Cholesterol is essential in formation and stabilization of raft-like structures in membranes. It is known with certainty that the formation of raft-like domains is due to preferential interaction of cholesterol with the saturated and unsaturated chains. In this study we computed the free energy of transfer of cholesterol in lipid bilayers with varying degrees of saturation. We used the weighted histogram analysis method (WHAM) to compute these free energy profiles. These simulations consisted of hydrated bilayers made up of 200 lipids of different chain saturations. In particular we used DPPC, POPC and DOPC lipid bilayers with two cholesterol molecules symmetrically transfered. Our calculations show energy and entropic components of free energy and demonstrate the role of lipid-lipid interactions in the transfer process.

3123-Pos Board B170

Influence of α-helical Transmembrane Peptides on the Affinity of Sterols for Phospholipid Bilayers

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It is well known that lipids can segregate laterally into nanoscopic domains or different phases. Yet very little is known about proteins can influence the lateral organization in cellular membranes. As most biomembranes contain relatively high concentrations of transmembrane proteins it is important to learn more about how lipid-protein interplay affects the lateral organization in membranes. Cholesterol is thought to have an important role in lateral organization of eukaryotic cell membranes. As cholesterol also has been implicated to take part in the sorting of cellular transmembrane proteins it is a good starting point to determine how transmembrane proteins influence the lateral sorting of cholesterol in phospholipid bilayers. Insight into this can be obtained by studying how cholesterol interacts with bilayer membranes of different composition in the presence of different transmembrane peptides, mimicking the transmembrane helices of proteins. For this purpose an assay, in which the partitioning of the fluorescent cholesterol analogue cholestatrienol (CTL) between large unilamellar vesicles (LUVs) and methyl-β-cyclodextrin (CD) can be measured, has been developed. The partition assay showed that CTL partition preferentially into fluid phospholipid bilayers with a more ordered acyl chain region, as has been observed previously with cholesterol. It is known that proteins can decrease or increase the order in lipid bilayers and that the nature of this effect is dependent on both the structure of the protein and the composition of the bilayer. In order to assess how such protein induced order changes in the lipid bilayer affects cholesterol partitioning we have measured CTL's affinity for bilayers with varying lipid composition and containing various transmembrane peptides.

3124-Pos Board B171

A 2h-nmr Study Of Popc/sterol Membranes: Some Exciting Anomalies

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In a recent article [1], Y-W Hsueh et al showed that the 2H-NMR order parameter, M1, of 1-[2H31]palmitoyl, 2-oleoyl, sn-glycero-3-phosphocholine (POPC)/ergosterol multi-bilayers at 25oC increased linearly as a function of ergosterol concentration to 25 mol%, but did not increase further when more ergosterol was added. By contrast, M1 for POPC/cholesterol bilayers increases linearly to at least 50% sterol. Now the structural difference between cholesterol and ergosterol is that ergosterol has an additional double bond in its fused ring (C7-8) and a trans double bond (C22-23) plus a methyl group (at C24) in its alkyl chain. The question then arises as to which of these structural changes is responsible for the observed saturation of the order parameter in POPC/ergosterol bilayers. In [1] it was shown that the M1 of POPC/7-dehydrocholesterol (7-DHC) multilayers behaves similarly to that of POPC/cholesterol, increasing linearly with [7-DHC]. Note that 7-DHC has an ergosterol fused ring structure but a cholesterol alkyl tail. To further explore this phenomenon, we determined the sterol concentration dependence of POPC containing brassicasterol, a phytosterol that has the same fused ring structure as cholesterol with the alkyl tail of ergosterol [2]. We found that POPC/brassicasterol bilayers exhibit the same saturation behavior in M1 at 25oC as POPC/ergosterol bilayers, but at a lower value of M1. We are in the process of examining POPC-campesterol bilayers to evaluate the role of the C22-23 trans double bond in the saturation effect. Other sterols are also being investigated in order to understand the sensitivity of POPC/sterol membranes to the sterol's alkyl tail structure.

[1] Y-W Hsueh et al., (2007) Biophys. J. 92:1606-1615.

[2] We are most grateful to Till Boecking for suggesting brassicasterol for this study.

3125-Pos Board B172

The Dynamic Stability of Cholesterol Clusters in DPPC Lipid Bilayers Studied by Molecular Dynamics Simulation

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The dynamic stability of cholesterol clusters in DPPC lipid bilayers was investigated by MD simulation. Two parallel simulations were performed at 20 mole % of cholesterol: in one system, cholesterols were initially arranged as clusters, and in the other, cholesterols were randomly placed. Any two cholesterol molecules in the same monolayer are assigned to the same cluster if their lateral separation is less than a predetermined cutoff distance. The results show that cholesterol clusters in DPPC bilayers are unstable and are ready to disperse into individual cholesterol even at the early stage of the simulation. In the cluster system, the average size of cholesterol cluster decreases monotonously and the total number of clusters increases with time, approaching the corresponding values of the random system. In addition, cholesterols in cluster experience more water exposure, and this unfavorable exposure is reduced when individual cholesterols are surrounded by DPPC molecules. The result is consistent with the Umbrella Model, which suggests that, driven by hydrophobic interactions, cholesterols have a strong tendency to avoid forming cluster in a lipid bilayer.

