Environment Responsive Fluorescent Probes for Visualization of Cellular Lipid Dynamics

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Lipid metabolism is an essential biological function for cells, including degradation and storage of lipids for energy production and membrane biogenesis for cell growth. Recent studies have revealed that the dysregulations in lipid metabolism are involved in various diseases such as obesity, diabetes, and cancer, thus it is increasingly important to understand the mechanism. Fluorescence imaging is a powerful technique for non-invasive analysis of lipid metabolism in living cells. However, the lack of molecular tools has limited the methodology of lipid-related research. Herein, I'd like to introduce some recently developed unique fluorescent probes that enable visualization of lipids in live cells.

1. Environment-sensitive fluorescent fatty acid (AP-C12)

Metabolic distribution of fatty acids (FAs) to organelles is an essential process for energy homeostasis as well as for the maintenance of membrane integrity, and the metabolic pathways are strictly regulated in response to environmental stimuli. To understand the exogenous FA metabolism, we have developed a new fluorescent FA probe, **AP-C12**, which bears an azapyrene (AP) dye that changes its photophysical properties depending on the microenvironment polarity of the transported organelle. Owing to the negative-solvatochromism of this AP dye,[1] the distribution of the metabolically incorporated FA probe in non-polar lipid droplets (LDs), medium-polarity membranes, and the polar mitochondrial matrix, can be visualized in different colors. Based on density scatter plots of the AP fluorophore, in nutrition-starved hepatocytes, we proved that the degradation of triacylglycerols in LDs occurs predominantly via lipolysis rather than lipophagy. Thus, this new tool can be expected to significantly advance our understanding of lipid metabolism in living organisms.

2. Super-photostable mitochondrial inner membrane marker (MitoPB Yellow and MitoPB Red)

Mitochondria play a central role in energy metabolism, and their functions are closely related to their membrane morphologies. Although super-resolution microscopy is a powerful technique for visualizing the mitochondrial ultrastructure such as cristae in living cells, it requires molecular tools that can specifically label the mitochondrial inner membrane with high photostability. We found that our newly developed fluorescent probe, **MitoPB Yellow**, possesses a long fluorescence lifetime, outstanding photostability, and

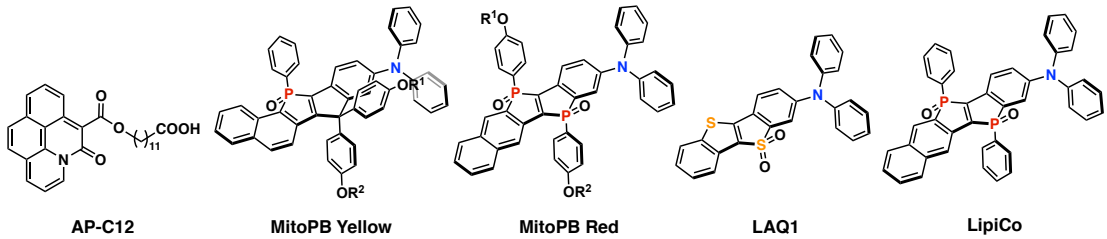
fluorogenic properties upon localization to the membrane.[2] In combination with a time-gated-STED microscopy, we successfully captured cristae structures with high signal-to-noise ratio as well as rapid inter-cristae mergence (< 2 s) in a single mitochondrion.

As a second-generation mitochondrial fluorescent marker, we recently developed a red-emissive superphotostable compound, **MitoPB Red**, which are based on a phospholo[3,2-*b*]phosphole-*P*,*P*'-dioxide scaffold. Because of stronger electron accepting nature of this building block, **MitoPB Red** shows higher environment sensitivity than MitoPB Yellow. We found that this probe can be applied to FLIM imaging for visualization of the inner membrane heterogeneity originating from the differences in lipid composition.

3. Probes for visualizing lipid droplets (LAQ1 and LipiCo)

Lipid droplets (LDs) are a universal organelle involved in various cellular functions, so understanding their roles in biological systems has been an important research subject. However, there are a limited number of fluorescent markers that satisfy all requirements including the selective staining of LDs, high photostability, and sufficient biocompatibility, have been developed. In this regard, we have developed a donor-π-acceptor compound **LAQ1** based on a thiophene-containing fused polycyclic scaffold [1]benzothieno[3,2-*b*][1]benzothiophene (BTBT), in which one thiophene ring is oxidized into thiophene-*S*,*S*-dioxide to form an electron-accepting building block.[3] Super-photostable **LAQ1** enabled recording the lipolysis of LDs and the concomitant lipogenesis, as well as long-term trajectory analysis of micro-LDs at the single-particle level in living cells.

Following this, we recently developed a red-emissive fluorescent probe for LD, **LipiCo**, which employs the same scaffold as **MitoPB Red**. Owing to the extremely high environment sensitivity of this probe, the lipid composition in LDs can be assessed from the fluorescence lifetime. Indeed, by combining **LipiCo** and FLIM, we could successfully visualize the differences in lipid composition among cell types as well as even in a single cell.



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