2368-Pos Intrinsic Bending and Structural Rearrangement of Tubulin Dimer: Molecular Dynamics Simulations and Quasiharmonic Analysis

Yeshitila Gebremichael[1], Jhih-Wei Chu[2], Gregory Voth[3]

[1] *Biomedical Engineering Department, Wayne State University, Detroit, MI, USA,*
[2] *Department of Chemical Engineering, University of California at Berkeley, Berkeley, CA, USA,*
[3] *Center for Biophysical Modeling & Simulation, Department of Chemistry, University of Utah, Salt Lake City, UT, USA.*

Board B483

Microtubules are long polymers of αβ-tubulin heterodimers. They undergo a process known as dynamic instability in which ends of microtubule switch stochastically between phases of slow growth and rapid shrinkage. The molecular mechanism inducing the depolymerization of microtubule has been attributed to the hydrolysis of GTP nucleotide bound to the b-tubulin. GTP hydrolysis is thought to cause microtubule instability by promoting outward curving of protofilaments constituting the microtubule lattice. The bending of the protofilaments is associated with the structural transformation of tubulin dimer from straight to curved conformations. However, the intrinsic bending of the dimer remains illusive. The present study employs molecular dynamics (MD) simulations and quasiharmonic analysis (QHA) to reveal the intrinsic bending as well as the local structural rearrangements of unassembled tubulin dimer as the dimer relaxes from its lattice constrained, straight conformation of zinc-induced tubulin sheet. The intrinsic bending of GDP-tubulin is examined by using the straight crystal structure of GDP-bound tubulin from tubulin sheet as a starting structure. The effect of the nucleotide state on the dimer bending is investigated by the introduction of g-phosphate into the b-tubulin to form GTP-bound tubulin. Both GDP-bound and GTP-bound tubulin dimers are found to have curved conformations, but with a smaller bending seen in the GTP-tubulin than in the GDP-tubulin. The perturbation induced through the introduction of g-phosphate is posited to play a role of straightening the intra-dimer bending. The local structural rearrangements due to the bending mode of motion of the dimer reveals that, one of the three functional domains, the intermediate domain, exhibits significantly lower bending deformation as compared to the others, signifying a dynamical connection to the functionally defined domains.

Visual Receptors

2369-Pos Influence of Lipid Membrane Composition on the Kinetics of Rhodopsin Activation by Time-Resolved UV-Visible Spectroscopy

James W. Lewis[1], Karina Martínez-Mayorga[2], Istvan Szundi[1], Jacqueline Epps[1], Victor U. Constantino[3], David S. Kliger[1], Michael F. Brown[4]

[1] *Department of Chemistry and Biochemistry, University of California, Santa Cruz, CA, USA,*
[2] *Torrey Pines Institute for Molecular Studies-PSL, Fort Pierce, FL, USA,*
[3] *Department of Biochemistry and Molecular Biophysics, University of Arizona, Tucson, AZ, USA,*
[4] *Department of Chemistry, University of Arizona, Tucson, AZ, USA.*

Board B484

The process of vision starts with *cis-trans* isomerization of the retinal cofactor of rhodopsin, which is a membrane protein prototype of the GPCR superfamily. Changes in lipid composition of membranes may crucially influence the activity of the embedded proteins [1]. Time-resolved UV-visible spectroscopy is a powerful technique where one can measure changes in the absorbance of a sample after perturbation by a light pulse. It has been employed to study the kinetic mechanism of rhodopsin photolysis[2]. Using an optical multichannel analyzer (OMA) we explore here the influence of the physicochemical properties of the membrane lipid bilayer on rhodopsin activation. Time-resolved absorbance changes from 1 microsecond to 50 milliseconds after excitation by 7 nanosecond pulses of 477 nm light were measured at 30 °C in several reconstituted membrane preparations of rhodopsin (DOPC, 25% DOPC:75%DOPE, egg PC, and POPC; all 100:1 lipid to rhodopsin ratio). Measurements were made at pH 5.5, pH 6.5 (high and low salt), and pH 8 in order to determine how the pK of the Meta I_{480}-Meta II equilibrium and other aspects of the photointermediate formation scheme were affected by the lipid membrane environment. Results show that membrane composition strongly affects the pK with all lipids studied having a lower equilibrium pK than for the native lipids. These differences are originate from membrane properties due to the polar head group and acyl chain composition of the lipid bilayer. Other aspects of photointermediate formation were more subtly affected by the changes in membrane composition, and no apparent heterogeneity was seen in any of these reconstituted samples.

