Supporting Information

An In Cellulo-Activated Multicolor Cell Labeling Approach Used to Image Dying Cell Clearance

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Content

Scheme S1. Synthesis and chemical deacetylation of compound 1………………………………..S3
Fig. S1 ¹H-NMR of compound 2……………………………………………………………………..S3
Fig. S2 ¹³C-NMR of compound 2……………………………………………………………………..S4
Fig. S3 ¹H-NMR of compound 3……………………………………………………………………..S4
Fig. S4 ¹³C-NMR of compound 3……………………………………………………………………..S5
Fig. S5 HRMS of compound 2 and compound 3……………………………………………………S5
Scheme S2. Synthetic routes for DHA-Red and AP-Red. …………………………………………S6
Scheme S3. Synthetic routes for DHA-Blue and DHA-Green. ……………………………………S6
Fig. S6 Esterase-triggered protein labeling with DHA-Red. ……………………………………..S6
Fig. S7 Incapability of AP-Red to label cellular proteins. ……………………………………….S7
Fig. S8  Dose- and Incubation time-dependent cell labelling with DHA-Red. ..........................S7
Fig. S9  Covalent cell labelling with DHA-Green. .................................................................S8
Fig. S10  Covalent cell labelling with DHA-Blue. .................................................................S8
Fig. S11 cytotoxicity of Dye-DHA diads. ..................................................................................S9
Fig. S12  Comparison of DHA-Dye diads to Cell-tracker CFSE. .................................................S10
Fig. S13 Fluorescence retention of DHA-mediated labeling in HeLa cells undergoing cell death...S11
Fig. S14 1H-NMR of compound 1. .........................................................................................S12
Fig. S15 13C-NMR of compound 1 .........................................................................................S12
Fig. S16 1H-NMR of DHA-Red...............................................................................................S13
Fig. S17 13C-NMR of DHA-Red.............................................................................................S13
Fig. S18 HRMS of DHA-Red.....................................................................................................S14
Fig. S19 1H-NMR of AP-Red....................................................................................................S14
Fig. S20 13C-NMR of AP-Red................................................................................................S15
Fig. S21 HRMS of AP-Red.........................................................................................................S15
Fig. S22 1H-NMR of DHA-Blue...............................................................................................S16
Fig. S23 13C-NMR of DHA-Blue.............................................................................................S16
Fig. S24 HRMS of DHA-Blue.....................................................................................................S17
Fig. S25 1H-NMR of DHA-Green.............................................................................................S17
Fig. S26 13C-NMR of DHA-Green..........................................................................................S18
Fig. S27 HRMS of DHA-Green.................................................................................................S28
Scheme S1. Synthesis and chemical deacetylation of compound 1.

Fig. S1 ¹H-NMR of compound 2
Fig. S2 $^1$C-NMR of compound 2

Fig. S3 $^1$H-NMR of compound 3
**Fig. S4** $^{13}$C-NMR of compound 3

**Fig. S5** HRMS of compound 2 and compound 3
Scheme S2. Synthetic routes for DHA-Red and AP-Red (A). Fluorescence properties of DHA-Red (4 μM) in PBS (pH 7.2) (B).

Scheme S3. Synthetic routes for DHA-Blue and DHA-Green.

Fig. S6 Esterase-triggered protein labeling with DHA-Red. Esterase or BSA (1.5 μg) were treated DHA-Red for 1 h and then subjected to SDS-PAGE. The gel was analyzed for fluorescence emission using λ<sub>ex</sub> = 520 nm (A) or stained with Coomassie Brilliant Blue (B).
Fig. S7 Incapability of AP-Red to label cellular proteins

Fig. S8 Dose- and Incubation time-dependent cell labelling with DHA-Red. (A) HeLa cells were co-stained with LysoTracker Blue (1 μM) and DHA-Green (2 μM) for 10, 30, 60, 120 min in DMEM and then imaged by confocal microscopy for intracellular fluorescence. (B) HeLa cells labeled with DHA-Green (1, 2, 4, 8 μM) for 60 min and then imaged by confocal microscopy for intracellular fluorescence. Scale bar: 10 μm.
**Fig. S9** Covalent cell labelling with DHA-Green. (A) HeLa cells were co-stained with LysoTracker Red (1 μM) and DHA-Green (4 μM) for 1 h. Cells were fixed with 4% Paraformaldehyde Fix Solution, washed and then imaged for intracellular fluorescence. (B) HeLa cells labeled with DHA-Green (2, 4, 8 μM) for 1 h and then fixed before confocal microscopy analysis. Scale bar: 10 μm.

**Fig. S10** Covalent cell labelling with DHA-Blue. (A) HeLa cells were co-stained with LysoTracker Red (1 μM) and DHA-Blue (4 μM) for 1 h. The cells were fixed with 4% paraformaldehyde Fix Solution, washed and then imaged for intracellular fluorescence. (B) HeLa cells labeled with DHA-Blue (2, 4, 8 μM) for 1 h and then fixed before confocal microscopy analysis. Scale bar: 10 μm.
Fig. S11 cytotoxicity of Dye-DHA diads.
Fig. S12 Comparison of DHA-Dye diads to Cell-tracker CFSE. HeLa cells were stained with CFSE (4 μM), DHA-Blue (4 μM), DHA-Green (4 μM), or DHA-Red (4 μM) for 60 min, respectively. The cells were further maintained in fresh DMEM for 0, 24, 48 h. A portion of the cells were harvested at indicated time points, rinsed with DMEM, and then analyzed by confocal microscopy (A) or flow cytometry (B) for intracellular fluorescence. (B) Chemistry of DHA-Green deacetylation in live cells. Scale bar: 10 μm.

S10
**Fig. S13** Fluorescence retention of DHA-mediated labeling in HeLa cells undergoing cell death. RIP3⁺ HeLa cells were stained with DHA-Blue (4 μM), DHA-Red (4 μM), or DHA-Green (4 μM) for 1 h, and then rinsed with PBS, and then treated with Smac/TNF to triggered apoptosis or with Smac/TNF/Z-VAD to trigger necrosis. The dying cells were maintained in fresh medium and then monitored by confocal fluorescence microscopy for intracellular fluorescence over time. Scale bars: 20 μm.
Fig. S14 $^1$H-NMR of compound 1

Fig. S15 $^{13}$C-NMR of compound 1
Fig. S16 $^1$H-NMR of DHA-Red

Fig. S17 $^{13}$C-NMR of DHA-Red
Fig. S18 HRMS of DHA-Red

Fig. S19 $^1$H-NMR of AP-Red
Fig. S20 $^{13}$C-NMR of AP-Red

Fig. S21 HRMS of AP-Red
Fig. S22 $^1$H-NMR of DHA-Blue

Fig. S23 $^{13}$C-NMR of DHA-Blue
Fig. S24 HRMS of DHA-Blue

Fig. S25 $^1$H-NMR of DHA-Green
Fig. S26 $^{13}$C-NMR of DHA-Green

Fig. S27 HRMS of DHA-Green