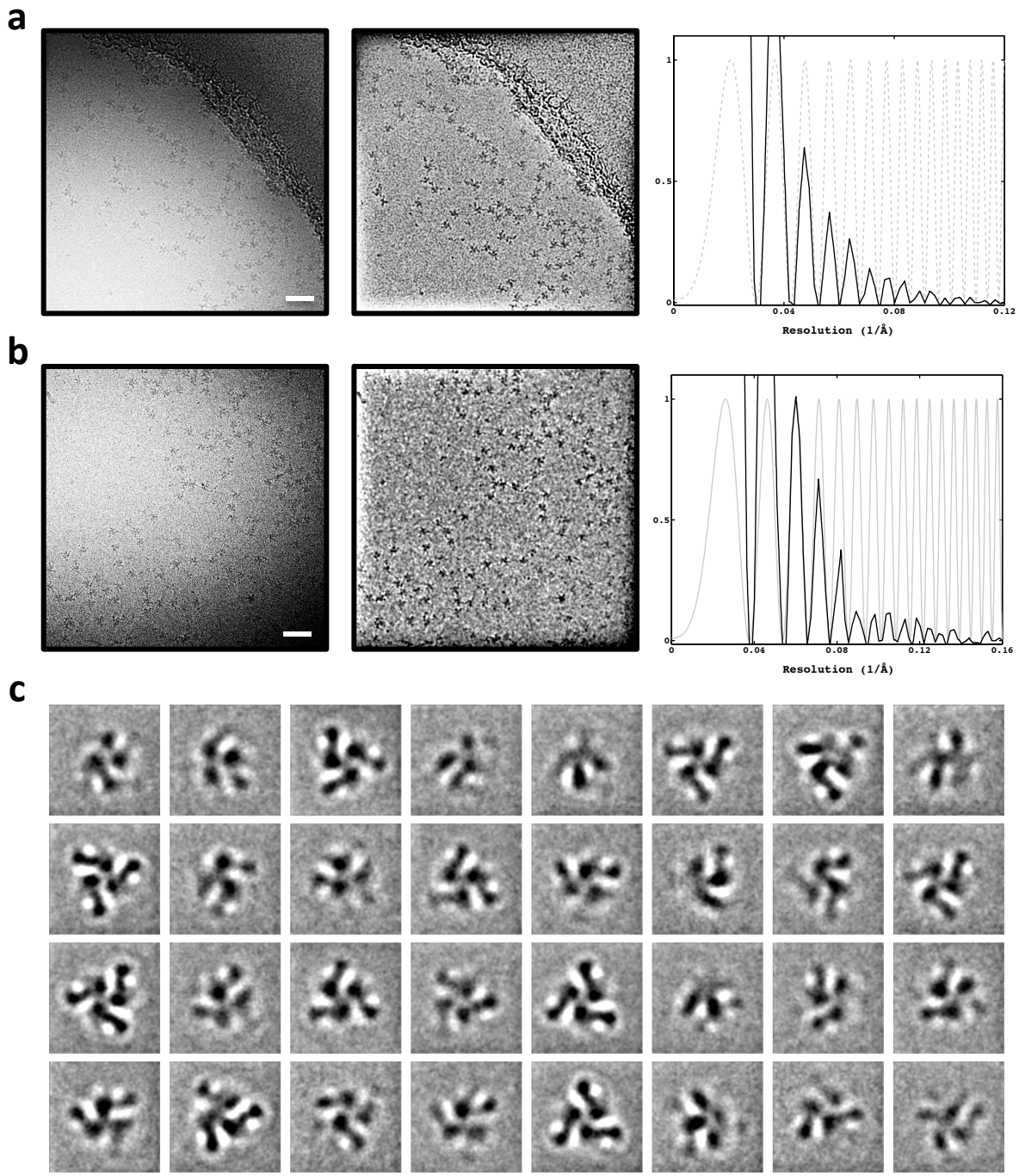
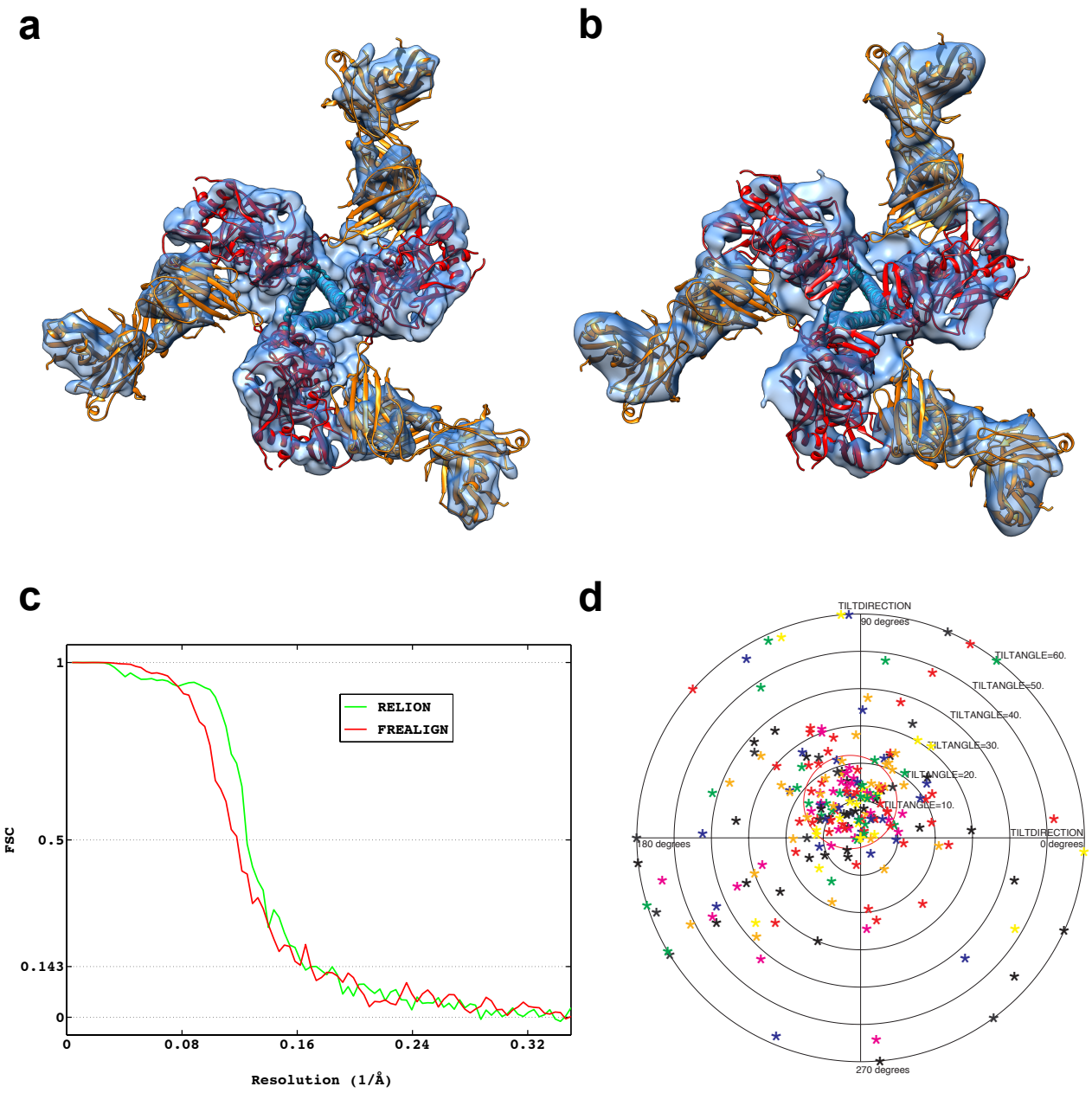
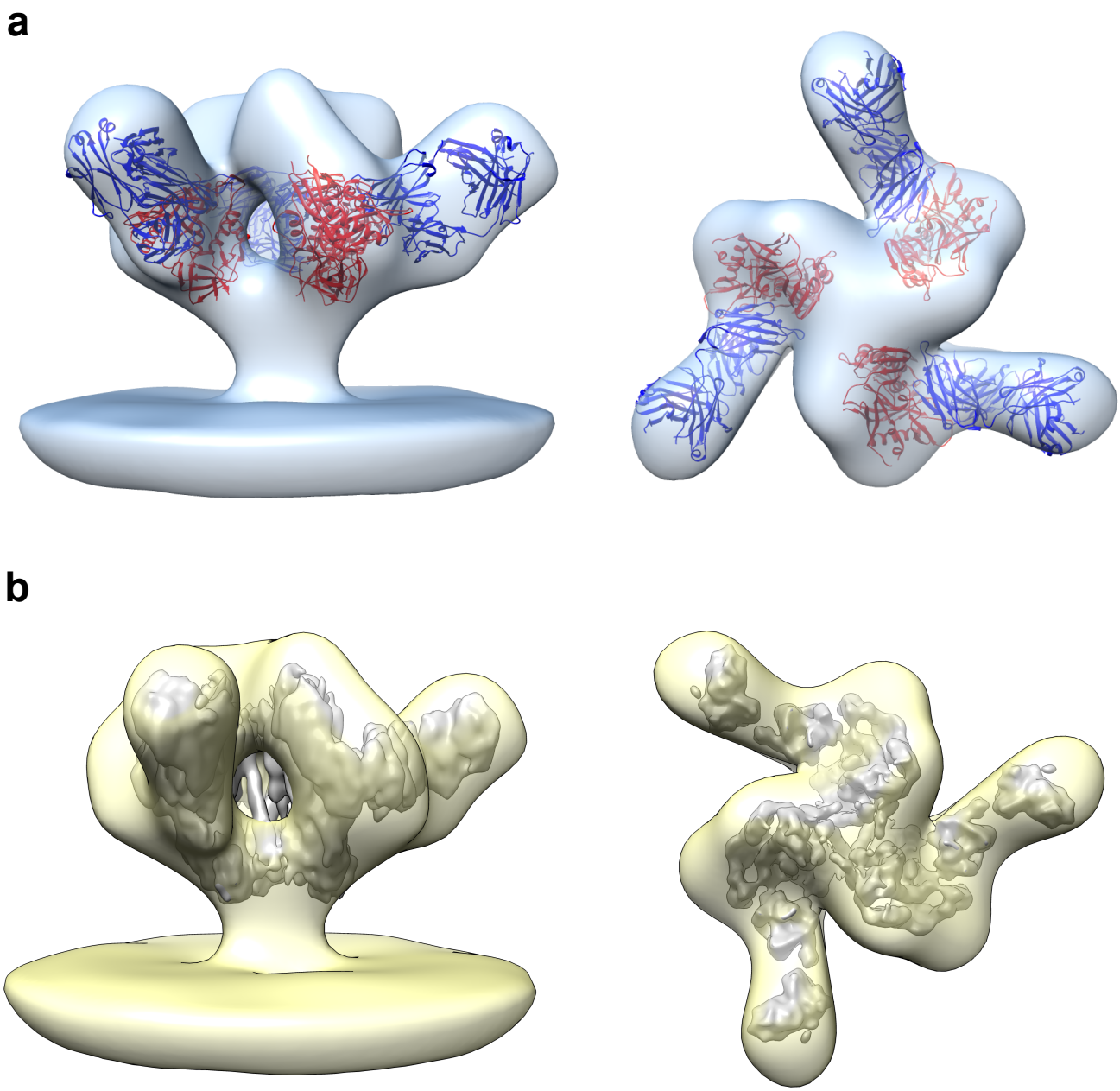


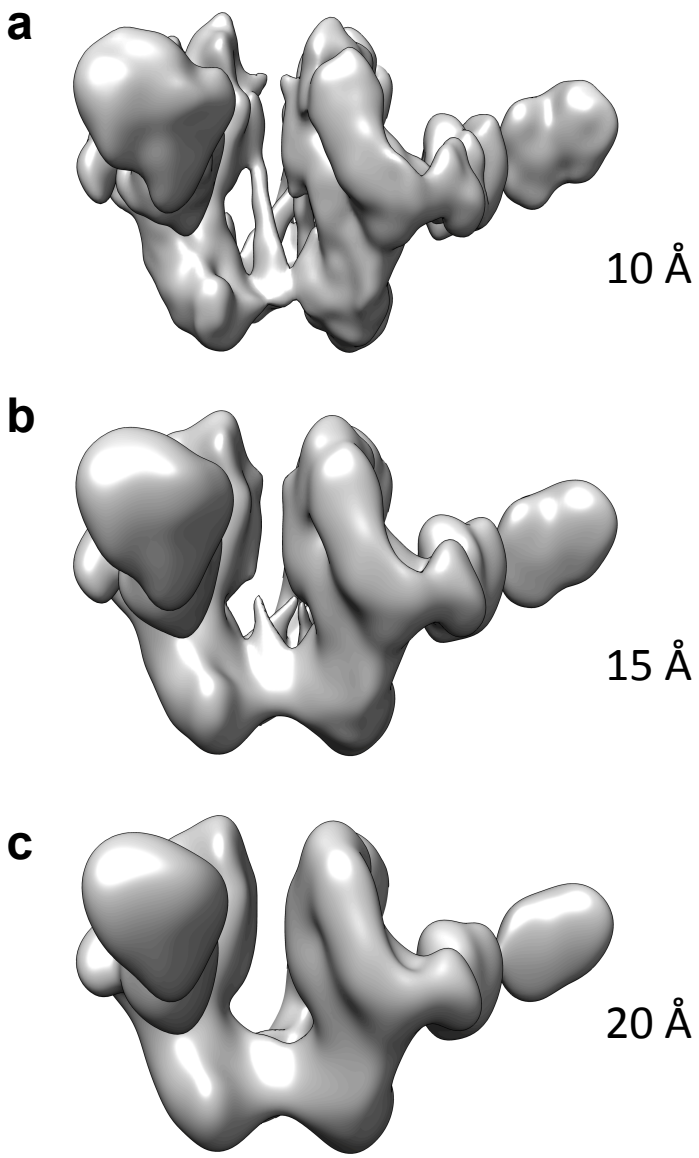
Supplementary Figure 1:Cryo-electron microscopic imaging of the Env-VRC03 complex. (a, b) Representative images recorded at a dose of ~ 10 el/Å^2 and underfocus values of ~ 2.6 μm (a) and 1.5 μm (b) (leftmost panels). Scale bar is 400 Å. Band-pass filtered versions of these images allows easier particle visualization (middle panels). Rotationally averaged FFTs of each image show signal from the Thon rings extending to ~ 8 Å for micrographs at higher defocus values and to ~ 6 Å for micrographs at lower defocus values (rightmost