

Applications for FRET

Application Note

Overview

FRET (Förster Energy Transfer or Fluorescence Energy Transfer) designates a mechanism describing a non-radiative energy transfer from one chromophore (fluorophore, fluorochrome) to another (Förster 1948). Two conditions are crucial for FRET to occur. Firstly, the chromophores have to be a very short distance from each other (few nm), and secondly, the emission spectrum of the excited chromophore (donor) must significantly overlap with the absorption spectrum of the second chromophore (acceptor). In the following experiment we chose the widespread FRET chromophore pair CFP-YFP (Heim 1996).

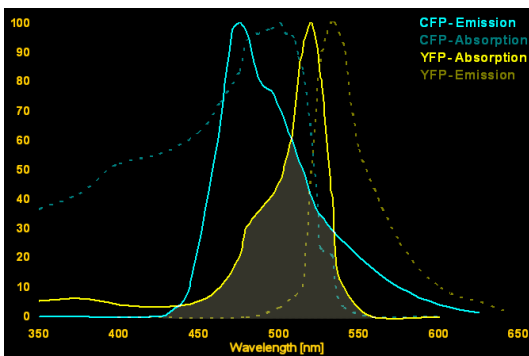


Figure 1
Normalized absorption and emission spectra of CFP and YFP (Clontech, USA). Designated Area shows the overlapping of the emission spectrum of CFP and the absorption spectrum of YFP.

FRET sensor

The guanine nucleotide exchange factor for Ras-like GTPases epac1 is directly activated by cAMP (Roscioni 2008). This epac1 has been modified in a way that CFP and YFP have been directly fused to the cAMP binding domain of epac1 (Nikolaev 2004) providing a reliable fluorescent cAMP indicator. As long as this cAMP indicator has no cAMP bound, the chromophores are very close to each other - FRET occurs. As soon as cAMP binds, the spatial conformation of the molecule changes and the chromophores are separated from each other terminating FRET. With this sensor spatial and temporal aspects of cAMP flux in the cell can be investigated.

Experimental Procedure

To influence the intracellular cAMP level, cells were stimulated with an adenosine-receptor agonist. Activation of adenosine receptors lead to an increase or decrease of the cAMP level in the cells, depending on the types of adenosine receptors present and the stimulus concentration applied, through activation or inhibition of adenylate cyclases (Ralevic 1998).

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HT 1080 cells were seeded on glass bottom dishes (35mm, 15mm inner diameter) and transiently transfected via lipofection (Lipofectamine 2000, Invitrogen, USA) with eCFP-epac1(human)-eYFP (pcDNA3-vector) four hours after seeding. FRET imaging was initiated 18 hours post transfection. Brightfield FRET Fluorescence Microscopy was performed using an iMIC microscope (TILL Photonics, Germany), UPlanApo 10x/0.4 objective (Olympus, Japan), Sencicam QE (PCO, Germany) LLD camera, FRET Filter Set Brightline CFP-YFP (Semrock, USA) and a Polychrome V light source (TILL Photonics, Germany). Images (50) were taken at 1 Hz, after five images (5/50) an adenosinereceptor-agonist was added to the medium.

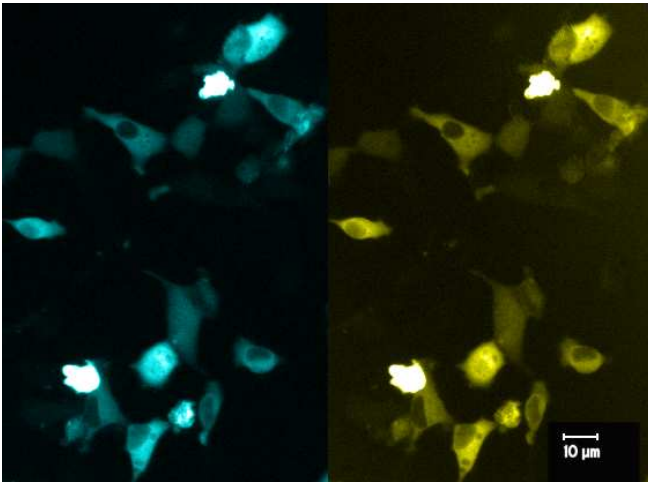
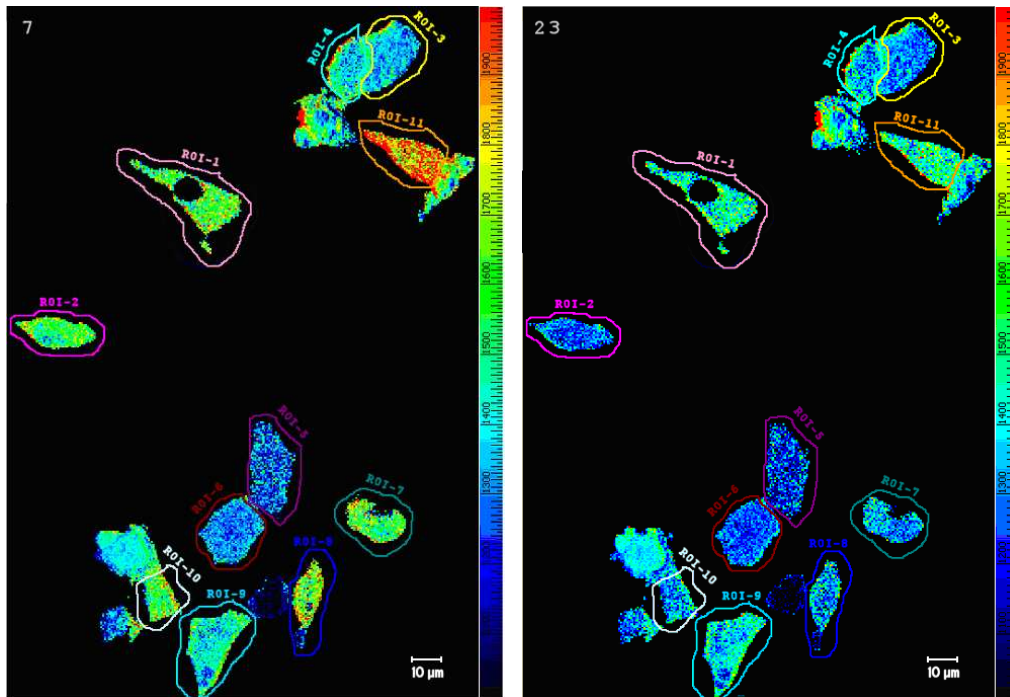


