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

Lucas M. URIARTE,[1] Cyril RUCKEBUSCH[1] and Michel SLIWA[1]

[1]LASIR, 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 published[1]. 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 sate 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.

In collaboration with: Institut de Biologie Structurale (Adam, Bourgeois, Byrdin, Colletier, Coquelle, Feliks, Field, Fieschi, Guillon, Schirò, Thepaut, Weik, Woodhouse), Max-Planck-Institut für medizinische Forschung (Barends, Doak, Foucar, Hilpert, Kovacsova, Nass, Roome, Schlichting, Shoeman), Departement of Physics Rennes (Cammarata), SLAC National Accelerator Laboratory (Aquila, Boutet, Hunter, Koglin, Liang, Robinson) and Riken Spring-8 Center (Iwata, Joti, Motomura, Owada, Tanaka, Togashi, Tono, Yabashi).

[1] Coquelle N, Sliwa M, Woodhouse J, Schirò G, Adam V, Aquila A, Barends TRM, Boutet S, Byrdin M, Carbajo S, De la Mora E, Doak RB, Feliks M, Fieschi F, Foucar L, Guillon V, Hilpert M, Hunter MS, Jakobs S, Koglin JE, Kovacsova G, Lane TJ, Lévy B, Liang M, Nass K, Ridard J, Robinson JS, Roome CM, Ruckebusch C, Seaberg M, Thepaut M, Cammarata M, Demachy I, Field M, Shoeman RL, Bourgeois D, Colletier J-P, Schlichting I, Weik M (2018) Chromophore twisting in the excited state of a photoswitchable fluorescent protein captured by time-resolved serial femtosecond crystallography. *Nature Chemistry* **10:** 31-37