liquid-ordered domains (rafts) that exist in the intact membrane prior to the addition of TR, the cause of formation of DRM is not fully understood. Our working hypothesis was that the formation of DRM is due to the established strong binding of SM to cholesterol. This results in the formation of SM=cholesterolcomplexes with a large negative spontaneous curvature, which stabilizes it against solubilization. This interpretation implies that under conditions of incomplete solubilization the solubilized (micellar) fraction arising from bilayers composed of PC and SM, will be rich in SM whereas in cholesterol-containing mixtures, the solubilized fraction will be rich in PC. To test these predictions, we have determined the SM/PC ratio in mixed micelles, using high resolution ^{31} P-NMR, to which non-solubilized phospholipids do not contribute, because of being broadened beyond detection. The results were as expected, thus supporting our working hypothesis (SM/PC$>$ 1.0 in the solubilized fraction of SM-PC liposomes; SM/PC$>$ 1.0 in the solubilization of liposomes made of PC, SM and cholesterol), This supports the hypothesis that the detergent-resistance of SM is due to the relative stability and large negative spontaneous curvature of the SM-cholesterol complexes. The results also demonstrate the strength of ^{31}p-NMR spectroscopy in the investigation of the partial solubilization of specific membrane phospholipids without having to separate the solubilized from non-solubilized fractions.

3117-Pos Board B164

Simple Phenomenological Model and Phase Behavior of Ternary Mixtures of Saturated and Unsaturated Lipids and Cholesterol

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We present a phenomenological theory for the phase behavior of ternary mixtures of cholesterol and saturated and unsaturated lipids, one which describes both liquid and gel phases. It leads to the following description of the mechanism of the phase behavior. In a binary system of the lipids, phase separation occurs when the saturated chains are well ordered, as in the gel phase, simply due to packing effects. In the liquid phase the saturated ones are not sufficiently well ordered for separation to occur. The addition of cholesterol, however, increases the saturated lipid order to the point that phase separation is once again favorable. Our theory addresses this last mechanism, the means by which cholesterol-mediated ordering of membrane lipids leads to liquid-liquid immiscibility. It produces, for the system above the main chain transition of the saturated lipid, phase diagrams in which there can be liquid-liquid phase separation in the ternary system but not in any of the binary ones, while below that temperature it yields the more common phase diagram in which a gel phase, rich in saturated lipid, appears in addition to the two liquid phases.

3118-Pos Board B165

Cholesterol-phospholipid Interactions: New Insights From Surface X-ray Scattering Data

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In cell membranes, cholesterol-enriched domains are presumably involved in a wide variety of cellular processes. Although a number of conceptual models exist, there is no consensus on the molecular mechanism of cholesterol-phospholipid interactions. Here we report on a systematic study of cholesterol-phospholipid interactions in lipid monolayers using Langmuir isotherms, epifluorescence microscopy, synchrotron X-ray reflectivity (XR), and grazing incidence X-ray diffraction (GIXD) techniques. Lipid monolayers consisted of cholesterol-DPPC mixtures with various cholesterol mole fractions (χCHOL from 0 to 1). XR results demonstrate that cholesterol tends to stay in the acyl chains region of DPPC with its hydroxyl group in a proximity to carbonyl groups of the phospholipid. Increase in cholesterol content promotes ordering of the phospholipid acyl chains. Moreover, X-ray and Langmuir isotherm data used in a complimentary manner indicate that in cholesterol-lipid mixture cholesterol molecule craves to grab an additional 10 Å2 of molecular area from the acyl chains directly above the phospholipid headgroups. These results provide a reasonable explanation for the well documented ''condensing effect'' of cholesterol in lipid mixtures. At high cholesterol concentrations the phospholipid headgroups tilt significantly, but even then appear to be incapable of providing an additional 10 Å2 required to enclose the cholesterol molecules. Interestingly, the critical cholesterol concentration at which phospholipids still shield cholesterol molecules is the same as that at which the phase transition from α- to β-region observed with the epifluorescence microscopy. GIXD data yield DPPC crystalline order only in the mixtures with χCHOL below 0.15. At higher χCHOL, cholesterol seizes the places of the acyl chains in the DPPC crystalline lattice at the same stoichiometry as cholesterol and DPPC in the mixture. Diffraction pattern of such mixtures yields a short-range hexagonal packing order with d-spacing increasing as a function of the χCHOL.