References

[1]. A. V. Botelho *et al.* (2006) *Biophys. J.* ***91***, 4464.
[2]. I. Szundi *et al.* (1998) *Biochemistry* ***37***, 14237.

2370-Pos Membrane Lipid Modification of Rhodopsin Activation Studied by FTIR Spectroscopy

Reiner Vogel[1], Karina Martinez-Mayorga[2], Mohana Mahalingam[1], Victor U. Constantino[3], Michael F. Brown[4]

[1] *Biophysics Group, Albert-Ludwigs University, Freiburg, Germany,*
[2] *Torrey Pines Institute for Molecular Studies-PSL, Fort Pierce, FL, USA,*
[3] *Department of Biochemistry and Molecular Biophysics, University of Arizona, Tucson, AZ, USA,*
[4] *Department of Chemistry, University of Arizona, Tucson, AZ, USA.*

Board B485

Rhodopsin is the prototype of the GPCR superfamily of membrane proteins, and its photochemical function is strongly infuenced by the lipid composition [1]. Structural changes of both the protein and retinylidene ligand are conveniently monitered by FTIR spectros-

copy [2]. Following exposure to actinic light, FTIR spectra were normalized and fitted to a linear combination of MetaI and MetaII spectra obtained at the pH extremes. For the Meta I state, changes in the amide I, carboxylic acid, and chromophore bands are evident with temperature. The Meta I conformation becomes "softer" with temperature whereas MetaII is little affected. We discovered effects of the lipid environment on the Meta I-Meta II equilibrium similar to previous flash photolysis studies [3]. Using FTIR we analyzed the influence of eggPC, POPC, or DOPC/DOPE mixtures of lipids on rhodopsin activation. Compared to the natural disk membranes, pK values for the MetaI-MetaII transition were decreased which depended on both the lipid acyl chains and head group composition. The different chain composition in PC, POPC, and DOPC membranes did not affect the conformation of MetaI; however changes were evident due to PE headgroups. Membranes with high content on DOPE favor the MetaII state. It is known that DOPC forms bilayers, whereas DOPE adopts the nonlamellar, reverse hexagonal phase. Hence rhodopsin function is associated with a balance of forces involving the curvature stress introduced into the bilayer by increasing amounts of PE lipids. A simple flexible surface model [1,3] accounts for these observations by coupling of the curvature free energy of the membrane lipid film to the activated MetaII conformation.

References

[1]. A. V. Botelho *et al.* (2006) *Biophys. J.* ***91***, 4464.
[2]. R. Vogel *et al.* (2006) *JACS* ***128***, 10503.
[3]. A. V. Botelho *et al.* (2002) *Biochemistry* ***41***, 6354.

2371-Pos Site-Directed ^{2}H NMR Reveals Functional Dynamics of Retinal Due to Light Activation of Rhodopsin

Andrey V. Struts[1], Gilmar F. J. Salgado[2], Karina Martínez-Mayorga[1], Avigdor Leftin[1], Constantin Job[1], Michael F. Brown[1]

[1] *Department of Chemistry, University of Arizona, Tucson, AZ, USA,*
[2] *Faculté de Pharmacie, Université René Descartes, Paris, France.*

