Sub-millisecond single-molecule fluorescence imaging enabled by intramolecular photostabilization

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Photophysical performances of organic dyes in modern imaging, including single-molecule and super-resolution fluorescence microscopy applications, are compromised by their transient excursions to dark triplet and radical states causing stochastic photo blinking and irreversible photobleaching. To circumvent these problems, we develop and study "self-healing" organic fluorophores, in which the dark triplet states are intramolecularly quenched by triplet state quenchers [1,2]. This intramolecular photostabilization approach dramatically increases fluorophore brightness, signal-to-noise ratio, and photostability, while simultaneously reduces phototoxicity by decreasing the generation of reaction oxygen species. A general photophysical framework is established to improve the performance of individual organic fluorophores across the visible spectrum. The performance enhancements of the fluorophores enable us to achieve robust, submillisecond recordings of protein dynamics using wide-field illumination and camera-based smFRET techniques [3]. These findings extend the potential to image single molecules in vitro and in live-cell applications in the absence of solution additives at physiological oxygen concentrations in the kilohertz regime.

[1] Altman et al., *Nat. Methods* **9,** 68–71 (2011).
[2] Zheng et al., *Chem. Soc. Rev.* **43,** 1044–1056 (2014).
[3] Pati et al., manuscript under review.