Photodynamic study of a novel near-iR photomodulatable Fluorescent Protein

Cédric MITTELHEISSER[1], Lucas URIARTE M[1], Daniel STUMPF[2], Nickels JENSEN[2], Martin WEIK[3], Stefan JAKOBS[2], Michel SLIWA[1]

[1] *LASIRE - UMR 8516, Université de Lille, Campus scientifique, Bâtiment C5, 59655 Villeneuve d'Ascq Cedex – France*

[2] *MPI, Max Planck Institute for Biophysical Chemistry, Am Fassberg 11, 37077 Göttingen – Germany*

[3] *Institut de Biologie Structurale, 71 avenue des Martyrs, 38044 Grenoble Cedex – France*

The development of novel photomodulatable fluorescent proteins, including reversibly switchable fluorescent protein (RSFPs) that are working in the red/NIR domain is one of recent challenges in superresolution fluorescence microscopy. We developed a new iR-RSFP engineered on *Deinococcus radiodurans* bacteriophytochrome (Dr-BphP). Bacteriophytochromes are proteins which incorporate the external chromophore biliverdin (BV), ubiquitous in many eukaryotic organisms as a product of heme degradation. The photocycle of this chromophore (bound to bacteriophytochrome proteins) involves the two photostable states P_r and P_{fr} which absorb red and far-red light respectively. Naturally occurring bacteriophytochromes are nevertheless weakly fluorescent in both P_r and P_{fr} forms, and Dr-BphP photo-switching dynamics take place on several order time-scales, ranging from hundreds of femtoseconds to a few milliseconds, involving several excited states and intermediates. The photo-mechanism is the sum of a cis-trans isomerization of the excited chromophore, deprotonation / protonation steps and structural changes in the protein.

The use and optimization of the new photo-switchable fluorescent BphP (iR-RSFP) requires thus understanding of its photo-dynamics. We investigated the multi-scale photo-dynamics of this new iR-RSFP using femtosecond pump-probe transient absorption spectroscopy, fluorescence single photon counting techniques, and nano-millisecond transient absorption spectroscopy. We compare its photo-dynamics with the wild type one and with a mutant which is fluorescent but not photo-switchable. We will then discuss the specific switching dynamics of this new iR-RSFP.

References:

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