Structural Analysis of Ionotropic Glutamate Receptors (iGluRs)

Background and Significance- Ionotropic glutamate receptors (iGluRs), such as AMPA, Kainate, and NMDA receptors, are activated by the primary endogenous neurotransmitter glutamate, mediate most of the excitatory neurotransmission in the mammalian nervous system, and regulate synaptic plasticity and neuronal functions. Binding of glutamate or an antagonist to the receptor regulates ion channel gating and thus ion permeation. In addition, auxiliary subunits tightly associated with native iGluRs are critically involved in receptor biogenesis, trafficking, and allosteric regulation. Since iGluRs play the central role in normal brain function, their dysregulation is associated with numerous pathophysiological conditions. Therefore, these receptors are considered an attractive target for drug development to combat various neurological and psychiatric disorders. However, drug development efforts are hindered by the limited understanding of the disease mechanisms and genetics of iGluRs as well as the lack of adequate structural information about different conformational states of ligand-bound and unbound receptors. Fortunately, in the past decade, cryo-EM has accelerated accumulation of the structural data for various iGluR conformations but only in the presence of auxiliary subunits. Structural information describing the conformational ensemble of the receptors alone remains limited and the available structures have low resolution (6-8 Angstrom). Previously, we have determined AMPA receptor structures in complex with the antiepileptic drug Perampanel (Yelshanskaya et 2016), the closed and desensitized state structures in complex with the auxiliary subunit GSG1L (Twomey et al. Neuron 2017), and the open, conducting state structure in complex with the auxiliary subunit STZ (Twomey et al. Nature 2017). Now, our efforts are focused on solving high-resolution cryo-EM structures of AMPA subtype receptor alone in different conformational states. We have expressed the protein in mammalian cells, purified it (Figure 1A) and used Vitrobot to make cryo-EM grids. The grids were screened on a Glacios microscope equipped with the Gatan K3 detector and yielded promising 2D class averages (Figure 1B and C). We therefore seek an opportunity to collect higher quality data on Krios microscopes to generate high-resolution iGluR reconstructions in different activation states.

Specific Aims-

1. Determine a high-resolution structure of the AMPA receptor.
2. Capture the receptor in different conformational states.

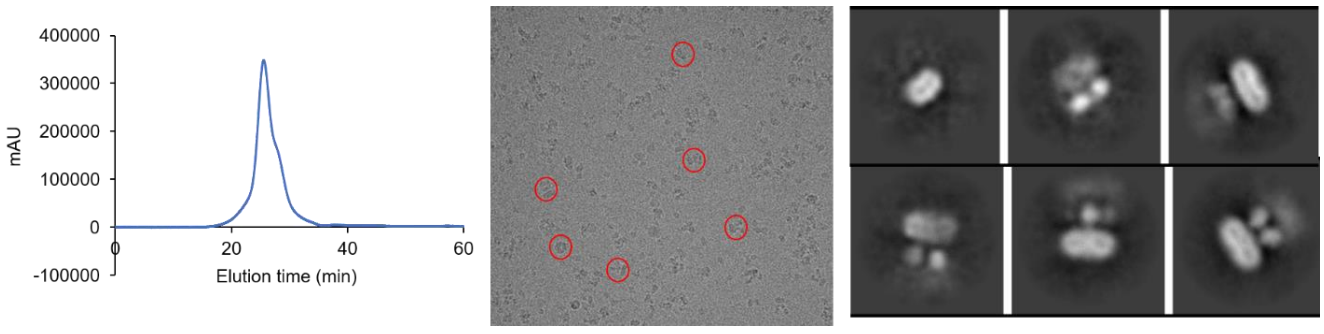

Figure1: (A) FSEC profile of purified AMPA receptor. (B) Exemplar micrograph. (C) 2D class averages.

The described research will help to characterize AMPA-subtype iGluRs structurally and functionally and, in doing so, will advance understanding of the ion channel gating mechanisms and inform new directions in structure-based drug design.