Poster Presentation

MS037.P09

Trapping a long-lived dark state in photoconvertible fluorescent protein mEos4b.

Elke De Zitter[1], Romain Berardozzi[2], Martin Byrdin[2], Virgile Adam[2], Peter Dedecker[1], Luc Van Meervelt[1], Dominique Bourgeois[2]
[1]*KU Leuven, Department Of Chemistry, Heverlee (leuven), Belgium,* [2]*Institut de Biologie Structurale J-P. Ebel, Groupe DYNAMOP, Grenoble, France*
E-mail: elke.dezitter@kuleuven.be

The dynamic behaviour of fluorescent labels plays a key role in advanced fluorescence imaging techniques. For example, superresolution fluorescence microscopy techniques, which provide access to the visualization of subcellular structures at the nanoscale, are fundamentally dependent on the photophysical properties of the employed labels.

In PhotoActivation Localization Microscopy (PALM), the most popular type of fluorescent proteins labels are green-to-red photoconvertible fluorescent proteins (PCFPs). As photoconversion is irreversible, these markers are in principle especially suited for "quantitative" PALM (qPALM) where molecular copy numbers and stoichiometry at the level of individual protein complexes and structures are investigated (1). In practice however, qPALM suffers from fluorescent protein blinking, a process in which the FP tends to repeatedly enter a reversible dark state. This blinking causes photoconverted (red) molecules to be potentially counted more than once, and may also compete with photoconversion of green molecules. Blinking is thus highly detrimental for accurate quantitative studies. In order to design a PCFP with reduced or even suppressed blinking, the associated mechanisms must first be understood (2,3).

Here, we used kinetic x-ray crystallography to investigate the structural nature of blinking in mEos4b, the latest member of the EosFP-family. With the aid of in crystallo microspectrophotometry to measure changes in absorbance and fluorescence emission upon laser illumination, we trapped both green and red mEos4b crystals in a long-lived dark state. After x-ray diffraction of both irradiated and non-irradiated parts of the crystals, we were able to calculate Fo-Fo difference maps and dark-state extrapolated electron density maps. Using these maps, we were able to determine the structures of the dark states. We show that blinking in both forms is accompanied by substantial conformational rearrangements of the chromophore and of the neighbouring residues Arg66, Ser142, His194 and Ile157. These residues are also found to move in reversibly switchable fluorescent proteins (RSFPs), which exhibit very efficient photochromism through cis-trans isomerization of their chromophore. However, in mEos4b, as the chromophore pocket is not adapted to isomerization, we observe a disturbed isomerization pattern. Altogether, this work reveals for the first time the blinking mechanism of a PCFP at the molecular level.

[1] Greenfield, D. et al.. (2009). PLoS Biol., 7, e1000137.
[2] Annibale, P. et al. (2010). J. Phys. Chem. Lett., 1, 1506–1510.
[3] Durisic, N. et al. (2014). Curr. Opin. Chem. Biol., 20, 22–28.

Keywords: Fluorescent protein blinking, Kinetic x-ray crystallography, in crystallo microspectrophotometry