In this study we have addressed the assembly of lipid bilayers in arrays and the stability of established membranes in different scaffold geometries.
To establish planar lipid membranes across large scale partition aperture arrays, we created a disposable single-use horizontal chamber design that supports combined optical-electrical measurements. Lipid bilayers could easily and efficiently be established across CO_2 laser micro structured 8×8 aperture partition arrays with average aperture diameters of 301 ± 5 μm.
To demonstrate the functionality of the lipid bilayers established across the 8×8 arrays, controllable reconstitution of the biotechnological and physiological relevant peptides valinomycin and gramicidin A, together with the membrane proteins α-Hemolysin and FomA were carried out. The results showed that the design supports low current (high sensitivity) recordings of membrane peptides and proteins by incorporating gramicidin A, α-Hemolysin and FomA into the established lipid bilayers.Finally, we tested the scalability of the assembly of lipid bilayers by creating rectangular 24×24 and hexagonal 24×27 lipid membrane arrays respectively. The two different geometries of the micro structured aperture arrays seem to support stable and functional membrane arrays, however, with somewhat different electrical properties. We propose that the presented design may be suitable for further developments of sensitive biosensor assays.

2534-Pos
Comparison of the Effects of Cholesterol or 3β-Hydroxy-5-Oxo-5,6-Secocholestan-6-Al on the Thermotropic and Structural Properties of Mixtures of Phosphatidylethanolamine and Phosphatidylcholine
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The oxidation of cholesterol with ozone produces 3β-hydroxy-5-oxo-5,6-secocholestan-6-al. This oxysterol has been implicated in a number of pathological conditions *in vivo* including atherosclerotic plaque formation and amyloidogenesis. We have shown previously that this oxysterol strongly modifies the physical properties of model membranes composed of different phosphatidylethanolamines or phosphatidylserine. In the present work we have extended our studies to ternary mixtures composed of phosphatidylethanolamine and phosphatidylcholine with sterols, either 3β-hydroxy-5-oxo-5,6- secocholestan-6-al or cholesterol. We use differential scanning calorimetry and small angle X-ray diffraction to characterize the phase behavior of mixtures of dipalmitoleoylphosphatidylethanolamine (diPoPE) and dipalmitoleoylphosphatidylcholine (diPoPC) or 1-palmitoyl-2-oleoyl-phosphatidylethanoloamine (POPE) and 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC). We compare the effect of the two sterols on the temperature of the transition of the ternary system from the liquid crystalline to the hexagonal phase (T_H) and on the curvature of the resulting cylindrical micelles. Addition of low concentrations of diPoPC increases T_H while adding cholesterol to this mixture significantly lowers T_H. The effect of 3β-hydroxy-5-oxo-5,6-secocholestan-6-al is much weaker than that of cholesterol. With regard to the curvature of the cylindrical micelles, the addition of diPoPC and 3β-hydroxy-5-oxo-5,6-secocholestan-6-al have opposing effects, while cholesterol does not effect the curvature at all. Low concentrations of POPC in POPE cause an increase in T_H and decrease the curvature of the cylindrical micelles, while cholesterol has the opposite effect.

2535-Pos
Self-Assembly Simulations of Membranes Containing Phospholipid Oxidation Products
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Products of phospholipid oxidation (OXPLs) are involved with the genesis or pathology of several diseases. OXPLs can modify the physical properties of biological membranes, thereby possibly altering several biological processes near membranes including signaling pathways. We have used atomistic and course grained simulations to investigate the properties of OXPL-containing lipid bilayers. We ran self assembly simulations of mixtures of palmitoyl-oleoyl-phosphatidylcholine (POPC) with two different OXPLs: PazePC, which is anionic, and PoxnoPC, which is zwitterionic. The total sampling time exceeds 1 millisecond. Despite having shortened and polar acyl chains, the two OXPLs POPC self assemble into stable lipid bilayers with POPC. The bilayers can accommodate at least 25% OXPL, although such bilayers have a lower area compressibility modulus. As as example of the modification of a membrane-associated biological process, we show that KALP-23 peptides partition differently in POPC-OXPL and POPC bilayers. The peptides adopt a transmembrane orientation more easily when OXPLs are present in the bilayers.

