Membrane Dynamics & Bilayer Probes II

1816-Pos Board B660

Lipid-Protein Interactions of Rhodopsin Investigated by Molecular Dynamics Simulations

Alexander Vogel[1], Alan Grossfield[2], Michael C. Pitman[3], Scott E. Feller[4], Michael F. Brown[5].
[1]Martin-Luther-Universität Halle-Wittenberg, Halle, Germany, [2]University of Rochester Medical Center, Rochester, NY, USA, [3]IBM TJ Watson Research Center, Yorktown Heights, NY, USA, [4]Wabash College, Crawfordsville, IN, USA, [5]University of Arizona, Tucson, AZ, USA.

Rhodopsin is currently one of two proteins of the G protein-coupled receptor (GPCR) family for which an atomic-resolution structure is available and therefore it serves as a prototypical GPCR. Over 50% of recently launched drugs are targeted to GPCRs so that these proteins are of outstanding pharmaceutical interest. Yet many aspects of the structure-function relations of rhodopsin are only poorly understood. Especially interesting is the important role played by highly unsaturated lipids in achieving full functionality. In this study we investigated lipid-protein interactions of rhodopsin through a series of molecular dynamics (MD) simulations. Rhodopsin was inserted into a membrane consisting of a 2:2:1 mixture of 18:0,22:6n3PE, 18:0,22:6n3PC, and cholesterol. MD simulations with a total trajectory of ~4 microseconds were conducted on the IBM Blue Gene/L supercomputer. Additional simulations were carried out for rhodopsin in membranes consisting of 18:0,18:1PC and 14:0,14:1PC. This allowed direct comparison of the lipid-protein interactions of rhodopsin in monounsaturated and polyunsaturated environments as well as bilayers of varying thickness. Our analysis focuses on the palmitoylations of two adjacent cysteins in helix 8 of rhodopsin which are a common structural motif in many proteins related to signal transduction. As seen in our earlier studies on lipid modifications of smaller proteins, these are generally highly flexible and best characterized by their dynamical structure. We calculated order parameters and correlation functions from the simulation trajectories and compared them to experimentally obtained results on lipid modifications of other proteins. In general we find the that the palmitoyl modifications of rhodopsin are highly flexible which can affect the binding free energy of the polypeptide to the membrane surface. Entropic contributions due to the configurational disorder of the posttranslational modifications reduce the binding energy so that doubly palmitoylated modifications are needed.

1817-Pos Board B661

Dynamic Structure Factors From Lipid Membrane Molecular Dynamics Simulations

Erik G. Brandt, Olle Edholm.
Depart ment Theoretical Physics, Royal Institute of Technology, Stockholm, Sweden.

The dynamics of biological membranes is present on many time- and length scales, from the pico-second vibrations of single lipid tail groups to micro-second collective undulations of hundreds of lipid molecules. These motions can be probed as local density fluctuations, and characterized and measured in different ways. Inelastic scattering experiments as well as computer simulations of biological membranes are usually theoretically interpreted within the framework of generalized hydrodynamics, where the dynamic structure factor, S(k,w), is the quantity of interest. S(k,w) is the space and time Fourier transform of the density-density correlation function and contains all the relevant information about the dynamics of a liquid system. We have performed large-scale molecular dynamics simulations from which we have calculated the dynamic structure factors for a lipid bilayer in the high temperature phase, enabling a thorough test of theoretical predictions, especially in the hydrodynamic limit of low wave vectors. The frequency and wave vector resolutions are considerably improved compared to traditional experiments, which make it possible to directly resolve the lines of the power spectrum. Membrane material constants have been determined and compared in fair agreement to experimental data. In addition, from the power spectrum we can distinguish two dispersive contributions to the elastic scattering. These correspond to two exponential relaxation processes on separate time scales. For low wave vectors, this analysis was impossible due to insufficient frequency resolution. To be able to fully decouple and analyze the nature of these dispersive modes from simulations, longer trajectories are required.

1818-Pos Board B662

Anomalous Diffusion Of Lipid Atoms And Molecules In Phospholipid Bilayers: A Combined Molecular Dynamics And Theoretical Study

Jhuma Das[1], Elijah Flenner[1,2], Maikel Rheinstädter[1], Ioan Kosztin[1].
[1]University of Missouri, Columbia, MO, USA, [2]Colorado State University, Fort Collins, CO, USA.

