Development of Photoswitchable Fluorescent Molecules for No-Wash Live Cell Imaging

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Photoswitchable fluorescent molecules (PSFMs) are valuable tools for tracking cellular dynamics and super-resolution imaging due to their photoswitching ability upon light irradiation. In recent years, photoswitchable cyanine[1] and diarylethene[2] have been applied to super-resolution imaging. However, these molecules require high concentrations of cytotoxic thiol for photoswitching or have poor cell membrane permeability, which hampers live cell applications. Despite the high demand for PSFMs that are suitable for live-cell imaging, no general method has been reported that enables reversible fluorescence control on proteins of interest in living cells.

Herein, we have established a platform to realize reversible fluorescence switching in living cells by adapting a protein labeling system. We have developed a new PSFM, named HTL-Trp-BODIPY-FF, which exhibits strong fluorogenicity upon recognition of Halo-tag protein and reversible fluorescence photoswitching in living cells (Figure 1). The fluorogenicity helps to minimize the fluorescence from unlabeled HTL-Trp-BODIPY-FF and allows no-wash labeling. As for the fluorescence photoswitching, we have used FF (furylfulgimide) as a photochromic FRET quencher[3,4]. The labeled HTL-Trp-BODIPY-FF exhibited reversible fluorescence switching upon light irradiation with higher photostability compared to the unlabeled one, assisted by the Halo-tag surface that prevents intermolecular aggregation. This is the first example of a PSFM that can be applicable to a general-purpose Halo-tag protein labeling system for no-wash live-cell imaging[4]. In this conference, we will report on the detailed molecular designs, photophysical properties, and biological experiments of HTL-Trp-BODIPY-FF.

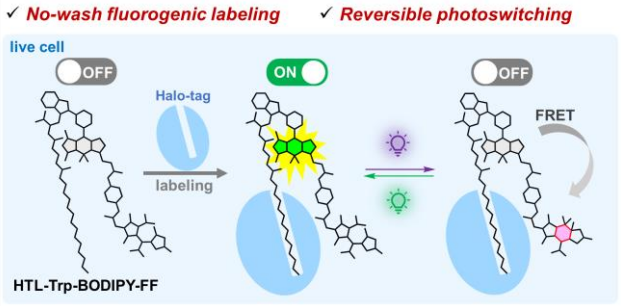

Figure 1. Schematic of this study

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