evrogen

# Enhanced red and far-red fluorescent proteins for *in vivo* imaging

GFP and its homologs are widely used as genetically encoded labels in many *in vivo* imaging applications. The fluorescent proteins emitting in the longer wavelength part of the spectrum provide even more opportunities, as they ensure less autofluorescence background, higher fluorescence resonance energy transfer efficiency and much more effective light penetration for deep imaging of animal tissues. Evrogen offers a set of exceptionally bright red and far-red fluorescent proteins optimized for different applications.

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Fluorescent proteins have proven to be indispensable tools for visualizing biological processes. Being genetically encoded, fluorescent proteins do not require additional chemicals to become fluorescent and can be precisely targeted to a specific tissue, cell or cell organelle. They can also be used for tagging proteins inside living cells, allowing *in vivo* studies of protein localization, movement and interactions.

Evrogen's fluorescent protein color palette covers the entire visible range from blue to far-red and consists of two main groups: TagFPs and TurboFPs. TagFPs are monomeric fluorescent proteins optimized for work with fusions. TurboFPs are dimeric fluorescent proteins that are not recommended for tagging proteins, but often perform better in other applications, such as labeling cells and cell organelles, tracking promoter activity, and whole-body imaging. In the long-wavelength part of the spectrum, monomeric TagRFP[1] and mKate2 (ref. 2) and dimeric TurboRFP[1] and TurboFP635 (Katushka[3]) have advantageous properties and excellent performance.

## Main properties

One of the most important properties to consider when choosing a fluorescent protein for most applications is the brightness. **Table 1** summarizes the brightness values for Evrogen red and far-red fluorescent proteins in comparison with their closest competitors.

All four Evrogen proteins are characterized by high resistance to low pH, allowing their use for labeling acidic organelles. Far-red TurboFP635 and mKate2 are extremely photostable under both wide-field and confocal illumination, which makes them ideally suited for long-term time-lapse imaging. The absence of cytotoxicity caused by overexpression of Evrogen's red and far-red fluorescent proteins has been confirmed in long-term experiments, including the generation of stable cell lines and transgenic animals.

All tags can be visualized using generally available light sources (either mercury arc lamp or appropriate laser lines) and most common filter sets.

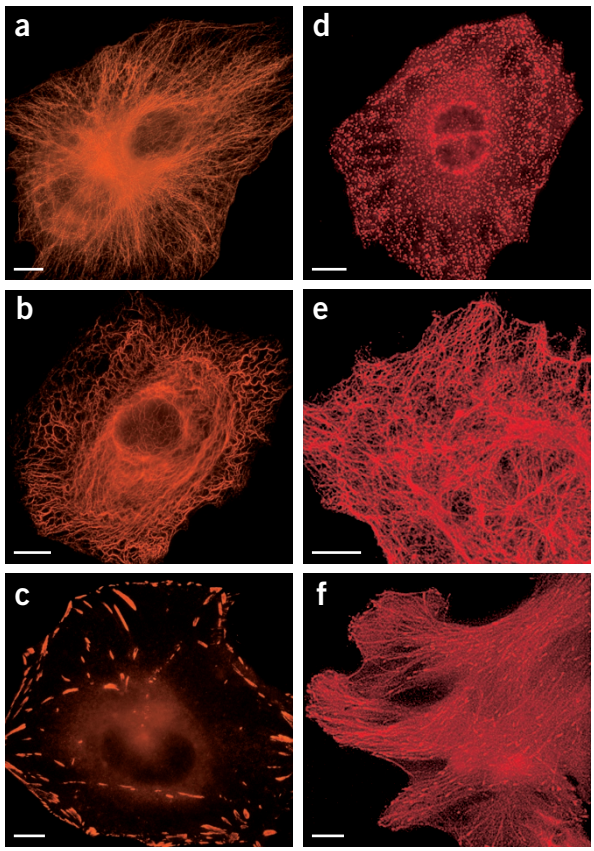


**Figure 1** | Fluorescence imaging of TagRFP and mKate2 fusions. (**a**–**c**) HeLa cells expressing fusions of TagRFP with α-tubulin (**a**), cytokeratin-18 (**b**) and vinculin (**c**). Images were kindly provided by Michael W. Davidson (Florida State University). (**d**–**f**) HeLa cells expressing mKate2 fusions with clathrin light chain (**d**) and vimentin (**e**), and FoLu cells transiently transfected with mKate2 fusion with microtubule-associated protein EB3 (**f**). Images modified from ref. 2. Scale bars, 10 μm.

