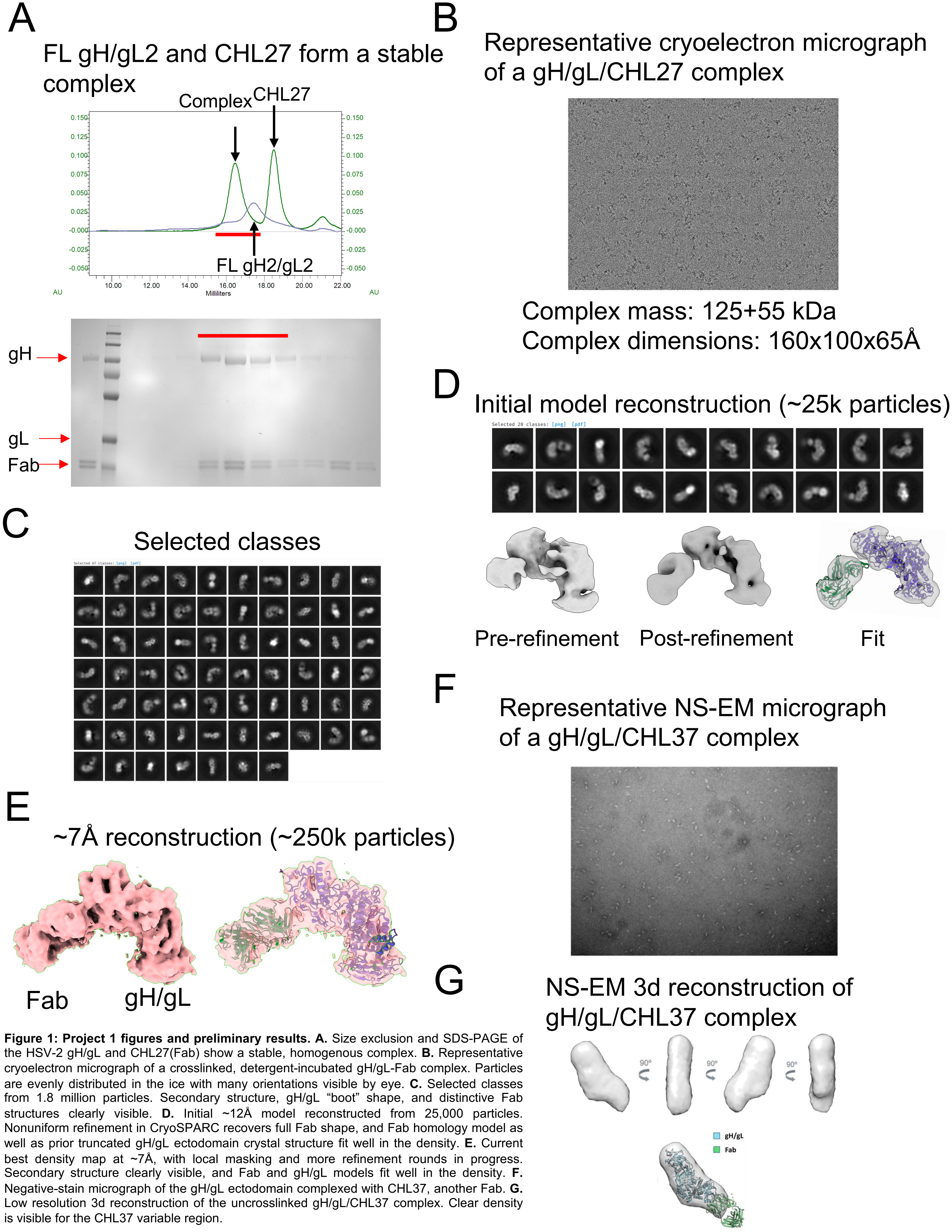
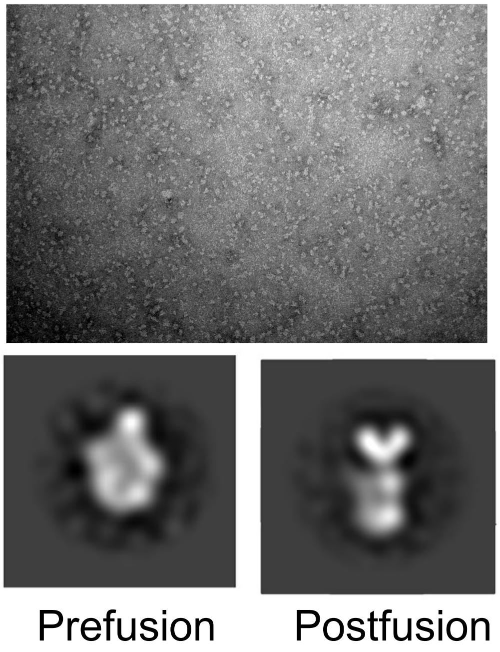
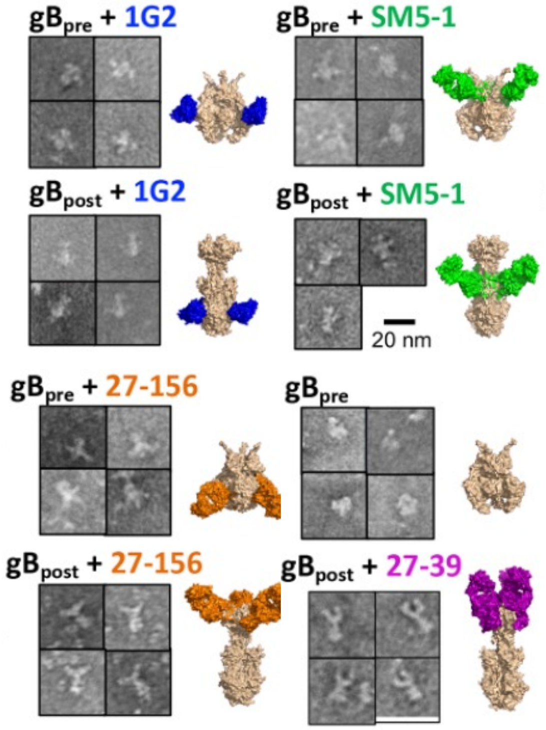


