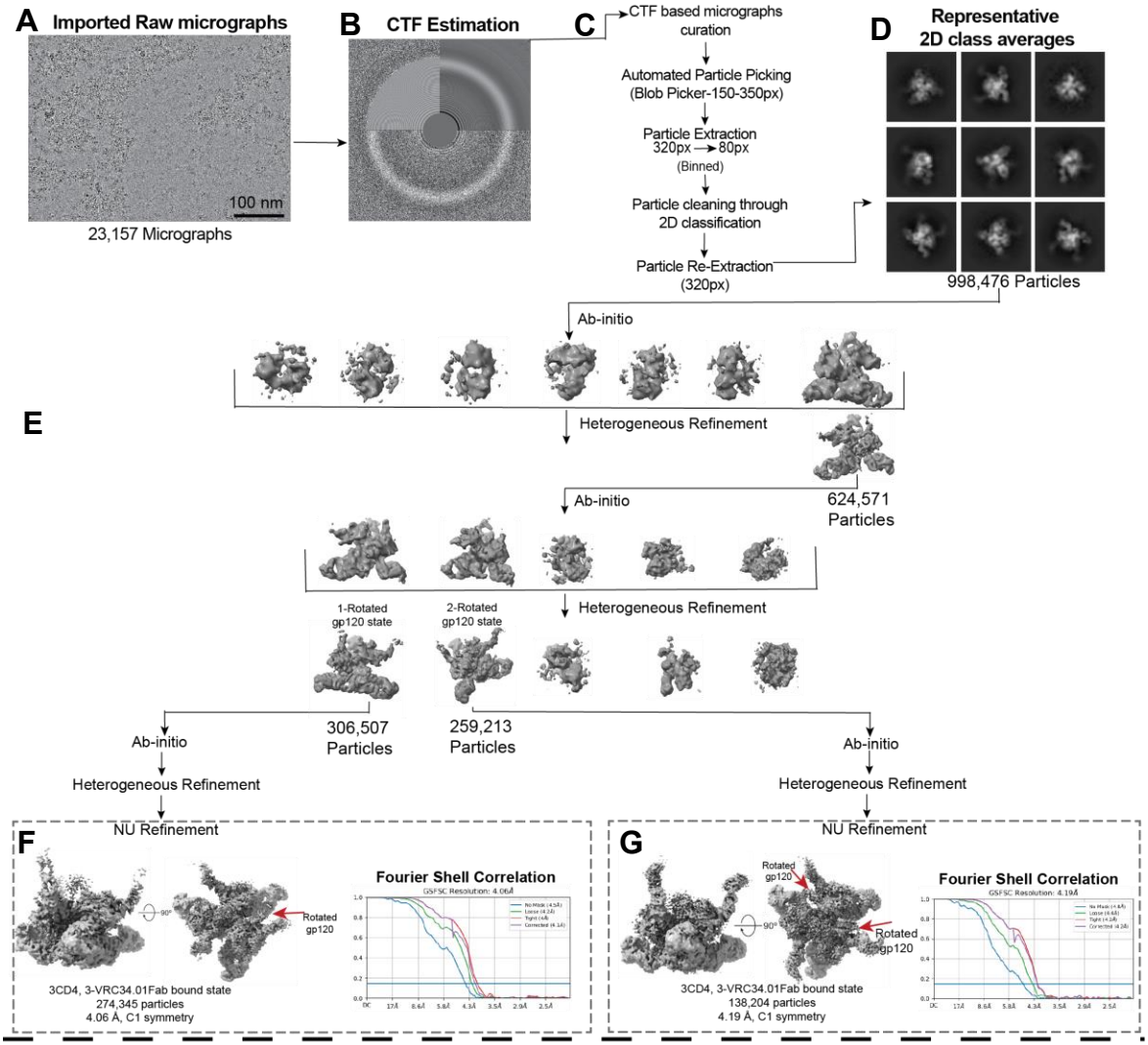
**Supplementary Figure 12. Visualizing VRC34-related conformational stabilization and activation of virus Env by smFRET of full-length Env on native virions from two structural perspectives**.

**A)** The domain organization of $Env_{BG505}$ sequence is depicted, with the fluorescent labeling sites used for smFRET and antibody recognition indicated. FRET-paired Cy3/Cy5 fluorophores were site-specifically labeled on V1V4 (N136* S401*) or gp120-gp41 (V4A1 R542*). A1 is a peptide tag, and *indicates an unnatural amino acid incorporation site for click labeling.

**B)** The conformational distribution-indicated FRET histograms of virus $Env_{BG505}$ probed from gp120 V1V4 (N136* S401*) structural perspectives in the absence or presence of different ligands. Virus $Env_{BG505}$ samples three primary conformational states (PT: Pre-triggered, PC: Prefusion Closed, and CO: CD4-bound open). PT predominates in the ligand-free condition (left panel), while VRC34 (two middle panels) shifts the conformational landscape differently from that of the CD4-bound opening (right panel). FRET histogram represents the mean + s.e.m. determined from three randomly assigned populations of FRET traces ($N_m$, number of traces).

