STUDY OF EGFR ACTIVATION AND DIFFUSION BY IMAGING TOTAL INTERNAL REFLECTION FLUORESCENCE CORRELATION SPECTROSCOPY

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Epidermal growth factor receptor, EGFR, is a tyrosine kinase that plays a fundamental role in a large variety of physiological and pathological processes in cells. As an important centre of signal transductions, its binding with downstream proteins and domains (including the phosphotyrosine binding domain, PTB) initiates various signaling pathways. Quantitative analysis of its diffusive behavior and binding to downstream molecules as well as its organization in the membrane is necessary for the understanding of signal transduction. In the past we have demonstrated that EGFR dimerizes on cell membranes [1] and can bind cytosplasmic phosphotyrosine binding domains [2] upon stimulation with epidermal growth factor (EGF).

Here we use Imaging Total Internal Reflection Fluorescence Correlation Spectroscopy (ITIR-FCS) to observe the diffusive behavior of EGFR and its organization on cell membranes. Using EMCCD or sCMOS cameras, ITIR-FCS can measure simultaneously up to 1,000,000 correlation functions at contiguous points with diffraction limited spatial resolution and a time resolution between 0.5 - 10 milliseconds. This provides very good statistics on the membrane behavior and gives access to information on the spatial organization of membrane components. In particular the FCS diffusion laws [3] can be easily used in ITIR-FCS to investigate the organization of EGFR in domains. The FCS diffusion laws imply that the measurement of the diffusion coefficient at various sizes of the observation area is not constant if the particle under investigation does not undergo free Brownian diffusion. Different observation areas in ITIR-FCS can be easily defined by combining the camera pixels into larger areas by pixel binning. The diffusion coefficient can then be determined for different observation areas in one single measurement. We use this advantage of ITIR-FCS to investigate the organization of EGFR in the membrane before and after activation by EGF and under conditions of cytoskeleton disruption and cholesterol removal to gain insights into EGFR function.

