are subjected to fluid shear stress or osmotic swelling induce substantial changes in lipid diffusion. These results suggest that tension directly causes changes in lipid diffusion, which may play a role in activation of integral membrane proteins. Conversely, this direct relationship may allow one to determine membrane stresses in cells from measured diffusion coefficients.

1019-Plat
Hydrophobic Mismatch: A universal Tool for Clustering, Demixing, and Sorting of Transmembrane Proteins
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Sorting of transmembrane proteins is a central task of eucaryotic cells, in particular in the secretory pathway.
Due to a lack of an organizing mastermind the decision whether a membrane protein participates in secretory transport or not has to be made by a self-organization process on the molecular scale, e.g. via cluster formation. We show by means of coarse-grained membrane simulations that hydrophobic mismatching can drive cluster formation of transmembrane proteins [1]. Also, proteins with different degrees of hydrophobic mismatching can segregate and form homo-oligomers. In addition, we show that proteins partition into the lipid phase with the smallest hydrophobic mismatch if the membrane has a heterogeneous composition. Our data thus indicate that hydrophobic mismatching may help to organize trafficking along the secretory pathway in living cells.
[1] U. Schmidt, G. Guigas & M. Weiss, Phys. Rev. Lett. 101, 128104 (2008).

1020-Plat
Backbone Conformation and Dynamics of the Lipid-Modified Membrane Anchor of Human N-Ras Investigated by Solid-State NMR and Molecular Dynamics Simulations
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Many proteins involved in signal transduction are anchored to membranes by covalently attached lipid modifications. In this study we investigated the conformation and dynamics of the backbone and side chains of the N-Ras membrane anchoring domain. Experimental solid-state NMR studies involved doubly lipid-modified uniformly ^{13}C and ^{15}N labeled heptapeptides representing the C-terminus of N-Ras, which were incorporated into DMPC bilayers. A structural model of the peptide was calculated on the basis of isotropic chemical shifts, explicit torsion angle measurements, and nuclear Overhauser effects determined by solid-state NMR. The amplitude of molecular motions was assessed by ^{1}H-^{13}C order parameter measurements using separated local field NMR. For determination of the correlation times of the motions, T_1 and T_2 relaxation times were measured and analyzed using a generalized relaxation approach. To further understand the dynamics of Ras, molecular dynamics simulations of the molecule in lipid bilayers were conducted. In generating starting conditions for the simulation, special attention was paid to the backbone conformation since transitions between conformations were found to be rare events in a previous simulation of 100 ns length on this system [1]. Therefore, the experimentally determined conformation of the peptide backbone was equilibrated using a replica exchange technique in an explicit membrane environment. This enabled us to identify different conformers and to assess their relative probability. The resulting distribution of conformations was used subsequently for a long conventional MD simulation that was analyzed with regard to the experimental data. The combined simulations and experimental approach enabled a detailed model of the dynamics of the peptide to be obtained.
[1] Vogel, A. Tan, K.-T. Waldmann, H. Feller, S.E. Brown, M.F. Huster, D. *Biophys. J.* **2007**, *93*, 2697-2712.

1021-Plat
Subdiffusion And Diffusion Of Lipid Atoms And Molecules: Relating The Dynamics Of Lipids To Neutron Scattering Experiments
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Inelastic neutron scattering (INS) experiments, based on latest generation of neutron sources, allow us to gain insight into the complex dynamics of lipid molecules in biologically relevant phospholipid bilayers. However, the proper interpretation of the INS scattering experiments requires theoretical and computational models that correctly capture the main features of lipid dynamics at atomic and molecular levels. To this end, here we use a 0.1 microsecond all-atom molecular dynamics simulation to investigate the dynamics of lipid atoms and molecules in a hydrated diyristoyl-phosphatidycholine (DMPC) lipid bilayer. First, as predicted by theories of polymer dynamics, we identify three well separated dynamic regimes in the mean square displacement of the lipid atoms and molecules: (1) a ballistic regime where the mean square displacement increases as the square of time for $t < 10$ femtoseconds; (2) a subdiffusive regime where the mean square displacement increases with a sub-linear power law for times between 10 picoseconds and 10 nanoseconds; and (3) a Fickian diffusion where the mean square displacement increases linearly in time for $t > 30$ nanoseconds. Next, we show that the cumulant approximation of the self-intermediate scattering function (which is the inverse Fourier transform of the dynamic structure factor measured in INS experiments) is in very good agreement with the simulation results, and allows us to connect the three time scales in the mean square displacement to the interpretation of neutron scattering results. Finally, we focus on the hydrogen atoms (which represent the main source of the incoherent INS signal) in the lipids and draw conclusions about the lipid dynamics by examining the wave-vector dependence of the intermediate scattering function.
Computer time was generously provided by the University of Missouri Bioinformatics Consortium.