2536-Pos
Study of the Cholesterol Umbrella Effect in DPPC and DOPC Bilayers by Molecular Dynamics Simulation
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The instability of cholesterol clusters in dipalmitoylphosphatidylcholine (DPPC) and dioleoylphosphatidylcholine (DOPC) lipid bilayers was investigated via atomistic Molecular Dynamics (MD) simulation. Cholesterol clusters in phosphatidylcholine (PC) bilayers are found to be very unstable and to readily disperse into cholesterol monomers. The instability may result from the difficulty for the system to prevent water exposure to cholesterol's aggregated hydrophobic bodies in a cluster. The system responds to artificially arranged cholesterol clusters in several interesting manners: (i) Cholesterol clusters quickly form a "frustum" shape to reduce water penetration through cholesterol headgroups; (ii) Many clusters bury themselves deeper into the bilayer interior, causing local bilayer deformation; (iii) Cholesterol fluctuates rapidly, both laterally and vertically to the bilayer plane, in order to escape from clusters. These fluctuations result in the disintegration of clusters, and in one incidence, a highly unusual flip-flop event of a cholesterol across the DOPC bilayer occurs. Our results show that cholesterols have a strong tendency to avoid forming clusters in lipid bilayers and that the fundamental cholesterol-cholesterol interaction is unfavorable. Furthermore, the radial distribution functions of cholesterol hydroxyl oxygen to various headgroup atoms of PC reveal that the PC headgroups surrounding cholesterol have a clear tendency to reorient and extend toward cholesterol. The range of this "Umbrella Effect" can reach up to 2-3 nm, larger than previously reported.

2537-Pos
Flip-Flop Motions of Lipid Molecules in Mixed Bilayer Systems
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The cell membrane is composed of a wide variety of lipid molecules, cholesterols, and membrane proteins. Lipid molecules in the membrane have several time scales of motions ranging from femtosecond to seconds. The flip-flop motion, in which lipid molecules move from one leaflet to the other, is known to be one of the slowest: it typically occurs within several tens of seconds, or much longer. Recently, experimental studies revealed that cholesterols (CHOL), diacylglycerols (DAG), and ceramides (CER) show fast flip-flop motions in some membranes. However, the molecular mechanisms underling the motions remain elusive.
In this work, we performed coarse-grained molecular dynamics simulations, using MALTINI force field parameters. We examined flip-flop motions of CHOL, DAG, and CER in phospholipid bilayer systems, composing of DAPC(di-20:4), SAPC(18:0-20:4), and POPC(16:0-18:1). In the simulations using DAPC membranes, we observed flip-flop motions of CHOL, DAG, and CER within a microsecond. The flip-flop rate of CHOL was the highest, whereas that of DAG was lower than CHOL. CER flipped only once during the simulation. This tendency of flip-flop motions is strongly correlated with the relative positions of the lipids to the bilayer membranes: CHOL stays almost at the center of the membrane, whereas the head group of CER is located at the water/membrane interface and interacted with solvent molecules strongly.
The flip-flop motions of lipids were also affected with the membrane environment. Within 1-microsecond simulations, CHOL flipped 257 times in DAPC, 196 times in SAPC, and 5 times in POPC. Thus, the flip-flop rate is strongly correlated with the number of double bonds in the acyl chains of bilayer phospholipids, suggesting the importance of the membrane fluidity. These simulation results qualitatively agree with existing experimental data and shed light on the molecular mechanisms underlying the dynamics of biomembranes.