3126-Pos Board B173

Effects of seaweed sterols fucosterol And desmosterol on lipid membranes

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All eukaryotes universally contain large amounts (20-30%) of higher sterols in their plasma membranes. It remains a mystery why different eukaryotic kingdoms have chosen different higher sterols for their membrane reinforcement, such as cholesterol in animals, ergosterol in fungi, phytosterols in plants, and e.g. desmosterol and fucosterol in algae. We have used a range of biophysical techniques, including calorimetry, fluorescence microscopy, atomic-force microscopy, and vesicle-fluctuation analysis, to assess the various physical effects of fucosterol on lipid membranes. Fucosterol and desmosterol induce acyl-chain order in liquid membranes, but less effectively than cholesterol in the order: cholesterol > desmosterol > fucosterol, reflecting the different molecular structure of the sterols. Fucosterol is much poorer that cholesterol to mechanically stiffen membranes. Both fucosterol and desmosterol are found to support liquid-ordered membrane phases and induce coexistence between liquid-ordered and liquid-disordered domains, a necessary requirement for forming small-scale domain structures which are believed to be important for membrane function.

3127-Pos Board B174

Making A Permanent Membrane Raft fromTethered Cholesterol

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It is well established that the presence of cholesterol increases the stability and rigidity of lipsomes by increasing their area-expansion modulus and bending energies. In nature, cholesterol molecules in the cell membrane are known to phase separate into cholesterol rich and cholesterol deficient domains, leading to the formation of ''rafts''. Here, we demonstrate the creation of a permanent raft, i.e., a robust supported lipid bilayer, using immobilized and dispersed cholesteryl groups covalently anchored to a hydrophilic polymer brush. This allows a uniform interaction of cholesteryl groups with the entire bottom leaflet of an supported lipid bialyer (SLB). When the surface cholesteryl concentration is 0.3 per square nanometer or higher, we obtain an air stable SLB while maintaining fluidity of the lipid membrane environment. The fluidic and air-stable SLB is not only a robust model for biophysical studies of membranes, but also an efficient cell-mimicking platform for high throughput analysis.

3128-Pos Board B175

Lateral Pressure Profile In Membrane With Lipids Interdigitation: Analytical Derivation

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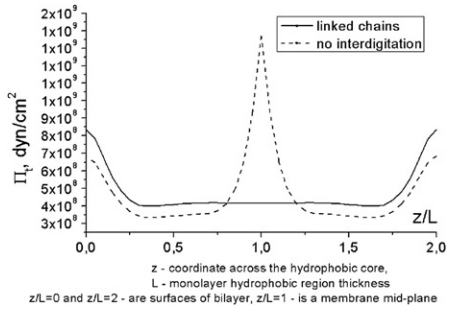
We derive analytically thermodynamic characteristics of a lipid bilayer membrane with interdigitation: lipid tails of the opposite monolayers interpenetrate. To allow for interdigitation, our microscopic model of bilayer treats lipids as semi-flexible chains with tails linked across the mid-plane of the membrane. We found striking difference between lateral pressure profiles for linked and not linked chains in the vicinity of the monolayers interface, see figure. Lateral pressure mid-plane peak disappears in the linked-tails case, while the free energy per chain increases by amount $\Delta F_{int} \sim 6k_BT$ (per chain) . This is purely entropic contribution to the free energy due to linking of the opposite chains. From this we deduced critical pressure capable of forcing interdigitation to a depth of a single lipid-chain CH_2-CH_2 segment of a volume $\Delta v \sim 70A^3$: $P_{int} = \Delta F_{int} / \Delta v \sim 3.5$MPa, in good agreement with experiment in DPPC bilayer (Chemistry Letters. Vol. 37 (2008) , p.604, Nobutake Tamai et al.). We also studied geometric constraints imposed by the balance between the

interdigitation free energy increase ΔF_{int} and energy of the hydrophobic mismatch in case of a liquid-condensed domain embedded in the liquid-expanded surrounding during e.g. the liquid-gel phase transition.



3129-Pos Board B176

Multiscale Modeling of supported bilayers

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Supported Lipid Bilayers are an abundant research platform for understanding the behavior of real cell membranes as they allow for additional mechanical stability. However, in computer simulations these systems have been studied only rarely up to now. Recently, we studied systematically the changes that a support induces on a phospholipid bilayer using coarse-grained molecular modeling on different levels.

We characterize the density and pressure profiles as well as the density imbalance inflicted on the membrane by the support. We also determine the diffusion coefficients and characterize the influence of different corrugations of the support. We then determine the free energy of transfer of phospholipids between the proximal (close to the surface) and distal leaflet of a supported membrane using the coarse-grained Martini model. It turns out that there is at equilibrium about a 2-3% higher density in the proximal leaflet.

