Substituted *meso*-Vinyl-BODIPY as Thiol-Selective Fluorogenic Probes for Sensing Unfolded Proteins in Living Cells

(*Graduate School of Engineering, Kyoto University*) ○Huiying Mu, Koji Miki, Kouichi Ohe

Keywords: thiol-reactive probe, BODIPY, unfolded protein, fluorescence

Cytotoxic aggregation of unfolded proteins causes a loss of protein function and endoplasmic reticulum (ER) stress which associated with cell apoptosis and neurodegenerative disorders. Thiol-reactive fluorogenic probes have recently found their use to sense protein unfolding process. Here we wish to report a new type of selective thiol-activatable fluorogenic probes (VBs) based on *meso*-vinyl-BODIPY dye, including **VB**, **VB1Cl** and **VB2Cl** (Figure 1a).[1] The electron-deficient BODIPY core confers thiol reactivity to the vinyl group, which was then further improved by introduction of electron-withdrawing groups at the BODIPY core. The monochloro-substituted derivative **VB1Cl** exhibiting high selectivity to thiols (Figure 1b), is applicable for sensing unfolded proteins with high sensitivity and large fluorescence enhancement (Figure 1c). In living cell imaging, we demonstrate the utility of **VB1Cl** for sensitively reporting the protein unfolding process under ER stress induced by antibiotic active tunicamycin and proteasome inhibitor MG132 in living cells (Figure 1d).

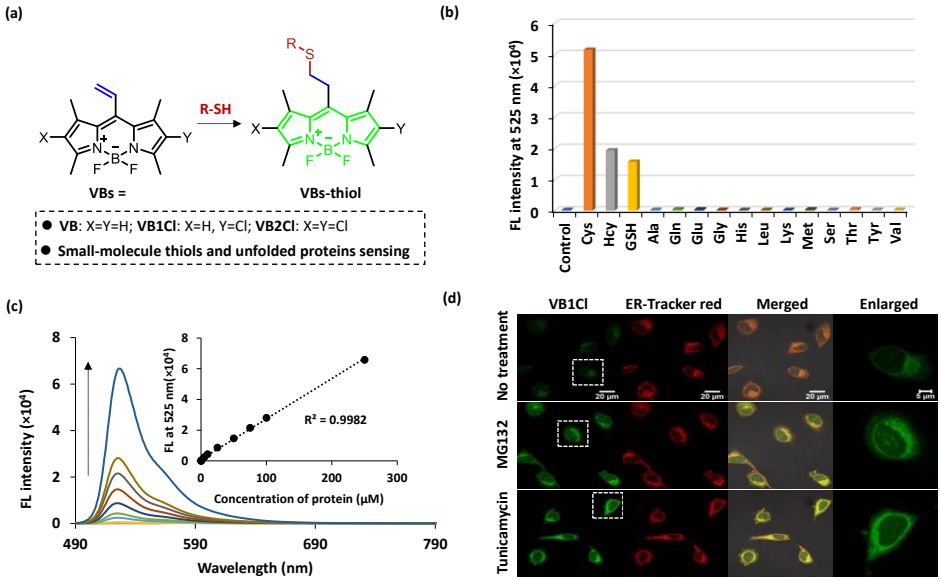

Figure 1. (a) Reaction scheme of **VBs** with thiol-containing compounds. (b) Selectivity of **VB1Cl** (5 μM) towards a variety of amino acids (1 mM). (c)Fluorescence spectra of **VB1Cl** upon the addition of unfolded protein *β*-lactoglobulin (LGB, 0–250 μM). (e) Confocal images of controlled and stressed HeLa cells stained with **VB1Cl** (5 μM) for 1 h and ER-Tracker red (1 μM) for 15 min, followed by methanol fixation.

[1] H. Mu, K. Miki, T. Kubo, K. Otsuka, K. Ohe. *Chem. Commun.* **2021**, *57*, 1818-1821.