The detailed characterization of protein-protein interactions is highly important to study the development of diseases, to identify new molecular and pharmacological targets and to detect subtle changes in protein interactions upon drug treatment. To achieve this, Fraunhofer IME has developed a systematically improved, flow cytometry-based Förster’s resonance energy transfer (FRET) assay for molecular verification of specific protein-protein interactions. This for the first time, combines identification and characterization of binding intensity and affinity in a high-throughput screening system in living cells. The improved sensitivity of this assay allows its use for identification of novel therapeutic targets for treatment of human diseases and for the detailed characterization of the binding profile of drugs.

**Field of application**
- Basic signaling pathway research
- Testing novel therapeutics and drugs
- Detailed characterization of the binding profile of drugs
- Molecular and pharmacological target identification

**Readout parameters**
- Flow cytometry-based FRET of protein-protein interactions in living cells
- Determination of the cellular localization by fluorescence microscopy (Fig. 1)
- Verification of mechanistic functionality by reporter gene analyses
- Detection of protein expression by western blot
Vector and cell line generation

- Cloning of expression vector(s), encoding for the gene(s) of interest fused with donor (Clover) and acceptor (mRuby2) FRET fluorophores
- Generation of human and murine cell lines transiently and stably expressing gene(s) of interest

Quality management and validation

Flow cytometry-based FRET measurements are performed using a BD LSRFortessa™ (BD Biosciences, Heidelberg, Germany) flow cytometer equipped with 488 nm and 561 nm lasers. To measure Clover and FRET, cells are excited with the 488 nm laser and fluorescence is detected with a 525/50 nm filter, while the FRET signal is measured with a 610/20 nm filter. The mRuby2 cells are excited with the 561 nm laser, while emission is also detected with a 610/20 nm filter. For each sample, a minimum of 10,000 Clover- and/or mRuby2-positive cells are analyzed. Cells stably expressing Clover/mRuby2 alone or in combination and Clover fused (63bp) mRuby2 are used as positive and negative controls. The binding affinity is measured based on the determination of the FRET intensity via the measurement of the mean relative fluorescence value.

Application example for drug-induced changes in protein-protein interactions analyzed by flow cytometry-based FRET

Fraunhofer IME investigated the effects of agonistic activation of a nuclear hormone receptor (NHR) or its antagonism on its protein-protein interactions with its co-repressor (CoR). For this, Clover-NHR and CoR-mRuby2 were stably expressed in HEK293T cells and treated either with a NHR agonist or a NHR antagonist or both added simultaneously. The measurement of FRET by flow cytometry in living cells resulted in roughly the same number of FRET-positive cells, expressing Clover-NHR and CoR-mRuby2 (Fig. 2), following solvent, agonist, antagonist or combined agonist/antagonist treatment. Additionally, the binding affinity of ligands based on the determination of the FRET intensity via the measurement of the mean fluorescence intensity (MFI) is determined. In relation to this important aspect, the FRET measurements revealed significant differences in FRET intensity and thus, in the binding affinity of the ligands. The total counts were highest in agonist-stimulated cells, followed by antagonist- and solvent-treated cells. The significantly strongest FRET was observed after stimulation with the NHR antagonist, alone or in combination with the NHR agonist (Fig. 3 and 4).

Selected publication


2 Comparison of the total percentages of treated FRET-positive living HEK293T Clover-NHR + CoR-mRuby2 cells © Goethe University Frankfurt, Fraunhofer IME / Verena Trümper, Andreas von Knethen, Tilo Knape.
3 Total FRET (488/610 nm) counts of treated FRET-positive living HEK293T Clover-NHR + CoR-mRuby2 cells © Goethe University Frankfurt, Fraunhofer IME / Verena Trümper, Andreas von Knethen, Tilo Knape.
4 FRET (488/610 nm) intensity of treated living HEK293T Clover-NHR + CoR-mRuby2 cells © Goethe University Frankfurt, Fraunhofer IME / Verena Trümper, Andreas von Knethen, Tilo Knape.