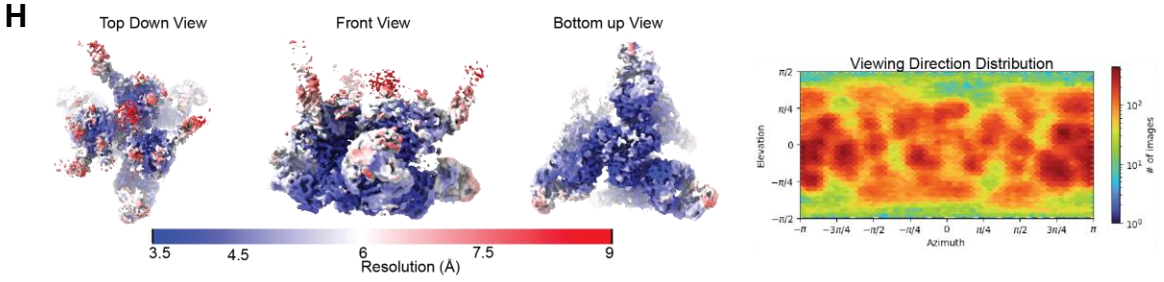
**C - E)** Indicated Cy3/Cy5 fluorophore attachment sites in the Env V1-V4 loops (**C**), three-dimensional presentation (**D**) and state occupancy quantifications **(E)** of FRET histograms in B of virus $Env_{BG505}$ observed from the gp120 V1V4 perspective. Relative state occupancies are presented as mean ± s.e.m. determined by estimating the area under each Gaussian curve of histograms in B. For state occupancies and parameters, see Table S4 and Methods.

**F)** The conformational distribution-indicated FRET histograms of virus $Env_{BG505}$ probed from the gp120 – gp41 (V4A1 R542*) structural perspectives in the absence or presence of different ligands. Virus $Env_{BG505}$ samples three primary conformational states (PT: Pre-triggered, PC: Prefusion Closed, and CO: CD4-bound open). PT predominates in the ligand-free condition (left panel), while VRC34 (two middle panels) shifts the conformational landscape differently from that of the CD4-bound opening (right panel). FRET histogram represents the mean + s.e.m. determined from three randomly assigned populations of FRET traces ($N_m$, number of traces). Three-dimensional presentation and quantification of FRET histograms observed from the gp120-gp41 perspective are shown in **Figure 4E-G**.

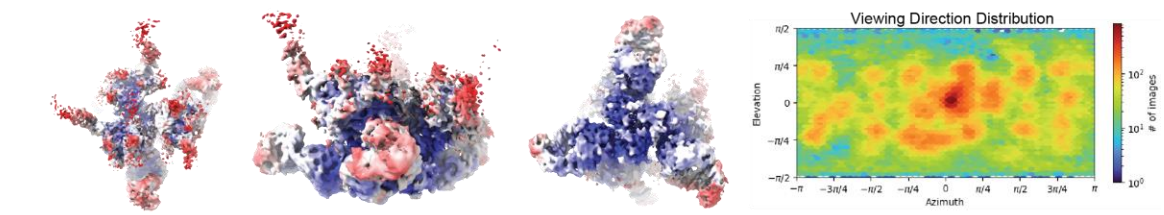



## Local Resolution Estimation

***Env in one-gp120 protomer rotated conformational state***




***Env in two-gp120 protomer rotated conformational state***



**Supplementary Figure 13. Cryo-EM data processing workflow for BG505 SOSIP.664 incubated with CD4 for 2 hours at 25 °C followed by incubation with VRC34.01 Fab for 30 minutes prior to vitrification**. **A)** Representative frame aligned micrograph. **B)** Representative contrast transfer function (CTF) fit. **C)** Data Processing workflow, leading to, **D)** Representative 2D class averages from 2D classification, followed by, **E)** 3D classifications and refinements. **F)** Refined map of Env bound to 3 CD4 molecules and 3 VRC34.01 Fabs, with one of the three gp120 protomers rotated (indicated with red arrow). **G)** Refined map of Env bound to 3 CD4 molecules and 3 VRC34.01 Fabs, with two of the three gp120 protomers rotated (indicated with red arrows). **H)** Left. Refined map of Env bound to 3 CD4 molecules and 3 VRC34.01 Fabs, with one of the three gp120 protomers rotated, colored by local resolution. Right. Particle distribution. **I)** Left. Refined map of Env bound to 3 CD4 molecules and 3 VRC34.01 Fabs, with two of the three gp120 protomers rotated, colored by local resolution. Right. Particle distribution.