3119-Pos Board B166

The Physical Properties Of Model Membranes Containing POPC, POPE And Sterol: A Deuterium NMR Study

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We have investigated the effect of sterol on the physical properties of lipid membranes containing 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC) and 1-palmitoyl-2-oleoyl-phosphatidylethanolamine (POPE). POPC/POPE/ergosterol, POPC/POPE/cholesterol, and POPC/POPE membranes were studied as a function of temperature using deuterium nuclear magnetic resonance (^{2}H NMR), with POPC and POPE deuterium labeled alternatively. It is found that the presence of ergosterol or cholesterol disorders gel-phase POPC/POPE (1:1) membranes, whereas orders lc-phase membranes. The modulation of lipid orders by ergosterol is less dramatic than that by cholesterol. In addition, the presence of ergosterol or cholesterol modulates the interaction between POPC and POPE. It is of interest that in POPC/POPE/cholesterol both lipid components display identical M_1(T) curves, which does not observed in POPC/POPE/ergosterol and POPC/POPE.

3120-Pos Board B167

Effects of Cholesterol and Unsaturated DOPC Lipid on Chain Packing of Saturated Gel-phase DPPC Bilayers

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Wide angle x-ray scattering (WAXS) from oriented lipid multilayers was used to study the effect of adding cholesterol (Chol) or 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) to gel-phase 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) bilayers. Small quantities (X<0.10 mole fraction) of both molecules disrupt the tight packing of tilted chains of pure gel-phase DPPC, forming a more disordered, untilted phase. The addition of larger quantities of DOPC causes the sample to phase-separate into a gel phase, characterized by a narrow WAXS peak, and liquid disordered phase, characterized by wide, diffuse WAXS scattering. In contrast, two WAXS peaks indicative of two coexisting phases were not observed in Chol/DPPC mixtures (X_{Chol} = 0.07 to 0.40). Instead, Chol caused a gradual increase in the width of the WAXS peak, consistent with a gradual change from a more gel-like to a more liquid-like state rather than passing through a region of two phase coexistence. Our WAXS data include a huge amount of information. A new method of analysis suggests that WAXS data may provide definitive results relating to the disagreements between previously published phase diagrams for Chol/DPPC.

3121-Pos Board B168

Role Of Membrane Cholesterol Content In The Activity Of Cyclooxygenase-2 (COX-2) In MCF-7 Human Breast Cancer Cells

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Cyclooxygenase-2 (COX-2) and its product PGE_2 are known to increase both angiogenesis and resistance to apoptosis (promoting tumor growth) and to enhance the penetration of cancer cells into adjacent tissues (causing metastasis). Thus, knowing how the activity of COX2 is regulated at the cellular level has implications for breast cancer therapeutic strategies. The goal of this research is to unravel a new molecular mechanism for regulating the activity of COX-2. The proposed molecular mechanism may be elucidated by using the sterol superlattice model. In plasma membranes, arachidonic acid (AA) is released by phospholipase A2 (PLA2). Cyclooxygenase then converts AA to prostaglandins (e.g., PGE_2). The activity of PLA2 is known to vary with membrane cholesterol content in an alternating manner, showing a local minimum at critical sterol mole fractions (C_r) for maximal superlattice formation. Hence, it is logical to hypothesize that the activity of COX (including the isoform COX-2) also varies with cholesterol content in a biphasic manner. In this study, the cholesterol content in MCF-7 human breast cancer cells was decreased systematically by using methyl-beta-cyclodextrin. A biphasic change in COX-2 activity, as monitored by the Cayman COX-2 assay with minor modifications, was observed at certain cell cholesterol content C_{rcell}. The cholesterol content near C_{rcell} could serve as a fine-tuning mechanism to regulate COX-2 activity and PGE_2 production, and consequently, cancer cell growth and metastasis. (supported by DOD, NSF and PDOH)

3122-Pos Board B169

Free Energy Of Cholesterol Transfer In Lipid Bilayers With Varying Degree Of Saturation

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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