Ligand-directed two-step labeling to quantify AMPA-type glutamate receptor trafficking

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AMPA-type glutamate receptor (AMPAR) is a subtype of the ionotropic glutamate receptors, which mediates fast excitatory neurotransmission in the central nervous system. The number of AMPARs on the postsynaptic surface changes dramatically during synaptic plasticity, a cellular mechanism of memory and learning. Impaired membrane trafficking of AMPARs causes cognitive impairment and psychiatric disturbance such as anxiety and depression. Therefore, analyzing the trafficking of AMPAR is indispensable for elucidation of the molecular mechanism of memory, learning and neurological diseases.

Our laboratory developed ligand-directed acyl imidazole (LDAI) chemistry, a chemical labeling method for cell-surface proteins in live cells[1]. Although this technique is powerful for labeling endogenous AMPARs[2], there are some restrictions for analyzing trafficking of AMPARs. First, live cells need to be kept at low temperatures (17 °C) during labeling to suppress the internalization of labeled AMPARs. Second, the culture medium needs to be exchanged for serum-free medium or buffered saline during labeling to decrease non-specific labeling of serum proteins such as albumin. The relatively long-term exposure (1–4 h) to these non-physiological conditions may interfere with neuronal activity or survival. Ideally, neurons should be kept under physiological conditions during chemical labeling.

Here, we develop a method for the rapid and selective labeling of AMPARs under physiological temperature in culture medium by ligand-directed two-step labeling combining LDAI chemistry and inverse-electron demand Diels-Alder (IEEDA) reaction[3]. This method allowed us to label AMPARs within a few minutes under cell-friendly condition. Furthermore, we successfully analyzed lifetime of AMPARs for long time without neuronal death, and quantified the fast recycling dynamics of AMPARs in the cultured neuron.

1) Fujishima, S.*et al.* Ligand-directed acyl imidazole chemistry for labeling of membrane-bound proteins on live cells. *J. Am. Chem. Soc.* 2012, **134**, 3961-3964

2) Wakayama, S. *et al.* Chemical labeling for visualizing native AMPA receptors in live neurons. *Nat Commun.* 2017, **8**, 14850

3) Ojima, K. *et al.* Ligand-directed two-step labeling to quantify neuronal glutamate receptor trafficking. *Nat. Commun.* (in press)