

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.

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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 ¹³C and ¹⁵N 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 ¹H-¹³C 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 diystoyl-phosphatidylcholine (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.

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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