Figure 2
Raw data image (1/50) of CFP channel (left) and YFP channel (right).

Results

A background signal was subtracted in each image of the time-lapse series. Thereafter the YFP-channel was divided by the CFP-channel resulting in a ratio-image-series without applying bleed-through correction. Regions of interests (ROIs) were drawn and for all ROIs the average was calculated for each image of the time lapse series. Figure 5 shows the curves of the normalized values where each line represents the ROI of the same colour (see figures 3 and 4). A few seconds after adding the stimulus to the medium, many cells show a decrease in FRET signal representing the increase of intracellular cAMP. The FRET signal decrease of “non-responding” cells after an application artefact (see figure 6) is likely based on a small response and/or asymmetric bleaching of the two chromophores.

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Figures 3 and 4
False color FRET images (frame 7/50) and (frame 23/50) of eCFP-epac-eYFP transfected HT-1080 cells after stimulus for adenosine receptors was applied. Frame 7 (left) shows FRET before the rise of the cAMP level, whereas in frame 23 (right) a substantial decrease of FRET and therefore an increase in cAMP can be observed.

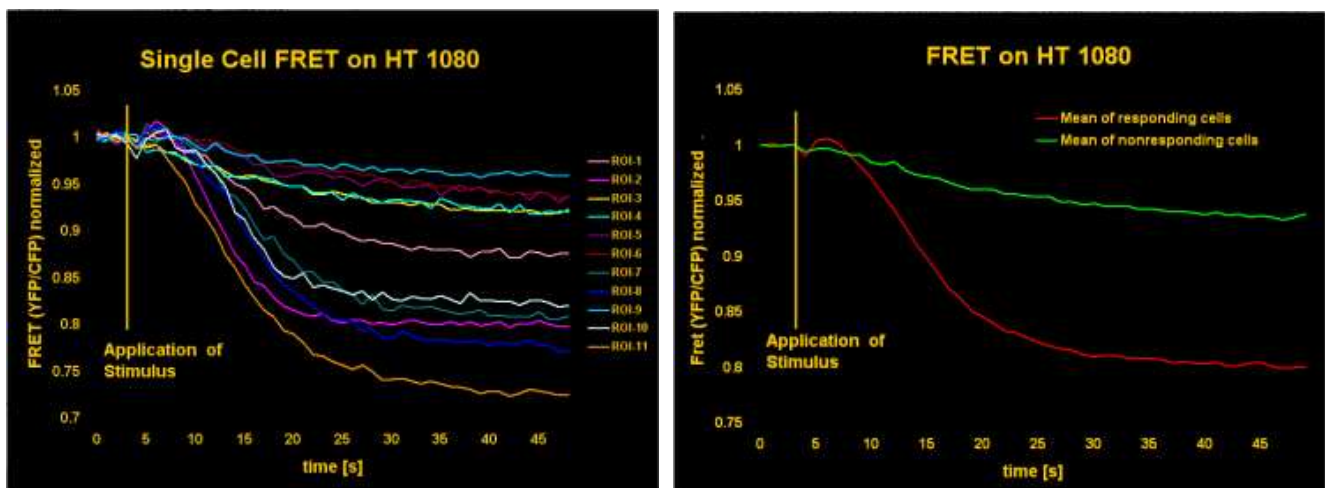


Figure 5 and 6
Normalized FRET kinetics of eCFP-epac-eYFP transfected HT-1080 cells. Cells were stimulated with an adenosine receptor agonist four seconds after the experiment was initiated. Activation of adenosine receptors led to an increase in cAMP level in the cytoplasm that can be observed in a decrease of FRET signal.

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