Board B486

Deuterium NMR relaxation methods were applied to elucidate the dynamics of the retinal ligand of rhodopsin in the dark state, cryo-trapped meta I, and meta II states. Site-specific ^{2}H NMR labels were introduced by deuteration of three methyl groups crucial for retinal function [1]. ^{2}H NMR spectra of aligned membranes containing rhodopsin indicated that retinal had significant mobility with order parameters of ≈ 0.9 for the rapidly spinning C-C^{2}H$_3$ groups, with off-axial motion of $\approx 15^o$. Analysis of the relaxation data showed that the dark state dynamics of the C9-methyl and C13-methyl groups are determined by intra-retinal interactions. Mobility of the C5-methyl is affected by interactions with Glu^{122}. Upon forming the meta I state, a significant decrease in the C9-methyl group rotational diffusion results from reduction of the polyene chain twist and steric interaction with the binding pocket. However, overall dynamics of the C9-methyl and C13-methyl groups in the meta I and meta II states indicate the absence of steric clashes with surrounding amino acids, consistent with the ^{2}H NMR structure of retinal in metarhodopsin I [2–3]. Little dynamic change of the C5-methyl resulted upon the transition to meta I and meta II, which together with the mobility of the C9-methyl and C13-methyl groups may indicate the absence of reorientation or displacement of the rhodopsin chromophore. Two minima observed in the T_{1Z} temperature dependence for the C5-methyl probably indicate two retinal conformers differing with respect to the C6-C7 torsion angle [4].

References

[1]. G. F. J. Salgado *et al.* (2004) *Biochemistry* ***43***, 12819.
[2]. G. F. J. Salgado *et al.* (2006) *JACS* ***128***, 11067.
[3]. A. V. Struts *et al.* (2007) *J. Mol. Biol.* ***372***, 50.
[4]. P.-W. Lau *et al* (2007) *J. Mol. Biol.* ***372***, 906.

2372-Pos Sequence Of Late Intermediates In The Activation Of Rhodopsin

Bernhard Knierim[1], Klaus-Peter Hofmann[1], Oliver P. Ernst[1], Wayne L. Hubbell[2]

[1] *Institut f. Medizin. Physik und Biophysik, Charité - Universitätsmedizin zu Berlin, Berlin, Germany,*
[2] *Jules Stein Eye Institute and Department of Chemistry and Biochemistry, UCLA, Los Angeles, CA, USA.*

Board B487

After light-induced *cis*/all-*trans* isomerization and early photointermediates, the visual pigment rhodopsin adopts an equilibrium between metarhodopsin I (meta I) and metarhodopsin II (meta II). Meta II formation is marked by deprotonation of the retinal Schiff base, resulting in a shift of the absorption maximum from 500 to 380 nm, and proton uptake by the protein [1]. At the cytoplasmic side of rhodopsin, meta II formation is accompanied by an outward movement of transmembrane helix (TM) 6 and smaller rearrangements at TM3 and TM7/H8 [for review see Ref. 2]. To determine the cause-and-effect relationship of these late activation events, site-directed spin labeling and flash photolysis were employed to monitor the kinetics of helix motion, Schiff base deprotonation and proton uptake. Measurements were performed with spin-labeled rhodopsin samples at different temperatures to yield Arrhenius activation energies (E_A). The motion of TM6 follows Schiff base deprotonation and is synchronous with the proton uptake (E_A = 62 kJ/mol). However, the pH dependence shows that helix motion can occur without proton uptake, suggesting that TM6 motion precedes proton uptake, exposing the D(E)RY motif, which is the locus of proton uptake from the cytoplasm [3]. Peptides corresponding to the C-terminal sequence of transducin bind to rhodopsin following helix motion and cause net proton release. Collectively, the data suggest the following temporal sequence of events involved in activation:

1. internal Schiff base proton transfer;
2. TM6 movement;
3. proton uptake from solution and binding of transducin.

References

1. Arnis, S., and Hofmann, K. P. (1993) *Proc. Natl. Acad. Sci. U.S.A.* ***90***, 7849–7853

2. Hubbell, W. L., Altenbach, C., Hubbell, C. M., and Khorana, H. G. (2003) *Adv. Protein Chem.* ***63***, 243–290.
3. Arnis, S., Fahmy, K., Hofmann, K.P., and Sakmar, T.P.(1994) *J. Biol. Chem.* ***269***, 23879–81 (1994).

