structural model of the interface between meta-II rhodopsin and the arrestin loop region is proposed based on molecular docking.

3502-Pos Board B549

Exploring the Thermodynamics of Activation Pathways of Bovine Rhodopsin with Fast Molecular Dynamics Simulations

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Recent progress made in the understanding of the structure and function of G-protein coupled receptors (GPCRs) points to compelling biophysical and crystallographic evidence for distinct conformational changes of the prototypical family member bovine rhodopsin/opsin upon illumination. To investigate possible activation pathways of bovine rhodopsin from an early inactive intermediate (lumirhodopsin) produced by the cis-trans photoisomerization of its retinal chromophore to an activated ligand-bound opsin-like conformation of the protein, we carried out several independent biased molecular dynamics simulations of the receptor in an explicit dipalmitoylphosphatidylcholine (DPPC) membrane bilayer. The simulations revealed significantly different activation pathways between inactive and opsin-like activated forms of bovine rhodopsin, whose thermodynamic behavior was evaluated by metadynamics using as reaction coordinates both the position along the identified representative pathways, and the distance from them. The results point to three common metastable states that are likely to correspond to a photoactivated deprotonated Meta I-380/Meta IIa, and two different Meta IIb-like intermediates of bovine rhodopsin. Inferences from these molecular models are expected to shed new light into mechanisms of allosteric modulation of rhodopsin function.

3503-Pos Board B550

Molecular Mechanism of Rhodopsin Photoactivation

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Rhodopsin is a highly specialized GPCR that is activated by the rapid photochemical isomerization of its covalently bound 11-cis retinal chromophore. Using two-dimensional solid-state NMR spectroscopy, we define the position of the retinal in the active metarhodopsin II intermediate and the protein conformational changes that couple retinal isomerization to breaking of the ''ionic lock'' between transmembrane (TM) helices H3 and H6. Retinal isomerization leads to steric strain within the retinal binding site between the β-ionone ring and helix H5, and between the C19/C20 methyl groups and EL2. These interactions trigger the displacement of EL2, deprotonation of the Schiff base nitrogen and protonation of Glu113. Motion of the β-ionone ring leads to rearrangement of the hydrogen bonding network centered on H5, while interactions of the C19 and C20 methyl groups are involved in rearrangement of the EL2. Motion of the β-ionone ring is also coupled to the motion of Trp265, which triggers the shift of helices H6 and H7 into active conformations and the rearrangement of the hydrogen bonding network centered on the conserved NPxxY sequence. Motion of helices H5, H6 and H7, in turn, is coupled to the rearrangement of electrostatic interactions involving the conserved ERY sequence at the cytoplasmic end of H3, exposing the G protein binding site on the cytoplasmic surface of the protein. The location of the retinal and reorganization of the protein upon receptor activation provides a structural basis for understanding the action of agonists and antagonists in the large family of class A GPCRs.

3504-Pos Board B551

Rhodopsin Activation in Membranes: Thermodynamic Model of the Two Protonation Switches

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Activation of the G protein-coupled receptor (GPCR) rhodopsin is initiated by light-induced isomerization of the retinal ligand, which triggers two protonation switches in the conformational transition to the active receptor state. Disruption of an interhelical salt bridge occurs by deprotonation of the retinal protonated Schiff base (PSB) together with uptake of a proton from the solvent by Glu134 of the conserved cytoplasmic E(D)RY motif. Using a combination of UV-visible and Fourier-transform infrared (FTIR) spectroscopy of rhodopsin, we access deprotonation of the PSB and activating conformational changes of the helix bundle separately, and study the pH-dependent activation mechanism of rhodopsin in different membrane environments. The data are analyzed using a thermodynamic framework based on the Hofmann-Hubbell scheme derived for rhodopsin in detergent, distinguishing between PSB deprotonation, activating conformational changes, and cytoplasmic proton uptake in the transitions from Meta I to Meta II_a, Meta II_b, and Meta II_bH^+, respectively. While a classical Henderson-Hasselbalch-like equilibrium between Meta I and Meta II_bH^+ is observed at 10 °C, more complex titration curves with non-zero alkaline endpoints are found above 20 °C. These reflect partial population of an entropy-stabilized Meta II_b state, in which the PSB salt bridge is broken and activating helix movements have taken place, but where Glu134 remains unprotonated. This partial activation is converted to full activation only by coupling to the pH-dependent protonation of Glu134 from the solvent, which enthalpically stabilizes the active receptor conformation. In a membrane environment, protonation of Glu134 is therefore a thermodynamic rather than a structural requirement for activating helix movements. In light of conservation of the E(D)RY motif in rhodopsin-like GPCRs, protonation of this carboxylate may serve a similar function in signal transduction of other members of this important receptor family.

