

Photoactivatable 光變式螢光蛋白

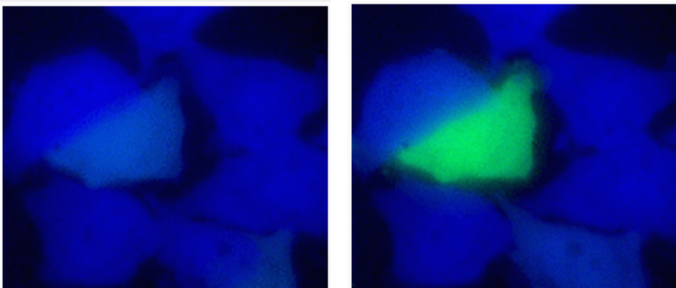
Photoactivatable fluorescent proteins (PAFPs)是一種監測細胞活動的工具。這種光變式螢光蛋白之特性是當外加以特定光源時，其光譜特性會隨之改變。大多應用在特定時間軸中追蹤活細胞、胞器或細胞類分子等研究。近年亦開發出可觀測蛋白質半衰期之應用。

應用一: PAFPs 追蹤細胞、胞器、蛋白質的運動軌跡

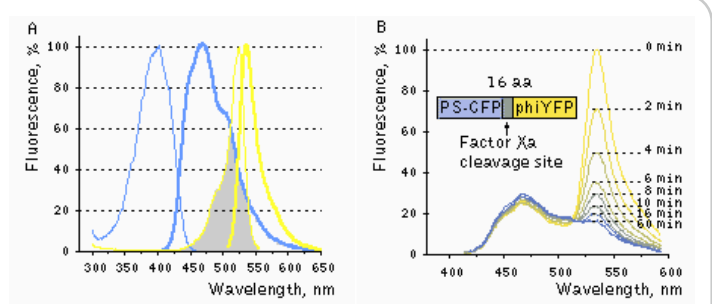
比起 photobleaching 技術 (如 fluorescence recovery after photobleaching (FRAP) or fluorescence loss in photobleaching (FLIP))，PAFPs 可以更精確、直接、無傷害性地監測細胞或蛋白質的運動軌跡。只需將 PAFPs tag 在特定的細胞或胞器或蛋白質，然後照射已特定光束，就可以觀察到目標物的活動變化。

應用二: 蛋白質降解的研究

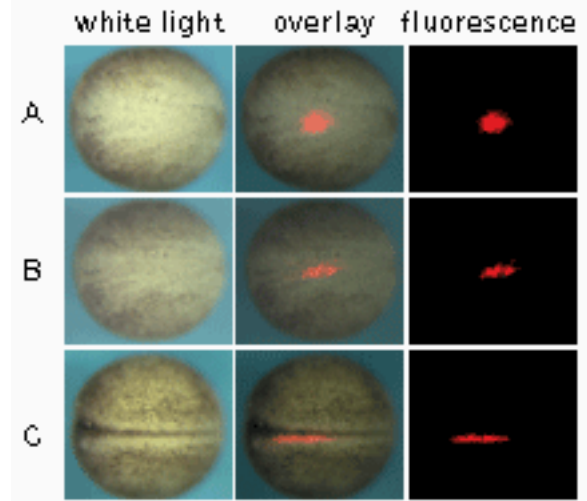
PAFPs allow careful determination of protein half-life. Cells are transfected with a construct coding for target protein fused with a PAFP. A steady-state concentration of the fusion protein and corresponding fluorescent signal depends on protein synthesis and maturation rates as well as protein degradation rate. After photoconversion of the PAFP in a whole cell, a pool of distinct fluorescent molecules appears, which is independent on the synthesis and maturation of the new PAFP molecules. Thus, the decay of the activated fluorescence directly corresponds to the degradation of the PAFP-tagged protein. Time-lapse imaging of the activated signal allows for quantification of degradation process in real-time at the single cell level.



