Detection of *lacZ*-positive cells in living tissue with single-cell resolution

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Selective imaging of targeted cells in living samples with chemical probes remains highly challenging. *LacZ* gene, which encodes *Escherichia coli* β-galactosidase, is widely used as a marker for cells with targeted gene expression or disruption. However, it has been difficult to detect *lacZ*-positive cells in living organisms or tissues at single-cell resolution, limiting the utility of existing *lacZ* reporters. Here we succeeded to develop a newly fluorogenic β-galactosidase substrate capable of labeling live *lacZ*(+) cells at a single-cell resolution in culture, as well as in living tissues and organisms.

We have established a rational and versatile molecular design strategy for hydrolase-sensitive fluorogenic probes based on intramolecular spirocyclization, and have developed various aminopeptidase-[1,2] and glycosidase-sensitive[3,4] probes. Among them, HMDER-βGal[3] has almost no color and fluorescence upon excitation with visible light due to preferred spirocyclized form in neutral pH condition, and sufficient cell permeability to visualize β-galactosidase activity in living tissue. However, its fluorescent hydrolysis product, HMDER, tends to leak out of cells during prolonged incubation. Thus, to retain the fluorescent product inside cells, we set out to integrate quinone methide chemistry into our design strategy. Quinone methide chemistry is widely used in the design of enzyme inhibitors, activity-based probes, prodrugs, and so on, and quinone methide intermediates are usually generated by the substrate-cleaving enzymatic activity, followed by release of leaving groups. Therefore, we aimed to incorporate a fluoromethyl group as a leaving group at 4th position of HMDER-βGal to develop a novel spiro-based **i**mmobilisable diethylrhodol-βGal (SPiDER-βGal) probe[5]. This probe was expected to be stable until cleaved by β-galactosidase. Enzyme-mediated cleavage of the β-galactoside moiety generates the reactive quinone methide intermediate that immediately reacts with intracellular nucleophiles such as proteins to form fluorescent adducts, so that the fluorophore is immobilized inside the cells. Thus, the enzymatic cleavage reaction simultaneously activates both fluorescence and binding ability to intracellular components.

SPiDER-βGal probe enabled selective and rapid detection of live *lacZ*(+) cells at single-cell resolution without perturbing cellular functions; not only can three-dimensional morphological information about the target cells and surrounding tissues be obtained with the aid of confocal microscopy, but also the firing activities of targeted neurons in living brain slices can be monitored by electrophysiological analysis[5].

References:

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