ligand class A GPCRs and that ''ionic-lock'' formation in ARs is directly correlated with the protonation state of a highly conserved aspartic acid, even though the two sites are located more than 20Å away from each other[5].
Additionally, following the real time evolution of the rhodopsin dimer up to microseconds after photoexcitation, we propose a tandem mechanism for signal transduction where one monomer is responsible for light detection while the other one serves as G-protein coupling site[6]. This interface-mediated pathway suggests oligomerization-aided signal transduction as a crucial biological mechanism to enhance activation of GPCRs.
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[2] Warne et al, Nature, 2008.
[3] Scheerer et al. Nature, 2008.
[4] Vanni et al. Biochemistry, 2009.
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[6] Neri et al. Submitted.

2166-Plat
Dynamics of Gq Protein Interactions with PLCβ3 Studied by TIRF Microscopy
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G protein-mediated activation of phospholipase Cβ (PLCβ) represents a primary mechanism to regulate many physiological events such induce smooth muscle contraction, secretion and modulation of synaptic transmission. Both Gαq- and Gβγ-subunits are known to interact and activate PLCβ enzymes, however little is known about the dynamics of this interactions and the relative contribution of the G protein subunits in intact cells. Using fluorescence resonance energy transfer- (FRET-) based assays in single intact cells we studies kinetics of receptor-induced interactions between Gβγ- and Gαq-subunits, interactions of both Gαq and Gβγ with PLCβ3 as well as interactions of regulator of G proteins signalling 2 (RGS2) with Gαq- and Gβγ-subunits. In order to restrict the protein/protein interaction studies to the cell membrane we applied total internal reflection (TIRF) microscopy. High temporal resolution ratiometric FRET imaging uncovered a markedly faster dissociation of Gαq and PLC upon withdrawal of purinergic agonists compared to the deactivation of Gq proteins in the absence of PLCβ3. This apparent difference in kinetics could be contributed to the GTPase-activating property of PLCβ3 in living cells. Furthermore we found that PLCβ3 modulated Gq protein kinetics to a similar extent compared to RGS2, which in vitro is about 100 fold more efficient in activating Gq-GTPase activity. We observed that both Gαq subunits and Gq-derived Gβγ-subunits interact with PLCβ3 in response to receptor stimulation. In the absence of receptor stimulation we did neither detect any specific FRET signals between Gq protein subunits and PLCβ3 nor did we detect any interactions between RGS2 and Gαq subunits. Finally we could not detect agonist- dependent FRET between RGS2 and Gβγ-subunits. Taken together, ratiometric FRET-imaging under conditions of TIRF allowed new insights into dynamics and interaction patterns within the Gq signalling pathway.

2167-Plat
Role of Plasma Membrane Structuring on Interferon Receptor Assembly & Signaling
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Signaling in cells is mediated through multi-protein complexes, and is triggered by recognition of a chemical molecule, the ligand, to membrane receptors at the extracellular side. Binding in turn leads to activation of intracellular signaling. Understanding of the dynamical behavior of receptors and its nanometric organization in the membrane is fundamental to understand the processes of cell signaling. We are studying type I interferon (IFN) receptor, a member of the cytokine family, which plays a key role in early innate and adaptive immune responses upon infection by pathogens. It is puzzling how different members of the ligand type-1 IFN family elicit differential responses while binding to only one surface receptor. The latter comprises of two proteins ifnar1 and ifnar2, present at very low surface concentration of 100-1000 molecules/cell. Upon ligand binding a ternary complex is formed and downstream signaling pathways activated. Cells quickly and effectively respond, despite the very low amount of receptor present. Using single-molecule wide-field fluorescence microscopy we follow individual receptor in the plasma membrane of living HeLa cells. Receptor subunits were labeled post-translationally with synthetic dyes, or with fluorescent proteins. Each of the receptor subunit was transfected and expressed, separately and simultaneously, allowing measurements on single component as well as on the ternary complex formed upon IFN binding. Through correlative analysis of Ifnar1 and Ifnar2 mobility we visualized association/dissociation events of the ternary complex upon stimulation, and obtained information on receptor's diffusion constants. We observed a switching in between fast and slow motility and vice versa, and a confinement of receptor components in domains. Upon stimulation an increase of cross-correlation between the components was observed as well as a change in mobility. Our findings suggest a role for membrane nanostructure as platform for differential cell signaling.

2168-Plat
Involvement of Transmembrane Helix Dimerization and Rotation in Signaling by the Thrombopoietin Receptor
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In this study, we investigate the role of the transmembrane (TM) domain in the activation of one member of the cytokine receptor family: the thrombopoietin receptor (TpoR). The TM domain is thought to play a key role in the activation by facilitating receptor homodimerization and by transmitting the ligand-induced re-orientation of the extracellular domain to the cytoplasmic domain through conformational changes. However, the precise mechanisms underlying these events are not fully understood. Here, we considered several unanswered questions: Is the homodimerization of TpoR TM domain important in receptor activation? Which TM residues are involved in stabilizing receptor interactions? Additionally, we investigated the role of a constitutively activating mutation (S505N) and the mechanism of action of a piperidine-4-carboxylic acid TpoR agonist (Compound 2). We show that the TM domain of the human TpoR dimerizes strongly and that the full-length receptors exist as homodimers on the surface of mammalian cells in the absence of ligand. Our results indicate that TpoR can adopt two different conformations involving two different sets of residues. One of the contact interfaces mimics an inactive unliganded TpoR dimer and the other corresponds to an active conformation that is compatible with the constitutive signaling induced by the S505N mutation. We also show that Compound 2 interacts specifically with the N-terminus portion of the active dimer conformation. Overall, our results give new insight into the role of the TM domain in the activation mechanism of TpoR and provide a more detailed model of cytokine receptor activation and modulation by small molecule agonists.

2169-Plat
Spatio-Temporal Patterns of Reactive Oxygen Species Production in PDGF Signaling Revealed by Nanoparticle Imaging in Living Cells
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Signaling by PDGF (Platelet Derived Growth Factor) is involved in cell migration, for metastasis formation or reparation of vascular lesions. Hydrogen peroxide (H2O2) is a known second messenger in this pathway and its intracellular concentration regulates the cell response. However, conventional methods are unable to measure quantitatively its temporal evolution. Here, we propose a new approach based on the imaging of YVO4:Eu nanoparticles. Their luminescence is indeed modulated by the oxidation state of doping europium ions. After photoinduced reduction, their chemical oxidation by H2O2 can be monitored by the nanoparticle imaging. We demonstrated in vitro that these particles are efficient probes to dynamically and quantitatively measure H2O2 concentration. By internalizing these nanoparticles in mamallian cells, we measured the oxidant response to a PDGF stimulation. We revealed the temporal pattern of H2O2 production and determined the effective affinity of PDGF receptors for their ligand. We thus proved that a persistent stimulation was necessary to trigger a significant H2O2 production: this intrinsic filtering could be of major physiological interest for understanding reliable cell migration. This response implies transactivation of EGF receptors, which we proved to be dominant at short times. The comparison of normal and tumoral cells revealed a faster and more important H2O2 production in tumoral cells. This likely relies on the different expression levels of proteins of the signaling cascade and points to the potential role of signal transduction dynamics for the regulation of metastasis formation.
This work proposes the first quantitative measurements of the oxidant signaling in cells by the use of new nanoprobes. It more generally opens new perspectives for the study of the spatio-temporal organization of the cell response and its physiological importance.