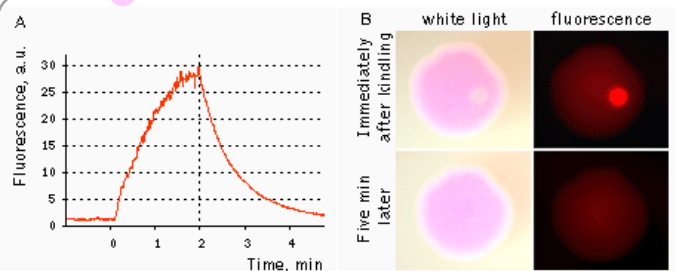
PS-CFP2 photoconversion in transiently transfected mammalian cells
L929 cells were transiently transfected with a plasmid encoding PS-CFP2 and tested under fluorescent microscope. Before photoswitching no detectable green fluorescence at FITC excitation was seen. In contrast, high-level signal was observed in cyan channel. Upon irradiation with 10-15 micro Joules (about 20-30 W/cm²) violet dye laser (404 nm) for a few seconds a fluorescence increase of more than 300-fold was observed in FITC channel.



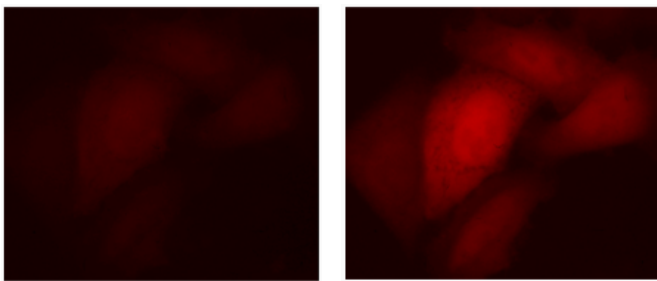
FRET between PS-CFP and PhiYFP.
(A) Excitation (thin lines) and emission (thick lines) spectra of PS-CFP (blue) and phiYFP (yellow) are shown individually. Spectral overlap is filled with gray. (B) Emission spectra of the PS-CFP-Xa-phiYFP fusion are shown before (yellow) and at various time points after commencing digestion with Factor Xa protease (yellow-blue hues of the spectral lines). When excited at 400 nm the uncleaved construct emitted mainly yellow light that gradually dimmed upon cleavage of the linker.



Monitoring of cell migration during Xenopus neural plate development using KFP-Red.
(A) At the early neurula stage, a round-shaped group of cells within the neural plate was irreversibly "kindled"; (B) longitudinal extension of the labeled group of cells after two hours after kindling; (C) thin stripe of the labeled cells at the end of neurulation.
Experimental data were presented by Dr. A. Zaraisky, Institute of Bioorganic Chemistry, RAS (Moscow, Russia).



KFP-Red reversible kindling and relaxation.
(A) Kindling and relaxation kinetics. Zero time is set at the commencement of irradiation with kindling light (532 nm laser light, 1% power). Kindling irradiation was stopped after 2 min. (B) Reversible photoactivation of KFP-Red in E. coli. The round-shaped part of the E. coli colony exp-ressing KFP-Red was irreversibly kindled with intense green light. This region fluoresces brightly, while its absorption is low. After several minutes, the kindled protein relaxed to the non-fluorescent state, while its absorption recovered.

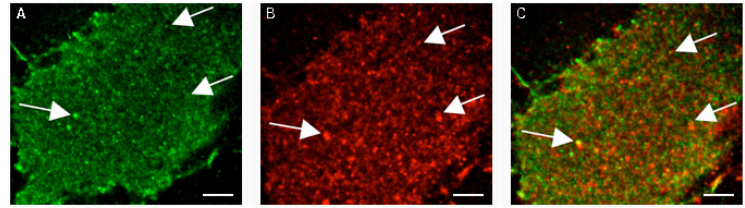


PA-TagRFP use for cell labeling.

