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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 CBI Specific Antibody
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The human cannabinoid receptor CBI 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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Forster 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-HT1A and 5-HT7, which tend to form a dynamic system of homodimers 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: $K_{d,5HT1A-5HT1A} > K_{d,5HT1A-5HT7} > K_{d,5HT7-5HT7}$. 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-HT1A 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:

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 phototop诊, 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, $\beta$arrestin 1 and $\beta$arrestin 2. Studies have shown that $\beta$arrestin 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 $\beta$arrestin 1 and/or 2. Using Forrester Resonant Energy Transfer (FRET) we will determine the degree with which melanopsin and arrestin interact. To date, we have successfully constructed melanopsin-GFP, $\beta$arrestin 1-eCFP, and $\beta$arrestin 2-eCFP 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 hydrophilic domain of its intact receptor. Now, we use a 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
Laigino Grasso, Romain Wyss, Joachim Piquet, Michael Werner, Pedro Pascoal, Ghérici Hassaine, Ruud Hovius, Horst Vogel.
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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 signaling 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” assembly and function in the membrane bilayer is not known. Using
a multidisciplinary approach combining computational and experimental work that leverages recent structural data, we present a model for the supramolecular structure of the rod cell disc membrane phototransduction machinery. We first show a comparison of multiple rhodopsin dimer interfaces in a model membrane using a coarse-grained molecular dynamics simulation approach accumulating more than a millisecond of simulation time. To characterize the preferred binding interface of a pair of rhodopsins, we determined the potentials of mean force as a function of the distance between two membrane-embedded receptors. The interfaces probed include helix 4 (H4), H4/H5, H5, H6 and H7. The results show that the H1/H2 dimer is an artifact of tail-to-tail tail conformations, with the interface comprising transmembrane H1 and H2 at the extracellular side and amphipathic H8 at the cytoplasmic one. The existence of the H1/H8 dimer was unambiguously corroborated by crosslinking experiments in which we identified CYS116 in H8 as the site of a chemical crosslink between two rhodopsins in native ROS disc membrane using proteolysis, CNBr cleavage, and high-resolution liquid-chromatography mass-spectroscopy (LC/MS). We then show how secondary interaction surfaces appear to stabilize extended “lubricated” rows of these dimers as seen earlier in atomic force microscopy studies. The synthesis of the new rhodopsin dimer orientation with the structures of the R*Gt complex in two different orientations and 3-D densities of the complex obtained earlier by electron microscopy argues for novel alternatives for the supramolecular organization of ROS membrane dimers. The model we propose suggests the possibility for an efficient one-dimensional mechanism for Gt to search for active receptor (R*) even under low light conditions.

2630-Pos Board B400
Dynamic Assembly of the Receptor-G-Protein Signaling Complex
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The recent crystal structure of the β2 adrenergic receptor-Gs protein complex (β2-AR-Gs) marks an important milestone in a two decades long race to decipher one of the most important and widely utilized signal-transduction mechanisms in biology. However, it is unclear how the relatively drastic and unexpected conformational changes evolve from the initial encounter complex. The number of compositional and conformational microstates that evolve along the reaction pathway from the initial encounter of agonist-activated receptor with the GDP-bound heterotrimeric G-protein to the nucleotide-free agonist-receptor-G protein complex. It illustrates how

2633-Pos Board B402
An Activating Helix Switch at the Rhodopsin-Transducin Interface
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Extracellular signals prompt G protein-coupled receptors (GPCRs) to adopt an active conformation (R*) and to catalyze GDP/GTP exchange in the z-subunit of intracellular G proteins (Gzβγ). Kinetic analysis of transducin (Gzβγ) activation has shown that an intermediary R*Gzβγ-GDP complex is formed which precedes GDP release and formation of the nucleotide-free R* G protein complex. Based on this reaction sequence we explore the dynamic interface between the proteins during formation of these complexes. We start with the R* stabilization by a Gz C-terminal peptide (GzCT) obtained from crystal structures of the GPCR opsins. Molecular modeling allows reconstruction of the fully elongated C-terminal z-helix of Gz (z5) and shows how z5 can be docked to the open binding site of R*. Two modes of interaction are found. One of them - termed stable or S-interaction - matches the position of the GzCT peptide in the crystal structure and reproduces the hydrogen bridge networks between the C-terminal reverse turn of GzCT and conserved E(D)RY and NPxY(x)5,6F regions of the GPCR opsins. The alternative fit - termed intermediary or I-interaction - is distinguished by a tilt and significant clockwise rotation of z5 relative to the S-interaction. It shows different z5 contacts with the NPxY(x)5,6F region and the second cytoplasmic loop of R*. From the two z5 interactions, we derive a ‘helix switch’ mechanism for the transition of R*Gzβγ-GDP to the nucleotide-free R* G protein complex. It illustrates how z5 acts as a transmission rod to propagate the conformational change from the receptor-G protein interface to the nucleotide binding site. The results are discussed in light of the recent structure of the β2 adrenergic receptor-Gs protein complex. A detailed mechanism of complex formation and GDP release is derived.

2633-Pos Board B403
Intermediates of the Channel Rhodopsin Photocycle
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Channelrhodopsins are sensory photoreceptors that mediate phototaxis in green algae (1, 2). Their function as ion channels renders them useful tools in the field of neuroscience. Due to the prolonged lifetime and light-sensitivity of the conducting state, mutants of cyanobium 128 are especially important. They can be switched on and off by light, and are therefore called step-function rhodopsins (3). Recent photocycle models have been proposed on the basis of visual and vibrational spectroscopy (4, 5), however, details of the photoautoreactions are still widely unknown. Here we present spectroscopic data on the slow cycling mutant C128T, with 200 fold extended lifetime of the conducting state. During the photocycle, a fraction of the proteins branches off into a side consisting of blue-shifted species which accumulate during prolonged illumination and play an important role in dark state regeneration (5). We will show that - depending on the illumination conditions - several structurally different dark states are regenerated. However, although such large structural alterations occur during the photocycle, they are not necessarily connected with on- and off-switching of the channel. A new model that connects chromophore isomerization and structural alterations of the protein will be discussed.