Development of versatile fluorescent protein labeling probes for live-cell imaging

Department of Applied Chemistry, Graduate School of Engineering

Professor Kazuya Kikuchi Researchmap https://researchmap.jp/kikuchilab315?lang=en

Associate Professor Masafumi Minoshima Researchmap https://researchmap.jp/cbatgcm_m33?lang=en

Abstract

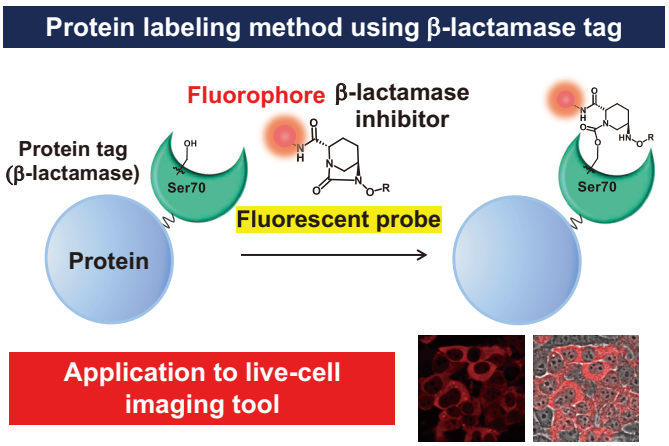
We herein developed a versatile chemical protein-labeling tool based on fluorophore-conjugated diazabicyclooctane β-lactamase inhibitors (BLIs) and wild-type β-lactamase protein tag. The fluorescent probes efficiently formed a stable complex with b-lactamase, and the labeled proteins were visualized over a long period of time in live cells. Moreover, a fluorophore-conjugated BLI prodrug enabled labeling intracellular proteins due to improved cell membrane permeability. Lastly, combining the labeling tool with a pH-activatable fluorescent probe allowed visual monitoring of lysosomal protein translocation during autophagy.

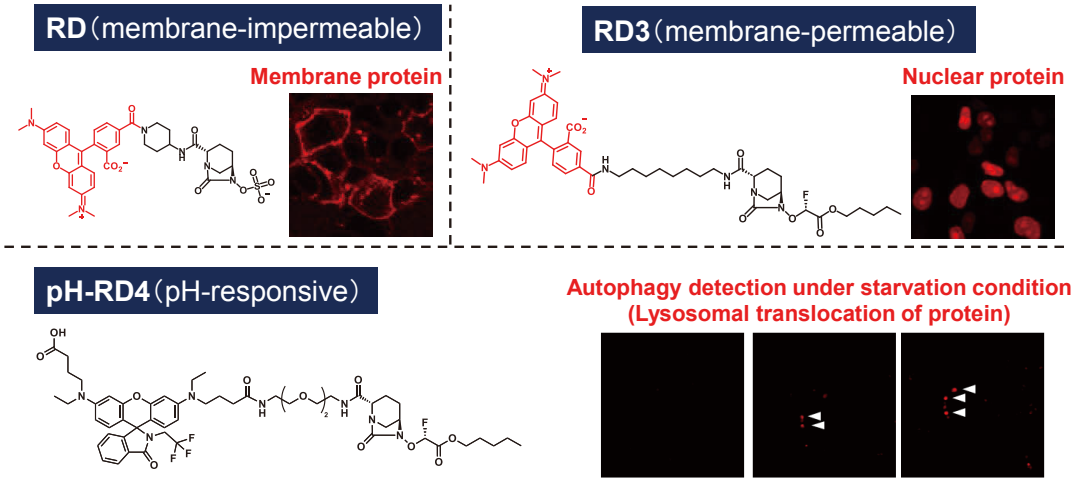
Background & Results

Real-time monitoring of protein dynamics and sensing of their surrounding environment are important methods to understand the function of protein in live cells. Fluorescent labeling tools are thus needed that possess fast labeling kinetics, high efficiency, and long-term stability. Our group has developed a covalent protein labeling system that uses the catalytically inactive mutant of β-lactamase as a protein tag. Meanwhile, the labile β-lactam ring of the substrates has pronounced chemical susceptibility and potential dissociation from the protein complex, which limits the prolonged imaging of intracellular protein dynamics. We initially conjugated relebactam, a piperidine analogue of BLI, with a tetramethylrhodamine (TAMRA) fluorophore to afford the new probe RD for labeling β-lactamase. RD efficiently labeled β-lactamase and formed a stable complex. In addition, RD labeled β-lactamase tag expressing in plasma membrane in living cells. We further developed a membrane-permeable fluorescent probe (RD3) using a BLI prodrug. RD3 showed membrane permeability and enabled intracellular protein labeling over a prolonged period of time in live cells. Then we applied the labeling method to imaging translocation of proteins into lysosomes in response to starvation-induced autophagy. Our imaging strategy relied on labeling intracellular proteins bearing a β-lactamase tag using a labeling probe with a pH-activatable fluorophore (pH-RD4). pH-RD4 increased fluorescence signals in acidic pH corresponding to lysosomal environment. After labeling intracellular protein in live cells with pH-RD4, the cells were starved by replacing the medium with an amino acid-depleted medium. The time-lapse images revealed that the fluorescence spots were found after two hours in the starvation medium, and the size and number then increased over time. This finding demonstrated that pH-RD4 is capable of long-term tracking of proteins and imaging the pH environment during the autophagy process.

Significance of the research and Future perspective

In this study, we developed a versatile fluorescent protein labeling system using a series of RD probes bearing BLI and its prodrug. This β-lactamase-based system has excellent protein labeling abilities in living cells, similar to existing protein labeling systems. Our protein labeling method can serve as a versatile tool for studying protein dynamics within living cells, which contributes toward advancing our understanding of molecular biology and medicine.





Patent

Treatise Minoshima, Masafumi; Umeno, Taro; Kadooka, Kohei et al. Development of a Versatile Protein Labeling Tool for Live-Cell Imaging Using Fluorescent β-Lactamase Inhibitors. Angewandte Chemie International Edition. 2023, 62, e202301704. doi: 10.1002/anie202301704

URL https://www-molpro-mls.eng.osaka-u.ac.jp/English/indexeng/indexENG.html

Keyword fluorescent probe, protein labeling, β-lactamase inhibitor, autophagy