panels). (c) Gallery of selected initial class averages obtained by classification of single particle projection images without any prior translational or rotational alignment reveals the characteristic views of the VRC03-bound gp120 trimer.



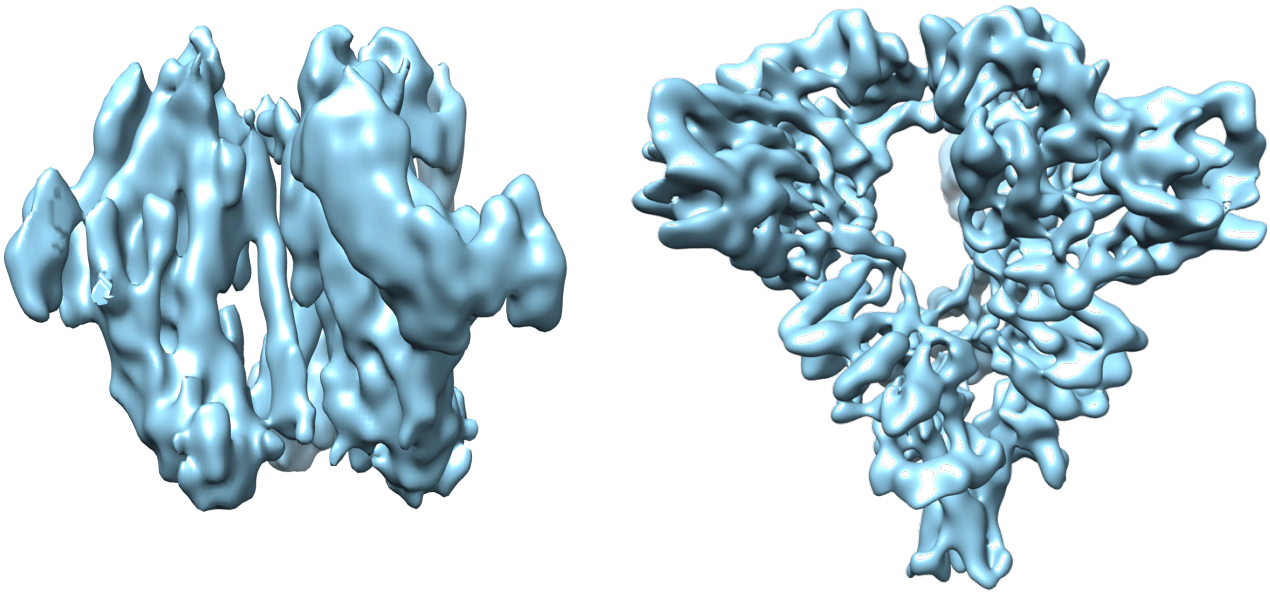
Supplementary Figure 2: Map resolution and validation. (a, b) Maps obtained from FREALIGN[20] (a) and RELION[21] (b) shown in top view, fitted with 3SE8[23] coordinates. (c) Plots of the Fourier Shell Correlation (FSC) coefficient for the structure of the Env-VRC03 complex. The curves show the "gold-standard" curve obtained using RELION and the curve obtained from the correlation of two halves of the data set