SENSITIVITY OF GREEN-STATE PHOTOPHYSICS OF MEOS2 TO INTENSE 561-NM LIGHT PERTURBS EFFICIENT GREEN-TO-RED PHOTOCONVERSION FOR QUANTITATIVE PALM

Daniel Thedié, Romain Berardozzi, Virgile Adam, Dominique Bourgeois,
Institut *de Biologie Structurale, Université de Grenoble Alpes, CEA, CNRS, F-38044 Grenoble, France*
Email: dominique.bourgeois@ibs.fr

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Green-to-red photoconvertible fluorescent proteins (PCFPs) such as the widely used EosFP derivatives play a central role in PhotoActivated Localization Microscopy (PALM). However, the complex photophysics of these genetically encoded markers complicates the extraction of quantitative information from PALM data. While blinking in the red form of PCFPs causes molecular overcounting errors, limited photoconversion efficiency induces undercounting errors. Mechanistic aspects of green-to-red photoconversion have traditionally been investigated under 405-nm light as the sole source of actinic light. However, intense 561-nm illumination is required to localize single molecules in PALM. Here, we show that readout 561-nm light considerably affects the green-state photophysics of mEos2 by populating a long-lived dark state. As a consequence we discovered an indirect green-to-red photoconversion pathway that, via this dark state, competes with direct photoconversion. The dark state is depopulated under violet light illumination, which also induces substantial irreversible bleaching of both the green and red forms of mEos2. Our data thus contributes to explain the ~60% photoconversion efficiency of mEos2 reported earlier (1), and reveals that the photophysics of PCFPs of anthozoan origin is substantially more complex than previously thought.

1. Durisic, N., et al., Single-molecule evaluation of fluorescent protein photoactivation efficiency using an in vivo nanotemplate. *Nature Methods*, 2014, 11, 156.