Acid-resistant Reversibly Switchable Green Fluorescent Protein for Super-resolution Imaging in Acidic Environments

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Reversibly switchable fluorescent proteins (RSFPs) are crucial tags for super-resolution observation of protein localization and dynamics inside living cells. However, their usage in acidic cellular environments (pH 4.5-6.0) has been limited, due to the high fluorescence pK_a (~5-6). Recently, we have developed a new acid-tolerant monomeric green fluorescent protein, designated Gamillus from Olindias formosa [1]. Here, we show the new photochromic mechanism in Gamillus, in which switching-off by green light irradiation is caused by trans-to-cis isomerization of the chromophore hydroxyphenyl ring that accompanies protonation. Through a combination of rational design and saturation mutagenesis, we developed two variants with enhanced switching contrasts and off-switching speeds, designated rsGamillus-S and rsGamillus-F, respectively. Changes in pH ranging from 4.5 to 7.5 exert almost no effect on the fluorescence intensity (pK_a = 3.6), switching-off speed and on/off switching contrast of rsGamillus. In addition, they exhibit 2-5 times higher on-switching speed than conventional green RSFPs. Exploiting these properties, we succeeded in high-contrast super-resolution imaging of cellular architectures in acidic conditions. Moreover, we found almost no thermal recovery from the off- to on-state in rsGamillus-S, which may make it applicable as a long-term information storage medium with the ability to record, erase or read information.

Reference: