

**Quantitative measurement of the YFP photoconversion to a CFP-like-species.
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Protein-protein interactions in cells are often studied with the method of Fluorescence resonance energy transfer (FRET). The commonly used FRET pair, CFP and YFP is often used in acceptor photobleaching measurements. After bleaching the acceptor (YFP) the donor (CFP) is unquenched and the resulting increase in donor intensity is measured. Previously it was reported [1] and discussed very controversially [2] that bleaching YFP produces a photoproduct with CFP-like properties. Although the relevance of such a conversion on acceptor photobleaching experiments is quite obvious, until now it has not been measured quantitatively.

We show that the amount of photoconversion is strongly dependent on the absolute concentration of YFP in the cell. Furthermore we measured the spectral properties of the photoproduct indicating that it is most prominently seen detected when using 405 nm as excitation light source.

In addition we quantitatively compare the amount of increase in the CFP detection channel after photoconversion (YFP to CFP-like species) in YFP expressing cells and after acceptor photobleaching in cells expressing a CFP-YFP FRET construct. Thereby it is possible to correct the obtained FRET signal for the contribution of the photoconversion. Using quantitative imaging condition provides a tool to increase the accuracy of CFP/YFP FRET measurements using the method of acceptor photobleaching.

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