Individual atoms and lipid molecules in biologically relevant phospholipid bilayers have an extremely rich dynamics that extend on a wide range of time and length scales. Computer modeling and simulations can be used effectively both to investigate the dynamics of such complex systems and to interpret the results from a variety of experimental techniques employed to probe such systems. Here we present an all-atom molecular dynamics (MD) simulation, combined with theoretical modeling, to investigate the dynamics of selected lipid atoms and lipid molecules in a hydrated diyristoyl-phosphatidylcholine (DMPC) lipid bilayer. From the analysis of a 0.1 microsecond MD trajectory we find that the time evolution of the mean square displacement (MSD) of lipid atoms and molecules exhibits three well separated dynamical regions: (1) for short times ($t <$ 10 fs) the motion is ballistic with a quadratic in time MSD; (2) for intermediate times (10 ps $<t$ 30 ns) the MSD is linear in time, corresponding to ordinary Fickian diffusion. The origin of the extended anomalous, sub-diffusive region is attributed to the polymeric nature of the lipid molecules, characterized by connectivity and flexibility. We propose a memory function approach for calculating the MSD over the entire time range, from the ballistic to the Fickian diffusion regimes. The lateral self-diffusion coefficient of lipid molecules determined by employing the memory function approach is found to be in good agreement with the one calculated directly from the long time MD trajectory of the lipid bilayer. The proposed memory function approach is a useful tool for interpreting neutron scattering experiments on lipid membranes.
Computer time was generously provided by the University of Missouri Bioinformatics Consortium.

1819-Pos Board B663

Fusion of Biomimetic 'Stealth' Probes into Lipid Bilayer Cores

Benjamin D. Almquist, Nicholas A. Melosh.
Stanford University, Palo Alto, CA, USA.

The ability to specifically and non-destructively incorporate inorganic structures into or through biological membranes is essential to realizing full bio-inorganic integration. This research explores a new method for interfacing inorganic structures with cellular membranes using nanometer-scale hydrophobic bands to specifically interact with the lipid bilayer core. Similar to the structure of transmembrane proteins, thin hydrophobic bands allow targeted formation of interfaces between lipid bilayers and macroscopic inorganic objects.
In this work, we show that by fabricating custom atomic force microscopy (AFM) probes that possess 2-10 nm hydrophobic bands formed through molecular self assembly, specific interaction with the hydrophobic core of lipid bilayers can be achieved. When hydrophilic probes are penetrated through stacks of lipid bilayers they exhibit distinct breakthrough distances of 2.9 +/− 0.3nm, corresponding to the hydrophobic core thickness of 2:1 SOPC:Cholesterol bilayers, followed by linear relaxation through the water layer between bilayers. In contrast, 'stealth' probes possessing a thin hydrophobic band exhibit strong association with the bilayer core, ''jumping'' 5.6 +/− 0.6nm from bilayer core to bilayer core, which corresponds to the compressed lamellar spacing of the bilayer stacks.
In addition, by using AFM, it is possible to examine how the properties of the hydrophobic band affect the adhesion strength between the bilayer and stealth probe. Molecular mobility, hydrophobicity, and orientation all have a role in determining the interface behavior, and in turn the potential applications for a given surface functionalization.

1820-Pos Board B664

Simulations of the Morphological Evolution of Lipid Bilayer Membranes Using a Phase-Field Method

Chloe M. Funkhouser[1], Francisco J. Solis[2], Katsuyo Thornton[1].
[1]University of Michigan, Ann Arbor, MI, USA, [2]Arizona State University, Glendale, AZ, USA.

We present a continuum-level simulation method for modeling phase separation and morphological evolution of multicomponent lipid bilayer membranes. Our objective is to investigate how various physical parameters input into the model, such as spontaneous curvature, phase fraction, and interleaflet coupling strength, affect the dynamics and equilibrium morphological phases formed in two-phase lipid bilayer membrane systems. The model applies to membranes with planar and spherical background geometries, simulating a nearly planar portion of membrane or entire vesicle, respectively. The compositions and shape of the membrane are coupled through a modified Helfrich free energy. The planar model treats the composition of each leaflet, and thus includes a term coupling these compositions across the bilayer. The compositional evolution is modeled using a phase-field method and is described by a Cahn-Hilliard-type equation, while shape changes are described by relaxation dynamics. For nearly planar bilayer systems with each leaflet having the same phase fraction, we find that domains in both leaflets align to reduce the interaction energy, as expected. When the coupling effect is stronger, this alignment occurs more

quickly and more precisely, showing that the coupling affects the dynamics. This leaflet coupling is found to heavily influence morphological evolution; in some cases the equilibrium morphological phase observed is very different from what was observed with our simpler monolayer model using similar conditions. We construct a phase diagram of equilibrium morphological phases in the composition space for a few values of the strength of the leaflet coupling. This model has been able to reproduce results found in lipid bilayer experiments probing interleaflet interactions, including the effect of domain registration across leaflets. For the vesicle model, we investigate how an ellipsoidal geometry imposed in the initial conditions affects the phase and morphological evolution.

