

(B) Surface plasmon resonance (SPR) demonstration that 12N, a rabbit mAb against the base of the BG505 SOSIP trimer (112), accesses its epitope on free RC1, but not on RC1-VLPs. RC1 or RC1-VLPs were injected over 12N IgG that was immobilized on a biosensor chip.

(C) A Coomassie-stained SDS-PAGE gel of conjugations of different immunogens to VLPs is shown. Note that the ratio of gp120 and gp41 to the VLP monomer subunit is lower in the RC1-4fill conjugation than the other conjugations.

(D) Left: Separation by size-exclusion chromatography (SEC) on Superdex 200 column of RC1-VLPs from unconjugated RC1 at small scale (100 µg SOSIP) is shown. The RC1-VLP peak is distinct from the peak for unconjugated RC1 trimer. Right: Coomassie-stained SDS-PAGE of SEC-purified RC1-VLP and RC1-4fill-VLP preps are shown.

(E) Left: Black trace: Separation by SEC on Superdex 200 column of RC1-VLPs from unconjugated RC1 at preparation scale of about 2 mg of SOSIP. The RC1-VLP peak is not distinct from the peak for unconjugated RC1 trimer. Red trace: Separation by SEC on Superose 6 column of RC1-VLPs from unconjugated RC1 at preparation scale ~2 mg. The RC1-VLP peak is distinct from the peak for unconjugated RC1 trimer. Right: nsEM images of RC1-VLPs purified using the Superdex 200 or the Superose 6 column are shown. Free RC1 trimer was observed in the RC1-VLP prep purified on the Superdex 200 column, but not in the prep purified on the Superose 6 column.