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

Hajime Shinoda, Ryosuke Nakashima, Tetsuichi Wazawa, Kosuke Noguchi, Tomoki Matsuda, and Takeharu Nagai*

‡The Institute of Scientific and Industrial Research, Osaka University, 8-1 Mihogaoka, Ibaraki, Osaka, 567-0047, Japan

***E-mail: ng1@sanken.osaka-u.ac.jp**

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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:

[1] Shinoda, H.; Ma, Y.; Nakashima, R.; Sakurai, K.; Matsuda, T.; Nagai, T., Acid-Tolerant Monomeric GFP from Olindias formosa. *Cell Chem Biol* **2018,** *25* (3), 330-338.e7.