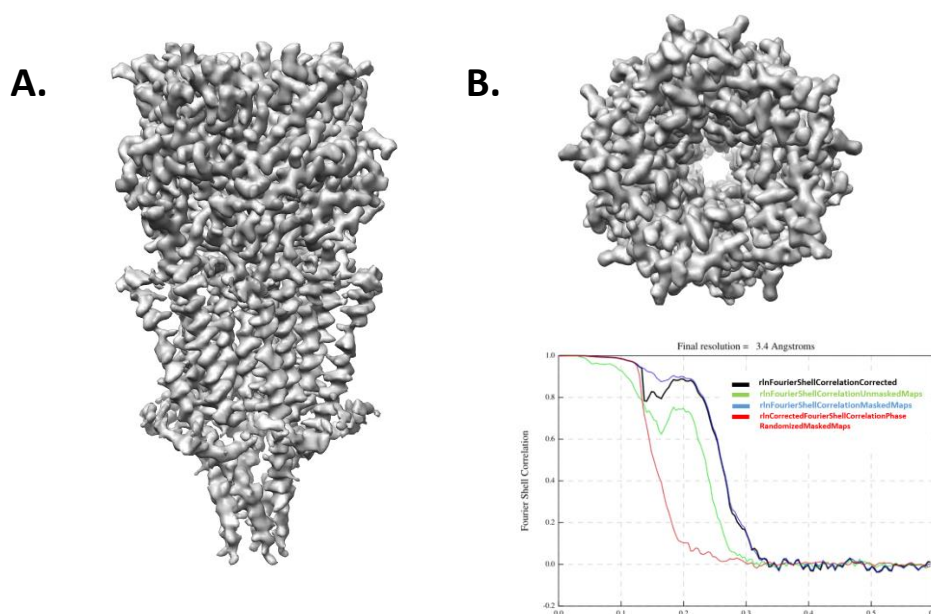


**Figure 1. Preparation of mutant 5-HT<sub>3A</sub>R for cryoEM imaging.** (A) A western blot showing recombinant expression of mutant 5-HT<sub>3A</sub>R in Expi Sf9 cells. Monomeric 5-HT<sub>3A</sub>R runs at ~50kDa, identified using Rho1D4 antibody which detects a C-terminally fused 1D4-tag built into construct. (B) A size-exclusion chromatography histogram showing mutant 5-HT<sub>3A</sub>R eluting at ~15mL. A Superose 6 Increase 10/300 GL column was used. (C) An example micrograph showing mutant 5-HT<sub>3A</sub>R particle dispersal. Sample was frozen with 100 $\mu$ M SMP100 and images were collected on a FEI Titan Krios with a Gatan K3 camera at 105k magnification. (D) 2D classes containing ~53k particles of mutant 5-HT<sub>3A</sub>R processed on RELION-5.0.



**Figure 2. Preliminary 3D reconstruction of mutant 5-HT<sub>3A</sub>R with 100 $\mu$ M SMP100.** (A) Side view of the mutant 5-HT<sub>3A</sub>R, positioned parallel to the plane of the cellular membrane. (B) Top view of same mutant 5-HT<sub>3A</sub>R structure, positioned perpendicular to the plane of the cellular membrane. This reconstruction has a resolution of 3.4 $\text{\AA}$ , with polishing and 3D refinement done in RELION-5.0. The map was visualized in Chimera. Preliminary structure obtained with ~15k particles (a subset of overall autopicked particle) from ~4.7k micrographs.