2373-Pos Retinal Relaxation in Rhodopsin Activation Viewed by Large-Scale Molecular Dynamics Simulations

Karina Martínez-Mayorga[1], Alan Grossfield[2], Jose L. Medina-Franco[1], Michael C. Pitman[3], Scott E. Feller[4], Michael F. Brown[5]

[1] *Torrey Pines Institute for Molecular Studies-PSL, Fort Pierce, FL, USA,*
[2] *Department of Biochemistry and Biophysics, University of Rochester Medical Center, Rochester, NY, USA,*
[3] *IBM TJ Watson Research Center, Yorktown Heights, NY, USA,*
[4] *Department of Chemistry, Wabash College, Crawfordsville, IN, USA,*
[5] *Department of Chemistry, University of Arizona, Tucson, AZ, USA.*

Board B488

The first event in the visual cascade involves the ultrafast photoisomerization of retinal, the cofactor of the G protein-coupled receptor rhodopsin. The intermediates through which rhodopsin reaches the pre-active and activated form (metarhodopsins I and II respectively) are well documented. Two mechanisms have been proposed for the counterion-based stabilization of the retinal protonated Schiff base (PSB) up to meta I formation, as derived from vibrational and UV-visible spectroscopy. In the first model, the PSB is stabilized by Glu^{113} in the dark and bathorhodopsin states, whereas Glu^{181} is the counterion in the meta I photointermediate. In the second model, the PSB is always stabilized by both residues. Combination of experimental ^2H NMR data with molecular dynamics simulations has proved to be a powerful approach to explore the atomic-scale behavior of membrane proteins [1, 2]. Here we compare these counterion models for rhodopsin within a 99-lipid bilayer in two trajectories of 1500 and 2000 ns respectively. We observe that the protonation state of Glu^{181} at the early stage of the simulation seems to direct the conformational behavior of retinal throughout the simulations. The orientations of the retinal methyl groups at the C5, C9, and C13 positions with respect to the membrane normal (z-axes) were different among the simulations. Comparison of the simulated results with the experimental solid-state ^2H NMR spectra [3] supports the complex-counterion switch model, in which both Glu^{113} and Glu^{181} stabilize the retinal PSB during meta I formation. Molecular simulations of the counterion stabilization mechanism gives input on the knowledge of the conformational pathways for the pre-active state meta I formation.

References

[1]. K. Martínez-Mayorga *et al.* (2006) *JACS* ***128***, 16502.
[2]. P.-W. Lau *et al.* (2007) *JMB* ***372***, 906.
[3]. A. V. Struts *et al.* (2007) *JMB* ***372***, 50.

2374-Pos Proteorhodopsin: Insight From AFM And Solid-state NMR

Clemens Glaubitz[1], Nicole Pfleger[1], Sarika Shastri[1], Adriana Klyszenko[2], Daniel Mueller[2]

[1] *Goethe University Frankfurt, Frankfurt, Germany,*
[2] *University of Technology Dresden, Dresden, Germany.*

Board B489

The proteorhodopsin family consists of hundreds of homologous retinal containing membrane proteins found in bacteria in the photic zone of the oceans. They are colour tuned to their environment and act as light-driven proton pumps with a potential energetic and regulatory function. Precise structural details are still unknown. Here, the green proteorhodopsin variant has been selected for a chemical shift analysis of retinal and Schiff base by solid-state NMR. Our data show that the chromophore exists in mainly all-trans configuration in the proteorhodopsin ground state. The optical absorption maximum together with retinal and Schiff base chemical shifts indicate a strong interaction network between chromophore and opsin. The analysis of 2D crystals did reveal a hexagonal protein packing under a very wide range of conditions indicating that PR might be also closely packed under native conditions. A low resolution 2D projection map did show a ring shaped oligomeric assembly of PR. Lee-Goldberg cross polarisation has been used to probe protein backbone mobility. Using atomic force microscopy (AFM) we directly observed the oligomeric assembly of native proteorhodopsin molecules. In contrast to all other vertebrate and archae rhodopsins characterized so far, we found that proteorhodopsin predominantly assembles into hexameric oligomers.

