

# Direct measurement of protein dynamics in single living cells using a rationally designed photo-convertible fluorescent protein

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## 1. INTRODUCTION

Application of fluorescent proteins for cell biological studies is expanding from conventional usage such as fluorescent tag for protein tracking or reporter for gene activity. Recently, several fluorescent proteins including PA-GFP (Photoactivatable-GFP), Kaede and Dronpa, which change in the emission spectra upon UV stimulation, have been reported [1]. We developed a new rationally designed Photo-convertible fluorescent protein, Phamret (**PH**otoactivation-**M**ediated **R**esonance **E**nergy **T**ransfer). Here, we show results of imaging and determination of diffusion coefficients to investigate protein dynamics in living cells using Phamret.

## 2. RESULTS

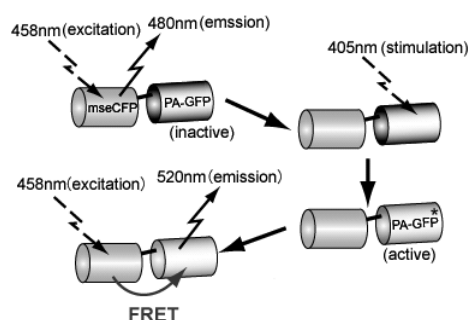


Figure 1: Photo-conversion mechanism of Phamret

Phamret is composed of two fluorescent proteins CFP (Cyan fluorescent protein) and PA-GFP. The photoconversion mechanism of Phamret is based on the photoactivation-mediated FRET from CFP to PA-GFP. After stimulation, PA-GFP is irreversibly activated to function as a FRET acceptor, by which fluorescence of Phamret change from cyan to greenish yellow by excitation at 458 nm.

We demonstrate images of mitochondria fusion and distribution of Histone-H2B during cell division as the successful application of Phamret to visualize the dynamics of proteins in living HeLa cells with laser scanning confocal microscopy.

We also developed a new method to determine diffusion coefficient by means of the analysis of fluorescent decay after photoactivation (FDAP). This method has ability to determine any diffusion kinetics ranging from  $< 0.1 \mu\text{m}^2/\text{sec}$  to approx.  $100 \mu\text{m}^2/\text{sec}$  that is wider than conventional methods FRAP and FCS. We successfully determined the diffusion coefficient of Phamret in solution and living cells.

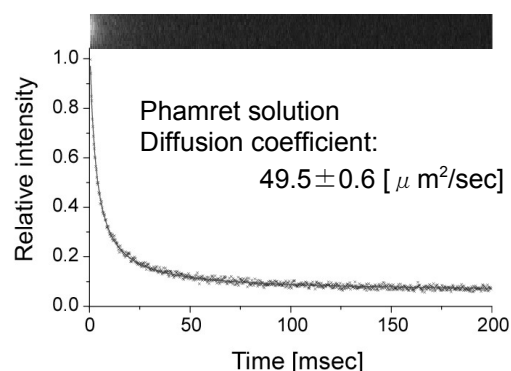


Figure 2: FDAP fluorescent decay curve

## 3. REFERENCE

[1] K.A. Lukyanov, D.M. Chudakov, S. Lukyanov and V.V. Verkhusha, "Innovation: Photoactivatable fluorescent proteins." *Nat Rev Mol Cell Biol.*, **6**, 885-891 (2005).