Long-Term, Single-Molecule Imaging in Live Cells with Photoregulated Fluxional Fluorophores

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Single-molecule localization microscopy (SMLM) imaging provides information about biological samples with spatial resolution exceeding the limit of diffraction. However, the improved resolution comes at the cost of increased phototoxicity, which limits the maximum duration of imaging. A large part of the phototoxicity stems from repeated irradiations necessary for controlled switching fluorophores to maintain the sparse labeling of the sample.[1] To achieve lower phototoxicity SMLM imaging, we synthesized molecules that can be photo-converted from a precursor state to an equilibrium state between a fluorescent and a non-fluorescent species. These so-called photoactivatable fluxional fluorophores offer control of emitter density during imaging with only a few irradiations, while most of the switching of single molecules is done thermally in a chemical equilibrium.[2] Previously reported photoactivatable fluxional fluorophores were limited for use in acidic media only. Here we report a systematic study of a set of probes which lead to achieving photoactivatable fluxional behavior at physiological pH in combined with HaloTag labeling. These developments allowed for the continuous observation of structure of endoplasmic reticulum for more than 20 minutes duration at single-molecule resolution.

[1] M. J. Rust, M. Bates, X. Zhuang, *Nat. Methods* **2006**, *3*, 793–795.
[2] E. A. Halabi, D. Pinotsi, P. Rivera-Fuentes, *Nat. Commun.* **2019**, *10*, 1232.