Synthesis and Characterization of Unsymmetric Thieno-Phospha-Rhodamine for Bioimaging

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Keywords: Bioimaging; Fluorescent Probe; Rhodamine; Near Infrared Imaging

Near-infrared (NIR) imaging has emerged as a highly promising modality due to its reduced tissue scattering and absorption, along with alleviated interference from autofluorescence in the NIR spectral region. However, conventional NIR dyes, such as cyanine and aza-BODIPY-based dyes, suffer from inherent limitations, including poor water solubility and low photostability. To overcome these challenges, NIR-emissive phospha-rhodamine dyes (PORs), in which an endocyclic oxygen atom of the rhodamine fluorophore is replaced with an electron-withdrawing phosphoryl group, have been developed.[1] We conceived that the substitution of benzene ring in PORs with an electron-donating thiophene ring would result in increasing in the HOMO energy level, leading to a bathochromic shift in the absorption and emission spectra, thereby enhancing deep tissue imaging capabilities.

In this study, we synthesized a series of unsymmetric thieno-phospha-rhodamine (**TPR**) dyes with varied amino substituents, such as pyrrolidyl (**TPR-py**), diethylamino (**TPR-Et**), and dimethylamino (**TPR-Me**) groups, on the thiophene ring (Figure 1a). These dyes exhibited similar photophysical properties, with absorption and emission maxima around 700 nm and 800 nm, respectively, resulting in large Stokes shift over 1600 cm^{-1} in the NIR region. Cell staining experiments with **TPRs** revealed that the electron-donating character of the amino groups significantly influenced the intracellular localization of the **TPR** dyes. Specifically, **TPR-py** primarily stained mitochondria (Figure 1b), while **TPR-Me** was predominantly localized in lysosomes (Figure 1c).

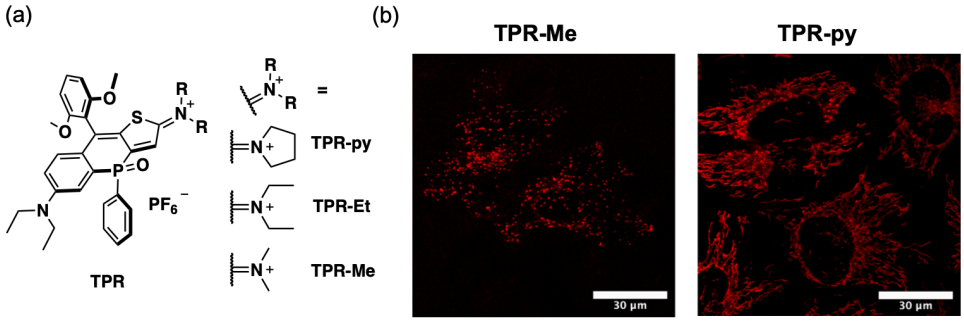

Figure 1. (a) Structure and (b) imaging result of **TPRs**.

1) Grzybowski, M.; Taki, M.; Senda, K.; Sato, Y.; Ariyoshi, T.; Okada, Y.; Kawakami, R.; Imamura, T.; Yamaguchi, *Angew. Chem. Int. Ed.* **2018**, *57*, 10137-10141.