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# APPLICATION NOTES

**Table 1** | Comparison of Evrogen red and far-red fluorescent proteins with closest analogs

| Color | Protein | Oligomerization | Excitation maximum (nm) | Emission maximum (nm) | Calculated brightness[a] | Brightness beyond 650 nm (% of mKate2)[b] |
|---|---|---|---|---|---|---|
| Red | TurboRFP | Dimer | 553 | 574 | 62 | 40 |
| | Product A | Tetramer | 563 | 582 | 24 (36) | 36 |
| | Product B | Tetramer | 557 | 579 | 17 | 22 |
| | TagRFP | Monomer | 555 | 584 | 48 | 32 |
| | Product C | Monomer | 556 | 586 | 3 | No data |
| | Product D | Monomer | 587 | 610 | 16 | 46 |
| Far-red | TurboFP635 | Dimer | 588 | 635 | 22 | 94 |
| | Product E | Tetramer | 594 | 649 | 4 | 19 |
| | mKate2 | Monomer | 588 | 633 | 25 | 100 |
| | Product F | Monomer | 598 | 625 | 13 | 50 |
| | Product G | Monomer | 590 | 649 | 4 (2) | 10 |

[a]Calculated brightness is a product of molar extinction coefficient and fluorescence quantum yield. The data for competitor products are from the manufacturer's website. Our data are given in parentheses when the difference is considerable. [b]Brightness beyond 650 nm is calculated as definite integrals over wavelength limits from 650 to 800 nm.

Among Evrogen fluorescent proteins, only TagRFP has been tested so far in two-photon laser scanning microscopy (TPLSM)[4]. This work showed that TagRFP has higher two-photon cross-section and brightness than all competitor proteins.

## Use for protein labeling

The exceptional brightness of TagRFP and mKate2 enables their use for *in vivo* protein labeling both independently and as additional colors in multicolor applications. We provide examples of fluorescence imaging of TagRFP and mKate2 fusions (**Fig. 1**). Neither tag affects normal localization of the fused protein, even in quite sensitive systems such as α-tubulin. No visible aggregates, nonspecific localization or cytotoxic effects were observed in the tested cell lines.

## FRET applications

Fluorescence resonance energy transfer (FRET) using a donor-acceptor pair of two fluorescent proteins fused to proteins of interest is a powerful technique that allows *in vivo* studies of protein interactions in living cells. The traditional cyan and yellow FRET partners have several substantial drawbacks limiting their utility for such application, such as relatively low dynamic range (donor/acceptor emission ratio change) and difficulties with spectral separation. Using TagRFP as an acceptor for the Evrogen green fluorescent protein TagGFP2 ensures higher FRET efficiency and more reliable spectral separation of the donor and acceptor fluorescence. Shifting the wavelengths toward the red part of the spectrum reduces input of cellular autofluorescence. The excellent performance of TagRFP in FRET applications had been demonstrated[5] both *in vitro* and *in vivo*.

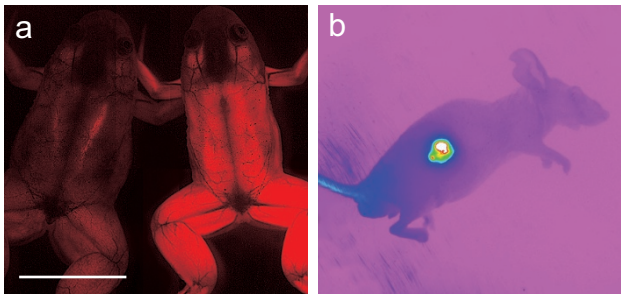


**Figure 2** | Use of TurboFP635 for whole-body imaging. (**a**) Fluorescence image of transgenic *Xenopus laevis* expressing TurboFP635 (frog on right) and product B (frog on left) under the control of cardiac actin promoter. This experiment clearly demonstrates the advantage of the longer-wavelength emission of TurboFP635 for whole-body imaging. Image from ref. 3. Scale bar, 10 mm. (**b**) Fluorescence image showing a melanoma implant expressing TurboFP635 in mouse xenograft model. Image was kindly provided by ChemDiv Inc.

## Whole-body imaging

Deep-tissue imaging using fluorescent proteins allows direct and non-invasive observation of the biological processes inside living organisms. The favorable 'optical window' for the visualization in living tissues is approximately 650–1,100 nm. Within this optical window, TurboFP635 and mKate2 are the brightest fluorescent proteins available so far (**Table 1**). An experimental study[6] shows that the signal coming from TurboFP635 located deep inside tissue is about 45 times stronger than the signal from GFP and 2 times stronger than the signal from the closest far-red competitor protein tested (Product F in **Table 1**). Together with excellent photostability and fast maturation, this makes TurboFP635 and mKate2 the proteins of choice for whole body imaging (**Fig. 2**).

## Conclusions

Using Evrogen red and far-red fluorescent proteins for *in vivo* imaging ensures bright signal and low background. Their excellent performance in protein labeling applications, FRET-based studies and whole-body imaging has been proven experimentally. Additional product information, images and movies are available on the Evrogen website, http://www.evrogen.com/.

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