Architecture of heteromeric AMPA-type glutamate receptors

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AMPA-type glutamate receptors (AMPARs) are neuronal ion channels that mediate fast excitatory neurotransmission and drive synaptic plasticity, the molecular mechanism underlying memory formation. In the brain AMPARs exist predominantly as heterotetramers of various combinations of four subunit subtypes (GluA1-4) and can associate with more than 30 proteins. Subunit composition and auxiliary proteins modulate gating kinetics, ion permeation, pharmacology, and trafficking. Here we combined X-ray crystallography, cryo-EM, normal mode analysis, crosslinking and electrophysiology to get insights into the architecture of heterotetrameric AMPARs. We initially focused on the N-terminal domains (NTDs), which comprise 50% of the receptor and are sequence-diverse between subunits, playing a role in receptor heteromeric assembly. Crystal structures of GluA2/3 and GluA2/4 NTD heterotetramers revealed a novel compact arrangement that we could trap in full-length receptors by cysteine crosslinking. We used the crosslinked protein to determine a cryo-EM structure of a full-length GluA2/3 heterotetramer, which showed a compact arrangement resembling the NTD crystal structures and deviating from GluA2 homomers determined previously. Cryo-EM models also showed two different conformations in the ligand binding domain, which binds glutamate and undergoes structural rearrangements that trigger channel opening. We finally used cysteine crosslinking experiments to investigate the conformation of other homo- and heterotetramers, which allowed us to dissect the rules of subunit positioning and confirmed that AMPARs are highly dynamic. Our data highlight the structural diversity of the NTD layer of AMPARs, a potential platform for synaptic protein binding, and illustrate the potential of cryo-EM to isolate conformational states of dynamic proteins.