dimerization of the receptor-ligand complex and then oligomerization. Past research, through predominantly biochemical methods, have concluded that EphA2 signaling depends on the degrees of multimerization of the proteins and the topology of the ligand presentation. However, clustering mechanisms of EphA2 proteins are not well understood because these signaling molecules function in the cell membrane, which is an environment that is difficult to characterize and manipulate. Our hypothesis is that the multi-scale organization of EphA2 in the cell membrane regulates its biochemical function. To mimic the cell-cell junction, we use a supported lipid bilayer - cell membrane hybrid system. Breast cancer cells presenting EphA2 are cultured on a fluid lipid bilayer consisting of ligand fusion proteins, which can stably interact with a subset of capturing lipids within the bilayer. This interaction allows us to control the protein density, precisely image it, and maintain molecular mobility so ligand-induced receptor clustering can occur. Receptor cluster size is varied by changing the cluster size and degrees of oligomerization of its ligand. On the nanometer length scale, antibodies are used to cross link monomeric forms of ligand fusion proteins and thereby vary the degrees of oligomerization. On the micrometer scale, patterned chromium substrates are used to segregate ligands into corrals of variable cluster sizes. Our results suggest that the spatial organization of receptor plays a role in orchestrating the cascade of signaling switches.

2561-Pos

Probing Mechanical Regulation of Receptor Signaling Using a Hybrid Live Cell-Supported Membrane Synapse

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Recent studies have shown that the spatial organization of cell surface receptors can exhibit regulatory control over their associated signal transduction pathways. The corollary that follows from this observation is that mechanical forces acting on ligands can influence receptor spatial organization and subsequent downstream signaling. Juxtacrine signaling configurations, in which receptor and ligand reside in apposed cell membranes, represent an important class of intercellular communication where physical restriction of ligand organization and movement is evident. Here, we reconstitute the juxtacrine signaling geometry using a hybrid synapse formed between a supported membrane displaying laterally mobile ligands that are natively membrane-anchored and live cells expressing cognate receptors for these ligands. Fluid membrane-tethered ligand presentation induces a global receptor reorganization phenotype. This phenotype is linked to the expression of a subset of proteomic and genomic biomarkers, which suggests an association with disease characteristics. Using nanopatterned substrates to impose mechanical barriers to lateral mobility, it is possible to restrict and guide this reorganization event. Mechanical perturbation of receptor transport within the cell membrane alters the cellular response to ligand, as observed by changes in cytoskeleton morphology and protease recruitment. Our results indicate that receptor reorganization may be a mechanism by which cells respond to the mechanical properties of their environment.

2562-Pos

FGFR1 Interaction with Co-Receptor Klotho-Beta at the Plasma Membrane

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FGF21/FGFR1 signalling modulates the survival and glucose sensitivity of fat and liver cells, properties that make this signalling pathway a potential target in the treatment of diabetes. The majority of FGFs interact with heparin proteoglycans in the matrix for presentation to high-affinity receptors such as FGFR1. In contrast, FGF21 exhibits negligible affinity for heparin. To activate FGFR1, FGF21 requires expression of an alternative co-receptor, Klotho-beta (KLB). To study the molecular interaction between FGFR1 and KLB at the cell membrane, we created fluorescent protein-tagged constructs of KLB and FGFR1. By using fluorescence recovery after photobleaching (FRAP), we show that KLB has a lower diffusion coefficient and mobile fraction than FGFR1. Subsequent addition of lactose, an inhibitor of non-specific galactoside binding in the matrix, increased mobility of KLB with no effect on FGFR1. To determine whether the addition of FGF21 induces FGFR1/KLB association, we are presently examining whether FGFR1 mobility slows to KLB levels in the presence of FGF21. We are also measuring homo-Förster Resonance Energy Transfer (homoFRET) on a Total Internal Refection Fluorescence (TIRF) microscope to reconcile these results by examining the oligomeric state of KLB and FGFR1 at the plasma membrane. Overall, these studies will determine whether FGFR1 associates with KLB in the presence of FGF21 revealing important mechanistic information of a novel endocrine factor.

2563-Pos

Caveolin-1 Boosts Clustering of Mu (μ) Opioid Receptors in the Plasma Membrane

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Caveolin-1 (Cav1), is a structural protein component of many mammalian cell plasma membrane and is known to be involved in lipid and protein sorting, receptor desensitization, receptor trafficking, cell migration and many other cellular events. Here we determine if stable expression of Cav1 in cells alters the receptor organization prototype on the membrane. We use two different cell lines for this study: Fisher Rat Thyroid (FRTwt) cells that do not express detectable level of Cav1 and a sister line that is stably transfected with canine Cav1 protein (FRTcav). We express μ opioid receptors (MOR) tagged with either YFP (MOR-YFP) or CFP (MOR-CFP) in cells for different experiments. Förster resonance energy transfer (FRET) measurement between MOR-CFP and Gαi-YFP in FRTwt and FRTcav cells shows receptor sequestration in the presence of Cav1. We find that diffusion of MOR-YFP in plasma membrane of FRTcav cells is slower compared to FRTwt cells by scanning fluorescence correlation spectroscopy (scanning-FCS) experiments. Photon counting histogram (PCH) analyses provide higher average brightness for MOR-YFP in FRTcav cells. Taken together these data provide evidence for caveolin-assisted enhanced clustering of G-protein coupled receptors on the plasma membrane.

2564-Pos

Combinatorial Live Cell Homo- and Hetero-FRET Microscopy of Membrane Proteins

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The structure-function-activity relationships of transmembrane receptors are often mediated not only by ligand-induced signaling but also homo- and hetero-philic binding interactions. Understanding the molecular basis for these interactions is therefore critical for elucidating receptor function. A powerful means of addressing these phenomena is to apply combinatorial microscopies that allow one to probe not only location but also orientation, association, and dynamics. By applying a coupled confocal-total internal reflection fluorescence (TIRF) microscopy imaging scheme, we are examining the distribution, association, and ligand accessibility of two families of transmembrane receptors: carcinoembryonic-antigen-related cell- adhesion molecule 1 (CEACAM1), and fibroblast growth factor receptor 1 (FGFR1), thought to associate with FGF21 co-receptor Klotho-beta (KLB). By using this coupled imaging platform, we can address differences in receptor behaviour, dynamics and structure on the free cell apical surface as well as in the cell itself by confocal microscopy and at the cell-substrate interface by TIRF microscopy. The use of homo- and hetero- Förster Resonance Energy Transfer (FRET) analysis provides us with a powerful means of examining real-time association kinetics of these systems and the effect of soluble ligands on receptor association.

2565-Pos

Studying the NF-κB Signalling Pathway with High-Resolution Fluorescence Microscopy

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Tumour Necrosis factor alpha (TNFα) has long been known to be an important mediator of inflammation, its secretion in cases of lesion or infection a main cellular event. Following activation of TNF receptors 1 (TNFR1) and 2 (TNFR2), the subsequent signal cascade can promote survival (by NF-κB activation) but also cell death (by activation of caspase-8). TNFR1 has been shown to form a trimeric structure in crystallographic studies, which corresponds to that of the native, homotrimeric TNFα. However, the dynamics of TNFR1 upon ligand binding are not yet fully understood.

Here, we use novel techniques from the toolbox of fluorescence spectroscopy and microscopy that enable high temporal and spatial resolution to study the dynamics of TNFα responses in eukaryotic cells. In particular, we use methods