

temperature before data acquisition. The presence of a Lc or Lc-like phase was recognized by lack of reversal of fluorescent properties at 25 or 15°C. The Lc phase was observed for pure DPPC vesicles by merocyanine 540, prodan, and patman but not laurdan or diphenylhexatriene; fluorescence properties of the latter two probes were completely reversible at all temperatures. Inclusion of cholesterol in the vesicles up to 20 mol% caused a linear reduction in the magnitude of the difference between membrane properties detected by these probes before and after vesicle heating. In contrast to previous reports using other techniques, the lack of reversal was not completely eradicated by cholesterol in the range of 20 to 45 mol% suggesting that an Lc-like phase exists for DPPC/cholesterol mixtures. The distinction between probes that can and cannot detect these phases has implications for interpreting the nature of them.

#### 438-Pos Board B207

##### Octyl- $\beta$ -D-Glucopyranoside Shows Composition Dependent Disordering Effects in Ternary Lipid Bilayers

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Solubilization of lipid bilayers by the nonionic detergent OG has been well characterized in one and two component lipid systems, but has not been extensively studied in ternary membranes containing mixed fluid phases. In order to understand the effects of membrane order on solubilization by OG, LUVs of pure POPC were compared to those composed of POPC plus varying fractions of sphingomyelin (SM) and cholesterol. Detergent partitioning from the aqueous phase into the bilayer and membrane solubilization were monitored by ITC. The fluorescent membrane probe, DPH, was observed by time-resolved fluorescence intensity and polarization decay in the presence of increasing detergent concentrations to determine the effects on membrane order and dynamics. The results of partitioning experiments showed approximately a 50% decrease in the partition constant between pure POPC and the most ordered samples. ITC solubilization experiments showed clear boundaries for the micelle-bilayer mixed aggregate coexistence region in samples of low initial order, but the distinct thermodynamic signature associated with this coexistence region was not present in the cholesterol and SM rich samples. The average excited state lifetime of DPH, an indicator of water penetration, in vesicles rich in POPC showed a rapid increase at the onset of solubilization, whereas vesicles rich in SM and cholesterol showed a corresponding rapid decrease. Dynamic fluorescence depolarization data analyzed in terms of a Brownian rotational diffusion model revealed an increase in the occupancy of the bilayer midplane by DPH prior to solubilization in less ordered membranes coupled with a constant rotational correlation time. Membranes with higher initial order showed little increase in midplane occupancy and increasing rotational correlation time in the same detergent concentration range, suggesting that two modes of membrane disruption are possible and depend on the lipid makeup of the bilayer.

#### 439-Pos Board B208

##### Ergosterol and Stigmasterol Interact with Phosphatidylcholine Lipid Bilayers Less Favorably than Cholesterol

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Sterols are a class of membrane lipids that is important to maintain plasma membrane structure and functions in eukaryotic cells. The maximum solubility of sterol in a lipid bilayer is the highest mole fraction of sterol that can be incorporated into a lipid bilayer before sterol precipitates from the bilayer to form crystals. A higher maximum solubility indicates more favorable interactions between the sterol and lipid bilayer. In this study, the maximum solubilities of ergosterol and stigmasterol in DOPC and DSPC lipid bilayers were measured using light scattering and further confirmed using optical microscopy. We found that correlation function of scattering intensities from two independent detectors can be used to sensitively determine the solubility limits of sterols. The validity of our new technique was confirmed by measuring the solubility limit of cholesterol in DOPC and DPPC lipid bilayers. We found that the maximum solubilities of ergosterol and stigmasterol are higher in PC lipid bilayers with saturated chains (DSPC) than that in PC bilayers with unsaturated chains (DOPC). Compared with cholesterol, ergosterol and stigmasterol both have much lower solubility limits in PC lipid bilayers. Our results suggest that minor differences in sterol structure could result in large differences in sterol-PC interactions.

