Cyan-to-green photoswitchable fluorescent protein PS-CFP2

- Monomer, succesful perfomance in fusions
- Irreversible photoconversion from a cyan to a green fluorescent form
- High contrast of photoconversion
- High pH stability allowing labeling of acidic organelles
- Recommended for tracking cell, organelle, and protein movement, monitoring the protein turnover and superresolution imaging

PS-CFP2 is an improved mutant of the photoswitchable monomeric fluorescent protein PS-CFP [D.M. Chudakov et al. 2004]. PS-CFP2 exhibits faster maturation and a brighter fluorescence both before and after photoswitching than its parental variant.

PS-CFP2 is capable of irreversible photoconversion from cyan to green fluorescent form in response to 405 nm light irradiation. It is recommended for real-time *in vivo* tracking movement of individual cells, organelles, and protein fractions [DM Chudakov, S. Lukyanov, and K. Lukyanov 2007]. It can also be applied for monitoring of the protein turnover at the single cell level [Zhang et al. 2007] and superresolution imaging by PALM [Shroff et al. 2007]. In addition, PS-CFP2 can be used as a routine cyan fluorescent tag (excitation maximum at 400 nm and emission maximum at 468 nm) at moderate excitation intensities and as a donor in FRET applications [Souslova and D.M. Chudakov 2006].

Main properties of PS-CFP2

Characteristic	
Fluorescence color	cyan I green
Excitation maximum, nm	400 I 490
Emission maximum, nm	468 I 511
Quantum yield	0.20 I 0.23
Extinction coefficient, $M^{-1}cm^{-1}$	43 000 I 47 000
Brightness*	8.6 I 10.8
pKa	4.3 I 6.1
Activating light	UV-violet (e.g. 405 nm)
Calculated contrast, fold	up to 2000
Structure	monomer
Cell toxicity	not observed
Aggregation	no
Maturation rate at 37°C	fast
Molecular weight, kDa	27
Polypeptide length, aa	238

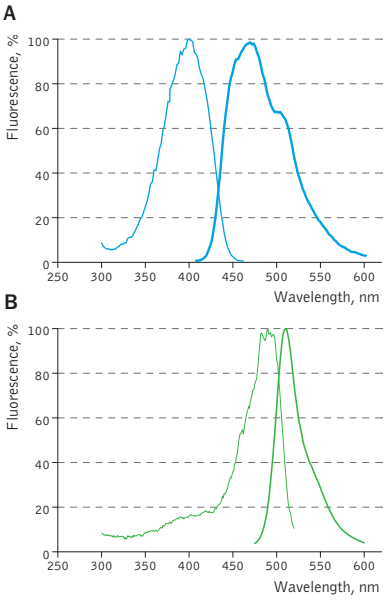
* Brightness is a product of extinction coefficient and quantum yield, divided by 1 000.

Performance and use

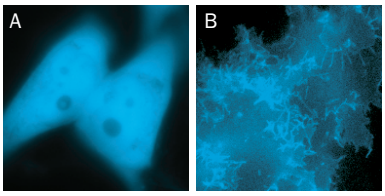
PS-CFP2 can be easily expressed and detected in a wide range of organisms. Mammalian cells transiently transfected with PS-CFP2 expression vectors display an evenly distributed cyan signal in 12-20 hrs after transfection. No cytotoxic effects are observed.

PS-CFP2 successful performance has been proven in many fusions including that with cytoplasmic β-actin, BH3 interacting domain death agonist (BID), nucleolar protein fibrillarin, and dopamine transporter (hDAT).

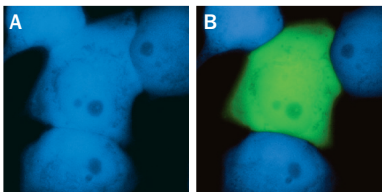
High pH stability: Before photoactivation, PS-CFP2 exhibits a high pH stability with a pKa of 4.3. No changes are observed neither in the shape nor in the amplitude of fluorescence spectra within a pH range of 5.0 and 9.0. This makes it possible to target PS-CFP2 to acidic organelles such as endosomes and lysosomes. After photoswitching, PS-CFP2 has a pKa of 6.1, similar to that of other GFP-like proteins with a phenolate anion chromophore (e.g. EGFP).



PS-CFP2 normalized excitation (thin line) and emission (thick line) spectra.
(A) before activation; (B) after activation. Complete PS-CFP2 spectra in Excel format can be downloaded from the Evrogen Web site at http://www.evrogen.com



PS-CFP2 expression in transiently transfected mammalian cells. (A) Whole-cell expression in HeLa cells; (B) Transiently transfected HEK239 cells expressing PS-CFP2 fusion with human dopamine transporter.



