Visualization of Lipid Metabolism Using an Environment-Sensitive Fluorescent Fatty Acid

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Lipid metabolism plays a key role in various biological systems such as energy homeostasis and cell proliferations. Because the metabolic pathways of fatty acids (FAs) are strongly related to cell functions, understanding how FAs are metabolized in cells has been an important research objective. Although fluorescent fatty acids are useful tools for monitoring intracellular FA dynamics in real time, they have several drawbacks such as low metabolic efficiency and different cellular distribution from those of natural FAs. Herein, we report a novel fluorescent fatty acid **AP-C12** based on 3a-azapyrene-4-on (AP) and demonstrate its practical utilities for visualizing FA metabolism in living cells.

The AP dye is an environment-responsive compound that shows a hypsochromic shift of absorption and emission maxima as the solvent polarity increases, while the fluorescence quantum yields are almost constant. Therefore, when **AP-C12** metabolites are transported to various organelles including mitochondria, lipid droplets (LDs), and endoplasmic reticulum (ER), differences in the polarity of the organelles can be detected as the differences in fluorescence properties, leading to the visualization of their subcellular distributions.

Upon incubation of oleate-treated HepG2 cells with **AP-C12**, we successfully visualized the cytosol, ER and mitochondrial membranes, and LDs, where **AP-C12** metabolites were located, in green, yellow, and red, respectively (Figure 1). We then analyzed the fatty acid metabolic pathways by using known inhibitors of the related enzymes. For example, when the cells were treated with a DGAT1 inhibitor T863, fluorescence signal of ER in yellow increased, indicating that diacylglycerol was highly accumulated in the ER membrane. Finally, we monitored the distribution of the metabolites in autophagic cells and found that autophagy-supplied FAs were re-metabolized and stored in LDs. These results demonstrated that **AP-C12** is a powerful tool for evaluating the fatty acid metabolism.

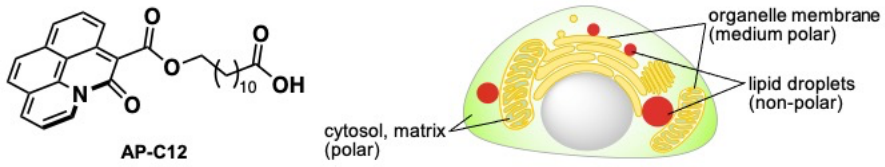

Figure 1. Schematic representation of the visualization of the distribution of fatty acid metabolites in cells stained with **AP-C12**.