to the ground state structure we originally used (PDB:1U19). This finding underlines the importance of loop flexibility in membrane protein interactions. Interestingly, when dimers of rho based on this new interface are arranged in rows-of-dimers, Gt placed on rho following the arrangement recently described for Gs on the β2AR correctly embed the lipid anchors of Gtα and Gtγ in the lipid phase. We also discuss the pros and cons of the different models of rho dimers for the visual phototransduction machinery, in the context of the rows-of-dimers arrangement, the functional implications of such supramolecular organization, the binding of Gt to rho and the structural, biochemical and physiological data available and the potential relevance for other GPCRs.

[1] Periole, et al.(2012)JAmChemSoc134,10959-10965.
[2] Knepp, et al.(2012)Biochemistry51,1819-1821.

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Cardioprotection by Cardiac Glycosides is Mediated by Signalosomes Acting on Mitochondrial P38-MAP Kinase to Open Mitokatp

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Preconditioning signals move from sarcolemma to mitochondria in signalosomes that contain the complete cardioprotective signaling cascade. The signalosome-mitochondria interaction causes opening of the mitochondrial ATP-sensitive K^+ channel (mitoK_{ATP}) and inhibition of the mitochondrial permeability transition (Quinlan, et al., Am J Physiol. 295, H953-61, 2008). Cardiac glycosides inhibit the Na,K-ATPase, and they also act as agonists for its non-canonical role in cell signaling. This interaction has been shown to mediate gene transcription and cardioprotection, and it is also required for the inotropic response. The aim of these studies was to determine whether ouabain causes signalosomes to be formed and delivered to mitochondria. Rat hearts perfused with ouabain yielded a signalosome fraction that was caveolar in nature and enriched with caveolin-3, Src, PKCε and the α1- and α2- subunits of the Na^+,K^+-ATPase. Electron microscopy of purified signalosomes revealed 140 nM vesicles decorated with immunogold-labeled caveolin-3. Ouabain signalosomes from heart opened mitoK_{ATP} in mitochondria isolated from untreated hearts. They also opened mitoK_{ATP} in liver mitochondria, suggesting that this is a general mechanism of cell signaling. The ouabain signalosome was found to interact with the mitochondrial outer membrane through the combined action of the terminal kinases Src and PKCε. The functional effects of the ouabain signalosome were blocked by the p38MAPK inhibitor SB203580, and the signalosome caused phosphorylation of an endogenous outer membrane p38MAPK. We infer that p38MAPK transmits the signal to the inner membrane PKCε that phosphorylates mitoK_{ATP} and causes it to open.

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Monte Carlo Study of the Association Rate Between Transducin and Photoactivated Rhodopsin at Disc Membranes

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High sensitivity of the vertebrate visual system requires rapid amplification of single photon absorption events into macroscopic signals at rod cells. The activation of many transducin proteins by a photoactivated rhodopsin (R*) is crucial for such amplification, and the speed of this process is limited by the association rate (k) between transducin and R* which depends on the lateral collisions of these proteins at the disc membranes. There is evidence suggesting that rhodopsin proteins (R) can be found forming crystalline structures made of rows of dimers in the membrane, however, the classic view where R rapidly diffuses laterally has not been discarded. We study how the organization of rhodopsin can influence the association rate between R* and transducin. We perform Monte Carlo simulations of the lateral diffusion and collisions of transducin and rhodopsin proteins in the membrane. Upon collision, a single R* will bind a transducin with a certain probability. We account explicitly for the crowding and confinement effects induced by three organizations of rhodopsin in the membrane: (a) diffusing monomeric R, (b) dispersed static crystals made of rows of R dimers, and (c) and scenario where the crystals are clumped together forming a paracrystalline structure. We observed that k differs between the studied configurations depending on whether the association process is controlled by diffusion or by the binding reaction. More precisely, the diffusive monomeric scheme results in lower k than the other studied configurations of R when the association rate is controlled by the binding reaction, and vice versa when k is controlled by diffusion. We also discuss the effect on the association rate of colocalization of transducin and rhodopsin due to the presence of membrane rafts in disc membranes.