1821-Pos Board B665

The Ionic State Of Ceramide 1-phosphate Affects Raft Domain Morphology And Fluidity

Zhiping Jiang, Arne Gericke.
Kent State University, Kent, OH, USA.

Ceramide 1-phosphate (Cer1P) is involved in cell survival, cell proliferation, inflammation and phagocytosis processes. Physiological processes that have been associated with Cer1P have been shown to be in some cases lipid raft dependent. Lipid rafts are proposed to exist in a liquid-ordered state it has been suggested that raft domains are involved in a variety of important biological processes. It has been shown that ceramide forms gel phase domains within the liquid-ordered raft domains and the question arises what kind of phase state Cer1P adopts when immersed in a raft domain. The physicochemical behavior of Cer1P is mainly routed in the protonation state of the phosphate headgroup. To investigate the phase behavior of Cer1P in raft domains, giant unilamellar vesicles (GUVs) composed of POPC/Sphingomyelin/Chol (1:1:1) with different concentrations of Cer1P were studied by fluorescence microscopy at buffers with different pH (pH5, pH7 and pH9). For a pH 7 buffer, the presence of Cer1P disrupted raft domains and induced lipid phase reorganization and the appearance of a Cer1P-enriched gel phase. In contrast to the large platforms reported for ceramide, the presence of Cer1P disrupts rafts. For pH 5 buffer, with increasing concentrations of Cer1P, the domain patterns were totally different from those observed for pH 7 buffer. The Cer1P gel phase disappeared completely and the raft type liquid disordered phase became dominant. In pH 9 buffer, the ability of Cer1P to disrupt rafts was attenuated. These experiments demonstrate that the protonation state of the phosphate headgroup affects the phase behavior of Cer1P within the raft. The headgroup of Cer1P might function as an electrostatic switch that drives the lipid in and out of gel phase domains which may modulate its availability to the relevant proteins.

1822-Pos Board B666

Interdigitation, Domains and Morphology, in Membranes of the Chain Asymmetric C24:1 Ceramide

Sandra N. Pinto[1], Liana C. Silva[1], Rodrigo F.M. de Almeida[2], **Manuel Prieto[1]**.
[1]Centro de Química-Física Molecular and IN, Lisbon, Portugal.
[2]Centro de Química e Bioquímica, Faculdade de Ciéncias, UL, Lisbon, Portugal.

Ceramide (Cer) is involved in the regulation of several biological processes, such as apoptosis and cell signaling. The alterations induced by Cer in the biophysical properties of membranes are thought to be one of the major routes of Cer action. To gain further knowledge about the alterations induced by Cer, membrane reorganization by the very long chain asymmetric nervonoylceramide (NCer) was studied. The application of an established fluorescence multiprobe approach, together with x-ray diffraction, differential scanning calorimetry, and confocal fluorescence microscopy, allowed the characterization of NCer and the determination of the phase diagram of palmitoyloleoylphosphatidylcholine (POPC)/NCer binary mixtures. Nervonoylceramide undergoes a transition from a mixed interdigitated gel phase to a partially interdigitated gel phase at 20°C, and a broad main transition to the fluid phase at 52°C. The solubility of NCer in the fluid POPC is low, driving gel-fluid phase separation, and the binary-phase diagram is characterized by multiple and large coexistence regions between the interdigitated gel phases and the fluid phase. At 37°C, the relevant phases are the fluid and the partially interdigitated gel. Moreover, the formation of NCer interdigitated gel phases leads to strong morphological alterations in the lipid vesicles, driving the formation of cochleate-type tubular structures.
Biophys. J. 2008 95: 2867-2879

1823-Pos Board B667

Interaction of Antimicrobial Oligomers with Lipids Studied by Solid-State NMR

Weiguo Hu, Abhigyan Som, Gregory N. Tew.
University of Massachusetts, Amherst, MA, USA.

