Optical regulation of protein translocation using a photo-reversible protein labeling system

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Artificial control of the dynamics of proteins with high precision provides valuable insight into complicated biological networks. Optogenetics and photo-responsive chemically induced dimerization (CID) are famous methods for spatiotemporal regulation of protein dynamics. However, both methods leave significant challenges for the fine-tuning at precise time and locations or cycle of repetition. In this study, we aimed at developing a novel chemical tool that allows photo-reversible control of subcellular protein localization, focusing on a photochromic compound. We previously developed a photochromic ligand, azoMTX, which binds to *Escherichia coli* dihydrofolate reductase (eDHFR) in a light-wavelength-dependent manner.[1] We thus designed a photochromic ligand for eDHFR conjugated with a HaloTag ligand to establish a photochromic CID system (**Figure 1**).

The designed photochromic dimerizer isomerized from *E* to *Z* isomer upon violet light irradiation and from *Z* to *E* isomer upon green light irradiation. The binding assay showed that the photochromic dimerizer had a higher affinity to eDHFR under violet light irradiation than dark conditions. HeLa cells co-expressing an organelle-targeted HaloTag and an eDHFR-fused fluorescent protein (FP) were treated with the photochromic dimerizer. When the cells were illuminated with violet and green light alternately, subcellular localization of the eDHFR-fused FP was photo-reversibly regulated.

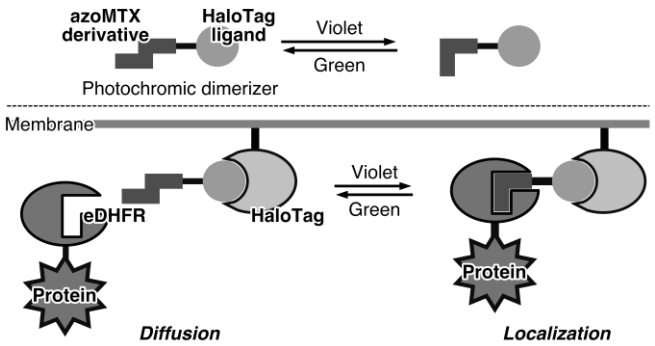

Figure 1. Schematic illustration of photochromism-based protein translocation.

1) Mashita, T.; Kowada, T.; Takahashi, H.; Matsui, T.; Mizukami, S. *ChemBioChem* **2019**, *20*, 1382–1386.