VISUALIZING THE FIRST STEPS OF CELL SIGNALLING.
MEMBRANE RECEPTOR CLUSTERING CHARACTERIZED WITH SUPER RESOLUTION MICROSCOPY.

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The epidermal growth factor receptor (EGFR) is a tyrosine kinase receptor whose signalling modulates cell growth, differentiation, survival, adhesion and migration. The deregulation of EGFR signalling leads to tumorigenesis and EGFR is found both overexpressed and mutated in a variety of human cancers.

Lysophosphatidic acid (LPA) is a lipid mediator, motility factor and chemoattractant for many cell types including a range of highly metastatic cancer cells. LPA signals through six specific G protein coupled receptors (GPCRs) which then couple to both distinct and overlapping signalling pathways.

Both EGF and LPA elicit signals at the plasma membrane and are then rapidly removed from the cell surface by endocytosis as a mechanism to terminate the production or activation of second messengers.

Clustering of molecules in the plasma membrane for endocytosis is a well-known process that until now was not easily quantitatively described due to the size of the clusters, close to the diffraction limit of visible light.

Recent developments in the field of optical super-resolution microscopy allow us to localize individual molecules with a precision of 10-20nm and give promise to quantify the number of labeled molecules.

In our study we use the super-resolution technique GSDIM to understand the spatial organization of EGF and LPA receptors at the molecular level in different time points following stimulation. Moreover we are interested in the existence of prearranged higher order structures of receptors (oligomers and clusters) that form specific signaling platforms as well as in the organization of the receptors in the plasma membrane of cells exposed to gradients of chemoattractants and different endogenous ligands.