2375-Pos Activation Of Rhodopsin At Physiological Temperatures: Structural And Thermodynamic Characterization Of The Two Protonation Switches

Reiner Vogel[1], Karina Martínez-Mayorga[2], Mohana Mahalingam[1], Victor U. Constantino[3], Michael F. Brown[4]

[1] *Biophysics Group, Albert-Ludwigs University, Freiburg, Germany,*
[2] *Torrey Pines Institute for Molecular Studies-PSL, Fort Pierce, FL, USA,*
[3] *Department of Biochemistry and Molecular Biophysics, University of Arizona, Tucson, AZ, USA,*
[4] *Department of Chemistry, University of Arizona, Tucson, AZ, USA.*

Board B490

Rhodopsin, the visual pigment for dim light vision, is considered a prototype of the G protein-coupled receptor (GPCR) superfamily and a complete understanding of its activation mechanisms is therefore of high relevance. FTIR spectroscopy as a non-invasive method can supply a wealth of structural and functional information on rhodopsin's activation mechanisms in its native membrane environment. Activation of rhodopsin is triggered by light-induced isomerization of the retinal ligand, which is covalently bound via a protonated Schiff base (PSB). This induces conformational transitions within the receptor protein, which involve two protonation switches leading ultimately to formation of the active receptor state

Meta II [1]: first a proton transfer from the PSB to its counterion, Glu113, thereby breaking the salt bridge between both groups; second the uptake of a proton by a cytoplasmic network between helices 3 and 6. As we could show previously the first step is the energetically most relevant step, with large enthalpic (ΔH) and entropic (ΔS) contributions [2], while the second step is enthalpically downhill. Below 20 °C, this second step drives the energetically unfavorable first reaction by an allosteric coupling mechanism mediated by the retinal chromophore [1]. Above 20 °C, the free energy change ΔG = ΔH -TΔS becomes favorable due to the increase of the entropic term, such that disruption of the PSB salt bridge proceeds also in the absence of the second protonation switch. The resulting state bears the full signature of an active state conformation, indicating that the second switch, cytoplasmic proton uptake, is relevant not in structural terms, but rather for thermodynamic stabilization of an otherwise electrostatically unfavorable conformation.

References

[1]. R. Vogel *et al.* (2007) Photochem Photobiol ***83***, 286.
[2]. R. Vogel *et al.* (2006) *JACS.* ***128***, 10503.

2376-Pos Calorimetry Reveals Different Rhodopsin Organization in Native Disk Membranes, Reconstituted Vesicles, and Detergent Micelles

Shui-Lin Niu, Drake C. Mitchell
NIH, Rockville, MD, USA.

Board B491

Dimerization and oligomerization of G protein-coupled receptor (GPCR) has become a new paradigm of signal transduction. Rhodopsin is a prototypical member of the GPCR family and often serves as a template in structural and functional studies of other GPCRs. Early studies depicted rhodopsin as rapidly diffused monomers, while recent studies described rhodopsin as highly ordered dimers in native disk membranes. Currently there is no clear understanding regarding the discrepancy among these studies. Our hypothesis is that the hydrophobic environment modulates the organization of rhodopsin. Here we investigated the thermal denaturation of rhodopsin in native disk membranes, reconstituted vesicles, and detergent micelles using differential scanning calorimetry (DSC). The transition temperatures and thermal denaturation profiles of rhodopsin differed significantly among these samples. The transition peak was much narrower in native disk membranes than that in reconstituted vesicles or detergent micelles, indicating different cooperativity among these samples. The ratios of van't Hoff enthalpy to calorimetric enthalpy from the equilibrium analysis and the activation energies of rhodopsin denaturation from the kinetic analysis are both consistent with dimeric structures in native disk membranes and monomeric structures in reconstituted vesicles or detergent micelles. These results suggested a weak association among rhodopsin molecules, which is readily disrupted when it is isolated in detergents or reconstituted in lipid vesicles. Our findings demonstrated a role of hydrophobic environment on rhodopsin dimerization and may help to clarify the current disagreement regarding rhodopsin dimerization.

2377-Pos Unfolding and Misfolding of Rhodopsin

Fernanda Balem, Arpana Dutta, Judith Klein-Seetharaman
University of Pittsburgh, Pittsburgh, PA, USA.

