Influence of temperature on fluorescence and photo-switching quantum yields for photo-switchable fluorescent proteins: the case of rsEGFP2

Xingjie Fu,[1] Lucas M. Uriarte,[1] Olivier Devos,[1] Guillaume Laurent[2], Rémi Métivier[2] Michel Sliwa[1]

[1] *Univ. Lille, CNRS, UMR 8516, 59000 Lille, France*
[2] *PPSM, ENS Paris-Saclay, CNRS, Université Paris-Saclay, 91190 Gif-sur-Yvette, France*
Email: xingjie.fu.etu@univ-lille.fr

Reversibly photo-switchable fluorescent proteins (rsFPs) can switch between a fluorescent (On) and a non-fluorescent (Off) state (Figure 1). The rsEGFP2 is the reference probe used in RESOLFT super-resolved microscopy. The fluorescence and photo-switching quantum yields of the probe are photo-physical parameters that affect the acquisitions time and the image resolution[1]. These parameters are generally characterized at room temperature. On the contrary, in vivo-imaging is done at 37.5 °C. Here we study the influence of temperature on these parameters. The FP On states are composed by several ground state species originated from the complex hydrogen bond network between the chromophore and the protein cage[2]. This species have different fluorescence lifetimes and therefore can be differentiated with time-resolved fluorescence measurements. Here we report the fluorescence lifetime (τ_{fl}) dependence with temperature to understand the ground state equilibrium of rsEGFP2, and correlate it with the fluorescence quantum yield. Interestingly, our measurements show that the fluorescence is coming from mainly two different conformers with different lifetimes. The longer lifetime is about 2.8 ns and responsible for around 90% of the total emission but represent only 40% of the decay amplitude. The shorter lifetime, which represent 50% of the decay amplitude, lasts 0.15 ns and can be considered as "non-emissive". Strikingly, our temperature dependence absorption spectra show no difference, revealing that the equilibrium between the two conformers is in the sub-millisecond range. Both lifetime constants decrease with increasing temperature, and the shorter lifetime had a three times higher temperature-dependence than the longer one. We could correlate the change in fluorescence with the On-to-Off switching yield. Overall, this work provides insights on the ground-state equilibrium between the two On conformers of rsEGFP2.

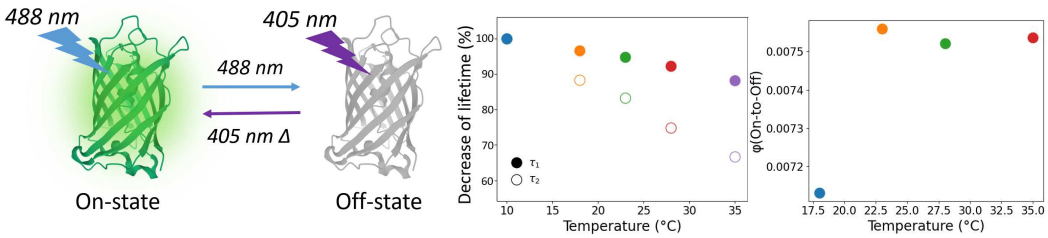

Figure 1. rsEGFP2 photo-switching and temperature-dependence of τ_{fl} and ϕ(On-to-Off)

[1] Hell, S. W.; et al. *Appl Phys A* **2003**, *77* (7), 859–860.
[2] Ghosh, A.; et al, N. *J. Phys. Chem. Lett.* **2017**, *8* (24),