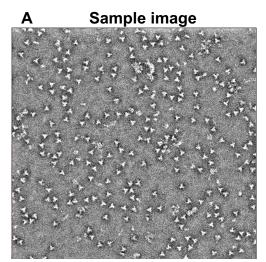


Figure 1. (Left) Size exclusion chromatography, (Middle) SDS-PAGE, (Right) Differential scanning fluorimetry profile of purified BG505 SOSIP sample

Figure 2

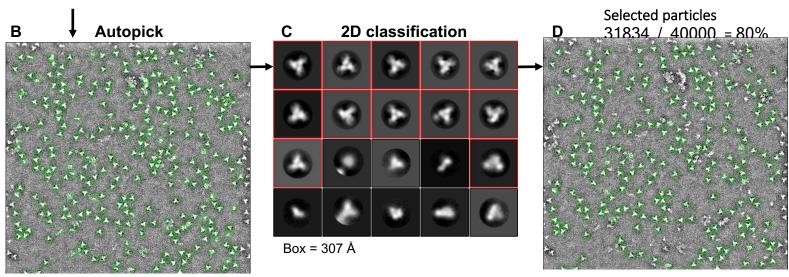


Lot: 013BT

Name: BG505gp140SOSIP/GnTI-/PBS

NSEM Methods:

A frozen aliquot from -80 °C was thawed at RT in Al block for 5 min. Sample was then diluted to 200 μg/ml with 5 g/dl Glycerol in HBS (20 mM HEPES, 150 mM NaCl pH 7.4) buffer containing 8 mM glutaraldehyde. After 5 min incubation, glutaraldehyde was quenched by adding sufficient 1 M Tris stock, pH 7.4, to give 75 mM final Tris concentration and incubated for 5 min. Quenched sample was applied to a glow-discharged carbon-coated EM grid for 10-12 second, then blotted, and stained with 2 g/dL uranyl formate for 1 min, blotted and air-dried. Grids were examined on a Philips EM420 electron microscope operating at 120 kV and nominal magnification of 49,000x, and 20 images were collected on a 76 Mpix CCD camera at 2.4 Å/pixel. Images were analyzed by 2D class averages using standard protocols with Relion 3.0 (Zivanov et al. 2018. *eLife*. 7:e42166).



100 nm

- Micrograph images may or may not be ideal for publication: They have been low-pass filtered @ 20 Å, high-pass filtered @ 500 Å, and down-sampled 2x.
- If higher quality images are need, please request from RJ or Katayoun, and indicate the NSEM Lot #
- Scale bar for micrographs is only accurate if image width = 3.0". To change size, group image and scalebar first, then resize

Figure 2. Negative Stain EM of BG505 SOSIP sample.

Cryo-EM data processing

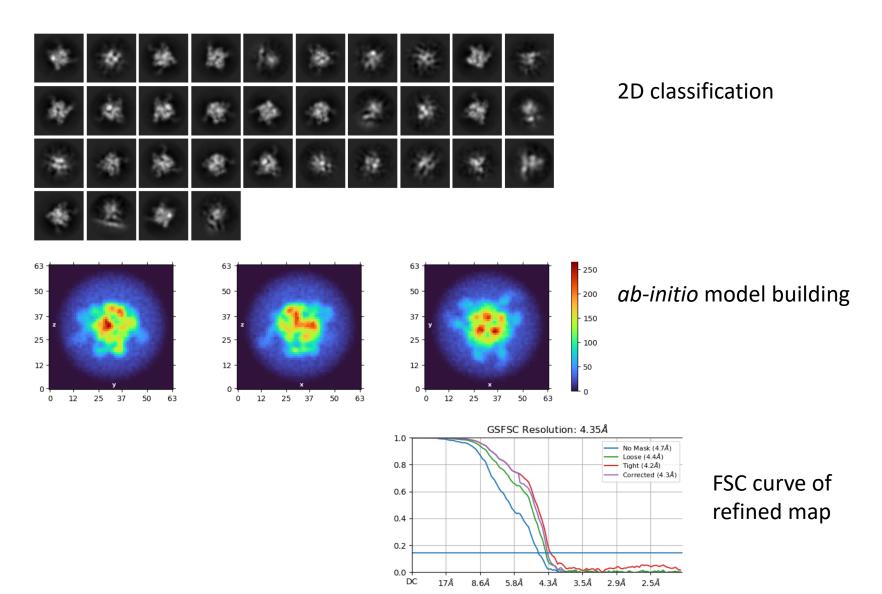


Figure 3. Cryo-EM of BG505 SOSIP sample.

Figure 4

BG505 SOSIP-CD4-17b-VRC34.01 cryo-EM refined map

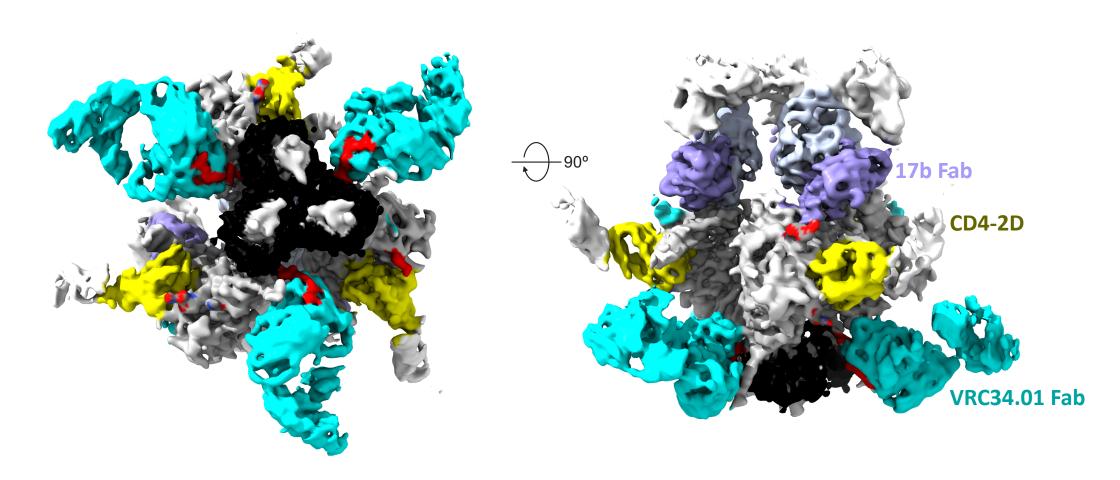


Figure 4. 3D reconstruction of partially open Env (grey: gp120, black: gp41) bound to CD4 (yellow), 17b (blue) and VRC34 (cyan)