

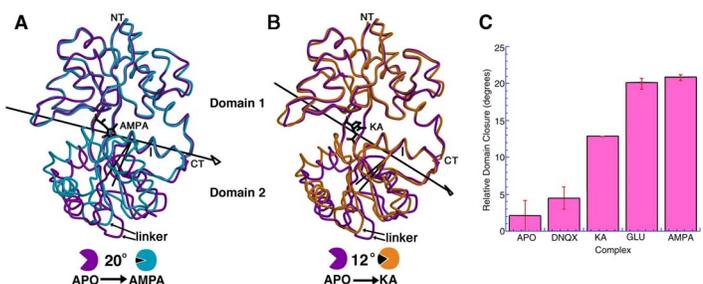
# Mechanisms for Activation and Antagonism of an AMPA-Sensitive Glutamate Receptor: Crystal Structures of the GluR2 Ligand Binding Core

N. Armstrong and E. Gouaux (Columbia U.)

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Beamline(s): X4A

Excitatory neurotransmission in the mammalian central nervous system is carried out primarily by the ionotropic glutamate receptor (iGluR) family of ligand gated ion channels. Binding of presynaptically released L-glutamate by iGluRs located in the postsynaptic terminal results in a brief (ms) opening of the ion channel. iGluRs are tetrameric integral membrane proteins with a ligand binding site located within each subunit. Regions of the receptor which comprise the ligand binding core are located in the ~150 residues (S1) preceding the first transmembrane segment and the ~150 residues (S2) preceding the second transmembrane region. A water-soluble construct (S1S2), which retains wild-type ligand binding affinities, can be generated by genetically replacing the membrane spanning regions, which separate S1 and S2 with a short hydrophilic linker<sup>1</sup>. We have determined the structures of GluR2 S1S2 in complex with two full agonists (AMPA and glutamate), a partial agonist (kainate), an antagonist (DNQX) and in the apo state.



**Figure 1.** A) Superposition of apo and AMPA structures. B) Super-position of apo and kainate structures. C) Plot of relative domain closure.

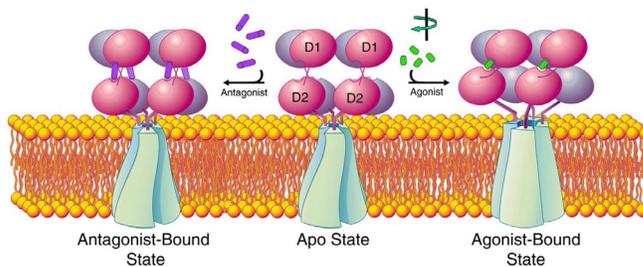
The AMPA and glutamate structures were solved by molecular replacement (MR) using the kainate structure, which was previously determined by MAD phasing using data collected at beamline X4A<sup>2</sup>, as the search probe. High resolution (1.7 Å) AMPA data was also collected at X4A. A promising MR solution was obtained for the DNQX crystal form. However, the structure could not be refined below an  $R_{\text{free}}$  of 0.4. Therefore, a three wavelength MAD data set was collected at X4A on a seleno-methionine derivitized DNQX crystal. The 40 selenium sites were located in anomalous differences fourier maps calculated using phases from the MR structure and the  $D_{\text{ano}}$  terms from the selenium peak data. The refined DNQX structure was used

as the search probe in the MR solution of the apo crystal form.

Superpositions show that the extent of separation between domain 1 and domain 2 differs significantly between these five structures. In the apo and DNQX structures the lobes of the ligand-binding core are expanded. Upon agonist binding domain 2 moves closer to domain 1, sequestering the agonist in the cleft. Relative to the apo state, AMPA and glutamate induce ~20° of domain closure and they maximally activate AMPA receptors, measured in terms of peak currents. In contrast, kainate, which activates ~90% less than AMPA or glutamate, induces only 12° of domain closure suggesting a positive correlation between domain closure and receptor activation.

Based on the spectrum of conformations seen in the full agonist-, partial agonist-, and antagonist-bound and apo states, we propose that the substantial degree of domain closure that occurs upon agonist binding initiates iGluR channel activation. Furthermore, comparison of the full agonist- and partial agonist-bound structures indicates that the channel activation level is dependent upon the conformation of the ligand binding domain and, more specifically, the extent of S1S2 domain closure. In contrast to the large conformational change induced by agonists, the binding of competitive antagonists, such as DNQX, produces minimal domain closure and therefore these ligands do not activate the wild-type receptor.

**References:** 1. G.Q. Chen, Y. Sun, R. Jin, and E. Gouaux, "Probing the ligand binding domain of the GluR2 receptor by proteolysis and deletion mutagenesis defines domain boundaries and yields a crystallizable construct," *Protein Sci.*, 7, 2623-2630, 1998; 2. N. Armstrong, Y. Sun, G.Q. Chen, and E. Gouaux, "Structure of a glutamate receptor ligand binding core in complex with kainate," *Nature*, 395, 913-917, 1998; 3. N. Armstrong and E. Gouaux, "Mechanisms for activation and antagonism of an AMPA-sensitive glutamate receptor: Crystal structures of the GluR2 ligand binding core," *Neuron*, 28, 165-181, 2000.



**Figure 2.** A Model for Glutamate Receptor Activation and Antagonism