
Acid-tolerant monomeric GFP derived from *Olindias formosa*

Hajime Shinoda^{*1}, Yuanqing Ma^{2,1}, Ryosuke Nakashima³, Keisuke Sakurai³, Tomoki Matsuda^{3,1}, and Takeharu Nagai^{†3,1}

¹Department of Biotechnology, Graduate School of Engineering, Osaka University – Japan

²EMBL Australia Node in Single Molecule Science, School of Medical Sciences, University of New South Wales – Australia

³The Institute of Scientific and Industrial Research, Osaka University – Japan

Abstract

Color pallet of fluorescent protein (FP) has made a great contribution to visualize molecular and cellular processes. However, most FPs lose fluorescence at pH lower than their neutral pK_a (≈ 6), and this has hampered their application in acidic organelles such as endosomes, secretory granules, lysosomes and vacuoles ($pH \approx 4.5-6.0$). To date, several acid-tolerant FPs are available for cyan and red color, however there is little report of acid-tolerant green FPs (GFPs) practically applicable to bioimaging. Here we developed an acid-tolerant monomeric GFP "Gamillus" from jellyfish *Olindias formosa* with excellent brightness, maturation speed and photostability. Results of X-ray crystallography and point mutagenesis suggest that the acid-tolerance is attributed to stabilization of deprotonation on chromophore phenyl ring in broad pH range by forming unique trans configuration. We demonstrated that Gamillus serves as a universal molecular tag, suitable for imaging in acidic organelles through autophagy-mediated molecular tracking to lysosomes. Multicolor imaging in combination of Gamillus with reported color pallet of acid-tolerant FPs is expected to unveil physiological phenomena in acidic environments.

Keywords: fluorescent protein, acid tolerance, pH insensitive, acidic organelles, lysosome, multicolor imaging, flower hat jellyfish

*Speaker

†Corresponding author: ng1@sanken.osaka-u.ac.jp