

## **Live imaging technology for the analysis of biological functions using fluorescent and chemiluminescent proteins**

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The discovery and development of green fluorescent protein (GFP) from the jellyfish *Aequorea victoria* and its color variants have revolutionized our ability to study biological function such as protein localization, dynamics and interactions in living cells. In addition to such a 1<sup>st</sup> generation of fluorescent proteins, a new class of photoactivatable fluorescent proteins (PAFPs) capable of pronounced light-induced spectral changes have been reported in the last few years. PAFP promise specific tissue, cell and protein labeling with high spatio-temporal resolution.

Here, we show a new member of PAFP, Phamret, composed of CFP and PA-GFP. The photo-conversion mechanism of Phamret is based on a photoactivation-mediated FRET (Förster resonance energy transfer from CFP to PA-GFP. Before UV or violet laser stimulation, PA-GFP in Phamret does not work as an acceptor for FRET, then cyan fluorescence is detected by excitation at 458 nm. After stimulation, PA-GFP is irreversibly activated to function as a FRET acceptor; thereby Phamret emits greenish yellow fluorescence by excitation at 458 nm. Fluorescence imaging of HeLa cells expressing Phamret with several targeting sequences showed the clear localization to the correct destination of subcellular organelles such as nucleus, nucleole, Golgi body, mitochondria and peroxisome, and were successfully photo-converted its fluorescent wavelength by 405 nm laser irradiation. We also used Phamret to visualize dynamics of several proteins including histone-H2B during cell division of HeLa cells. When the nuclear halves were photo-converted on the cells expressing histone-H2B-Phamret, these patterns were preserved through cell division. We demonstrate the suitability of Phamret for protein labeling and tracking to quantitatively study dynamics of the protein in mammalian cells.

In addition, development and application of a high performance chemiluminescent protein will be also introduced.