These results are in favorable agreement with recent data obtained by very large scale modeling using a water free model where flip-flop can be observed directly. We compare results of the free energy of transfer obtained by pulling the lipid across the membrane in different ways. There are small quantitative differences but the overall picture is consistent. We are additionally characterizing the intermediate states which determine the barrier height and therefore the rate of translocation. Simulations in atomistic detail are performed for selected systems in order to confirm the findings. Calculations on unsupported bilayers are used to validate the approach and to determine the barrier to flip-flop in a free membrane.

3130-Pos Board B177

Optical Birefringence of Multi-Lamellar Vesicles with Anisotropic Internal Structure

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Multi-lamellar vesicles (MLV) which are shrunk under the osmotic condition can form a small nano-layered structure. When the internal structure of the shrunken MLV(SMLV) is geometrically anisotropic, it has an optically birefringence property; known as form birefringence. When the SMLV is trapped by the optical tweezers with a polarized laser beam, it can rotate due to the optical birefringence. We characterize the geometrical anisotropy of this internal structure using the relation between the rotational motion and the optical birefringence. We also present a simple model to describe the anisotropic internal structure of SMLV, where the layered structure is parameterized with the thickness of each lipid bi-layer and the internal distance between the next bi-layers.

3131-Pos Board B178

Nonlinear Deformations of Bilayer Lipid Membranes

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Novel continuum models that describe the equilibrium configurations of planar lipid bilayers will be presented. The models are derived within the nonlinear theory for smectic A liquid crystals proposed by Stewart (IW Stewart 2007 Contin. Mech. Thermodyn. 18:343) in which the usual director and unit layer normal do not always necessarily coincide. The total energy of lipid bilayers consists of an elastic splay term, smectic layer bending and compression terms, a coupling term between the director and layer normal, a surface tension term, and a surface anchoring term. Nonlinear equilibrium equations are obtained by using variational methods and are then solved by analytical and numerical methods. The solutions illustrate the nonlinear deformations of lipid bilayers including the misalignment of lipid molecules at their interface with other media such as, for example, proteins and surface substrates.

3132-Pos Board B179

Orientational Order and Raft Interactions in Lipid Bilayers

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Model and reconstituted membranes undergo a mixing-demixing phase transition, and, at low temperatures, the membrane is often a two-dimensional emulsion of liquid ordered and liquid disordered phases. This phase separation may be important for many functions of cellular membranes, including vesicle trafficking and signaling, and it has been implicated in a number of human diseases. We describe here the effects of orientational order (tilt, nematic, or hexatic) in one of the low-temperature phases on the behavior of the two-dimensional emulsion. We found that the orientational order in the continuous component of the emulsion can lead to the formation of companion singularities in the order parameter around the inclusions of isotropic liquid disordered phase. The orientational order parameter and strong anchoring boundary conditions also give rise to long-range interactions between the inclusions. The interaction is attractive at large separations and is repulsive at short separations. This interaction stabilizes the emulsion and leads to the formation of inclusion dimers. The sizes of the dimers depend only on the type of the orientational order and the sizes of the inclusions; hence, our calculation of this size can be used to test for the presence of a hidden order parameter in liquid ordered phases. The behavior in the presence of strong thermal fluctuations will be discussed as well.

3133-Pos Board B180

Line Tension Of Membrane Domains Calculated From Chemical Interactions Betweem Lipids And Elastic Splay And Tilt

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Model is developed to calculate the line tension of a domain in bilayer lipid membrane from both the chemical interactions between lipid molecules and the elastic deformations of the membrane. Two-component lipid bilayer is used to display the essential physics that determines line tension, without being obscured by complexities of the multi-component bilayer. Means to expand the approach to multi-component system has been formulated. The domain is assumed to be in thermodynamic equilibrium with the surround. Chemical interactions are incorporated by using regular solution theory, mean field approximation. Whenever a height mismatch exists at the boundary between the domain and the surround, the membrane deforms so as to prevent exposure of hydrophobic surfaces to water. The deformation energy is calculated by assuming that deformations occur through splay and tilt. The calculated line tension can be written as a sum of ''mechanical'' and ''chemical'' terms; each term is implicitly dependent on the other. For height mismatch of only a few Angstroms, line tension is accurately determined from the chemical interactions between lipids alone. For greater height mismatch, both chemical interactions and elastic deformations contribute. The calculated line tension is a function of temperature. Differences in spontaneous curvatures of the membrane lipid components lowers the effective critical temperature for domain formation. Below the critical temperature, the characteristic thickness of the transitional zone between the phases is several nanometers; it rapidly increases as the critical point is approached. If line tension and compositions of domains and surround are known for one temperature, they can be calculated over the entire temperature range. The model therefore allows values of line tension and domain composition that is experimentally measured at one temperature to be theoretically extended to a large range.

3134-Pos Board B181

Lamellar-Hexagonal Phase Transition Kinetics Depend Strongly on Degree of Saturation

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Phase transition temperatures of lipid-water systems have long been known to exhibit a strong dependence on the rate of heating or cooling. The difference between the phase transition temperatures seen on heating and cooling is