PS-CFP2 photoconversion in transiently transfected mammalian cells.
Central cell expressing PS-CFP2 was irradiated by intense 405 nm light that resulted in PS-CFP2 photoswitching. Before photoswitching no detectable green fluorescence at FITC excitation was seen in cells expressing PS-CFP2. In contrast, high-level signal was observed in cyan channel. Upon irradiation with a 10-15 micro Joules (about 20-30 W/cm^2) violet dye laser (405 nm) for a few seconds a fluorescence increase of more than 300-fold was observed in FITC channel. (A) before photoconversion; (B) after photoconversion.

High contrast of photoconversion: In response to intense 400 nm light irradiation, PS-CFP2 undergoes irreversible photoconversion expressed in a decrease in cyan fluorescence and appearance of a 490 nm excitation peak with emission maximum at 511 nm. After complete photoconversion, green fluorescence of PS-CFP2 increases more than 400 times, whereas the level of cyan fluorescence drops more than 5.5 times lower. The ratio of green fluorescence of the activated and inactivated form is four times higher for PS-CFP2 than for PA-GFP [Patterson and Lippincott-Schwartz 2002]. Unlike that of PA-GFP, emission spectrum of PS-CFP2 changes completely, switching from cyan to green fluorescence. Thus, the increase in the green-to-cyan fluorescence ratio accounts for more than a 2000-fold contrast. Considerable decrease of cyan fluorescence during PS-CFP2 photoconversion provides a molecular tool for simultaneous tracking of both the movement of the photoactivated protein and its replacement with the non-activated form.

Suitability for tracking protein traffic has been demonstrated on example of PS-CFP (the parental variant of PS-CFP2) fused with the human dopamine transporter, hDAT [D.M. Chudakov et al. 2004]. PS-CFP-tagged hDAT was expressed in HEK293 cells. As expected, the fusion protein was localized in the cellular membranes. Then PS-CFP-hDAT was selectively photoswitched in the middle parts of two filopodia by short pulse of 404 nm laser irradiation. High contrast of photoconversion allowed monitoring hDAT movement precisely within thin filopodia in the vicinity of a big non-switched PS-CFP-hDAT pool at the filopodia base. At the same time, a decrease in the cyan fluorescence during photoswitching allowed monitoring non-switched PS-CFP-hDAT molecules entering the activated region.

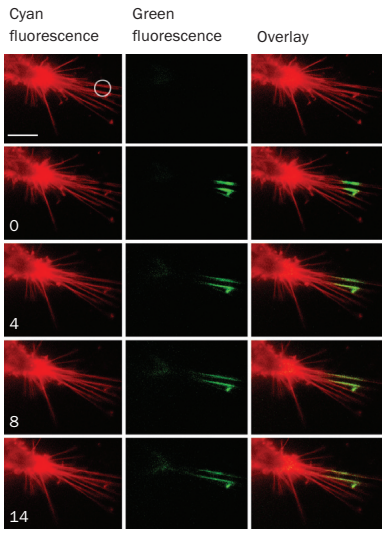
When expressed heterologously, hDAT is capable of endocytosis. To test whether early endosomes are able to exchange cargo proteins such as hDAT, PS-CFP-hDAT fusion was selectively photoswitched in several endosomes. Endosomes (both photolabeled and intact) were monitored within the whole cell for more than an hour after the photoactivation. They exhibited fast and rather chaotic intracellular movement. Two endosomes drew together to form a doublet. One of them contained photoswitched PS-CFP-hDAT and soon after their contact PS-CFP-hDAT mutual exchange between the endosomes was occurred: cyan fluorescence of the activated endosome recovered while green fluorescence of the second endosome grew.

Suitability for FRET applications has been demonstated using a fusion construct comprising fluorescent donor (PS-CFP) and acceptor (PhiYFP) proteins with a linker containing Factor Xa protease cleavage site [Souslova and D.M. Chudakov 2006]. In these fusions energy from the excited donor protein migrates partly to the acceptor due to FRET. Incubation of purified fusion constructs with Factor Xa protease eliminates FRET and leads to a gradual increase in the donor emission peak and a simultaneous decrease in the acceptor emission.

