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Establishment of a new method for long-term single-molecule fluorescence imaging and its application to synaptic molecules

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Single-molecule fluorescence imaging (SMFI) is a promising method to unvail dynamic features of molecules underlying physiological and pharmacological responses of cells. However, the time window for SMFI is limited due to photobleaching of the labeled dye, precluding detailed analysis. Here we aim to overcome the limitation of SMFI by developing a protein tag system named DeQODE tag system, consisting of two components: Quenched Organic Dye Emission (QODE) probe, a small organic dye coupled with a quencher moiety, and DeQODE tag, a single-chain antibody that turns on fluorescence emission of the QODE probe by binding the quencher. We expected that reversible and repeatable cycles of association and dissociation between QODE probe and DeQODE tag enable virtually perpetuating fluorescent tagging of the target protein. We tested the applicability of DeQODE tag system to SMFI in COS7 cells expressing DeQODE tag-fused syntaxin 1A and a synaptic protein Munc13-1. Fluorescent spots of QODE probe repeatedly appeared and disappeared for at least an hour. Also, the decrease in syntaxin 1A mobility was detected upon the application of phorbol ester recruiting Munc13-1 to plasma membrane. These results show that the DeQODE tag system-based long-term SMFI enables detailed analysis of molecular dynamics of signaling proteins in living cells.