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

Davide Provasi[1], Krzysztof Palczewski[2], Marta Filizola[1].
[1]Mount Sinai School of Medicine, New York, NY, USA, [2]Case Western Reserve University, Cleveland, OH, USA.

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

Steven O. Smith.
Stony Brook University, Stony Brook, NY, USA.

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

Reiner Vogel[1], Mohana Mahalingam[1], Karina Martínez-Mayorga[2], Michael F. Brown[2].
[1]University of Freiburg, Freiburg, Germany, [2]Department of Chemistry, University of Arizona, Tucson, AZ, USA.

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

Kristina Kirchberg[1,2], Tai-Yang Kim[1], Martina Möller[1], Ramona Schlesinger[2], Georg Bueldt[2], **Ulrike Alexiev**[1].
[1]Physics Department, Freie Universitaet Berlin, Berlin, Germany, [2]Institute of Neuroscience and Biophysics, Forschungszentrum Juelich, Juelich, Germany.

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.

[1] Kim T.-Y., Winkler K., and U. Alexiev (2007). Photochemistry and Photobiology 83, 378-384
[2] Alexiev, U., I. Rimke, and T. Pöhlmann (2003). J. Mol. Biol. 328, 705-719
[3] Lehmann N., Alexiev U., and Fahmy K. J (2007). Mol. Biol. 366, 1129-1141

3506-Pos Board B553

pH Dependent Interaction of Rhodopsin with Anthocyanin Plant Pigments

Naveena V. Yanamala, Fernanda Balem, Kalyan C. Tirupula, Judith Klein-Seetharaman.
University of Pittaburgh, Pittsburgh, PA, USA.

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

of detergent micelle resonances, a problem overcome by the selective excitation approach.

3507-Pos Board B554

An Important Double Bond: Effects of 22:5n-6 vs. 22:6n-3 on Visual Signal Transduction

Drake C. Mitchell[1], Shui-Lin Niu[2].
[1]Portland State University, Portland, OR, USA, [2]NIH/NIAAA, Bethesda, MD, USA.

In a normal, healthy retinal rod outer segment 40% to 50% of the phospholipid acyl chains consist of docosahexaenoic acid (DHA, 22:6n-3). Diets that are deficient in n-3, or ω-3, fatty acids lead to the replacement of 22:6n-3 with 22:5n6. Dietary n-3 deficiency leads to a spectrum of developmental disorders associated with learning, memory, intelligence, and visual function. We examined rhodopsin, transducin (G_t) and phosphodiesterase (PDE) function and acyl chain packing in large unilamellar proteoliposomes consisting of phosphatidylcholines with *sn-1* = 18:0, and *sn-2* = 22:6n-3, 22:5n-6 or 22:5n-3. Rhodopsin activation and binding to G_t was assayed with steady-state and time-resolved UV/vis spectroscopy, acyl chain packing was assessed via time-resolved fluorescence of diphenylhexatriene (DPH) and PDE activity was determined from the change in pH due to hydrolysis of cyclic GMP. The motion of DPH in the membrane was slower in 22:5n-6 than in 22:6n-3 and overall acyl chain packing was more constrained. The most significant structural difference between the 22:5n-6 containing bilayer and bilayers containing both n-3 polyunsaturates was in the bilayer mid plane where 22:5n-6 produced much higher DPH orientational order. At physiological temperature the formation of both the active metarhodopsin II conformation (MII) and the MII-G_t complex was much slower in 18:0,22:5n-6 PC than in 18:0,22:6n-3 PC and the equilibrium amount of MII formed was 50% higher in 18:0,22:6n-3 PC. In 18:0,22:5n-6 PC PDE activity at a physiologically relevant level of rhodopsin activation is only about 60% of that observed in either 18:0,22:6n-3 PC or 18:0,22:5n-3 PC. Taken together, these results demonstrate that the subtle change in bond configuration from 22:6n-3 to 22:5n-6 produces more structured acyl chain packing in the bilayer midplane, leading to delayed and reduced MII-G_t interaction and PDE function.

3508-Pos Board B555

Dynamics of the Internal Water Molecules in Squid Rhodopsin

Eduardo Jardón-Valadez, Ana-Nicoleta Bondar, Douglas J. Tobias.
UCI-Irvine, Irvine, CA, USA.

G-protein coupled receptors (GPCRs) are major parmaceutical targets because of their key role in a diverse array of physiological functions. The visual rhodopsin is the prototype for the family A of GPCRs. Upon photoisomerization of the covalently-bound retinal chromophore, visual rhodopsins undergo a large-scale conformational change that prepares the receptor for a productive interaction with the G protein. The mechanism by which the local perturbation of the retinal cis-trans isomerization is transmitted throughout the protein is not well understood. The recently reported crystal structure of squid rhodopsin (M. Murakami and T. Kouyama, Nature 453, 363, 2008) displays new features that may provide additional insight into the mechanism of the signal transduction in GPCRs. It has been suggested, based on the location of water molecules in the interhelical region extending from the retinal towards the cytoplasmic side, that a water-mediated hydrogen-bond network may play a role in the activation process. As a first step towards understanding the role of water in rhodopsin function, we have performed a molecular dynamics simulation of squid rhodopsin embedded in a hydrated bilayer of polyunsaturated lipid molecules. Here we report results from the simulation that show that the water molecules present in the crystal structure participate in favorable interactions with side chains in the interhelical region, and form a persistent hydrogen-bond network in connecting Tyr315 to Trp274 via Asp80. We also present preliminary results from a simulation study of the changes in the structure and dynamics of the hydrogen-bond network that accompany the photoisomerization of the retinal chromophore.

