

The fluorescence size exclusion chromatography (FSEC) profile illustrates that the wild type and mutant GLR proteins were adequately purified and confirms that a pure protein sample is added to the grid in sample preparation (Figure 1A). Figure 1A shows pure protein peaks for both the wild type and mutant GLR, with the highest intensity at a 25-minute elution time, which is what is expected for this size of tetramer as the WT resulted in a 3.57 angstrom resolution cryo-EM reconstruction (Green et al. Molecular Cell 2021). Figure 1B demonstrates an exemplary micrograph from screening the mutant protein sample on the 200 kV Glacios microscope. The particle dispersion and particle shape in the micrographs obtained from screening this mutant protein (Figure 1B) appears similar to what was observed for the WT. The limited number of micrographs obtained from screening this mutant on the Glacios resulted in promising 2D class averages (Figure 1C) that further support the claim that these grids are ready to be used for collecting a large data set to obtain the structure of this GLR.