#### 440-Pos Board B209

##### A New Phase Boundary in Phosphatidylcholine/Cholesterol Bilayers (In the Dimyristoylphosphatidylcholine/Cholesterol Bilayer): EPR and DSC Studies

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A new phase boundary, at cholesterol/dimyristoylphosphatidylcholine (Chol/DMPC) mixing ratio of  $\sim 1$ , was observed by saturation-recovery electron paramagnetic resonance (EPR) spin-labeling method in a multilamellar suspension of DMPC and Chol prepared using a rapid solvent exchange method. With spin-labeled cholesterol analogues (androsterane spin label [ASL] and cholesterol spin label [CSL]) it was shown that the upper limit of the cholesterol concentration in the liquid-ordered phase of the DMPC membrane is  $\sim 50$  mol%, above which the excess of cholesterol forms the pure cholesterol bilayer domain (CBD). Thus, the phase boundary at a Chol/DMPC mixing ratio of  $\sim 1$  separates the region with a single liquid-ordered phase from the region with a coexisting liquid-ordered phase and the CBD. Because the pure cholesterol cannot form the free bilayer in the buffer, the CBD has to be supported by the surrounding phospholipid bilayer saturated with cholesterol. The next phase boundary is defined by the cholesterol solubility threshold (CST) that indicates the amount of cholesterol which can saturate the DMPC bilayer and form the CBD. The excess of cholesterol above the CST forms monohydrate cholesterol crystals which precipitate outside of the lipid bilayer. It was shown by differential scanning calorimetry (DSC) that the CST, which separates the region with a coexisting liquid-ordered phase saturated with cholesterol and the CBD from the region in which cholesterol crystals are formed, is located at the Chol/DMPC mixing ratio of  $\sim 2$  ( $\sim 66$  mol% cholesterol). The extended phase diagram for the DMPC/cholesterol membrane, covering the region where the membrane is saturated and oversaturated with cholesterol, is proposed.

#### 441-Pos Board B210

##### The Effect of Electrostatics on the Line Tension at the Edge of a Bilayer

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For bilayers composed of charged lipids placed in an aqueous medium, Betterton and Brenner (Phys Rev. Lett., 2000) showed that the line tension  $\lambda$  at the bilayer edge comprises two contributions: a positive hydrophobic line tension,  $\lambda_h$ , corresponding to the elastic deformation of lipids in the line, and a negative electrostatic line tension,  $\lambda_e$ , corresponding to the capacitive energy of double layer charging. They showed that  $\lambda_e$  depends on a single dimensionless parameter,  $P$ , which is the ratio of  $\lambda_h$  and a characteristic scaling of  $\lambda_e$ . For values of  $P$  below a critical value of 2, the net line tension becomes negative, implying that fluid bilayers with high surface charge densities should be unable to close up to form vesicles, and that vesicles should be unstable structures. However, we have prepared and imaged stable vesicles with surface charge densities and pH/salt conditions corresponding to  $P \ll 2$ ; this is inconsistent with the above prediction.

To understand this discrepancy, we revisit the calculations of Betterton and Brenner, with the new, key inclusion of details of the geometry of the bilayer. This results in two additional dimensionless parameters in the problem:  $\alpha$ , the ratio of the bilayer thickness to the Debye length, and  $f$ , the surface charge density in the edge of the bilayer relative to the planar region of the bilayer. If the surface charge density is uniform over the entire bilayer ( $f=1$ ) and  $\alpha$  is  $O(1)$  or higher, we show that  $\lambda_h$  can be positive, i.e. even electrostatics would favor a decrease in the perimeter of an open hole in the bilayer! We delineate conditions under which the net line tension becomes positive, and show that this can happen even when  $P \ll 2$ , in agreement with our experimental observations.

#### 442-Pos Board B211

##### Simulations of the Rupture of Liposomes Near Solid Surfaces

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The behavior of lipid membranes near solid surfaces has a great significance both in medicine and in technology. In spite of the widespread use and study of such membrane phenomena, their theoretical analysis is rather scarce. Our main goal here is to understand the process during which membrane vesicles first adhere to solid surfaces, then rupture (or go through a series of transient ruptures) due to the mechanical tension induced by the adhesion (not only between the membrane and the surface, but also between two adjacent membrane areas), and finally spread along the surface forming a supported lipid bilayer. In our theoretical description we simultaneously consider the dynamics of spontaneous pore opening and closing; volume loss via leakage through the pores; and the advancement of the adhesion fronts. All these processes are supposed to follow an overdamped dynamics and are coupled to each other through membrane tension. Here we identify under which conditions the dynamics leads to the formation of hat shaped geometries with a projecting brim, and compare our results with experimental observations.