S32-1 **Behavior of membrane receptors in living cells visualized by using a new tag-probe fluorescence labeling method**

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To investigate behavior of membrane receptors in cell membranes, detection methods in living cells are essential. The specific labeling of proteins in living cells using a genetically encodable tag and a small synthetic probe targeted to the tag has several advantages over widely used fluorescent proteins. We recently developed a method using a high-affinity coiled-coil formation between the E3 tag $(EIAALEK)_3$ attached to the target protein and the Kn probes $(KIAALKE)_n$ (n = 3 or 4) labeled with a fluorophore [1]. The coiled-coil method is suitable for membrane proteins and having advantages such as 1) smaller size (1/5 of fluorescent proteins), 2) no impairment of receptor function, 3) easy control of labeling ratio, 4) cell-surface specific labeling 5) high specificity (K_d = 6 nM and 64 nM for K4 and K3, respectively), 6) quick labeling within 1 min. The agonist-induced internalization of β2-adrenergic receptor (β2AR) and EGF receptor could be clearly visualized. Following internalization, pH of endosomes is known to decrease to 5–6. Fluorescence ratiometric detection of β2AR internalization was examined. The detection simplicity compared with the conventional granularity analysis is useful for high throughput screening. [1] Yano, Y., Yano, A., Oishi, S., Sugimoto, Y., Tsujimoto, G., Fujii, N., Matsuzaki, K. (2008) ACS Chem. Biol. 3, 341–345.