Board B492

Retinitis Pigmentosa (RP) is an inherited disease that progressively leads to blindness. The rod photoreceptor rhodopsin was first identified as a genetic cause of RP, and more than 100 mutations in rhodopsin are known. 70% of the mutations studied were found to cause misfolding of rhodopsin, but this knowledge has not yet been leveraged in developing treatments for RP. Through an understanding of the folding mechanism of rhodopsin, it may be possible to identify avenues for treatment of RP. Towards this goal, thermal and chemical (SDS) unfolding studies were performed. A maximal decrease of about 50% of total helical content was observed. Furthermore, six cysteines become reactive to sulfhydryl reagents on addition of SDS indicating unfolding of the helical bundle. However, with further increase in SDS concentration only two of them remained reactive, possibly due to formation of a non-native core structure in the denatured state. Stabilizing native and destabilizing non-native conformations by addition of small molecules may be a way to prevent formation of misfolded forms of rhodopsin. Recently, rhodopsin has been shown to bind anthocyanin and porphyrin compounds. Here, we present preliminary evidence suggesting that porphyrins may stabilize native rhodopsin. Experiments in tissue culture are being conducted to investigate whether enhanced stability can rescue correctly folded rhodopsin. The addition of porphyrin along with retinal at early stages in tissue culture appears to increase rhodopsin regeneration. Furthermore, secondary effects on cell growth also occur as evidenced by increased cell pellet weights. The results suggest that porphyrin treatment may be a future avenue for patients with RP.

2378-Pos The Protonation State of Glu142 Differs in the Green and Blue Absorbing Variants of Proteorhodopsin

Joel M. Kralj[1], Vladislav B. Bergo[1], Jason J. Amsden[1], Elena N. Spudich[2], John L. Spudich[2], Kenneth J. Rothschild[1]
[1] *Boston University, Boston, MA, USA,*
[2] *University of Texas Medical School, Houston, TX, USA.*

Board B493

Proteorhodopsins are a recently discovered class of microbial rhodopsins, ubiquitous in marine bacteria. Over 4000 variants have thus far been discovered, distributed throughout the world's oceans. Most variants fall into one of two major groups, green absorbing or blue absorbing proteorhodopsin (GPR and BPR, respectively) based on both the visible absorption maxima (530 vs. 490 nm) and photocycle kinetics (~20 vs. ~200 ms). These differences appear

to be determined by the identity of a single residue at position 105 (leucine/GPR, glutamine/BPR). We find using a combination of visible and infrared spectroscopy that a second difference is the protonation state of a glutamic acid residue located at position 142 on the extracellular side of helix D. In BPR, Glu142 (the GPR numbering system is used) is deprotonated and can act as an alternate proton acceptor, thus explaining the earlier observations that neutralization of the Schiff base counterion, Asp97, does not block formation of the M intermediate. In contrast, Glu142 in GPR is protonated and cannot act in this state as an alternate proton acceptor for the Schiff base. These findings support the recent prediction that Arg94 in BPR forms a salt-bridge with Glu142. Disruption of this salt bridge during the BPR photocycle but not in GPR may be a fundamental difference in their respective molecular mechanisms. Furthermore, since the pK_a of Glu142 is near the pH of its native marine environment, changes in pH may act to modulate its function in the cell.

2379-Pos The Effect of Bleaching on the Thermal Stability of Native and Cross-linked Rhodopsin

Thomas Edrington[1], Michael Bennett[2], Arlene Albert[1]

[1] *University of Connecticut, Storrs, CT, USA,*
[2] *NIH, Bethesda, MD, USA.*

Board B494

The photoreceptor protein rhodopsin is a GPCR that has been proposed to exist as a dimer or higher order oligomer in native rod outer segment disk membranes. After activation by light, the dark-adapted form of the receptor rhodopsin is converted to the bleached form opsin. Rhodopsin exhibits considerably greater thermal stability than opsin. This stability is reflected in the thermal denaturation temperature (Tm), which is approximately 15oC higher for rhodopsin than for opsin. In this study we investigate the effect of partial bleaching on the Tm and on the activation energy for thermal denaturation (Eact) of opsin and of rhodopsin to determine if opsin alters the stability of rhodopsin. If mixed oligomers are present we expect the Tm to be perturbed. Rhodopsin in disk membranes was also cross-linked to generate stable dimers to allow us to determine the effect of bleaching on the Tm if dimers were known to be present. Both sets of disk membranes were exposed to light to achieve between 0 and 100% bleaching. Differential scanning calorimetry (DSC) experiments were performed using a MicroCal VP-DSC microcalorimeter. Samples were scanned at rates of 15, 30, 60 and 90o/hr. The Tm for rhodopsin and opsin in native disks were determined in the same resulting excess heat capacity curve. The Tm remained constant for native rhodopsin and opsin regardless of the level of bleaching. The Eact was calculated from the dependence of the protein transition temperature (Tm) on the rate of the increase of temperature (scan rate). In contrast, the thermal characteristics of cross-linked rhodopsin were profoundly altered. The Tm of cross-linked rhodopsin exhibited a strong dependence on the extent of bleaching. We conclude that native rhodopsin behaves as a monomer in isolated disk membranes.

