Fluorescence lifetime mapping of lipid compositions using an environment-responsive lipid droplet probe

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Lipid droplet (LD) is an essential organelle, which consists of lipid esters core covered with a phospholipid monolayer. Much attention has recently been paid to the effect of the composition of neutral lipids, especially a ratio of triacylglycerol (TAG) and cholesterol ester (CE), in LDs on cell functions. However, although many fluorescent probes for LDs have been developed to date, none of them can evaluate lipid composition. Therefore, it is a challenge to develop a new fluorescent probe that has high LD selectivity as well as high environmental sensitivity that can discriminate between TAG-rich and CE-rich LDs. Herein, we report a phosphole-oxide-based LD probe, **LipiCo** (Figure 1a) and its practical applications to the LDs imaging by fluorescence lifetime imaging microscopy (FLIM).

The fluorescence lifetime (τ) of **LipiCo** is very sensitive to the solvent polarity, which allows us to distinguish even a slight difference in polarity between toluene and CH_2Cl_2 with the τ values of 7.7 ns and 6.8 ns, respectively. Because its fluorescence is significantly quenched in more polar solvents such as DMSO, non-polar LDs is clearly visualized using **LipiCo** with high signal-to-noise ratio, even at ultra-small sizes. Indeed, LDs were selectively detected with **LipiCo** in a variety of cell types.

First, using FLIM, we measured the fluorescence lifetime of **LipiCo** in artificial LDs consisting of triolein and cholesterol oleate. The τ values increase with increasing a ratio of cholesterol oleate, indicating that **LipiCo** enables the evaluation of lipid composition in cells. We then stained Huh-7 cells and recorded the LDs by FLIM. Importantly, even in a single cell, the fluorescence lifetime of each LD showed a heterogeneous distribution in the range of 5.5 ns to 6.8 ns (Figure 1b). Taking advantage of these unique properties of **LipiCo**, we monitored the metabolic accumulation process of cholesterol into the LDs in hepatocytes. Upon the addition of cholesterol, longer τ values were observed, as the incubation time was increased (Figure 1c). The wide distribution of τ values in this case suggest that cholesterol is not taken up uniformly by all LDs and each LD plays a different role in the metabolic process.

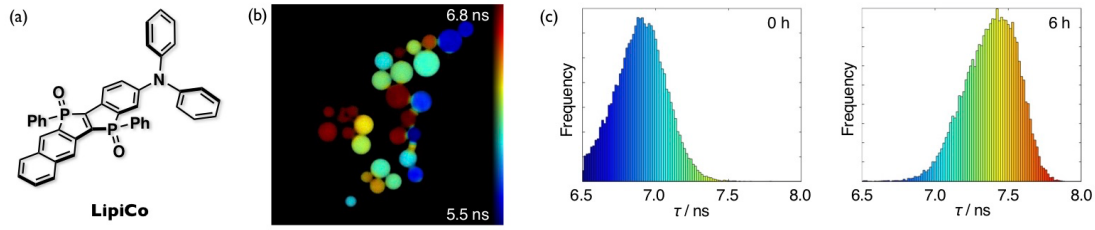

Figure 1. (a) A molecular structure of **LipiCo**. (b) A FLIM image of LDs in one HuH-7 cell stained with **LipiCo**. (c) Distributions of fluorescence lifetime in LDs of HuH-7 cells 0 h (left) and 6 h (right) after the addition of cholesterol.