obtained using FREALIGN, both indicating a resolution value of ~ 6 Å as measured by the 0.143 FSC cutoff criterion. (d) Validation of density map using tilt-pair parameter plot as suggested by R. Henderson and colleagues[44]. The spread in orientational assignments around the known goniometer settings is within ~ 12.5° for > 62% of the selected particle pairs, consistent with the ~ 6 Å resolution reported for the maps.



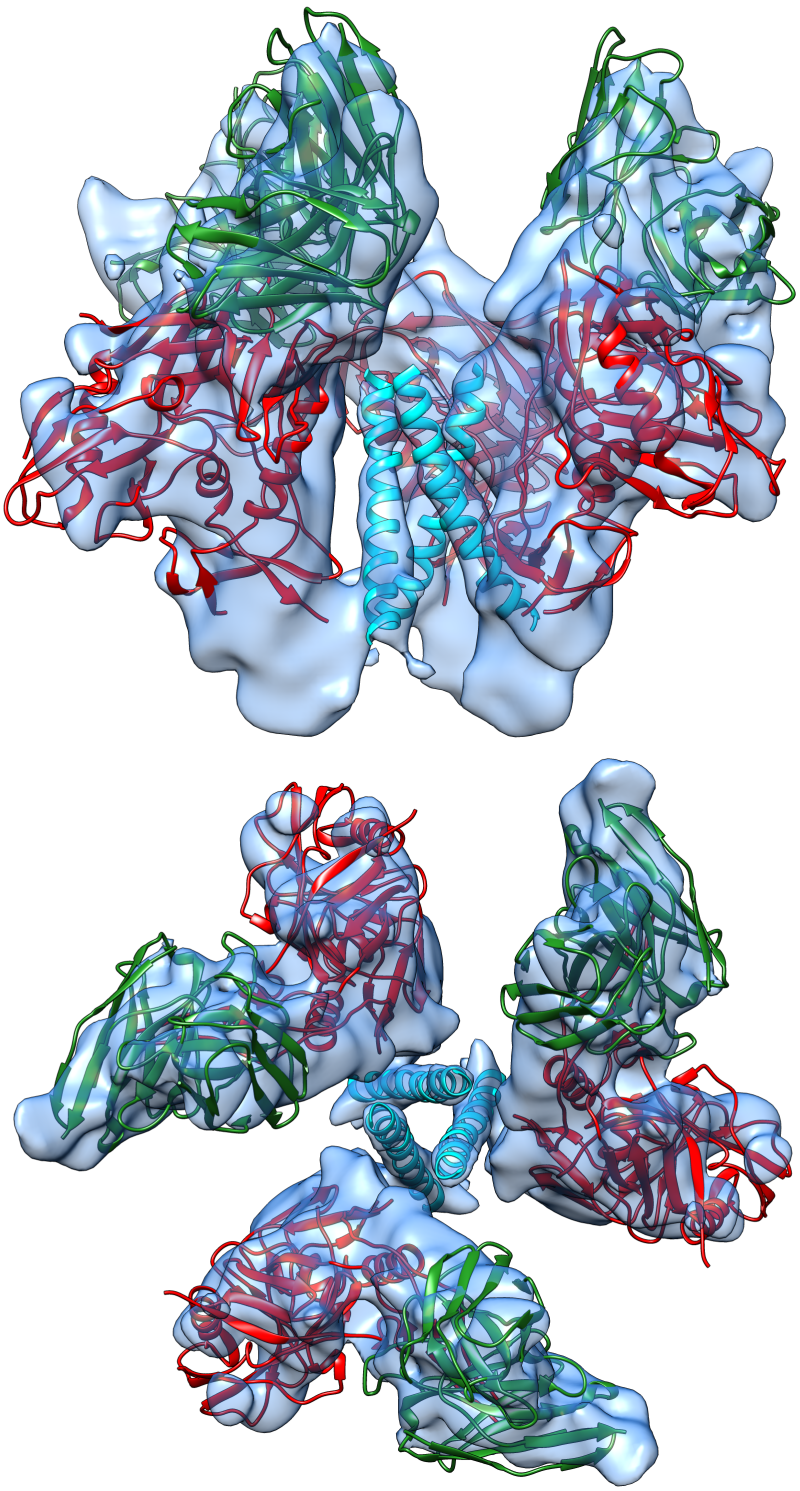
Supplementary Figure 3: Correspondence between 6 Å and 20 Å density maps. (a) 20 Å structure of the complex of VRC03 with native trimeric Env obtained using cryo-electron tomography of intact HIV-1[14]. Front perspective (left) and