PHOTOSWITCHING DYNAMICS OF THE REVERSIBLE PHOTOSWITCHABLE FLUORESCENCE PROTEIN RSEGFP2: CRYSTAL AND SOLUTION

Lucas M. URIARTE,1 Cyril RUCKEBUSCH1 and Michel SLIWA1

1LASIR, CNRS UMR 8516, University of Lille, 59655 Villeneuve d'Ascq, France
lucasmuriarte@gmail.com, https://lasir.univ-lille1.fr

RESOLFT super resolution microscopy is based on the ability of individual fluorophores to reversibly switch between a bright (on) state and a dark (off) state. The resolution in RESOLFT depends among other parameters on the amount of excitation-deexcitation cycles that a fluorophore can perform before photobleaching. Reversibly photoswitchable fluorescent proteins that have been proven to be useful in RESOLFT, such as rsEGFP and rsEGFP2, are characterized by a low switching fatigue compared to for example Dronpa, which was the first reversibly photoswitchable fluorescent protein to be discovered. rsEGFP2 is a negative photoswitcher in which the reversible switch from on to off involves a cis-trans isomerization and protonation change of the chromophore. Recently, the excited state dynamics of the photoswitching mechanism of rsEGFP2 from the off to the on-state was published1. Using time-resolved pump-probe absorption spectroscopy (TA) in solution, the existence of several intermediate states on the picosecond time scale has been shown. Using an X-ray free-electron laser, time-resolved serial femtosecond crystallography (TR-SFX) on the picosecond timescale showed that the hydroxybenzylidene imidazolinone chromophore in the excited state adopts a near-canonical twisted conformation with the two cyclic moieties perpendicular to each other. Formation of this twisted chromophore conformation, halfway between the trans and cis isomers, is accommodated by a shift in the central α-helix and restricted by the close proximity to the V151 side chain. Mutation of the latter into an alanine increases the off-to-on photoswitching quantum yield. The ground state evolution after excitation has not been studied yet and the proton transfer mechanism thus remains unclear. Here we present time-resolved pump-probe UV-visible spectroscopy on the nanosecond to millisecond timescale of rsEGFP2 in the crystalline state and in solution that reveal the deprotonation time of the chromophore in the ground state.

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