Visualization and dissection of intracellular signaling events

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Our lab is interested in monitoring and manipulating intracellular signaling pathways. In order to visualize intracellular events, we prepare fluorescent sensors, either genetically encoded or based on small molecule probes prepared in the chemistry lab. In the past, we generated several genetically encoded kinase sensors (PKC, PKA, CaMKII) as well as FRET substrates for phospholipases and proteases. The work shown here focuses on growth factor signaling, namely essential features of epidermal growth factor (EGF) signal transduction such as receptor dephosphorylation and internalization.

We report on a synthetic peptide-based FRET sensor for phosphotyrosine phosphatase 1B, a key enzyme for terminating EGF signaling in cells. In a novel approach, sensor peptide interaction with the donor-labeled enzyme monitors the formation of the enzyme-substrate complex with the help of fluorescent lifetime imaging (FLIM). This provides new insights in EGF receptor signaling. The method demonstrates that it is necessary and possible to measure populations of enzyme activity with temporal and spatial resolution.

Stimulation of cells by EGF generates significant changes in phospholipid composition. Here it is demonstrated that a small group of phospholipids represent a sufficient signal to induce clathrin-coated pit formation and EGF receptor endocytosis, as is monitored by realtime microscopy. By using membrane-permeant derivatives of single phosphatidylinositols, it is demonstrated that altered phosphoinositide levels govern most of the downstream events in tyrosine receptor kinase signaling, thereby manifesting the second messenger function of these lipids. In addition, the selectivity of the endocytosis process for receptor types is shown by multi-channel imaging.

Both projects would not have been possible without the uncompromised interaction of chemists and biologists and the extensive use of microscopy.