caveolae domains. Fluorescence Correlation Spectroscopy studies on live cells show that the presence of caveolae changes the distribution of the apparent diffusion coefficients of B2R and Gαq but not μOR and Gαi. Our results suggest that Gαq can localize its associated receptors to caveolae domains to enhance their signals.

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Probing the Stoichiometry and Geometry of M3 Acetylcholine Receptors at the Plasma Membrane

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G-protein-coupled receptors (GPCRs) are the largest family of transmembrane proteins in nature. GPCRs cascade signals from outer environment into cells and hence, are the target of more than 60% of modern clinical drugs. Determination of oligomerization of GPCRs is subject to significant controversy in the literature. We have investigated the quaternary structure of human muscarinic acetylcholine receptor type 3 (hM3) in living cells using Förster Resonance Energy Transfer (FRET) and two-photon excitation in an optical microspectroscopic set-up [Raicu et al, Nature Photonics, 2009]. The wild-type form of the hM3 receptor was fused to Cerulean while its mutated form, activated solely by a synthetic ligand (RASSL), was fused to Citrine and expressed constitutively in Flp-InTM T-RExTM 293 cells [Alvarez-Curto et al, Journal of Biological Chemistry, 2010]. When WT-hM3-Citrine and RASSL-hM3-Cerulean were co-expressed in same cells, excited donor (Cerulean), transferred its energy to nearby acceptors (Citrine) through FRET. Apparent FRET efficiency (Eapp) distribution maps were obtained for the imaged section of the cells by unmixing acquired spectrally resolved images using elementary donor and acceptor spectra. By selecting pixels in the Eapp image corresponding to the plasma membrane, a FRET efficiency histogram was obtained displaying the number of pixels in a particular range of Eapp values versus the corresponding Eapp value. The Eapp histograms were analyzed using a FRET theory [V. Raicu, Journal of Biological Physics, 2007], predicting various numbers and positions of peaks in the histograms for various sizes and geometry of oligomers. Eapp histograms of all cells were best fitted by a rhombus tetramer model. By also simulating the amplitudes of the peaks, we determined that hM3 receptors form both dimers and rhombus tetramers at the plasma membrane, and that their proportion remained largely unaffected by CNO binding.

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GPCR Activation on the Microsecond Timescale in MD Simulations

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G-protein coupled receptors (GPCRs) play a central role in signal transduction and consequently represent a major drug target. Structural similarities suggest a common working mechanism, which is typically deduced from crystal structures of activation intermediates. The latter have become available recently, in particular for the light sensing GPCR rhodopsin and other members of the rhodopsin-like GPCR family. These crystal structures provide snapshots along the activation pathway, however, the transitions between those remain speculative.

Time resolved spectroscopy is able to provide crucial insights into dynamic details. However, another promising route to study receptor dynamics was opened by the recent occurrence of special purpose computers for molecular dynamics (MD) simulations, which allow to observe conformational transitions related to receptor activation/deactivation directly in unbiased MD simulations on timescales of tens of microseconds.

The activation kinetics for GPCRs, such as rhodopsin, typically feature time constants on the order of milliseconds, which is still beyond reach for direct simulation. However, here we present microsecond simulations for a special case, squid rhodopsin from Todarodes pacificus. While it shares many structural features with its bovine counterpart, activation occurs with a time constant of 12.5 microseconds at room temperature. We analyze simulations of the light activated receptor spanning up to 20 microseconds, allowing us to identify activation events and focus specifically on the interactions between the retinal ligand and the receptor, that trigger the activation process.

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Characterizing the Effect of $A_{2A}R$-D_2R Heteromeric Complex Formation on Homomeric $A_{2A}R$ and D_2R Signaling

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G protein-coupled receptors (GPCRs) are the targets of many drugs used in clinical practice because G proteins mediate a plethora of physiological functions. Appreciation of the existence of oligomeric GPCR complexes with distinct signaling properties from their monomeric counterparts is growing. Yet, the effect of heteromerization on the pharmacology and signaling of many GPCR monomers remains unknown. We have undertaken the task to examine the effect of heteromerization on the G_s signaling through the adenosine 2A receptor ($A_{2A}R$) and G_i signaling through the dopamine receptor type 2 (D_2R). Signaling through the $A_{2A}R$-D_2R heteromeric complex is of great interest as this heteromer is a pharmacological target for pathologies associated with dysfunctional dopaminergic signaling, such as in Parkinson's disease. In order to analyze $A_{2A}R$-D_2R heterocomplex cross signaling through G_i, we are using an electrophysiological assay with heterologously expressed channels serving as reporters for GPCR signaling. Preliminary data suggest that heteromer formation decreases the dopamine elicited G_i signaling through the D_2R. We are in the process of assessing the effect of D_2R on the G_s signaling of the $A_{2A}R$, using a tritiated cAMP assay. We hypothesize that G_s and G_i signaling through the $A_{2A}R$-D_2R heterocomplex are inversely coupled, in a manner similar to the serotonin 2A- metabotropic glutamate type 2 receptor heteromer (Fribourg et al. 2011). Thus, if our hypothesis is correct, heteromerization is expected to increase adenosine elicited G_s signaling through the $A_{2A}R$. Furthermore, we expect that dominant agonists and inverse agonists for the $A_{2A}R$ and D_2R can be used to manipulate the G_i and G_s signaling through the heteromer. Characterization of the signaling pathway through the $A_{2A}R$-D_2R heteromer could lead to novel therapeutics for Parkinson's disease.

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Incorporation of Fluorescently Tagged Chemokine Receptor 5 (CCR5) into Membrane Nanoparticles

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Nanoscale apolipoprotein bound bilayers (NABBs) are soluble phospholipid bilayers encapsulated by the amphipathic helical protein apolipoprotein A-I (Apo A-I). NABBs, unlike detergent micelles and liposomes, provide a homogenous, native-like membrane environment to study structure-function relationships of membrane proteins. NABBs have previously been employed to study G protein-coupled receptors (GPCRs) such as rhodopsin and CCR5, but the approach was limited by poor expression of Apo A-I and low yields of GPCR incorporation. To address these shortcomings, we have engineered several synthetic genes of Apo A-I derived from zebrafish that express at higher levels in E. coli as compared to older Apo A-I variants. The size of the NABBs can be controlled by changing the length of Apo A-I, an additional advantage that will enable study of receptor oligomerization. We intend to use this platform in conjunction with single molecule fluorescence techniques to study GPCR-ligand interactions, focusing on the chemokine receptors CCR5 and CXCR4. The endogenous ligands of these receptors - including RANTES (CCR5) and CXCL12 (CXCR4) - have been shown to prevent HIV particle entry, but the kinetics and structural determinants of binding are poorly characterized. We show receptor labeling with Alexa 488 and 647 using unnatural amino acid mutagenesis and SNAP-tag technologies followed by incorporation into NABBs. The assemblies have been characterized by fluorescence correlation spectroscopy. Future experiments will use multicolor total internal reflection fluorescence (TIRF) microscopy and fluorescence cross-correlation spectroscopy (FCCS) to determine the kinetics of ligand binding and the stoichiometry of the signaling complex. These parameters will shed light on the mechanism of class A GPCR activation and could potentially inform future development of HIV entry blockers.

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Functional Signaling Changes Resulting from GPCR Heteromerization: Relevance to Psychosis

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Functional Signaling Changes Resulting from GPCR Heteromerization: Relevance to Psychosis

A broad spectrum of symptoms has been evidenced in psychotic disorders, while nearly 70 years of research has produced anti-psychotic drugs (APDs) that alleviate only certain ones of these symptoms and may cause the