Fluorescence lifetime imaging microscopy (FLIM)-based determination of FRET to visualize receptor signalling in intact cells

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Receptor-initiated signalling events are key elements in regulating cellular behaviour. In particular, protein tyrosine kinase (PTK)-mediated signalling is known to trigger the assembly of protein complexes critical for downstream cellular events such as induction of gene expression, cell growth, cytoskeletal reorganization and complex cellular responses such as cell migration or phagocytosis. Propagation of PTK signalling depends on the recognition of phosphorylated tyrosine (pY) residues via protein-protein interaction domains (e.g. Src homology 2 (SH2) domains), which guide the formation of productive signalling complexes. While biochemical approaches allow the identification of PTK phosphorylation sites and pY-binding proteins, they often disrupt the cellular context. On the other hand, conventional fluorescence microscopy can not resolve direct protein-protein interactions.

Using the phagocyte receptor CEACAM3 as our model system, we have demonstrated that several SH2-domain containing cellular proteins, including the Src family kinase Hck, the adaptor protein Grb14, and phosphatidylinositol-3' kinase (PI3K) act as transient, pY-dependent CEACAM3 binding partners [1-3]. By laser-scanning confocal microscopy and acceptor photobleaching-based FRET measurements of CyPet-labeled CEACAM3 and YPet-labeled SH2-domains we were able to detect active signalling complexes at sites of ligand-induced receptor clustering in fixed cells [1, 2]. To observe receptor signalling in living cells, we have now started to employ CEACAM3-GFP and different SH2-domains coupled to mCherry as donor/acceptor pairs in FLIM-FRET measurements [3]. Time-resolved photon counting and fluorescence lifetime determination enabled the detection of CEACAM3-Grb14 complexes upon ligand binding and allowed the calculation of binding stoichiometry in subcellular regions.

As pY-CEACAM3 is able to associate with several binding partners in a mutually exclusive manner, we will present our strategy to resolve the temporal and/or spatial regulation of CEACAM3-signaling complexes via FLIM-FRET. Importantly, our experimental approach is not restricted to CEACAM3-mediated signalling, but can be broadly applied to visualize and quantify receptor-induced protein-protein interactions in intact cells. Therefore, this kind of quantitative microscopy holds promise to integrate biochemical findings into the cellular and tissue context.

