that explores how chelating agents such as ethylenediaminetetraacetic acid (EDTA) modify the interactions between membrane. EDTA was shown to induce a phase coexistence in multilamellar systems of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) [1]. In this study we show that the effect of EDTA depends on other mobile ions in solution and lipid type. These data require a reevaluation of theoretical models of lipid bilayer interactions. [1] Johnson et al, Langmuir 2014.

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Hydrocarbon Thickness Dictates Cholesterol's Location, Orientation and Motion in a Phospholipid Bilayer

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The lateral sequestration of lipids with polyunsaturated fatty acid (PUFA) chains into membrane domains depleted of cholesterol has been hypothesized to have an important role in neurological function. This effect has long been attributed to a strong aversion of the disordered polyunsaturated fatty acid (PUFA) chains to the rigid smooth alpha face of cholesterol. Previously, we have performed neutron diffraction studies of deuterated cholesterol incorporated into bilayers composed of diarachidonoyl phosphatidylcholine (di-20:4 PC, DAPC), a lipid with two omega-6 PUFA chains. It was found that cholesterol sequestered at the bilayer midplane, in contrast to its usual upright orientation with the hydroxyl group located near the lipid/water interface. To date it remains unclear whether or not cholesterol's aversion to PUFA chains is a universal property of PUFAs, a behavior unique to omega-6 PUFAs, or the product of membrane disorder.

Using different physical characterization techniques (i.e., neutron diffraction, NMR) and MD simulations, we obtained detailed structural data that rationalize much of the previously inexplicable data regarding cholesterol's behaviour in PUFA bilayers. We are able to demonstrate that cholesterol's mass distribution in the center of a PUFA bilayer is the same for both omega-6 and omega-3 PUFAs. In addition, neutron, 2H NMR and MD data suggest cholesterol's sequestration into the bilayer center may in fact be driven by hydrophobic thickness mismatch, and not necessarily membrane disorder.

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Membrane Domain Interactions by Monte Carlo Type Analysis of Osmotic Stress Data

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Diverse physiological processes in living systems depend on fundamental interactions of physical origin on the nanoscopic length scale. Of particular interest are forces acting between membrane domains/rafts across the aqueous phase governing their mutual alignment. Besides bare interactions, such as van der Waals attraction or solvation (hydration) forces, also membrane bending fluctuations, which relate to domains' bending rigidities, need to be considered. We have developed a method based on Monte Carlo simulations and global small-angle X-ray scattering analysis, allowing us to scrutinize osmotic stress data of coexisting liquid-ordered (Lo)/ liquid-disordered (Ld) domains for interdomain interactions. We report results for DSPC/DOPC/cholesterol and DPPC/DOPC/cholesterol lipid mixtures and focus in particular on the bending rigidities of Lo/Ld phases. Results are discussed with respect to effects on membrane-mediated partitioning of proteins in different lipid environments, domain line-tension and size-dependent alignment of like-domains.

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How do Cholesterol and Saturated Sphingolipids Affect Acyl Chain Order in the Fluid Phase of Binary POPC Bilayers - a Study with 1-oleoyl-2-propionyl-DPH-sn-glycero-3-phosphocholine

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It is known that cholesterol increases the order in the fluid phase of a lipid bilayer but it has remained unclear whether or not various sphingolipids also have an ordering effect on the bulk phase ordering properties in the fluid phase. The acyl chain order in the gel phases has previously been reported with trans-parinic acid (tPA; steady state anisotropy or lifetime analysis) but acyl chain order in the fluid phase phase separated systems has been mostly measured indirectly, e.g. with 1,6-Diphenyl-1,-3,5-hexatriene (DPH) or N-rhodamine.dipalmitoylphosphatidyletanolamine (Rho-DOPE). By using a 1-oleoyl-2-propionyl-DPH-sn-glycero-3-phosphocholine (18:1-DPH-PC) as a reporter molecule the order in the fluid phase could be measured since the unsaturated probe has preferential partitioning into the disordered phase. The systems we studied were: POPC with 50 mol% palmitoyl sphingomyelin (PSM), POPC with 29 mol% of either palmitoyl ceramide (PCer), palmitoyl galactosyl ceramide (PGalCer) or palmitoyl glucosyl ceramide (PGluCer). In the above bilayer systems (with coexisting disordered and ordered phases present), 18:1-DPH-PC steady state anisotropy reported no gel phase melting during temperature ramps. This contrasts with tPA, whose anisotropy in binary bilayers reported meltings of the ordered phase. 18:1-DPH-PC steady state anisotropy measurements at 23°C showed that PCer, PGlcCer, PGalCer addition to a fluid POPC bilayer had a very minor ordering effect compared to cholesterol addition. These results indicate that cholesterol is superior to saturated sphingolipids to increase order in the fluid phase and that 18:1-DPH-PC is a fluorophore suitable to measure membrane order in the fluid phase.

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Partitioning of the Transmembrane Peptide GWALP23 between Lo and Ld Phases in Macro and Nanoscale Domains. Nanometer-Scale Domains can be Treated as a Phase

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If liquid-ordered (Lo) and liquid-disordered (Ld) domains coexist in plasma membrane (PM), then the domain size, shape, and morphology can influence membrane behavior. For multi-component lipid mixtures that model the PM, this phase domain morphology can be controlled by lipid composition. Here we find the partition coefficient of a transmembrane peptide (GWALP23) between Lo and Ld phases, for mixtures forming macro domains (bSM or DSPC/DOPC/Chol) or nanoscale domains (bSM or DSPC/POPC/Chol). The WALP family of peptides has proven to be a useful model for investigating the fundamental principles governing protein-lipid interactions. GWALP23 presents 23 amino acid residues with the sequence (GGAF FLALALALALALWLAGA). By use of Forster Resonance Energy Transfer (FRET), we measured the phase preference of GWALP23, finding favorable partition into Ld phase for all four systems. However, care must be taken when comparing the absolute values of the partition coefficient obtained for macro and nanoscale domains, since the small domain size is a key factor in the measured FRET. In addition, we used the fluorescence emission of 18:1,18:1-LR-PE to measure the partition coefficient of this dye between Lo and Ld phases in the case of macro domains (DSPC/DOPC/Chol) and nanodomains (DSPC/POPC/Chol), finding similar values in each case. Taken together, our results imply that nanodomains can be treated as a phase.

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Cell Cycle Position Determines Critical Temperatures in Plasma Membrane Vesicles

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Giant plasma membrane vesicles (GPMVs) isolated from RBL-2H3 cells appear uniform at physiological temperatures, contain coexisting liquid-ordered and liquid-disordered phases at low temperatures, and experience micron-sized critical fluctuations close to their critical temperature. Individual vesicles have well-defined critical temperatures yet there is significant vesicle to vesicle variation even when GPMVs are isolated from a plate of seemingly identical cells. In this study, we explore if heterogeneity in critical temperatures arise, at least in part, from cells being at different stages of the cell cycle. Populations of cells were synchronized at S, G2, M, and G1 stages using a double