

Figure 1. Construct design, expression, purification, and cryo-EM of heterotetrameric AMPARs. **A.** Topology of GluA1 and GluA2 constructs labeled with mCherry and GFP fluorophores and containing His and Strep affinity tags, respectively. **B.** Bright-field, mCherry and GFP fluorescence images of HEK 293 cells co-expressing GluA1 and GluA2. **C.** FSEC traces for solubilizate of HEK 293 cells co-expressing GluA1 and GluA2 followed by mCherry (red) and GFP (green) fluorescence (dual wavelength detection during a single run) and purified material followed by Tryp fluorescence (black). Note, rightward shift of the tetrameric peak after removal of tags using thrombin. **D.** SDS-PAGE for the protein at intermediate and final stages of purification. Note, the two bands correspond to GluA1 and GluA2. **E.** 2D-class averages. **F.** Cryo-EM density for LBD-TMD, with subunits GluA1 and GluA2 colored light orange and blue, competitive antagonist ZK200775 red, and lipids purple. **G.** Model of ZK200775 with cryo-EM density shown as blue mesh. **H.** Closeup view of the pore-forming domains M2 and M3 in GluA1 and GluA2 subunits.

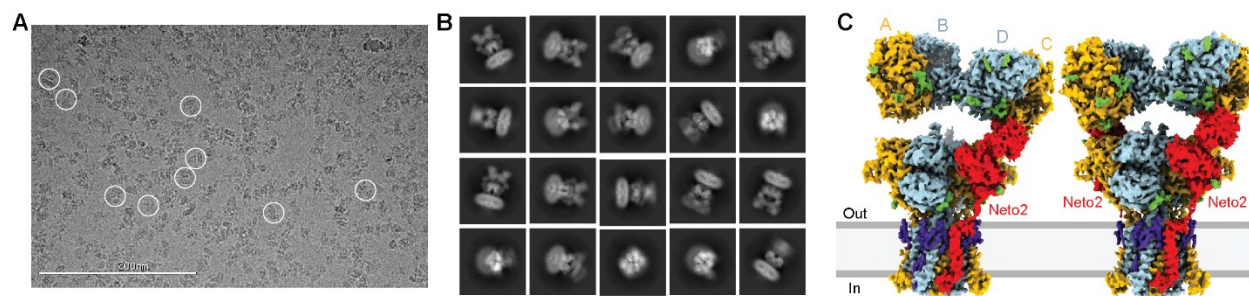


Figure 2. Preliminary single-particle cryo-EM data for GluK2-Neto2 complex. **A**, Representative micrograph with example particles circled in white. **B**, Reference-free 2D class averages illustrating different particle orientations. **C**, Preliminary cryo-EM reconstructions of GluK2-Neto2 in the apo condition with one or two molecules of Neto2 bound, GluK2 subunits colored orange and light blue, lipids in purple, carbohydrates in light green and Neto2 in red.

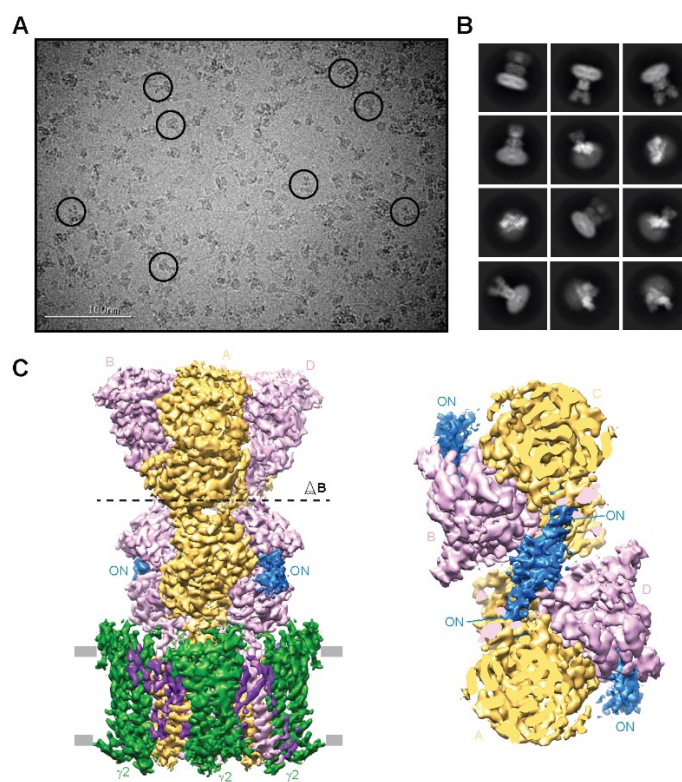


Figure 3. Preliminary single-particle cryo-EM data for GluA2- γ 2 in complex with synthetic oligonucleotide. **A**, Representative micrograph with example particles circled in black. **B**, Reference-free 2D class averages illustrating different particle orientations. **C**, Preliminary reconstruction of GluA2- γ 2 in complex with oligonucleotide (ON), with density for GluA2 subunits shown in yellow (A and C) and pink (B and D), γ 2 in dark green, lipids in purple, and ON in blue. View parallel to the membrane is on the left, while extracellular view on the LBD layer from the level indicated by dashed line is on the right.