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.

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

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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.

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

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

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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.

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

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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.

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

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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