Live HeLa cells transiently transfected with the PA-TagRFP-C expression vector were imaged during the photoactivation.

PA-TagRFP use in PALM imaging techniques: high brightness, photostability and absence of initial fluorescence signal from PA-TagRFP make it a protein tag of choice for super resolution two-color PALM/single-particle tracking PALM imaging techniques. The excellent performance of PA-TagRFP in two-color single-particle tracking PALM experiments was demonstrated for several PA-TagRFP-tagged and PAGFP-tagged fusions in live COS-7 cells [Subach et al., 2010].

An example for the tracking of PA-TagRFP-tagged epidermal growth factor receptor (EGFR-PATagRFP) and PAGFP-tagged vesicular stomatitis virus G protein tsO45 (VSVG-PAGFP) in live COS-7 cells by two-color single-particle tracking PALM is shown below.



(A,B) The separate and (C) merged distribution of VSVG-PAGFP (green) and EGFR-PATagRFP (red) in PALM images. Arrows indicate areas of apparent colocalization between the VSVG and EGFR molecules. Scale bars are 2µm.

訂購資訊

PRODUCT	CAT.#	DESCRIPTION	SIZE
pPS-CFP2-C	FP801	Mammalian expression vector encoding humanized PS-CFP2 and allowing its expression and generation of fusions to the PS-CFP2 C-terminus	20 µg
pPS-CFP2-N	FP802	Mammalian expression vector encoding humanized PS-CFP2 and allowing its expression and generation of fusions to the PS-CFP2 N-terminus	20 µg
pKindling-Red-N	FP301	Mammalian expression vector encoding humanized KFP-Red and allowing its expression and generation of fusions to the KFP-Red N-terminus	20 µg
pKindling-Red-B	FP302	Bacterial expression vector; source of the KFP-Red coding sequence	20 µg
pKindling-Red-mito	FP401	Mammalian expression vector encoding humanized KFP-Red targeted to mitochondria	20 µg
pPA-TagRFP-C	FP811	Mammalian expression vector encoding humanized PA-TagRFP and allowing its expression and generation of fusions to the PA-TagRFP C-terminus	20 µg
pPA-TagRFP-N	FP812	Mammalian expression vector encoding humanized PA-TagRFP and allowing its expression and generation of fusions to the PA-TagRFP N-terminus	20 µg
pPA-TagRFP-actin	FP813	Mammalian expression vector encoding humanized PA-TagRFP fused with human cytoplasmic β-actin	20 µg
pPA-TagRFP-tubulin	FP814	Mammalian expression vector encoding humanized PA-TagRFP fused with human α-tubulin	20 µg
pPA-TagRFP-H2B	FP815	Mammalian expression vector encoding humanized PA-TagRFP fused with human histone H2B	20 µg
Vector set			
Mito-tracker	FPM01	Mammalian expression vectors set for fluorescent labeling of mitochondria: pTurboGFP-mito, pPhi-Yellow-mito, and pKindling-Red-mito	

螢光特性	產品-PS-CFP2	產品-KFP-Red	產品-PA-TagRFP
Fluorescence color	cyan / green	NO / red	cyan / green
Excitation max (nm)	400 / 490	580 / 580	- / 562
Emission max (nm)	468 / 511	600 / 600	- / 595
Quantum yield	0.2 / 0.23	<0.001 / 0.07	nd / 0.38
Ext. coeff. (M-1cm-1)	43 000 / 47 000	123 000 / 59 000	nd / 66 000
Brightness*	8.6 / 10.81	0 / 4.1	0 / 25.1
Activating light	UV-violet (e.g. 405 nm)	green (530-560 nm)	UV-violet (e.g. 390-420 nm)
Calculated contrast, fold	up to 2000	35-70	~540
Structure	monomer	tetramer	monomer
Cell toxicity	not observed	not observed	not observed
Aggregation	no	no	no
Maturation rate at 37°C	fast	medium	fast
Molecular weight (kDa)	27	26	27