Dual color single molecule imaging to track the GPCR-G protein interactions

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INTRODUCTION: Single molecule imaging has become an attractive method to reveal dynamic and heterogenous nature of signaling proteins, including G protein coupled receptor (GPCR), *in vitro* and in living cells. Recent studies reported the dynamic changes in GPCR oligomerization, but its importance in signal transduction is still poorly understood. Here, we visualized single molecules of GPCR and G protein simultaneously, aiming to reveal the relation between the transient oligomer formation and G protein activating property of GPCR.
METHODS: GPCR and G protein were genetically fused with self-labeling tags, SNAP-tag and Halo-tag, respectively, being labeled with organic fluorescent dyes in living cells. The probes were imaged with single molecule resolution using a total internal reflection fluorescence microscope (TIRFM).
RESULTS AND DISCUSSION: The two probes were visualized as diffusing individual fluorescent spots on the cell membrane at a frame rate of 55 Hz. Fluorescence intensity and location of the fluorescent spots were automatically calculated from the raw movies. First, the analysis of the fluorescence intensity of GPCR revealed that ligand stimulation induced an increase in the number of brighter fluorescent spots, which were assigned to be oligomers of GPCRs. Second, the colocalization events of GPCR and G protein were detected, and their duration times were analyzed. The result showed that ligand-activated GPCRs interacted with G proteins longer than the basal state GPCRs. Finally, the relation between oligomerization of GPCR and elongation of GPCR-G protein interaction was investigated. Ligand stimulation increased the duration of oligomer GPCRs with G protein and the G protein interaction of monomer GPCRs was not affected. Therefore, oligomer formation of GPCRs would be a key step in signal transduction in living cells. In conclusion, we established a dual color single molecule imaging system and revealed that the activated GPCRs formed oligomers that stably interacted with G proteins.