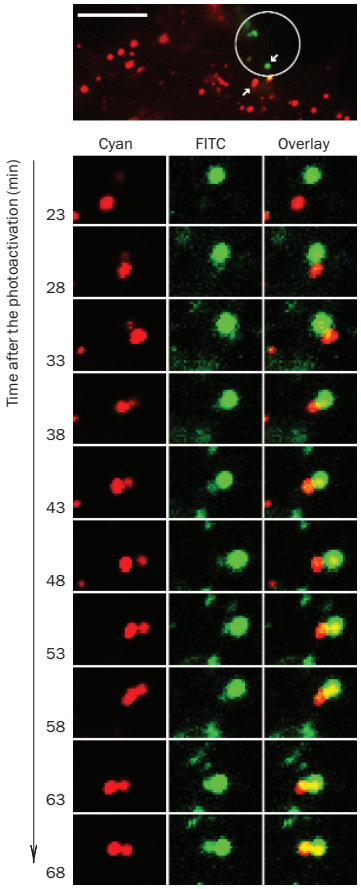
Excitation at 402 nm resulted in a substantial yellow fluorescence emission, showing effective FRET. Digestion of the linker between the two fluorescent proteins separated fluorophores and disrupted FRET. Increase in the donor-to-acceptor emission ratio after their separation reached 6.85-fold, which is better than that for any other reported GFP-like protein pairs.

PS-CFP2 can be used for careful determination of protein half-life as it has been described for Dendra2 in [Zhang et al. 2007]. In the method proposed, cells are transfected with a construct coding for target protein fused with a photoswitchable tag. 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 photoswitchable tag in a whole cell, a pool of distinct fluorescent molecules appears, which is independent of 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 as a label for the dual-color superresolution imaging: Photoactivated localization microscopy (PALM) allows imaging of intracellular proteins at nanometer spatial resolution [**id-ref175**]. Now it is possible to perform dual-color superresolution imaging using PS-CFP2 and second, green-to-red photoswitchable protein (like Dendra2) [Shroff et al. 2007]. It has been demonstrated that cyan-to-green photoactivation of PS-CFP2 works perfectly and, importantly, "higher spatial resolution can be obtained with PS-CFP2 than with Dronpa". Dual-color superresolution imaging using PS-CFP2 can reveal the spatial relationship between two proteins in whole fixed cells down to the nanometric level.



Tracking of PS-CFP-hDAT fusion within filopodia of HEK293 cells. Signals in cyan and FITC channels are shown in red and green pseudocolors, respectively. Circle outlines the photoswitched region. Scale bar, 10 μm. Time after the photoactivation (min) is indicated on the left.



PS-CFP-hDAT interchange between two endosomes. Signals in cyan and FITC channels are shown in red and green pseudocolors, respectively. Circle outlines the photoswitched region. Arrows point to the endosomes tracked. Scale bar, 10 µm. Time after the photoactivation (min) is indicated on the left.

Recommended antibodies, filter sets and laser lines

PS-CFP2 can be recognized using Anti-Tag(CGY)FP antibody (Cat.# AB121) available from Evrogen.

Visualization before photoswitching

Before activation, PS-CFP2 produces cyan fluorescence with excitation and emission maxima

at 400 and 468 nm, respectively. Standard levels of excitation do not cause significant photoswitching of cyan or photobleaching of green fluorescence.

PS-CFP2 excitation spectrum is absolutely different from that of common cyan fluorescent proteins, such as TagCFP, ECFP or Cerulean. Because of the shorter excitation wavelength, cyan fluorescence of PS-CFP2 can be easily separated from green fluorescence of its photoactivated state as well as from any green fluorescent protein. Therefore, common CFP filter sets are not optimal for PS-CFP2 visualization and photoactivation.

Recommended filter sets are

XF119-2*, XF131, XF06, XF03, XF11, XF129-2, XF05-2 (Omega Optical); DAPI-5060B* and DAPI-1160A (Semrock); 31037, 31041, 31016, 31021, 31000v2, 1009v2, 31013v2, 11005v2, 31047 (Chroma Technology Corp.).

* - preferred filter sets

Photoswitching

PS-CFP2 undergoes irreversible photoconversion (in response to intense 405 nm light irradiation) expressed in a decrease in cyan fluorescence and appearance of a 490 nm excitation peak with emission maximum at 511 nm.

Visualization after photoswitching

For visualization of green fluorescence of photoactivated PS-CFP2, filter sets used for routine GFPs visualization are recommended with excitation about 470-500 nm and emission collected at about 500-550 nm (e.g. Omega Optical XF100-2). Importantly, excitation wavelength must not be below 450 nm to avoid cross-excitation of non-photoactivated PS-CFP2.

Note: To avoid undesirable photoactivation of PS-CFP2 and photobleaching of its photoactivated form it is recommended that excitation light intensities and exposition times be minimized during visualization of both protein forms.

