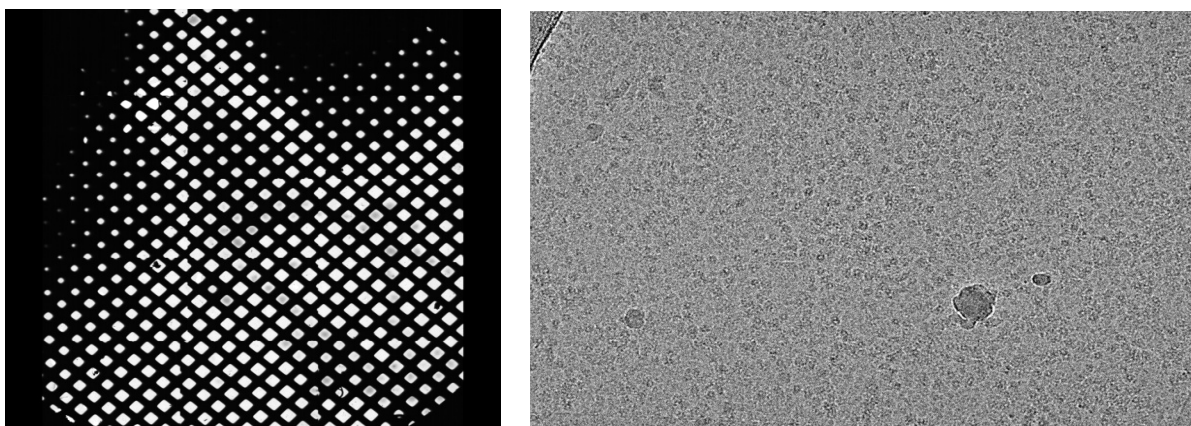
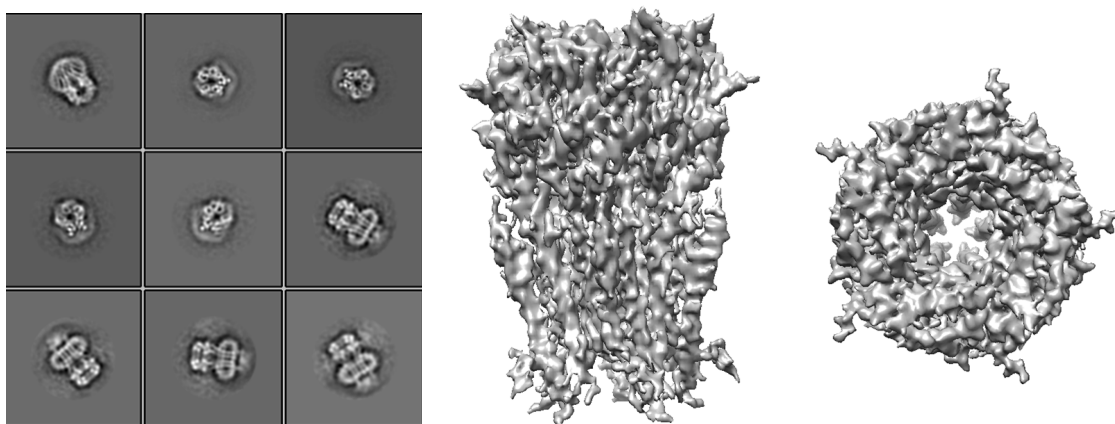


**Figure 1.** (Left) A Western blot using anti-histidine antibody, recognizing a 6x-his tag placed on the C-terminus of the protein. The protein monomer is ~50kDa. (Right) A size-exclusion chromatography histogram showing the GlyR peak at ~16 mL, which is shifted about 1 mL to the left when the protein is reconstituted in a lipid nanodisc.



**Figure 2.** (Left) Atlas of a graphene-oxide grid frozen with GlyR and glycine and a GlyR-specific Fab. (Right) An 81k magnification image of the GlyR and glycine + Fab sample. Images from a Titan Krios with a G3i K3 camera.



**Figure 3.** (Left) Selected 2D classes from the data collected from grids shown in figure 2. Pixel size is 1.1Å, 136k particles. (Right) 3D reconstruction from the 2D classes shown, resolution of 3.6Å. Data was processed in both RELION and CryoSPARC.