2380-Pos Mutations in Channelrhodopsin 2 (ChR2) reveal a new mechanism for ion conductivity and pH sensitivity

Andre Berndt[1], Katja Stehfest[1], Oliver Ernst[2], Dietrich Gradmann[3], Peter Hegemann[1]

[1] *Humboldt University, Berlin, Germany,*
[2] *Charité Berlin, Berlin, Germany,*
[3] *Albrecht von Haller Institut, Goettingen, Germany.*

Board B495

In the last few years the light activated ion channel ChR2 from Chlamydomonas reinhardtii became a useful tool for Neurophysiologists. However, the mechanisms of ion conductivity and channel activation are largely unknown. We investigated single mutations of ChR2 by two electrode voltage clamp (TEVC) technique and flash photolysis. It is well known that the channel conducts beyond protons also cations with variable efficiency. Hence, it is not unusual that current amplitudes and reversal potentials of the wild type are depend on the external pH and the cation composition at both sites of the membrane. We present here data from single mutations that cause modified pH sensitivities and reversal potentials. The results were fitted to a transport model in order to calculate relative rate constants for cation and proton transport, respectively. We identified residues that are candidates for ion selectivity and/or pH-sensing and responsible for the overall activity of ChR2. Two mutations resulted in outward currents under external acidic conditions suggesting that the ChR2 mechanism is related ion pumps than transport ions actively across the membrane. The results are compared with kinetic studies (photocycle measurements) on purified ChR2 wild type and mutants.

2381-Pos The Role of Cholesterol in the Membrane Environment of the Rhodopsin Dimer Suggests Involvement in the Structural and Functional Asymmetry of G Protein-Coupled Receptor Oligomers

George Khelashvili[1], Marta Filizola[2], Harel Weinstein[1]

[1] *Weill Medical College, New York, NY, USA,*
[2] *Mount Sinai School of Medicine, New York, NY, USA.*

Board B496

Recent experimental findings indicate that the occurrence of asymmetric conformational changes in agonist-bound G protein-coupled receptor (GPCR) dimers depends on their interaction with G proteins [Damian M. et al., 2006, EMBO J., *25*:5693–702]. Since no asymmetric conformational changes have been observed in the absence of G protein, the current hypothesis of GPCR dimer activation is that the direct G protein/receptor dimer interaction is responsible for structural and functional asymmetry in GPCRs. We

present the results of a Molecular Dynamics (MD) simulation study suggesting that the surrounding membrane composition contributes to the propensity for asymmetric changes in GPCR dimers. This inference is reached from a comparison of the prototypic rhodopsin dimer simulated in explicit POPC lipid-containing membrane [Filizola et al., 2006, JCAMD *20*:405–16] with new MD simulations of the same dimeric system in 2:1 POPC/Cholesterol (CHOL) mixed membranes. Specifically, the dynamic behavior of the two interacting protomers of the rhodopsin dimer is found to be different in the two simulations and leads to structural differences resulting from the asymmetric distribution of CHOL around the protomers. The protomer surrounded by the larger number of CHOL molecules is more rigid compared to the other subunit, which may affect the ability to undergo the specific conformational changes required for activation. These results are in line with recent experimental findings [Niu et al., 2002, J. Biol. Chem. *277*(23):20139–20145] showing that high cholesterol content in cell membrane has a suppressing effect on the activation of rhodopsin.

2382-Pos Imaging Heptahelical Receptors in Nanoscale Apolipoprotein Bound Bilayers

Sourabh Banerjee, Thomas Huber, Thomas P. Sakmar

The Rockefeller University, New York, NY, USA.

