Characterization of photoswitchable fluorescent proteins at cryogenic temperatures

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Keywords: cryoPALM, fluorescent proteins, RSFPs, microspectrophotometry

One of the current directions in the rapidly evolving field of single-molecule localization microscopy is to perform imaging at cryogenic temperature. CryoSMLM offers several advantages: native structure of samples preserved by flash-freezing, improved photostability [1] and photon yield of fluorescent markers [2], and potential combination with cryoEM (cryo super-resolution CLEM) [3], [4]. The major challenge of cryoSMLM, however, is the reduced ability of fluorophores to switch below the glass transition temperature [5]. Indeed, switching of organic dyes cannot be induced by switching buffers in the absence of diffusion at low temperature, and while several studies have demonstrated the possibility of using fluorescent proteins (e.g. Dronpa [6], PAmKate [7]) in cryoPALM experiments, their switching rate was much slower than at room temperature, resulting in long acquisition times. A key issue is also the largely incomplete switching of a protein pool to the ON state [5], affecting the effective labeling density and final image resolution. A better understanding of the switching mechanisms of fluorescent proteins at cryo temperatures is therefore necessary for the development of new and improved variants or illumination protocols.
Here, we investigated cryo-photoswitching of the fluorescent protein rsEGFP2 and some of its mutants at 100 K at the ensemble level. Using microspectrophotometry, we analyzed their absorbance and fluorescence emission spectra along switching cycles. By blue shifting the wavelength of the employed activation laser we achieved a significant improvement in the amount of protein that could be back switched to the ON state after complete ON-OFF switching. If confirmed at the single-molecule level, this finding will open the door to an improved cryoSMLM data collection strategy.

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