3505-Pos Board B552

Real-Time Dynamics Changes of Helix 8 upon Phosphorylation of Rhodopsin and Arrestin Binding

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The G-protein coupled receptor (GPCR) rhodopsin transduces light signals in photoreceptor cells. For a rapid recovery of the receptor, efficient signal termination is mandatory. On the molecular level, this termination process starts with the phosphorylation of serines and/or threonines in the C-terminus of rhodopsin by rhodopsin kinase. Subsequently, the phosphorylated photoexcited rhodopsin is recognized by visual arrestin, which binds to the cytoplasmic loops of the receptor.

In this work we focus on the real-time dynamics of helix 8, a putative cytoplasmic surface switch region in class-I GPCRs. Using multidimensional time-correlated single photon counting [1] and site-specific fluorescence labelling [2], we measured the time-resolved anisotropy changes of phosphorylated and photoactivated phosphorylated rhodopsin upon interaction with arrestin. Distinct real-time dynamics changes of helix 8 were observed after phosphosphorylation and interaction with arrestin. From real-time dynamics studies of helix 8 in rod outer segment membranes we concluded that a reduced mobility of helix 8 is a prerequisite for rhodopsin activation [2]. FTIR-Fluorescence cross-correlation measurements indicate further that changes in the environment of helix 8, as measured with a fluorescent reporter group, may correlate with a partial loss of secondary structure upon Metarhodopsin II formation [3]. The observed mobility changes after phosphorylation highlight the importance of helix 8 as a surface element, which is involved in activation, molecular recognition, and deactivation of the receptor.

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3506-Pos Board B553

pH Dependent Interaction of Rhodopsin with Anthocyanin Plant Pigments

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Anthocyanins are a class of natural compounds common in flowers and vegetables. Because of the increasing preference of consumers for food containing natural colorants and the demonstrated beneficial effects of anthocyanins on human health, it is important to decipher the molecular mechanisms of their action. Previous studies indicated that the anthocyanin cyanidin-3-glucoside (C3G) modulates the function of the photoreceptor rhodopsin. Here, we show using selective excitation ^{1}H NMR spectroscopy that C3G binds to rhodopsin. Ligand resonances broaden upon rhodopsin addition and rhodopsin resonances exhibit chemical shift changes as well as broadening effects in specific resonances, in an activation-state dependent manner. Molecular docking studies also support this conclusion. Thermal denaturation and stability studies using circular dichroism (CD), fluorescence and UV/Visible absorbance spectroscopy show that C3G exerts a destabilizing effect on rhodopsin structure while it did not significantly alter G-protein activation and the rates at which the light-activated Metarhodopsin II state decays. These results indicate that the mechanism of C3G enhanced regeneration may be based on changes in rhodopsin structure promoting access to the retinal binding pocket. The results provide new insights into anthocyanin-protein interactions and may have relevance for the enhancement of night vision by this class of compounds. This work is also the first report of the study of ligand binding to a full-length membrane receptor in detergent-micelles by ^{1}H NMR spectroscopy. Such studies were previously hampered by the presence