2538-Pos
The Effect of Cholesterol on Membrane Chain-Chain Packing
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Membrane structure is very important issue for cell. Membrane is not only the ''wall'' for protecting cell but also the interface for exchanging signal, ions and molecules. Many evidences show that membrane protein will fold to functional structure by associating with suitable membrane structure. Lipid chain-chain packing is one of important structures and will affect membrane thickness, lipid lateral diffusion and membrane domain formation. We will use grazing incident X-ray diffraction to probe lipid chain-chain packing. The 12keV X-ray light source in BL13A beam line of NSRRC and home-made humidity-temperature controlled chamber will be applied in the measurements. Cholesterol will

be used to interact with model membrane to study their effect on chain-chain packing.

2539-Pos

Sterol Affinity for Bilayer Membranes is Affected by their Ceramide Content and the Ceramide Chain Length

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It is known that ceramides can influence the lateral organization in biological membranes. In particular ceramides have been shown to alter the composition of cholesterol and sphingolipid enriched nanoscopic domains, by displacing cholesterol, and forming gel phase domains with sphingomyelin. Here we have investigated how the bilayer content of ceramides and their chain length influence sterol partitioning into the membranes. The effect of ceramides with saturated chains ranging from 4 and 24 carbons in length was investigated. In addition, unsaturated 18:1- and 24:1-ceramides were also examined. The sterol partitioning into bilayer membranes was studied by measuring the distribution of cholestatrienol, a fluorescent cholesterol analogue, between methyl-beta-cyclodextrin and large unilamellar vesicle with defined lipid composition. Up to 15 mol% ceramide was added to bilayers composed of DOPC:PSM:cholesterol (60:20:20), and the effect on sterol partitioning was measured. Both at 23 and 37 °C addition of ceramide affected the sterol partitioning in a chain length dependent manner, so that the ceramides with intermediate chain lengths were the most effective in reducing sterol partitioning into the membranes. At 23 °C the 18:1-ceramide was not as effective at inhibiting sterol partitioning into the vesicles as its saturated equivalent, but at 37 °C the additional double bond had no effect. The longer 24:1-ceramide behaved as 24:0-ceramide at both temperatures. In conclusion, this work shows how the distribution of sterols within sphingomyelin-containing membranes is affected by the acyl chain composition in ceramides. The overall membrane partitioning measured in this study reflects the differential partitioning of sterol into ordered domains where ceramides compete with the sterol for association with sphingomyelin.

2540-Pos

Importance of Head Group Methylation on Sphingomyelin Membrane Properties and Interactions with Cholesterol

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Sphingomyelins (SMs) are important constituents of the plasma membrane and have, together with other sphingolipids and sterols, been identified as lipid components in membrane rafts. Interactions between sphingolipids and sterols have been shown to be important for the formation of ordered domains in model systems and also suggested to be a driving force in the formation of membrane rafts. The structure of sphingomyelins is important for interactions with sterols and in this study we have investigated the importance of the methyl groups in the head group of SMs upon membrane properties and interactions with cholesterol. Using specifically synthesized SM-analogues, having a stepwise decreasing number of methyl groups in the phosphocholine head group, we are able to systematically study how the size of the head group affects membrane properties and sterol interactions. The sphingomyelin analogues were composed of sphingosine having palmitic acid in the N-linked position. Using the anisotropy of 1,6-diphenylhexatriene we have determined the transition temperature between gel and liquid crystalline phases (Tm). Decreased methylation of the head group was shown to increase the Tm from 42°C (PSM), 53°C (dimethyl), 61°C (monomethyl) to 65 °C for ceramide-phoshoethanolamine (no methyl groups). Initial experiments using fluorescence quenching of cholestatrienol in complex model systems, shows that the ability to form sterol containing ordered domains also is affected by the degree of methylation in the head group. We are currently analyzing sterol interaction for the SM-analogues using DPH anisotropy and further prospects include determination sterol partitioning to vesicles containing each of the SM-analogues and differential scanning calorimetry studies.