PS-CFP2 visualization, photoactivation and tracking in a confocal microscope

The following parameters are recommended for Leica microscope DMIRE2, confocal TCS-SP2, equipped with 25 mW 405 nm diode laser, 125 mW Ar laser, objective HCX-PL-APO-63x/1.40-0.60/OIL (parameters may vary for different experimental systems):

For visualization of PS-CFP2, we recommend the use of 405 nm laser excitation. In a scanning mode, 1-5% power of 405 nm laser causes only negligible PS-CFP2 photoactivation, and can thus be used for preliminary visualization. Fluorescence emission of nonphotoactivated PS-CFP2 can be collected from about 420 nm to about 510-530 nm to gain maximum signal.

For the fast PS-CFP2 photoactivation (which is required to track dynamics of rapidly moving protein of interest), we recommend using 50-100% power of 405 nm laser, 50-2000 ms irradiation in a point, using "point-bleach" mode in Leica confocal software. From our experience, "point-bleach" mode allows much faster photoactivation than any scanning modes, probably because of the continuity of irradiation. Tracking of a protein characterized by slow dynamics does not require fast photoactivation. In this case, high-power scanning of the ROI works perfectly well for PS-CFP2 photoactivation.

For photoactivated PS-CFP2, you can use 488 nm laser excitation and collect fluorescence emission between 500 nm to 550 nm. To avoid crosstalk, we recommend the use of sequential mode if you need to visualize both initial and photoactivated PS-CFP2. Use low intensity excitation light to avoid photobleaching.

Please refer also to the detailed protocol is published in DM Chudakov, S. Lukyanov, and K. Lukyanov 2007.

Available variants and fusions

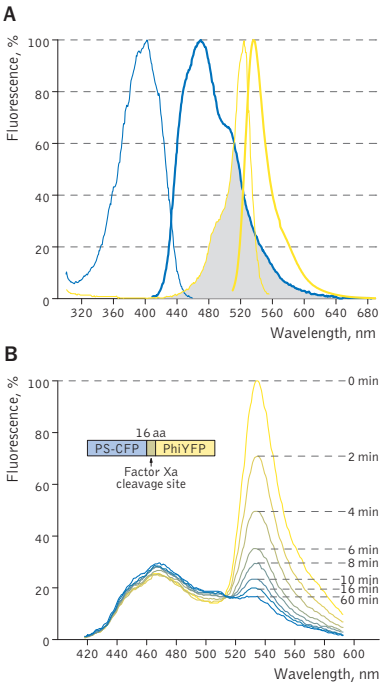
PS-CFP2 mammalian expression vectors contain PS-CFP2 coding sequence with codon usage optimized for high expression in mammalian cells, i.e. humanized [Haas, Park, and Seed 1996]. Humanized PS-CFP2 can also be expressed in *E. coli* and some other heterological systems upon subcloning into appropriate vector.

The available vectors encoding PS-CFP2 are listed below in the section PS-CFP2-related products. For most updated product information, please visit Evrogen website www.evrogen.com.

If you need PS-CFP2 codon variant or fusion construct that is not listed on our website, please contact us at product@evrogen.com.

Licensing opportunities

Evrogen technology embodied in PS-CFP2 is available for expanded and commercial use with an adaptable licensing program. Benefits from flexible and market driven license options are offered for upgrade and novel development of products and applications. For licensing information, please contact Evrogen at license@evrogen.com.



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.

References

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Chudakov, D.M. et al. (2004). Nat Biotechnol, 22 (11): 1435–1439 / pmid: 15502815

Haas, J., E. C. Park, and B. Seed (1996). Curr Biol, 6 (3): 315–324 / pmid: 8805248

Patterson, G.H. and J. Lippincott-Schwartz (2002). Science, 297 (5588): 1873–1877 / pmid: 12228718

Shroff, H. et al. (2007). Proc Natl Acad Sci USA, 104 (51): 20308–20313 / pmid: 18077327

Souslova, E.A. and D.M. Chudakov (2006). Microsc Res Tech, 69 (3): 207–209 / pmid: 16538627

Zhang, L et al. (2007). Biotechniques, 42 (4): 446–450 / pmid: 17489230

PS-CFP2-related products

Product	Cat.#	Description	Size
Antibodies against PS-CFP2			
Anti-Tag(CGY)FP	AB121	Rabbit polyclonal antibody against TagCFP, TagGFP, TagGFP2, TagYFP, PS-CFP2, Case12, HyPer, and EGFP	100 µg

Please contact your local distributor for exact prices and delivery information.