Manipulating photoblinking in red fluorescent proteins: localization microscopy with ordinary labels and longer tracks in single-particle tracking

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Fluorescent proteins are essential tools in the life sciences, yet are also known for their varied and rich photochemistry, including the presence of short- and long-lived intermittencies ('blinking'). This blinking can be detrimental but also essential for the imaging: in localization microscopy, for example, one desires long-lived blinking to ensure emitter sparseness, while in single-particle tracking these intermittencies limit the length of the tracks that can be required, restricting the available information.
In this talk I will discuss two recent investigations into the structural changes underlying long-lived intermittencies in red fluorescent proteins, and how these can be manipulated to enhance the performance of advanced imaging. In the first investigation, we discovered that the fluorescence of mCherry can be reversibly caged using addition of beta-mercapto ethanol. We investigated the mechanism underlying this caging using ensemble spectroscopy and crystallography, and found that it arises from a direct reduction of the chromophore as well as a reversible Michael addition of beta-mercapto ethanol onto the chromophore. We then showed how this caging could be used to perform localization microscopy in samples labeled with unmodified mCherry [1].
The second study investigated long-lived intermittencies in the red state of a popular photo-activable fluorescent protein, mEos4b. We identified a long-lived dark state in this protein using immobilized single-molecule spectroscopy, measured the absorption and emission spectra of this state, and determined its crystal structure. Based on this structure, we rationalized that weak illumination with 488 nm light would likely revert it back to the emissive state, and indeed found recovery of the red fluorescence. We demonstrated how this can be used to achieve considerably longer and more informative tracks in SPT-PALM. Our blinking suppression strategy should be similarly useful in increasing the accuracy of molecular counting in quantitative SMLM imaging [2].

References

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