A family of synthetic mimic of antimicrobial peptides (SMAMP), amphiphilic *meta*-phenylene ethynylene (mPE) molecules show a wide range of antimicrobial activity and specificity. The interaction of a specifically active mPE molecule (AMO-2) with mixed DOPE/DOPG lipid was studied by solid-state NMR. The AMO-2 molecules do not preferentially interact more strongly with either lipid component, but rather are well dispersed in the lipid matrix. AMO-2 intimately interacts with all parts of lipid molecules, including head groups. Magic-angle spinning sideband analysis indicated that in samples with co-existing lamellar and inversed hexagonal phases (H_{II}), neither lipid component aggregate in either phase. The presence of AMO-2 molecules causes dynamic disorder in lipid head groups, as evidenced by the broadening of both static and MAS ^{31}P spectra. AMO-2 molecules do not massively transform the lamellar lipid into H_{II} phase.

1824-Pos Board B668

Phase Separation in Binary Mixtures of Bipolar and Monopolar Lipid Dispersions Revealed by Solid-State 2H NMR Spectroscopy and Small Angle X-ray Scattering

Andrey Struts[1], David P. Holland[2], Gabriel Longo[2], Matthew J. Justice[3], Igal Szleifer[2], Avigdor Leftin[1], Horia I. Petrache[3], Michael F. Brown[1], David H. Thompson[2].
[1]University of Arizona, Tucson, AZ, USA, [2]Purdue University, West Lafayette, IN, USA, [3]Indiana University-Purdue University, Indianapolis, IN, USA.

Binary mixtures of C20BAS and POPC membranes have been studied by 2H NMR spectroscopy and small angle X-ray scattering (SAXS) over a wide range of concentrations and at different temperatures. Experiments tested the possibility of formation of phase-separated lipid domains predicted by the mean field theory [1]. Membranes composed of three specifically deuterated C20BAS derivatives [1,1,20,20-2H4]C20BAS, [2,2,19,19-2H4] C20BAS, and [10,11-2H2] C20BAS with protiated POPC and with membranes containing POPC-d31 and fully protiated bolalipid were used in NMR experiments to obtain structural information for the mixture. The 2H NMR spectra of 10,11-2H2-C20BAS:POPC membrane dispersion reveal that the bolalipid is predominantly in the transmembrane conformation at high bolalipid concentrations. At 50 mole percent C20BAS and lower, components appear in the spectra with smaller quadrupolar couplings, most likely indicating the presence of U-shaped conformers. The proportion of U-shaped bolalipids becomes more prominent as the amount of POPC in the membrane increases. However, the transmembrane component is still the dominant bolalipid conformation in the membrane even at 45 °C and 10 mole percent C20BAS, where it accounts for roughly 50% of the bolalipid population. The large fraction of C20BAS transmembrane conformers regardless of the C20BAS:POPC ratio together with POPC-bolalipid hydrophobic mismatch can be explained by co-existence of bolalipid-rich domains separate from the POPC-rich domains. In SAXS experiments only a single distinct lamellar repeat distance was observed, corresponding roughly to the average of bolalipid-rich and POPC-rich domains. These observations are consistent with the presence of microphase-separated domains in the mixed membrane samples. [1] G.S. Longo et al. (2007) Biophys. J. 93, 2609.

1825-Pos Board B669

Interactions of Ceramide and Sphingomyelin Quantified in Mixtures with an Unsaturated Phosphatidylcholine

Bruno M. Castro[1], Rodrigo F.M. de Almeida[2], Liana C. Silva[1], Manuel Prieto[1].
[1]CQFM and IN, IST, Lisboa, Portugal, [2]CQB, FCUL, Lisboa, Portugal.

Over the past two decades, the discovery of sphingolipid signaling pathways has stimulated research on the biochemistry and biophysics of these molecules. The involvement of membrane biophysical changes is important but difficult to assess. A detailed characterization of these changes can be obtained in systems where the global lipid composition is controlled and known, as in the case of model membranes.
To better understand how sphingolipids modulate the biophysical properties of the membrane, the interactions between palmitoyl-ceramide (PCer) and palmitoyl-sphingomyelin (PSM) were studied in the presence of the fluid and naturally abundant phospholipid palmitoyl-oleoyl-phosphatidylcholine (POPC) in membrane model systems [1]. The use of two fluorescent membrane probes, distinctly sensitive to lipid phases allowed a thorough biophysical characterization of the system. In these mixtures, PCer recruits POPC and PSM in the fluid phase to form extremely ordered and compact gel domains. Gel domain formation by low PCer mol fraction (up to 12 mol %) is enhanced by physiological PSM levels (20-30 mol % total lipid). For higher PSM content, a three-phase situation, consisting of fluid (POPC-rich)/gel (PSM-rich)/gel (PCer-rich) coexistence, is clearly shown. To determine the fraction of each phase a quantitative method was developed. This allowed establishing the complete ternary phase