**Figure 1: Project 1 figures and preliminary results. A.** Size exclusion and SDS-PAGE of the HSV-2 gH/gL and CHL27(Fab) show a stable, homogenous complex. **B.** Representative cryoelectron micrograph of a crosslinked, detergent-incubated gH/gL-Fab complex. Particles are evenly distributed in the ice with many orientations visible by eye. **C.** Selected classes from 1.8 million particles. Secondary structure, gH/gL "boot" shape, and distinctive Fab structures clearly visible. **D.** Initial ~12Å model reconstructed from 25,000 particles. Nonuniform refinement in CryoSPARC recovers full Fab shape, and Fab homology model as well as prior truncated gH/gL ectodomain crystal structure fit well in the density. **E.** Current best density map at ~7Å, with local masking and more refinement rounds in progress. Secondary structure clearly visible, and Fab and gH/gL models fit well in the density. **F.** Negative-stain micrograph of the gH/gL ectodomain complexed with CHL37, another Fab. **G.** Low resolution 3d reconstruction of the uncrosslinked gH/gL/CHL37 complex. Clear density is visible for the CHL37 variable region.

**A** Representative NS micrograph of prefusion HCMV gB



**B** NS-EM images of pre- and postfusior HCMV gB in complex with Fabs



**C** Initial cryoEM studies of uncrosslinked prefusion HCMV gB

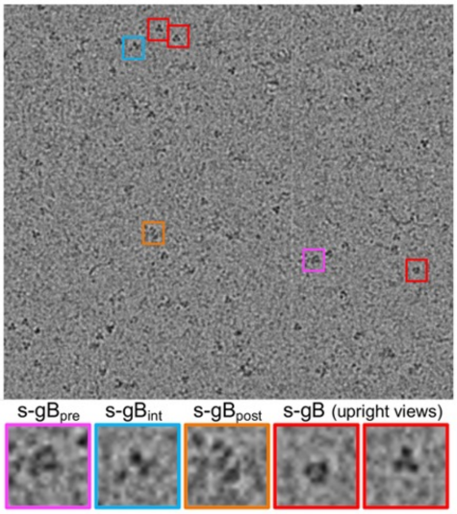


**Figure 2: Project 2 figures and preliminary results. A.** NS-EM of our stable prefusion HCMV construct. Most particles are in a globular, prefusion state rather than elongated, postfusion state when compared to previous 2D class averages. **B.** Selected NS-EM particle images of prefusion and postfusion HCMV gB in complex with gB-specific Fabs. Corresponding 3D representations of Fab binding modes modeled manually. **C.** Initial cryoelectron micrographs of uncrosslinked HCMV gB reveals a preferred orientation problem we aim to resolve using glutaraldehyde crosslinking and octyl β-D-glucopyranoside detergent incubation as determined by our Project 1 preliminary data.