Development of Fluorogenic Probes for Monitoring Protein-protein Interactions with Reversible Labeling Strategy

○ Jiatong Li,[1] Masafumi Minoshima,[1] Kazuya Kikuchi.[1,2] (1. Graduate School of Engineering, Osaka University, 2. Immunology Frontier Research Center, Osaka University)

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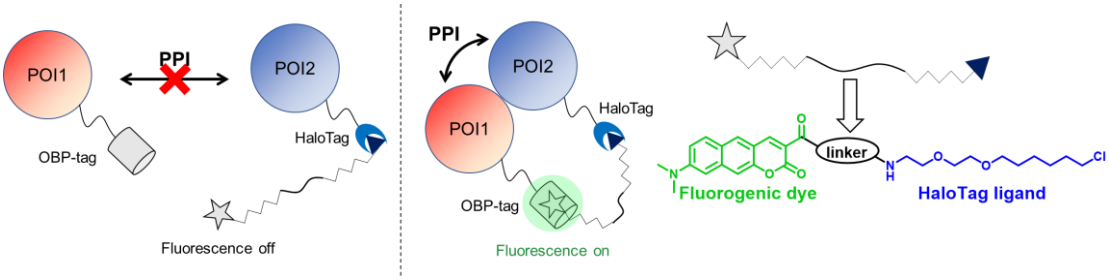
Protein-protein interactions (PPI) play essential roles in signal transduction and molecular transportation in cells[1]. Understanding PPI reveals the network of gene products and function in cells. Fluorescence imaging is a powerful tool for visualizing biomolecules in living cells, enabling researchers to study biological processes with high spatiotemporal resolution. Fluorescence Resonance Energy Transfer (FRET) [2]-based techniques and Bimolecular Fluorescence Complementation (BiFC)[3] methods have been widely used for investigating PPIs. Nevertheless, interpreting PPIs by these approaches may be confounded by various factors, such as fluorescence crosstalk and fluorescence artifact by spontaneous complementation, respectively.

To address these challenges and advance the detection of PPIs, our research focuses on a reversible labeling strategy utilizing chemical fluorescent probes. This study introduces an innovative imaging approach for visualizing PPIs based on a fluorogenic and reversible protein labeling system. The key of this method is the OBP-tag, a protein tag designed to be fused with the protein of interest (POI), enabling dynamic labeling and visualization with reversibility.

This approach represents a novel method for PPI detection. A series of fluorescent probes were synthesized and characterized, and their irreversible binding to HaloTag and reversible interaction with OBP were successfully validated. Significant fluorescence shifts were observed in response to PPIs. Using N-Cadherin interactions as a model in live-cell imaging experiments, we demonstrated visualization at cell adhesion sites and confirmed the reversible labeling capabilities.



(1)Lu, H. et al. *Sig Transduct Target Ther* **2020**, *5* (1), 213.
(2)Park, S.-H. et al. *J. Am. Chem. Soc.* **2019**, *141* (10), 4273–4281.
(3)Wang, S. et al. *ACS Chem. Biol.* **2018**, *13* (5), 1180–1188.