Trans to cis isomerization photo-dynamics in rsEGFP2 starting from OBF or HT conformer

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Reversibly photo-switchable fluorescent proteins (RSFP) find growing applications in cell biology for super-resolution fluorescence microscopies. These proteins can be switched between a fluorescent (*on*) state and a non-fluorescent (*off*) state, which mainly involve a *cis-trans* isomerization around a double bond and a *change in protonation state* of a chromophore within a protein cage. Recent publications have shined light on the photo-switching dynamics of an RSFP used commercially, the rsEGFP2[1,2]. The authors have combined time-resolved pump-probe absorption spectroscopy (TA), where the protein is studied in solution, with time-resolved crystallography (SFX) where protein is studied in the crystalline state. These measurements revealed for the *off* (*trans* neutral) to the *on* (*cis* anionic) switching the existence of a twisted chromophore, halfway between the *trans* and *cis* isomers on the picosecond time-scale[1] followed by a microsecond protein rearrangement and a millisecond deprotonation in the ground state[2]. These measurements allowed us to design two variants that are characterized by two different *off* isomers originating presumably from a hula-twist (HT, volume conserving) and a one-bond-flip (OBF, free volume demanding) *on*-to-*off* switching.

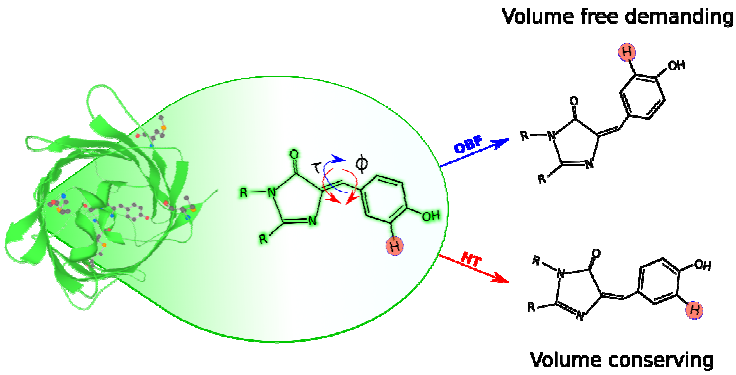

Figure 1. Schematic representation of rsEGFP2 chromophore cis-trans isomerization, via OBF involving a rotation around Φ, or via HT involving a rotation around Φ and τ.

These two variants are perfect tools to clarify the *trans-cis* isomerization dynamics in rsEGFP2. Indeed, details on the mechanism of double bond isomerization within RSFP remain still elusive; in particular the existence (multiple twisted conformers) and nature of excited and ground intermediate states[3]. Using time resolved spectroscopy and crystallography, we characterized the entire *off*-to-*on* photo-dynamics (intermediate states and their lifetime) starting from both *off* conformers. An important result is that independently of the *trans* isomers the isomerization has the same yield, which is determined by a common sub-picosecond step assigned to a HT-type dynamics. These results may help in designing new RSFPs for super-resolution microscopy.

[1] Coquelle N, Sliwa M, et al. *Nature Chemistry* **2018**, 10**:** 31-37, 2018.

[2] Woodhouse J, Nass-Kovacs G., Coquelle N., Uriarte, L. M, et al. *Nature communications* **2020**, *11*, 741.

[3] Gozem S. et al. *Chemical. Reviews.* **2017**, 117, 22, 13502–13565