RED rsFPs FOR SUPER-RESOLUTION MICROSCOPY

Francesca Pennacchietti[1], Konstantin A. Lukyanov[2], Vladislav V. Verkhusha[3], Alexander S. Mishin[2], Ilaria Testa[1]

[1] KTH Royal Institute of Technology/Science for Life Laboratory, Stockholm, Sweden
[2] Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences, Moscow, Russian Federation
[3] Department of Anatomy and Structural Biology and Gruss-Lipper Biophotonics Center, Albert Einstein College of Medicine, Bronx, NY, USA.

E-mail: francesca.pennacchietti@scilifelab.se

Key words: super-resolution fluorescence microscopy, RESOLFT, reversible photoswitchable fluorescent proteins, red FPs.

Reversibly photo-switchable fluorescent proteins (rsFPs) play a key role as tag in optical nanoscopy. Their characteristic state transition between a long-lived dark state (OFF) and a fluorescent state (ON) allows to decrease the intensities required for imaging by several order of magnitude compared to short-lived electronic transitions. As a consequence, techniques as reversible saturable/switchable optical fluorescence transition microscopy (RESOLFT) or saturated depletion nonlinear structured illumination microscopy (NL-SIM), when combined to rsFPs switching, result ideal for non-invasive investigation of live systems at the nanoscale.
Therefore, the switching properties of the rsFPs are the major driving forces in the development of new imaging schemes for faster and gentler RESOLFT and NLSIM nanoscopes. Like in the case of MoNaLISA (Molecular Nanoscale Live Imaging with Sectioning Ability) [1] where a sequence of different and highly modulated light patterns is used to confine simultaneously the emission volume to nanosized spots, which are then recombined in a super-resolved image of 50 μm recorded in less than a second.

Here we present the further development of the nanoscope in the red region of the spectra to image the rsFusionReds, new reversible switchable proteins, which emit at 600-630 nm [2]. Additionally, the rsFusionReds switching photocycle can be elicited with wavelengths above 500 nm, opening new possibility for multi-colours and minimal invasive live cell imaging in light-scattering sample.

[1] Masullo, L. et al. “Enhanced photon collection enables four dimensional fluorescence nanoscopy of living systems”, *Nat. Commun*, 2018.

[2] Pennacchietti et al. “Fast reversibly photoswitching red fluorescent proteins for live-cell RESOLFT nanoscopy” *Nat Methods*, 2018.