

FLUORESCENCE CORRELATION SPECTROSCOPY TO PROBE MULTISTATE CIS-TRANS PHOTOISOMERIZATION OF A REVERSIBLY SWITCHABLE FLUORESCENT PROTEIN VARIANT

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Reversibly photoswitchable (i.e. photochromic) fluorescent proteins open the way to a number of advanced bioimaging techniques applicable to living-cell studies such as innovative FRET schemes and nanoscopy [1]. In most reported cases a *cis-trans* photoinduced isomerization of the protein chromophore is the underlying mechanism accounting for the reversible optical switching [2]. We recently developed a new set of very efficient photochromic proteins from *Aequorea Victoria*. These photochromic variants share all the same optical behaviour: illumination at the B band wavelength of the chromophore (anionic band, 480-510 nm) leads to population shift to a new non-fluorescing state (D) absorbing around 400 nm; illumination of D at 400 nm quickly restore the protein fluorescence by repopulating the B state. By means of combined theoretical-Raman studies, some of us were able to attribute the dark state to a neutral *trans* form of the chromophore [3]. Hence, photoswitching involves not only a *cis-trans* isomerisation but also a change in the chromophore protonation. Fluorescence correlation spectroscopy (FCS) is an ideal technique to unveil complex kinetic mechanisms, and it is often applied to fluorescent proteins [4]. Here, we shall present our FCS studies on a new yellow photochromic AFP mutant at different pH and illumination conditions. We shall show that this protein is characterized by two fast (μ s time-scale) photoprocesses solely attributable to *cis-trans* photoisomerization and protonation exchange. Our findings highlight the existence of a third state, possibly *trans* anionic, that becomes populated upon illumination and retains pH dependence. We believe that these studies provide useful data for the engineering of photochromic proteins tailored to specific *in vivo* applications.

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