Effect of Molecular Stereoisomerism in Developing Highly Photostable and Fluorogenic NIR Probes for Long-term Imaging

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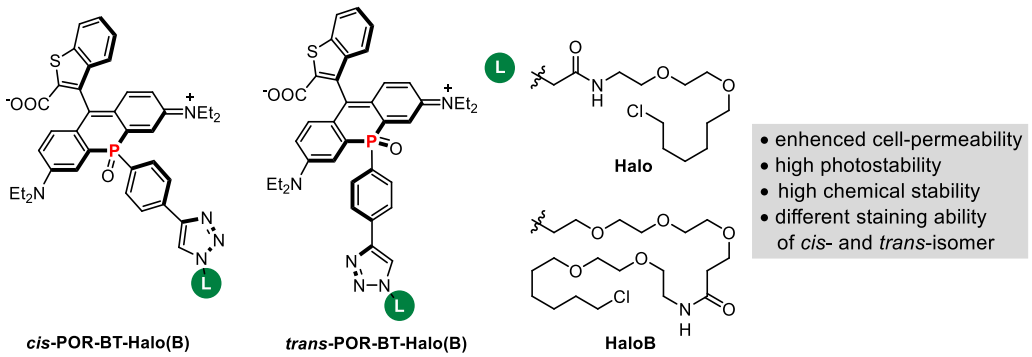
Prominent progress of fluorescence microscopy rising in recent years, such as multi-photon, super-resolution, and single-molecule microscopies, opens a new door to visualize the cellular morphology and dynamics for an extended period of time with high spatiotemporal details. However, these advances put forward higher demands of fluorescent labelling reagents, including high photostability and brightness, good cell-membrane permeability, target specificity, and suitable excitation and emission wavelengths. These demands, it should be noted, are usually difficult to meet at the same time.

Recent studies on rhodamine-based probes have shown their advantages to overcome these obstacles. The dynamic equilibrium between a fluorescent zwitterion and a non-fluorescent spirolactone enables the tunability of cell-membrane permeability. Inspired by our previous works on PREX (photoresistant xanthene) dyes,[1,2] we developed new phospha-rhodamine dyes with improved performance for protein labeling by introducing a benzo-thiophene-2-carboxylic acid at the 9-position of the xanthene motif. The resulting *cis*- and *trans*-isomers, namely *cis*- and *trans*-POR-BT, were successfully separated, and structurally identified by X-ray diffraction. The corresponding dyes with a HaloTag ligand showed different staining ability in living cell imaging. *Cis*-POR-BT-Halo non-specifically stained membrane structures of cellular organelle, such as endoplasmic reticulum, mitochondria, lysosome *etc.*, while the *trans*-isomer selectively labeled to HaloTag-fused proteins, which allowed long-term imaging of cell division and four-color imaging in the vis-NIR region.



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