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

that provide subdiffraction optical resolution [3]to study the relation of biomolecular structure and function of TNFa binding to the receptor at the nanometer scale.

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2566-Pos
Computational Modelling of the Drosophila Phototransduction Cascade
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This work presents detailed modelling of the single photon response, the quantum bump, of fly photoreceptors. All known components participating in the primary phototransduction process are taken into account, and estimates have been obtained for the both the physical and the chemical parameters. The result is a detailed analysis of the first, crucial step in fly vision. The same model can be used for multiphoton response, i.e. in the case of higher light intensity stimuli.
The model successfully reproduces the experimental results for the statistical features of quantum bumps (average shape, peak current average value and variance, the latency distribution, etc), arrestin mutant behaviour, low extracellular Ca cases, etc. The TRP channel activity is modelled using the Monod-Wyman-Changeux (MWC) theory for allosteric interaction, which led us to a physical explanation of how Ca/calmodulin regulates channel activity. The model can combine deterministic and stochastic approaches and allows for a detailed noise analysis. The computational model was coded in Matlab using the Parallel Computing Toolbox, which allows computations on multicore computers and computer clusters. An appropriate graphic user interface was developed which gives very convenient and instructive presentation of the parameters used in the modelling and could easily be expanded to other G-protein coupled cascade processes.

2567-Pos
Mathematical Model of Basal and Agonist-Dependent GIRK Channel Activity
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We developed a model of GIRK channel activity. The channel activation scheme was based on sequential non-cooperative binding of 4 Gβγ molecules to channel protein (generating 5 closed states) and Gβγ independent channel opening. The kinetics of Gβγ interaction with subsequent change of channel conformation were adjusted to generate activation time of ~ 1 s for a step rise in Gβγ concentration. The kinetics of switch from closed to open conformation were derived from single-channel analysis of GIRK1/2 recordings in *Xenopus leaves* oocytes. For simulation of agonist-dependent channel activation we incorporated the above scheme into a general model of G-protein cycle. This model was derived from that of Thomsen-Jaquez-Neubig. Several features were added: a) receptor was allowed to couple to G-protein in agonist-bound and in free state; b) finite affinity of Gα to Gβγ was assumed in GTP- and GDP-bound states; c) microscopic reversibility was obeyed in cyclic schemes containing reversible reactions; d) the assumption that G-protein concentration exceeded the receptor concentration was relaxed in order to enable simulation of titration experiments. We simulated the time-course of channel activation induced by step change in agonist concentration in presence and in absence of Gβγ-scavenging protein. We also simulated receptor-titration experiments. The results of simulations were compared to whole-cell experiments in *Xenopus leaves* oocytes. Our model produced realistic time course of channel activation and also demonstrated decremental dependence of activation time on receptor concentration. Comparing the simulation results with those expected from binary shuttle model of channel activation based on considerations of free diffusion of membrane proteins lead to the conclusion that G-protein activation by receptor is probably of catalytic collision-coupling type, while the channel and G protein were either in a tight complex or diffused in a restricted membrane domain.

2568-Pos
Electrophysiology and Live Fluorescence Imaging to Monitor the Effects of Potassium Channel Blockade on Lipopolysaccharide-Induced Immune Signaling
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Studies have shown that ion-channel function in immune cells such as macrophages can influence pathogen-induced immune signaling. Thus, ion channels are viewed by some researchers as potential therapeutic targets for developing novel strategies for regulating immune response on demand when standard anti-pathogen therapies such as antibiotics and vaccinations fall short. However, the direct contribution of ion-channel function to the complex and interconnected signaling pathways in immune response has proved elusive, largely due to the difficulty in tracking multiple signaling nodes in these pathways in real-time. Toward this end, we tracked the real-time inflammatory response to *E. coli* derived lipopolysaccharide (LPS) in a mouse macrophage-like cell-line (RAW 264.7) with electrophysiology to measure potassium channel currents and live imaging with fluorescent fusion reporters of crucial events involved in immune signaling. We developed two reporter constructs: 1) GFP fused to the NFκB transcription factor subunit RelA (GFP-RelA) to track early (<30 min) immune response, and 2) a TNFα promoter driving expression of mCherry with a terminal PEST sequence construct to track later (>2 hours) cytokine induction. In RAW264.7 cells, a 100 nM LPS challenge produces two waves of GFP-RelA translocation from the cytoplasm to the nucleus while gradually increasing the expression of mCherry (TNFα promoter activity). Continuous exposure of LPS-challenged cells to the BK- and Kv-channel blocker tetraethylammonium modifies the translocation dynamics of GFP-RelA and the induction of the TNFα promoter in a dose-dependent manner. Thus we provide evidence in support of a BK- and/or Kv-channel contribution to both early and later LPS induced inflammatory signaling.

2569-Pos
Release of ATP Through Hemichannels Affects Basal Ciliary Activity in the Human Airways
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The frequency of ciliary beat (CBF) is the main factor that determines the effectiveness of mucociliary clearance in the airways. ATP is a known agonist of the CBF, since addition of ATP (10 μM) to the extracellular medium, increases the CBF in different ciliated epithelial cells. There is evidence that epithelial cells constitutively secrete ATP in the airways; however the contribution of extracellular ATP to the control of basal CBF has not been studied. We propose that the airway epithelium release ATP through hemichannels followed by an activation of purinergic receptors, contributes to the control of basal CBF. **Methods**: CBF was recorded using microphotodensitometry technique using primary cultures of human adenoid explants. We also used Western Blot analysis to determinate the expression of $P2Y_2$ purinergic receptor, Pannexin 1 and Connexin 43 hemichannels and used different channel blockers to determinate the contribution of each channel to the control of CBF. **Results**: The spontaneous basal CBF in the cultures was 9.3 ± 0.1 Hz (n=91) and the extracellular ATP concentration was 1.04 ± 0.36 nM in 1.5 mL (n=3). Apyrase (50 U/mL), an extracellular ATP ectonucleotidase, decrease the basal CBF in 19.4% ± 7.0 (n=7). Suramine, a purinergic receptor antagonist, reduce the basal CBF in a 12% and the hemichannels blockers 18β-Glycyrrhetinic acid (50 μM), Carbenoxolone (50 μM) and La^{3+} (100 μM), reduce the basal CBF in a 33.5% ± 4.9, 7.9% ± 1.3 and 21.74% ± 4.3 respectively (n=3). These results provide evidence that affecting the channels or hemichannels associated to the release of ATP or the paracrine/autocrine effects of ATP on the epithelium affects the CBF and suggest that extracellular ATP concentration might contribute to the control of basal CBF in the airways. FONDECYT 1080679.

2570-Pos
Metabotropic Purinergic Receptors in Satellite Glial Cells
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Objective: The dorsal root ganglia (DRG) contains the pseudo-unipolar neurons of sensory input. Neuron somata is enveloped by satellite glial cells (SGC) whose functions is still unknow. To further unveil the sinalization between neurons and glia in DRG we have investigated the expression of purinergic metabotropic receptors (P2Y) by SGC of DRG.