2541-Pos

Spin Trapping the Oxidized Products of PUFA in Model Membranes: The Protection Conferred by Vitamin E

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Electron paramagnetic resonance (EPR) spectroscopy is recognized as the most sensitive and noninvasive means to quantify free radicals of biological relevance such as reactive oxygen species (ROS). In spin trapping a molecule (the spin trap) reacts with the free radical producing a spin adduct that is sufficiently stable to be detected by EPR. Here we apply a novel spin trapping technique to investigate the protection that α-tocopherol (vitamin E), the major lipid soluble antioxidant in membranes, confers on polyunsaturated lipids in model membranes. Polyunsaturated fatty acids (PUFA) readily oxidize because they have a *cis, cis*-1, 4-pentadiene motif that renders the central methylene group vulnerable to attack by ROS. Our method quantifies the oxidized products of PUFA in lipid vesicles that have been exposed to a physiologically relevant, oxidizing enzyme that initiates the free radical chain reaction. By measuring the reduction in lipid peroxidation due to the presence of α-tocopherol, we test the hypothesis that the vitamin co-localizes with polyunsaturated lipids in membrane domains to ensure close proximity to the most vulnerable lipid species.

2542-Pos

Cholesterol Displaces Ceramide from its Tight Packing with Sphingomyelin

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The present study deals with a combination of fluorescence spectroscopy, atomic force microscopy (AFM), differential scanning calorimetry (DSC) and confocal microscopy to study two different effects: i) lateral segregation in pSM/Chol binary mixtures, and ii) the effects of pCer incorporation into pSM/Chol mixtures. The data reveals the segregation of large cholesterol-enriched microdomains within the range X_{Chol} = 0-0.25 in the binary pSM/Chol mixtures. In comparison with the pSM/pCer mixture (Busto et al. 2009), sphingomyelin shows a higher preference for ceramide than for cholesterol. In ternary pSM/Chol/pCer mixtures, an immiscibility between cholesterol- (pSM/Chol) and ceramide-enriched (pSM/pCer) phases at high pSM/(Chol+pCer) ratio is observed, where no ceramide over cholesterol nor cholesterol over ceramide displacement is detected. Furthermore, the calorimetric and confocal microscopy data concur in showing an inability of pCer to displace cholesterol both at low and high cholesterol concentrations. Interestingly, an inverse cholesterol-mediated ceramide displacement from its tight packing with sphingomyelin is clearly observed. These observations in model membranes in the absence of the lipids commonly used to form a liquid-disordered (L_α) phase support the proposed role of raft-like domains (Silva et al, 2007) rather than ceramide, in regulating ceramide-induced platform formation within cell membranes.

* Busto J.V. et al. (2009) Coexistence of immiscible mixtures of palmitoylsphingomyelin and palmitoylceramide in monolayers and bilayers. *Biophys. J.*, in press.
* Silva L.C. et al. (2007) Ceramide-domain formation and collapse in lipid rafts: membrane reorganization by an apoptotic lipid. *Biophys. J.*, 92(2): 502-516.

2543-Pos

Material Properties of Lipid Membranes from Molecular Dynamics Simulations

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Experimental data for many important properties of lipid bilayers are scarce and uncertain. This includes for instance area per lipid, area and volume compressibilities, area expansion coefficients and heat capacity.

Present simulation techniques can often give such properties more easily and sometimes with better accuracy than experiments. Simulation results depend, however, upon potential parameters that yet are not enough tested and validated. Still, the time is getting ripe for systematic calculation of membrane properties using simulation techniques. This is useful both since it will provide better values for a number of membranes properties but also since a more serious test of the membrane force field parameters will push the refinement and development on this side.

Initially, the study has concentrated on area per lipid, area compressibility and bending modulus. We have shown that the inverse apparent area compressibility modulus obtained from the area fluctuations of the system shows a linear variation with system size. From this, the the true area compressibility modulus can be obtained by extrapolating to small areas. From the term that varies linearly with system size and is due to undulations, the bending modulus of the bilayer can easily be calculated. The method has been applied to several lipids including DPPC and DMPC.