1022-Plat
Towards Subcellular Tissue Sampling by Near-Field Laser Ablation: A 'Protein Microscope' to Map Peptide Distributions in Cells
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We report on the development of a new instrument, dubbed a 'Protein Microscope,' that uses near-field optical techniques to increase the spatial resolution of atmospheric pressure matrix-assisted laser desorption and ionization (AP-MALDI). This functions as a novel front-end for time-of-flight mass spectrometry. Standard protein identification techniques involve homogenization of a tissue sample, which destroys all spatial and temporal information about the expressed proteins. Our new NSOM-based instrument will allow the identification and mapping of proteins expressed in intact cells and tissues, which is of great interest as protein expression connects genomic information with the functioning of an organism. This poster will focus on the development of near-field-based ablation of sub-cellular-sized regions of tissue and plant samples.

1023-Plat
A Biomolecular Photodiode For Imaging Of Cell Membrane Potential
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Despite the recognized importance of electrical signals in many biological systems, there has been very limited success in the creation of a robust fluorescent voltage sensor. Using standard molecular biology techniques, we have created a biomolecular photodiode consisting of a membrane-bound cytochrome c protein fused with a GFP (green fluorescent protein) variant. A similar photodiode assembly has been shown to produce unidirectional photocurrent in vitro with the cytochrome acting as an acceptor of excited electrons from the FP donor upon excitation with visible light. Electron transfer between the cytochrome and the FP is a highly voltage dependent process. By embedding this assembly in the plasma membrane of living cells, it is subjected to the same electric potential as the membrane. As the membrane potential of the cell changes over ~100 mV, as in an action potential, the extent of electron transfer should vary significantly, manifesting as a change in fluorescence intensity of the FP donor. As this is a very fast process with a high sensitivity to changes in electric potential, this biophotodiode is expected to form a robust sensor of electrical activity in cells. The feasibility of the sensor is investigated in several ways, including modeling, electrophysiology, and direct application of current to purified membrane fragments.

Platform O: Phototransduction: Signaling Events Downstream of Photon Absorption

1024-Plat
Structure and Dynamics of Signal Transducing Membrane Complexes
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We have used electron cryo-microscopy (cryo-EM) of single particles (individual protein complexes) and two-dimensional crystals along with fluorescence resonance energy transfer (FRET) and fluorescence recovery after

photobleaching (FRAP) to determine structures and dynamics of complexes formed by the photoreceptor G protein transducin, and its effector enzyme, cGMP phosphodiesterase, PDE6. Cryo-EM studies of complexes tagged with Fab fragments of monoclonal antibodies revealed that the inhibitory gamma subunit of PDE6, PDE6γ, stretches from the catalytic domain of PDE6 where its carboxyl-terminal region binds, to the GAFa domain, where its amino terminus binds, consistent with previous photo-crosslinking studies. FRET reveals that in unbound PDE6γ the amino and carboxyl termini are fairly close to one another. Upon addition of the catalytic subunits of PDE6, there is an initial very fast (near diffusion limit) binding to the catalytic domain, followed by a very slow (minutes) stretching out and binding of the amino terminal region to the distant GAFa domain. FRAP measurement of diffusion in living rod cells of transgenic *Xenopus laevis* revealed free diffusion of transducin along the long axis of cell, and the presence in disk membranes of cholesterol-dependent lipid microdomains, into which transducin complexes segregate upon activation.