top views (right) of the density map, fitted with three copies of the structure of the complex of VRC03 Fab with gp120 (PDB 3SE8[23]). The 2D cryo-electron microscopic images in Figure 1 can be recognized as projections of the propeller-shaped 3D structure of the Env-VRC03 complex shown here. (b) Front (left) and top views (right) of the superposition of the density map of soluble trimeric HIV-1 Env-VRC03 Fab complex (obtained using cryo-electron microscopy at ~6 Å resolution) with density map of the native trimeric HIV-1 Env-VRC03 complex (obtained using cryo-electron tomography at ~ 20 Å resolution).



Supplementary Figure 4: Change in appearance of density map with filtering to lower resolutions. (a-c) Front views of the 6 Å density of map of the complex of VRC03 Fab with soluble trimeric HIV-1 Env, filtered to resolutions of 10 Å (a), 15 Å (b) and 20 Å (c). As the resolution is progressively lowered, the overall shape of the map is unaltered, but the central helices which are prominently resolved at 10 Å resolution, fade into the background at resolutions of 20 Å or lower, resulting in what has the appearance of a central cavity that is observed in the tomographic density maps of unliganded and VRC03-bound trimeric Env.



Supplementary Figure 5: Comparison of the structure of the closed, pre-fusion conformation of soluble trimeric HIV-1 Env presented in this work (left) with that reported by Mao, Sodroski and colleagues[24] for unliganded, full-length, trimeric HIV-1 Env (right). The map shown in the left panel is identical to that presented in Figure 3.



Supplementary Figure 6: Structure of the open, activated trimeric Env conformation[14]. Side (top) and top views (bottom) of the 9 Å density map of the open state of trimeric Env stabilized in a complex with the Fab fragment of the 17b monoclonal antibody. The map is fitted with three copies of PDB 3HMG[39] for the gp41 central densities (cyan) and three copies of gp120 (red) and the Fv fragment of 17b (green) derived from the coordinates for the structure of the gp120-sCD4-17b complex (PDB 1GC1[2]).