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Lipid Nanotubes and Double-Membrane Sheets Induced by Osmotic Deflation of Giant Unilamellar Vesicles Encapsulating Aqueous Two-Phase Solutions

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Membraneless organelles and liquid-liquid condensates in cells are gaining increasing interest in the last years. Here, we mimic them using giant vesicles encapsulating aqueous two-phase polymer solutions. The vesicles undergo morphological transformations upon osmotic deflation, accompanied by phase separation of the encapsulated solution (Adv. Mater. Interfaces 4:1600451, 2017). The vesicles exhibit budding, inward nanotube and double-membrane sheet formation. The structures are confined to the liquid-liquid interface and have a typical thickness below conventional optical resolution (ACS Nano 10:463, 2016). We use super-resolution microscopy (2D and 3D STED) combined with a microfluidic technique (Lab Chip 19:626, 2019) to resolve their shape and dimension. The nanotubes can transform into double-membrane sheets reminiscent of endoplasmic reticulum cisternae. They are connected to the mother vesicle surface via a small membrane neck which allows medium exchange with the external environment. The tube-to-sheet transformation can either start from the highly curved end of a nanotube, or proceed via coalescence of the orifices of multiple nanotubes on the mother vesicle surface. The transformation process is prohibited when tubes intertwine into knots. The double-membrane sheets provide a more efficient way of storing excess membrane area compared to nanotubes. Understanding the coexistence of nanotubes and double-membrane sheets as in the endoplasmic reticulum and the tube-to-sheet transformation may help to shed light on the origin and evolution of similar structures in the cell. This work is part of the MaxSynBio consortium, jointly funded by the Federal Ministry of Education and Research of Germany and the Max Planck Society.

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The Thermotropic Behavior of Saturated Phosphocholines in the Presence of Steroid Saponins

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Steroidal saponins are used in the preparation of corticosteroids and other hormonal drugs of a steroidal nature. Saponins are characterized by pronounced surface activity and many of their biological effects might be related to the changes in the elastic properties of the cell membranes. The present study focuses on effects of digitonin, betulin, and diosgenin on the thermotropic behavior of saturated phosphocholines using differential scanning microcalorimetry. Large unilamellar vesicles were prepared by electroformation methods from pure dipalmitoylphosphocholine (DPPC) or distearoylphosphocholine (DSPC). Addition of the digitonin to the liposome suspension up to the ration 1:50 to the lipid led to the elimination of the pre-transition, a decrease in the maximal temperature of main transition on about 1 degree C and did not practically change the transition cooperativity of both DPPC and DSPC. Betulin and diosgenin did not affect the pre-transition and cooperativity of main transition, but slightly decreased the its maximal temperature on the 0.3 and 0.5 degree C independently of the lipid composition of liposomes, respectively. Possible intercalation of saponins into lipid bilayer and membrane dehydration due to hydrogen bonding between saponin glycosides and lipids are discussed. The study was supported by RSF (#19-74-00093) and scholarship SP-484.2018.4.

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Coupling of Leaflet Structure in Asymmetric Lipid Vesicles

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Lipid asymmetry is a hallmark of biological membranes. In particular, prototypical mammalian plasma membranes are known to be composed of an outer leaflet enriched in cholinephospholipids, while the majority of the aminophospholipids are confined to the inner leaflet. Asymmetric large unilamellar lipid vesicles (aLUVs), produced via cyclodextrin-mediated lipid exchange, are a new platform for more realistic mimics of biological membranes. These systems were shown to be stable over several days and have already been investigated by elastic scattering techniques (small-angle neutron and X-ray scattering; SANS/SAXS), providing insight into structural properties of the individual leaflets. One of the enduring questions concerning plasma membrane architecture and lipid asymmetry is the possibility of bilayer leaflets being coupled to each other, which may influence a number of physiological processes that require communication between interior and exterior of the cell. However, in the physiologically relevant fluid phase no evidence of structural coupling has yet been reported from scattering studies. In this work, we explore the role of hydrocarbon chain interdigitation as a potential trigger for transleaflet coupling. We use combinations of dipalmitoylphosphatidylcholine (DPPC) in the inner leaflet and mixed lipids with varying chain length mismatch in the outer leaflet, in particular C16:0/C18:1 PC (POPC), C18:0/C18:1 PC (SOPC), C18:0/C14:0 (SMPC), C14:0/C18:0 (MSPC) and C16:0/C14:0 PC (PMPC). This entails different interdigitation states of the mixed-chain lipids into the inner leaflet. We present consequences on transbilayer coupling as observed from leaflet specific structural data and thermotropic behavior of these systems.