1025-Plat
Mouse Cone Opsins Require An Arrestin For Normal Inactivation
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Arrestins are a family of proteins that arrest the activity of phosphorylated G-protein coupled receptors (GPCRs). While it is well established that rhodopsin, the GPCR of rod phototransduction, requires an arrestin (ARR1) for normal inactivation, the requirement for an arrestin for cone opsin inactivation has been disputed. We established that mouse cones express two distinct visual arrestins, Arr4 (alias "cone arrestin") and Arr1, and by recording electrical responses of the cones of WT mice, and mice with one or both the arrestins knocked out, established that an arrestin is required for normal inactivation of both mouse S-opsin and M-opsin. We also estimated the expression levels of Arr1 and Arr4 in cones, and established that both arrestins at their normal expression levels are competent to support inactivation. The complete absence of Arr1 and Arr4 from cones, however, slows down cone inactivation much less than does the absence of Arr1 from rods.

1026-Plat
Light-Dependent Translocation of Arrestin in Rod Photoreceptors is Signaled through a Phospholipase C Cascade and Requires ATP
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Light adaptation of rod photoreceptors induces translocation of arrestin from inner segments (IS) to outer segments (OS). Our study suggests that components of the G-protein linked phosphoinositide pathway play a role in signaling the initiating events of arrestin translocation. We show that arrestin translocation can be stimulated by activators of phospholipase C (PLC) and protein kinase C (PKC) in the absence of light. Conversely, arrestin translocation to the OS is significantly slowed by inhibitors of PLC and PKC.
In the second part of this study, we investigated the mechanism by which arrestin translocates in response to light. Other investigators have suggested that arrestin translocation between the OS and IS is a passive process, resulting from arrestin's binding affinity for light-activated, phosphorylated rhodopsin in the outer segments and affinity for microtubules in the inner segments. The central tenet of this model is that arrestin's translocation is an energy independent process. In our investigation of this process, we found that treatment of Xenopus retinas with potassium cyanide inhibits arrestin translocation to the OS in response to light, but that translocation can be restored by the removal of cyanide and addition of ATP. These results were confirmed in the mouse retina and clearly suggest that at least one step in arrestin translocation requires ATP. We also found that an arrestin with scrambled C-terminal 30 amino acids retained its binding for both activated rhodopsin and microtubules, but yet was unable to translocate in response to light. The results obtained from both investigating the signaling cascade and the mechanisms of arrestin translocation indicate that arrestin translocation between the IS and OS is more complex than previously proposed, and likely involves both diffusion and motor-assisted processes.

1027-Plat
NCKX Reaction Cycle: ATP, Voltage And Ion Regulation
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Ca^{2+} concentration in photoreceptor rod outer segment (OS) strongly affects the generator potential kinetics and the receptor light adaptation. The response to intense light stimuli delivered in the dark produce voltage changes exceeding 40 mV: since the Ca^{2+} extrusion in the OS is entirely controlled by the Na^+:Ca^{2+}, K^+ exchanger (NCKX), it is important to assess how the exchanger ion transport rate is affected by the voltage and, in general, by intracellular factors (like Mg-ATP, known to regulate the Na^+:Ca^{2+} exchanger). The NKCX regulation was therefore investigated in isolated OS, recorded in whole-cell configuration, using ionic conditions that activated maximally the exchanger in both forward and reverse mode. In all species examined (amphibia: *Rana esculenta* and *Ambystoma mexicanum*; reptilia: *Gecko gecko*), the forward (reverse) exchange current increased about linearly for negative (positive) voltages and exhibited outward (inward) rectification for positive (negative) voltages. Since hyperpolarization increases Ca^{2+} extrusion rate, the recovery of the dark level of Ca^{2+} (and, in turn, of the generator potential) after intense light stimuli results accelerated. Mg-ATP increased the size of forward and reverse exchange current by a factor of ~2.3 and ~2.6, respectively, without modifying their voltage dependence. This indicates that Mg-ATP regulates the number of active exchanger sites and/or the NCKX turnover number, although through an unknown mechanism. The ion transport mechanism was further investigated by using voltage and Ca^{2+} jumps (achieved via photolysis of caged-Ca^{2+} or fast solution changes) and by studying the NCKX selectivity in different ionic conditions. Ca^{2+} jumps, but not voltage jumps, produced current transients, possibly originating from electrogenic partial reactions. No monovalent cation substituted for Na^+ at the NCKX binding sites, but Rb^+ substituted for K^+, while Sr^{2+}, Ba^{2+}, Mg^{2+} substituted for Ca^{2+} with an apparent permeability ratio of 0.78, 0.20, $<$0.05:1, respectively.

