

A STROBOSCOPIC APPROACH FOR FAST PHOTOACTIVATION-LOCALIZATION MICROSCOPY WITH DRONPA MUTANTS: S-PALM

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The photophysical properties and photoswitching scheme of the reversible photoswitchable GFP-like fluorescent proteins Dronpa-2 and Dronpa-3 were investigated by means of ensemble and single-molecule fluorescence spectroscopies, and compared to the precursor protein Dronpa [1]. The faster response to light and the faster dark recovery of the new mutants observed in bulk holds at the single-molecule level. Analysis of the single-molecule traces allows us to extract the efficiencies and rate constants of the pathways involved in the forward and backward switching, and we find important differences when compared to Dronpa. We rationalize our results in terms of a higher conformational freedom of the chromophore in the protein environment provided by the β -can. The thorough understanding of the photophysical parameters has allowed us to optimize the acquisition parameters for camera-based subdiffraction-limited imaging. We show that Dronpa and its mutants are useful for fast photoactivation localization microscopy (PALM) using common wide-field microscopy equipment, where individual Dronpa molecules can be localized several times. We provide a new approach to achieve fast photoactivation-localization microscopy by introducing simultaneous two-color stroboscopic illumination (S-PALM) [2]. The generic character of the approach is proven by simultaneous imaging of Dronpa-mutants and the red emitting photo-activatable protein mKikGR [3]. The first biological applications of S-PALM will be reported.

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