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Plasma Membrane Packing Asymmetry Drives Transmembrane Protein Localization

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Phospholipid asymmetry between the two plasma membrane leaflets has been discovered 40 years ago and since then, various physiological processes have been associated with asymmetric lipid distributions and changes to lipid asymmetry. Nevertheless, it remains unclear how lipid asymmetry affects the biophysical properties of individual leaflets and whether this putative biophysical asymmetry affects transmembrane proteins. To address these questions, we conducted a detailed analysis of asymmetric plasma membrane leaflet lipidomes and leaflet-specific biophysical properties. We further investigated whether distinct leaflets are maintained in intracellular organelles and how such intracellular membrane asymmetry may affect transmembrane protein localization and structure. Lipidomics revealed a striking disparity in lipid acyl chains, with the inner plasma membrane leaflet containing two-fold more acyl chain unsaturations than the outer leaflet. Consistent with the difference in unsaturation, we observed that the outer leaflet was highly packed, resembling a liquid ordered phase, whereas the inner leaflet was much more disordered. A bioinformatic analysis revealed that transmembrane domains (TMDs) of single-pass transmembrane proteins in the plasma membrane are broadly asymmetric in shape, with smaller accessible surface areas in the outer leaflet than the inner (i.e. thinner outside half of the TMD, fatter inside). We inferred that this shape asymmetry should facilitate insertion into the asymmetrically packed plasma membrane. We verified this hypothesis by creating TMD constructs with several shapes and discovered that proteins with a small accessible surface area in the outer leaflet preferred to localize at the plasma membrane regardless of their inner leaflet counterpart. This study delivers new insights into the structural organization of cell membranes and reveals that outer leaflet packing can drive protein localization to the plasma membrane.

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Lipid Bilayers Influenced by Taurin and Betain

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We studied the influence of natural osmolytes Taurin and Betain on the stability and fluidity of lipid model membranes. They components of daily products aim at our well-being, such as energy drinks or baby creams. We used a combination of simultaneous SAXS/WAXS/DSC measurements, being able to identify the structures, their dimensions and the phase transition temperatures. Thermal scans show morphologies similar to aqueous solution of the pure lipids, although with different dimensions and transition temperatures. In POPE, we observe structures with high curvature, and upon temperature cycling, the induction of possible cubic phases. Fully hydrated POPE hydrated with solutions of such osmolytes surprisingly lacks anomalous diffraction patterns over a wide temperature range on cooling from the hexagonal phase. The diffraction peaks in the last patterns are much less intense than at the beginning of the scan. The DSC shows a higher phase transition temperature on heating than for pure aqueous POPE, while during cooling this is not seen. DSC of POPE in on these samples shows a transition at a different temperature than purely hydrated POPE. The SAXS scan indicates a less organized stack of bilayers and the hexagonal phase, normally following the lamellar upon heating, could not be clearly seen up to 85^{o}C. Moreover, no well-organized structure was formed during cooling. On combining SAXS/WAXS and DSC results obtained from dispersions of POPE in either aqueous solution of Taurin and Betain, one can see their effect from a structural perspective, that is, their influence on the macro organization of the self-assembled mesogenic units. Both osmolytes seem to require high temperatures (weaker interactions between headgroups of neighbouring lipids) to effectively interact with the POPE (headgroups) and influence the dispersion.