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Mutagenesis Study of Retinal Entry Pathway of Rhodopsin

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The visual photoreceptor rhodopsin binds a photosensitive ligand 11-cis-retinal to respond to light stimulation. The regeneration of rhodopsin with 11-cis-retinal completes the photocycle of visual signaling. The molecular determinants of retinal entry can be assessed by measuring the retinal uptake kinetics of site-directed rhodopsin mutants. Here we report an assay for this purpose based on the energy transfer between 11-cis-retinal and a fluorescent probe covalently attached to the receptor. Previously, we developed an approach for site-specific labeling of G protein-coupled receptors by combining amber codon suppression technology and strain-promoted alkyne-azide cycloaddition reaction[1]. We have demonstrated robust fluorescent labeling of receptors without generating a cysteine-free background. We utilized this approach to label rhodopsin with Alexa488 as the FRET donor. Compared to tryptophan fluorescence spectroscopy, our assay exhibits a greater sensitivity and enables the characterization of mutant with slow retinal uptake kinetics. We showed that the heterologously expressed rhodopsin labeled at the second intracellular loop retains the wild-type retinal uptake kinetics. We further introduced a series of substitutions for the residues located in proximity of the retinal β-ionone binding pocket. We observed three orders of magnitude changes in retinal uptake kinetics. By varying the residue substitution we further studied the effect of hydrogen bonding and sterical hindrance. The insights into retinal entry not only shed light on a physiologically relevant process, but also facilitate understanding of retinal diseases.

[1] H Tian, TP Sakmar, T Huber. Site-specific labeling of genetically encoded azido groups for multicolor, single-molecule fluorescence imaging of GPCRs. Methods in Cell Biology. In press.

1560-Pos Board B290

Quantum Logic Gate Model for G Protein Coupled Receptors

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Presented is an investigation into how G protein coupled receptors (GPCR) derive their function from information encoded in their structure. Embedded in GPCRs, are two potentially coupled aspartate residues that are fundamental to receptors' ligand response properties. To better understand the interaction picture of these residues, a model was developed. In the model, protein geometry was treated as a lattice described by Gaussian network theory. Lattice nodes are defined as the positions of Cα atoms, and the wavefunction describing their pairwise interactions represents a multilevel system, with a logical qubit embedded in the ground state. Increasing the population of the ground state is achieved through a reduction of local lattice vibrational entropy. Since lattice vibrational entropy is modulated by the degree of correlation between nodes, it is therefore dependent upon equilibrium fluctuations of nodes in a given network arrangement. To investigate equilibrium fluctuations in different lattice configurations, molecular dynamics simulations were performed on different GPCR conformations. Results from the simulations demonstrated that the structure of an intermediate agonist bound conformation has reduced vibrational entropy. Further statistical analysis suggests that the intermediate conformation has a high potential to induce proton coherence through delocalization in a water wire. Since NMR experiments support this mechanism, it is proposed that the experimental GPCR free energy surface describes a biological quantum logic gate.

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Preferred Homo- and Hetero-Dimeric Configurations of All Major Opioid Receptor Subtypes as Predicted by Simulated Self-Association in Explicit Lipid-Water Environment

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Recent crystallographic structures of G protein-coupled receptors, including members of the opioid receptor subfamily, have shown inter-protomer contacts between parallel orientations of the receptors within the crystal. Although it is tempting to speculate that these parallel arrangements are indicative of physiologically relevant dimeric configurations, it is unclear whether they would preferentially form within a lipid environment. We have performed a series of unbiased, coarse-grained molecular dynamics simulations to compare the ability of mu-, kappa-, and delta-opioid receptors (MOP, KOP and DOP, respectively) to freely associate into homo- or heteromeric configurations in