Board B497

Apolipoprotein A-I and phospholipids self-assemble into discoidal complexes ideally suited for studies of transmembrane proteins including heptahelical G protein-coupled receptors (GPCRs). We have used zebrafish apo A-I to form such discoidal bilayers, called Nanoscale Apolipoprotein Bound Bilayers (NABBs), containing GPCRs in a controlled, soluble, native-like membrane-mimetic environment. We demonstrate a rapid and quantitative method of incorporation of GPCRs into NABBs using bovine rhodopsin as a model system. We developed methods to control the ratio of receptor to NABB and imaged the rhodopsin-NABBs using electron microscopy with two independent labeling techniques to measure stoichiometry and receptor orientation. Rhodopsin reconstituted into NABBs is dramatically more stable than rhodopsin solubilized in commonly used detergents. We conclude from fluorescence G protein activation assays of rhodopsin-containing NABBs that a single rhodopsin is necessary and sufficient for coupling to the G protein transducin. We are currently investigating the feasibility of rapid incorporation of heterologously-expressed receptors into zebrafish apo A-I NABBs in order to avoid long-term exposure to detergents and maximize functional receptor reconstitution.

2383-Pos Are PUFAs G-protein Signal transducers on a Microsecond Timescale?

Thomas H. Haines

Rockefeller University, New York, NY, USA.

Board B498

Polyunsaturated fatty acids (PUFAs), ubiquitous in eukaryotes, are absent in prokaryotes as are G-proteins and G-protein coupled receptors (GPCRs). I propose that GPCRs use PUFAs to signal G-proteins. Rhodopsin is an example. Over 80% of the lipids chains in its disc membrane are 22-carbons long with six double bonds (DHA). The dominant membrane-spanning protein in the rod outer segment (ROS) membrane is rhodopsin. It signals to transducin, a G-protein. One photon activates 300 to 500 transducins. GPCRs and G-proteins always have lipid anchors. The low energy state of PUFAs is helical. In the ROS the main PUFA is DHA (C22:6) It may be stretched to full length (22 carbons) expanding the bilayer locally. Let's say rhodopsin expands perpendicular to the bilayer as a consequence of absorbing a proton. Aided by Helix VIII and its 2 adjacent lipid anchors Helix VII may expand and drag lipids out of the bilayer. This unstable state would then collapse and sends a soliton across the membrane. As the soliton (tsunami) peak hits transducins it separates their α- from their $\beta\gamma$-subunits. The erect PUFAs would amplify such a signal. In this model, GTP's cleavage destabilized the G-protein making it inactive and sensitive to cleavage by the wave. The wave crosses the entire ROS disc in 2 microseconds. Transducin site-specific mutations near the lipid anchor on the α- subunit interfere with G-protein cleavage. The model would presumably also apply to hormone GPCRs. The hormones bind to the interior of the GPCR even the large peptide hormones. The PUFAs, primarily on the inner monolayer, need only activate a few G-proteins to activate an enzyme. The wave need not travel as far.

Photosynthesis

2384-Pos Charge And Protein Dynamics In The Reaction Center Of Purple Bacteria *Rhodobacter Sphaeroides* Studied By Ultrafast Mid-infrared Spectroscopy

Natalia P. Pawlowicz[1], Ivo H. M. van Stokkum[1], Michael R. Jones[2], Jacques Breton[3], Marie-Louise Groot[1], Rienk van Grondelle[1]

[1] *Vrije Universiteit, Amsterdam, The Netherlands,*
[2] *University of Bristol, Bristol, United Kingdom,*
[3] *CEA-Saclay, Saclay, France.*

Board B499

Time-resolved visible pump/mid-infrared probe spectroscopy was applied to investigate electron transfer and radical pair/protein relaxation at room temperature in the *Rb. sphaeroides* RC. Wild-type RCs both with and without the quinone electron acceptor Q_A, the Alanine M260W and Tyrosine M210W mutants, were excited at 600 nm, 800 nm, 794 nm and 860 nm. The region between 1600 cm^{-1} and 1800 cm^{-1} encompasses absorption changes associated with carbonyl stretch vibrational modes of the cofactors and protein. A sequential analysis and simultaneous target analysis of the data showed a free energy drop in P_LP_M bands due to charge-transfer