1028-Plat
Regulation Of Photoreceptor Guanylyl Cyclase By Ca2+/Mg2+ Exchange In GCAPs
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Photon absorption by rods and cones activates transduction cascade that shuts down cGMP-gated channels and thus decreases free Ca^{2+} concentrations in outer segment. The Ca^{2+} feedback, which activates guanylyl cyclase (retGC) through guanylyl cyclase activating proteins (GCAPs), accelerates cGMP resynthesis in photoreceptors and thus expedites their recovery. GCAP1 and GCAP2, two ubiquitous among vertebrate species GCAPs that sequentially activate retGC during physiological response of rods to light, are Ca^{2+}/Mg^{2+}-binding proteins. They have one non-metal binding EF-hand, EF1, and three metal-binding EF-hands - EF2, EF3, and EF4. For each metal-binding EF-hand in GCAP1 we found point mutations that can block binding of Ca^{2+}, but not Mg^{2+}, and those that can block both Ca^{2+} and Mg^{2+} binding. We tested their effects on activation of retGC at physiological Mg^{2+} and either low Ca^{2+} (conditions representing light adaptation) or high Ca^{2+} (dark adaptation). Mg^{2+} binding in EF-2 and EF3 was essential for activation of retGC in the conditions of light adaptation. Mg^{2+} in EF2 was especially critical for the binding of GCAP1-GFP to retGC1 in co-transfected HEK293 cells, as revealed by confocal fluorescence microscopy. Mg^{2+} binding in EF4 contributed to neither retGC1 docking nor its activation. Instead, the replacement of Mg^{2+} by Ca^{2+} in this domain in the conditions of dark adaptation was the key event that switched the cyclase off. The Mg^{2+}/Ca^{2+}exchange in EF3 was required for the subsequent binding of Ca^{2+} in EF4. Contrary to EF3 and EF4, Mg^{2+}/Ca^{2+} exchange in EF2 was not essential for retGC inhibition. Binding of Mg^{2+} *versus* Ca^{2+} causes characteristic changes in the intrinsic Trp fluorescence of GCAP1 corresponding to its activator *versus* inhibitor states, including the non-metal binding EF1.

1029-Plat
Phototransduction Cascade Inactivation Kinetics Depend on Experimental Solutions
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Significant variation exists in published measurements of the kinetics and sensitivity of mouse rod photoreceptors; this variation includes ~2× differences in the amplitude of the single-photon response and the half-maximal flash strength, and ~1.5× differences in the time-to-peak of the dim flash response. The most obvious discrepancy between these studies is in the conditions used to store and perfuse the retina during the course of the experiment. Here we characterize changes in phototransduction produced by different recording conditions. Consistent with past work, the sensitivity and response kinetics depended strongly on recording conditions. These differences resulted, at least in part, from an apparent change in relative time constants of rhodopsin and transducin inactivation. These results underscore the importance of identifying experimental conditions that closely resemble the physiological environment of the retina to relate properties of phototransduction to downstream processing and visually-guided behavior.