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FRET-based method for rapid screening of PDE-inhibitors in living cells

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Inhibitors of cAMP- and cGMP-phosphodiesterases (PDEs) are important substances which are in clinical use or under development for the therapy of plethora of diseases, such as erectile dysfunction, heart failure and memory disorders. However, there are very few methods available to screen new substances for their PDE inhibitory activity in intact living cells. Current methods using reporter cell lines are rather indirect and based e.g. on calcium imaging of cyclic nucleotide gated channel activity [1].

To develop a new strategy for PDE-inhibitor screening, we used fluorescence resonance energy transfer (FRET)-based sensors for cAMP and cGMP, which consist of a single cAMP/cGMP-binding domain flanked by a pair of green fluorescent protein mutants [2,3]. To directly monitor the activity of a PDE of interest, we fused these sensors to the N-termini of several PDEs: cAMP sensor *Epac1-camps* was fused to the N-terminus of PDE4A1 or to PDE3A with deleted N-terminal hydrophobic region 1 (amino acids 1–279), cGMP sensor *cGES-DE2* was fused to the N-terminus of PDE5A1. Control experiments with such chimeric proteins demonstrated that the fusion of PDE did not affect the binding properties of the sensors and, conversely, the catalytic activity of the PDEs was not altered by the presence of the sensors on their N-termini.

Fusion proteins transfected into HEK293a cells were uniformly distributed in the cytosol and reacted to the addition of subtype-specific PDE inhibitors or IBMX with a robust decrease (*Epac1-camps*-PDE4A1 and *Epac1-camps*-PDE3A sensors) or increase (*cGES-DE2*-PDE5 sensor) of FRET, which was measured in a 96-well plate using high-throughput iMIC microscope (Till Photonics). The IC_{50}-values for various PDE inhibitors measured in our system correlated well with the published data from *in vitro* assays. *Epac1-camps*-PDE3A sensor was also able to report a cGMP-dependent inhibition of PDE3A-activity.

In addition to well established techniques, our assay allows to perform a rapid and direct screening of PDE inhibitors for various enzyme isoforms in living cells and gives kinetic information about whether inhibitors can enter the cell and how fast they block certain PDE.

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