3509-Pos Board B556

Single-Molecule Photoactivation and Localization of Signaling Complexes in T cells

Chih-Jung Hsu.
University of Pennsylvania, Philadelphia, PA, USA.

In T cells, membrane receptor and ligand engagement initiates a signaling cascade. Ligand binding is relayed through specific membrane receptors, protein tyrosine kinases, and critical adaptors to regulate downstream activation of transcription factors, cytokine production and cell proliferation. The membrane segregation and relocalization of these signaling components display highly dynamic protein networks in response to distinct stimulations. However, the membrane-protein and protein-protein interactions involved in the formation of signaling complexes are still poorly understood. We are currently using a high-resolution imaging technique to visualize the early stage of signal transduction events at the single-molecule level with photoactivatable or photoconvertable fluorescent markers. The photoactivation and localization of T-cell signaling components allow measurements of molecular spatial and temporal correlation in response to different stimulations. With dual-color application, molecular correlation functions will enhance the understanding of the protein-protein interactions in signaling complexes.

3510-Pos Board B557

Molecular un-stability of peptide-MHC complex and activation of T cell

Naoki Ogawa[1], Yuji C. Sasaki[2], Osami Kanagawa[1].
[1]Research Center for Allergy and Immunology, RIKEN, Yokohama, Japan, [2]Biomedical Group, SPring-8/JASRI (JST/CREST Sasaki-team), Sayou-gun, Japan.

CD4+ T cell responses require the recognition of specific peptide-MHC complexes displayed by APC. It is important to determine how Ag presentations affect the ensuing T cell response. Imunization of B10.BR mice with immunodominant peptide 48-61 of Hen egg lysozyme elicit two different types of T cell responses. First type of T cell (type A termed by Unanue et. al) respond to APC pulsed with either peptide or whole HEL protein. Second type of T cell (termed type B) respond to APC incubated with peptide but showed no response to APC with whole protein. Some of the type B T cell clone exhibit unusual response to the variant of 48-61 peptide and responded better to poor MHC binding peptide that to strong MHC binding peptide. In contrast, reactivity of the type A of T cell clones correlate well with the affinity of the peptide to the MHC molecules. Since weak MHC binding peptides form unstable complex, we hypothesize that T cells, like type B T cell, respond well to unstable MHC peptide complex by interacting with one of multiple transitional conformations. To test this hypothesis, we observed the movement of peptide/MHC complex at the single molecular level by using diffracted X-ray tracking (DXT) method. It was found that movement of the low affinity peptide MHC complex was greater than that of high affinity peptide MHC complex. However, the difference between two complexes was mainly due to the overall movement of the molecule rather than the different movement of the peptides in the MHC groove. Thus, peptide bound to the MHC greatly influence the movement of the whole MHC peptide complex and this movement strongly affect the recognition by T cell.

3511-Pos Board B558

The Influence Of Plasma Membrane Order On TCR Signalling

Ben de Wet, Brid Cronin, Mark Wallace, Thomas Harder.
University of Oxford, Oxford, United Kingdom.

The signalling events that follow T-cell receptor triggering are mediated by multi-molecular complexes consisting of both membrane-associated and cytosolic proteins. Formation of these complexes is driven by a network of protein-protein and protein-lipid interactions. We study how the selective partitioning of signalling components into separate ordered/raft or disordered phases of the plasma membrane controls the assembly and activity of these signalling complexes.

Previous work from our laboratory shows that T cell plasma membrane domains engaged in TCR signalling specifically undergo condensation, and that treatment of Jurkat T-cells with polyunsaturated fatty acids specifically disrupts condensed membrane rafts at TCR activation sites. We have also shown that disruption of membrane order by 7-ketocholesterol leads to reduced recruitment of key TCR signalling components as measured biochemically and by TIRF microscopy of fixed cells. We aim to understand the influence of this biophysical phenomenon on the formation and composition of TCR signalling protein/lipid complexes.

To this end, we are currently studying the effects of raft disruption by 7-ketocholesterol and polyunsaturated fatty acids on the kinetic behaviour of TCR signalling components. We employ single molecule real time TIRF microscopy of fluorescently labelled TCR signalling proteins as well as biochemical and proteomic analysis of TCR activation domains upon raft disruption.

3512-Pos Board B559

Dissecting T cell receptor nanocluster signaling

Boryana N. Manz[1,2], Bryan L. Jackson[1], Rebecca S. Petit[1], Michael L. Dustin[3], Jay T. Groves[1,4].
[1]Department of Chemistry, UC Berkeley, Berkeley, CA, USA, [2]Biophysics Graduate Group, UC Berkeley, Berkeley, CA, USA, [3]Molecular Pathogenesis Program, Skirball Institute of Biomolecular Medicine, NYU School of Medicine, New York, NY, USA, [4]Howard Hughes Medical Institute, Chevy Chase, MD, USA.

The early steps of T cell activation involve the spatial rearrangement of T cell receptors (TCR) and associated molecules into small nanoclusters at the