2624-Pos Board B394

Site-Directed Fluorescence Studies of Purified, Functional Cannabinoid Receptor Cb1: Agonist-Induced Conformational Changes in TM6 are Blocked by an Allosteric Modulator and Enhanced by a Novel CB1 Specific Antibody

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The human cannabinoid receptor CB1 is one of the most highly expressed GPCRs in the central nervous system, and a promising target for therapeutic applications. However, structural and biophysical information about this receptor have been limited due to difficulties in purifying the receptor in a functional form and working with its hydrophobic ligands. Here we report on our ability to purify and study a detergent-solubilized functional form of the CB1 receptor. Our site-directed fluorescence labeling studies show specific conformational changes in CB1 in response to different cannabinoid ligands. This work involved attaching a specific fluorescent label to transmembrane helix 6 (TM6) of CB1. We then studied this labeled sample using various fluorescence approaches. Our data shows specific spectral changes of the attached probe upon addition of various cannabinoid ligands, which we can clearly interpret to be due to drug-induced conformational changes in the receptor. We have subsequently used this labeled CB1 mutant to explore the effect of allosteric ligands and conformationally sensitive antibodies on TM6 movements in CB1.

2625-Pos Board B395

Model for the Oligomer Formation of Serotonin Receptors Based on Quantitative lux-FRET Measurements

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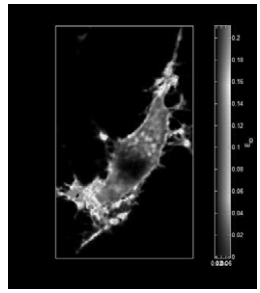
Förster Resonant Energy Transfer (FRET) is often used in experimental methods which aim at investigating the interaction of molecules at distances beyond diffraction limited resolution. Here we apply 'linear unmixing FRET' (lux-FRET) [1]. By using the lux-FRET we can obtain three important quantities, the FRET-efficiency multiplied by the fraction of donors and acceptors in FRET state as well as measures for the total donor and acceptor concentrations. In the present study we applied lux-FRET to analyse the oligomerisation behaviour of two serotonin receptors 5-HT_{1A} and 5-HT_7, which tend to form a dynamic system of homo- and hetero-dimers. From the modelling of measured lux-FRET data we conclude that the receptors have significantly different affinities to form oligomers with the dissociation constant order: $K_{5\text{-}HT1A\text{-}5\text{-}HT1A} > K_{5\text{-}HT7\text{-}5\text{-}HT1A} > K_{5\text{-}HT7\text{-}5\text{-}HT7}$. Quantitative FRET measurements on a custom tailored spinning disk system at single-cell level confirmed these results and also allowed to visualize distribution of mono- and dimers within the cell (Figure 1). Together with observation that the 5-HT_{1A} receptor-mediated signalling is significantly impaired in hetero-oligomers, our data suggest that receptor functions can be modulated by the dynamic interaction within oligomeric complexes.



REFERENCE:

[1] Wlodarczyk et al. Biophysical J, 94:986-1000. (2008)

2626-Pos Board B396

Understanding the Interaction Between Melanopsin and Arrestin using FRET

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A small subset of retinal ganglion cells known as the ipRGCs regulate several non-visual processes including pupillary light reflex, circadian rhythmicity, and sleep. These processes are mediated by the photopigment, melanopsin, expressed in the ipRGCs. Upon illumination, melanopsin initiates a signaling transduction cascade within the cell. This signal induces a depolarization resulting in the firing of action potentials that carry light information to higher order processing centers in the brain. Like most G-protein coupled receptors (GPCRs), melanopsin signaling is attenuated by G-protein coupled receptor kinase (GRK) phosphorylation. This phosphorylation is a cue for arrestin binding which terminates the signal. However, it is unknown if arrestin deactivates melanopsin. In mammals, three isoforms of arrestin are expressed: visual arrestin, βarrestin 1 and βarrestin 2. Studies have shown that βarrestin 1 and 2 are co-expressed with melanopsin, while visual arrestin localizes exclusively in the rods and cones. Therefore, we hypothesize that melanopsin is deactivated by either βarrestin 1 and/or 2. Using Forester Resonance Energy Transfer (FRET) we will determine the degree with which melanopsin and arrestin interact. To date, we have successfully constructed melanopsin-eGFP, βarrestin 1-eRFP, and βarrestin 2-eRFP expression vectors. We are currently attempting to express these constructs in HEK-293 cells and verify their expression and localization by Western blot assay and confocal microscopy.

2627-Pos Board B397

Mapping Interactions Between the Amino-Terminal Region of Secretin and its Receptor using Disulfide-Trapping

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While NMR and crystal structures have defined the molecular basis for docking the carboxyl-terminal region of natural peptide ligands with the disulfide-bonded amino-terminal tail of class B GPCRs, the structural basis for docking the biologically critical amino-terminal regions of these ligands is much less clear. We previously utilized photoaffinity labeling to define spatial approximations between distinct positions within this region of secretin and residues within the core domain of its intact receptor. Now, we use a more powerful disulfide-trapping approach to systematically explore spatial proximities between cysteine residues incorporated into the amino terminus of secretin and into each of the extracellular loops (ECLs) of its receptor. This approach is less disruptive, due to the use of cysteines in place of the large photolabile moieties necessary for photolabeling, and the spatial information is more useful due to the shorter length of the disulfide bonds formed. Cysteines were incorporated into each of the six amino-terminal positions of secretin, with only positions 2 and 5 tolerated to yield reasonable binding affinities and biological activities. These two peptides were used to probe 61 ECL mutants in which cysteine residues were incorporated into the ECLs. The patterns of disulfide formation were quite distinct for the two probes. The position 2 probe predominantly labeled residues in the carboxyl-terminal region of ECL1 and amino-terminal regions of ECL2 and ECL3, while the position 5 probe labeled those within the carboxyl-terminal region of ECL2 and throughout ECL3. These data add substantial new insights for refining our understanding of secretin binding and activation of its receptor.

2628-Pos Board B398

Cell-Derived Vesicles as a Minimal Cell Prototype

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Cellular signaling reactions are classically investigated by measuring optical or electrical properties of individual living cells or suspensions of cells. Here we show how the binding of ligands to cell surface receptors and the subsequent activation of signaling reactions can be monitored in single, sub-micrometer sized native vesicles with single molecule sensitivity. The native vesicles are derived from live mammalian cells either by incubation with chemicals (e.g. cytochalasin) known to destabilize the interaction of the cytoskeleton with the plasma membrane, or by micromanipulation using optical tweezers. Such native vesicles comprise parts of the plasma membrane and the cytosol of the mother cell. They represent the smallest autonomous containers capable of performing cellular signaling reactions thus functioning like minimal cells. To demonstrate this, we measured in individual vesicles derived from individual cells with single molecule resolution the different steps of G protein-coupled receptor mediated signalling like ligand binding to receptors, subsequent G protein activation and finally receptor deactivation by interaction with arrestin. The observation of cellular signalling reactions in individual (sub)micrometer sized vesicles opens the door to downscale the analysis of cellular functions to the atto- and femtoliter range for multiplexing single cell analysis or investigating receptor mediated signalling in multiarray format.

2629-Pos Board B399

Probing the Receptor Dimer Interfaces of G-Protein Coupled Receptor in Model Membranes

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How the components of the G protein-coupled receptor (GPCR) ''signalosome'' assemble and function in the membrane bilayer is not known. Using