DISSECTING THE ALLOSTERIC MODULATION OF THE METABOTROPIC GLUTAMATE RECEPTOR 2 USING SINGLE MOLECULE FRET

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Metabotropic glutamate receptors (mGlu) are dimeric, multidomain neuroreceptors belonging to the class C of G protein-coupled receptors. The 8 mGlu family members represent important drug targets for different brain diseases. Positive allosteric modulators that display selectivity among the 8 mGlu subtypes are considered highly promising compounds to target mGlu related brain diseases. To date the mechanism by which they increase agonist potency and efficacy remains to be understood. Here, we elucidated how a small molecule allosteric modulator regulates agonist action by monitoring the structural dynamics of full-length mGlu2 using single molecule FRET at submillisecond resolution. We established a robust solubilization strategy that allows to maintain full functional integrity of receptors in solution for several days. We further employed genetic code expansion to incorporate the non-canonical amino acid p-Propargyloxyphenylalanine and fluorescently labeled the extracellular domains at selected positions using click chemistry. Using single molecule FRET, we monitored ligand-dependent inter- as well as intrasubunit conformational changes. We demonstrate a fast (subms) oscillation of the extracellular domain between the inactive and the active states, that is only partially stabilized by the natural full agonist glutamate. The addition of a positive allosteric modulator or purified, heterotrimeric Gi protein, both binding to the 7 transmembrane domains, are necessary to fully reorient the extracellular domains to adopt the fully active state. Furthermore, our results show that different orthosteric ligands exert different degrees of conformational changes and highlight the allosteric effect on agonist efficacy and potency, mediated through a long-range interdomain communication.

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