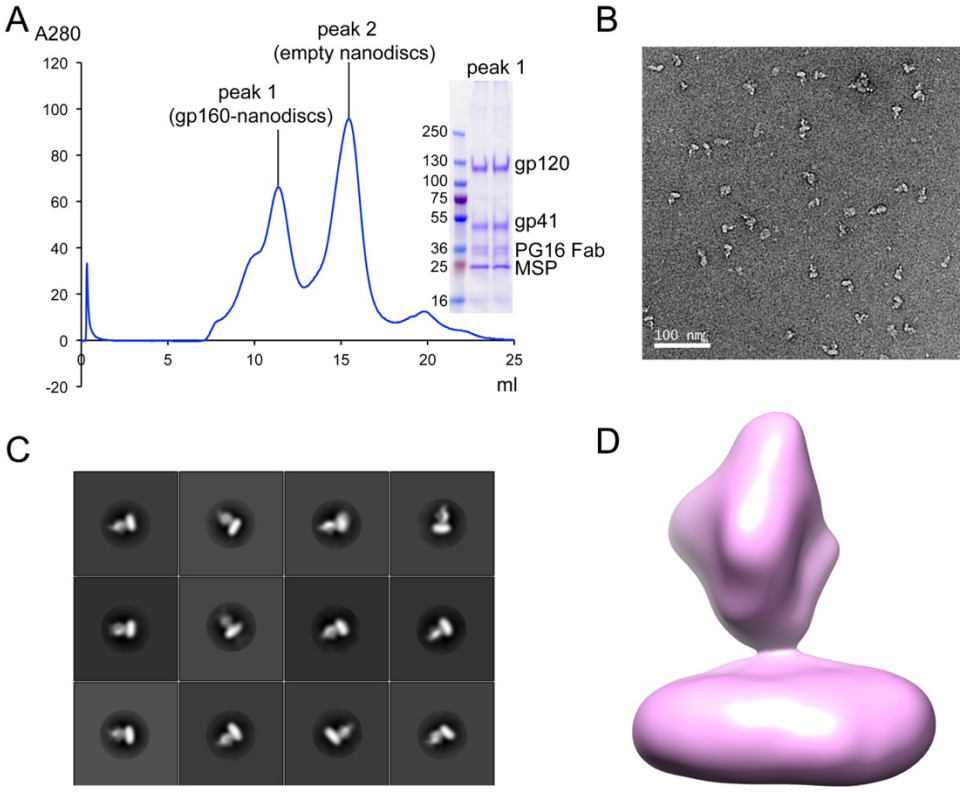

Figure 1. Purification and negative-stain electron microscopy of the HIV-1 Env-nanodiscs. (A) The purified Env-nanodisc sample was resolved by gel-filtration chromatography on a Superose 6 column. Peak 1 and 2 contain the Env-PG16-nanodiscs and empty nanodiscs, respectively. Inset, fractions of the peak 1 were analyzed by Coomassie stained SDS-PAGE. Labeled bands were confirmed by western blot. (B) Raw image of the Env-nanodiscs by negative stain. (C) 2D class averages of the negatively stained Env-nanodiscs. The size of the class average square is ~330 Å. (D) 3D reconstruction from the negatively stained images of the Env-nanodiscs.

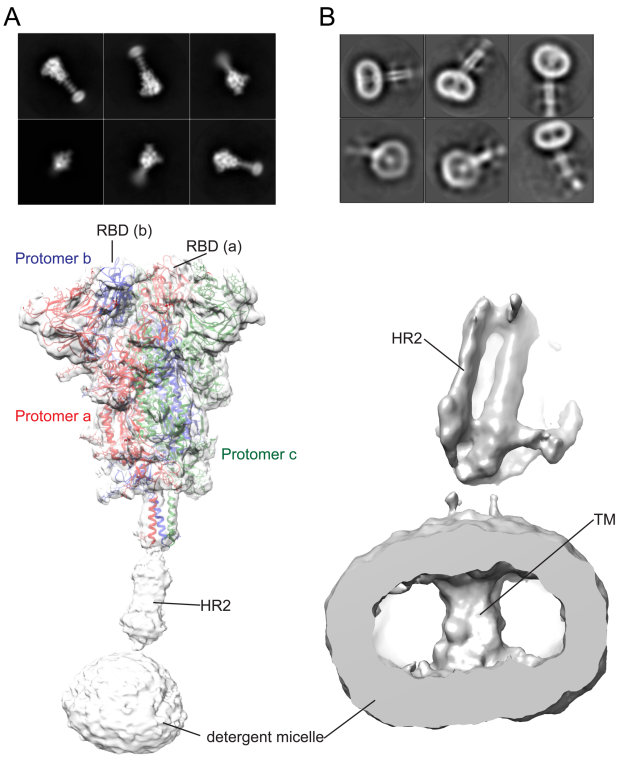

Figure 2. Data set for the G614 S trimer sample in thicker ice. (**A**) Top, 2D averages of the cryo-EM particle images of the G614 S trimer in thicker ice. Bottom, the reconstruction of the G614 S trimer from the new data set, representing a closed, three RBD-down conformation, fitted by the model for the G614 trimer. Three protomers (a, b, c) are colored in red, blue and green, respectively. RBD locations are indicated. (**B**) Top, 2D averages of the re-extracted images of the membrane regions of G614 S trimer. Bottom, the reconstruction of the G614 S trimer with local mask to exclude the ectodomain, showing the HR2 and TM are structured.

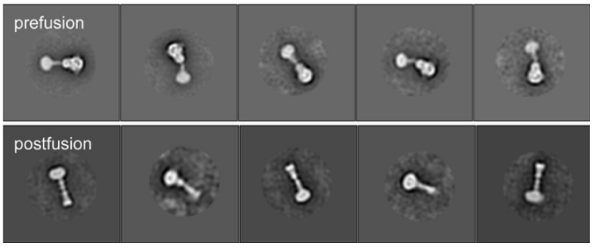

Figure 3. Negative stain of the SARS-CoV-2 S-nanodiscs. 2D class averages of the negatively stained Env-nanodiscs. Top, the prefusion conformation; bottom, the postfusion conformation. The size of the class average square is ~850 Å.