Literature Report

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Article

Simultaneous Two-Color Visualization of Lipid Droplets and Endoplasmic Reticulum and Their Interplay by Single Fluorescent Probes in Lambda Mode

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研究领域

功能荧光材料的设计、合成及其在生物医学成像中的应用



Simultaneous dual-colour tracking lipid droplets and lysosomes dynamics using a fluorescent probe





Chem. Sci., 2019, 10, 2342–2348.

So far, a few probes have been developed for simultaneous LD and ER imaging with one emission color.



Ac-2010



Figure 3 Intracellular localization of Ac-2010. (a) HepG2 human hepatocellular carcinoma cells were double-stained with the blue fluorescent Ac-2010 (10 µM) pseudocolored as red, and **(b)** endoplasmic reticulum-specific dye (ER-TrackerTM Green). **(c)** Orange color derives from colocalization of ER-TrackerTM and Ac-2010. Red spots correspond to lipid droplets as ER-tracker does not stain oil bodies, while Ac-2010 does. Scalebar is 10 µm.

Nagy et al. Lipids in Health and Disease 2013, 12:175.



Label (Ex/Em): Bis-BODIPY® FL C11-PC (~488/530 nm)



Fig. 5 Dynamics of lipid droplets. A Merged image of a cell stained with Bodipy PC (green) and Nile red (red). Note co-localisation of dyes in the lipid droplets (yellow).

Protoplasma (2009) 238:47-58.



Scheme 1. Schematic Illustration of Two-Color Visualization of Lipid Droplets (LDs) and ER Using ESIPT-Active Fluorescent Probes PPC and EPC and the Control Probe MNC.



Figure 1. Optimized structures and normalized absorption (dashed lines) and fluorescence emission (solid lines) spectra of (A) PPC, (B) EPC, and (C) MNC in 1,4-dioxane. For PPC and EPC, $\lambda ex = 405$ nm; for MNC, $\lambda ex = 385$ nm.



Figure 2. Fluorescence photos under 365 nm excitation of a hand-held ultraviolet lamp, fluorescence spectra, and CIE1931 coordinates of (A) PPC, (B) EPC, and (C) MNC in 1,4-dioxane/water mixtures with different water fractions (f w). For PPC and EPC, $\lambda ex = 405$ nm; for MNC, $\lambda ex = 385$ nm.



Figure 3. (A) Fluorescence spectra and (B) CIE1931 coordinates of PC in 1,4-dioxane/water mixtures with different water fractions (f w). The CIE1931 coordinates of PPC were also plotted as a contrast. (C) 1H NMR chemical shifts (in units of ppm) of the hydroxyl hydrogen and the distances (in units of Å) between the hydroxyl H and ketonic O of PPC, EPC, MNC, and PC. $\lambda ex(PC) = 360$ nm.



Figure 4. Real-color fluorescent images and in situ fluorescence spectra of LDs (indicated by the red circles) and the ER (indicated by the green circles) in live HeLa cells stained with PPC, EPC, and MNC for 2 min. $\lambda ex = 405$ nm. Scale bar: 20 μ m.



Figure 5. (A) Confocal fluorescent images of HeLa cells stained with PPC. Colocalization experiments of HeLa cells stained with PPC and (B) BODIPY 493/503 or (C) ER Red. Rr is the average colocalization coefficient of these ROIs indicated by the white circles. $\lambda ex(PPC) = 405$ nm; $\lambda ex(BODIPY) = 473$ nm; $\lambda ex(ER Red) = 543$ nm. Scale bar: 20 µm.



Figure 6. Real-color fluorescent images of live SiHa and MSCs stained with PPC, EPC, and MNC. The ROIs indicated by the red and green circles are analyzed for the in situ fluorescence spectra of LDs and the ER, respectively. $\lambda ex = 405$ nm. Scale bar:10 µm (SiHa cells) or 20 µm (MSCs).



Figure 7. Real-color fluorescent images of oleic acid pretreated HeLa cells stained with PPC or EPC and the corresponding normalized in situ fluorescence spectra in nascent LDs (indicated by the blue arrows), mature LDs (indicated by the red arrows), and the ER (indicated by the green circles). $\lambda ex = 405$ nm. Scale bar: 10 µm.





In situ and real-time Lambda mode based fluorescence imaging of PPC in oleic acid pretreated HeLa cells.



In situ and real-time Lambda mode based fluorescence imaging of EPC in oleic acid pretreated HeLa cells.

Figure 8. (A) In situ dynamic monitoring of LD formation in oleic acid pretreated HeLa cells stained with PPC at different time points. (B) Enlarged fluorescence images of the ROIs indicated by the white rectangles in (A). (C) In situ fluorescence spectra of mature LDs (indicated by the red arrow), ER (indicated by the green circles), and nascent LDs (indicated by the blue arrows). $\lambda ex = 405$ nm. Scale bar: 20 µm.



Figure 9. Real-color fluorescent images of oleic acid pretreated HeLa cells stained with PPC and anti-TIP47 antiserum and the corresponding normalized in situ fluorescence spectra in nascent LDs (indicated by the blue arrows), mature LDs (indicated by the red arrows), and ER (indicated by the green circle). PPC, $\lambda ex = 405$ nm; anti-TIP47 antiserum (fluorescence from Cy3-conjugated goat antirabbit IgG), $\lambda ex = 561$ nm. Scale bar: 20 µm.