Thymidine block that arrests cells at the border between G1 and S phases. Critical temperatures were elevated in GPMVs isolated from cells in cell cycle phases that precede cell division (G2 and M) compared to other stages (G1 and S). In unsynchronized cells, critical temperatures were found to be inversely proportional to cell density, suggesting that contact inhibition and associated arrest of cell growth results in lower plasma membrane critical temperatures. Lower critical temperatures were also observed when growth was arrested through overnight serum starvation, and elevated critical temperatures were restored 24h after the addition of serum containing medium. Transition temperatures are also lowered in GPMVs prepared from cells undergoing apoptosis through the application of TRAIL, and vesicles contain a more rigid liquid-ordered or gel phase at low temperature. These results are in agreement with past studies that have indicated that plasma membrane composition varies within the cell cycle. Since GPMV critical temperatures are hypothesized to reflect on the magnitude of lipid-mediated heterogeneity in intact cells, these results suggest that membrane heterogeneity is greatest in cells undergoing rapid growth and cell division and is suppressed in cells under low growth conditions.

440-Pos Board B220
Molecular and Mechanical Manipulation of Membrane Domains in Planar Supported Bilayers
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Nano-membrane domains are hypothesized to play an integral role in many cell signaling pathways. Their transient nature and biocomplexity underlies a myriad of fundamental questions about lipid-lipid and lipid-protein interactions and their roles in cellular functions. As a result, there is a need for innovative approaches for understanding different biophysical aspects of membrane assemblies and their underlying, multiscale dynamics. Here, we integrate dynamic holographic optical trapping (HOT) and fluorescence imaging with fluorescence correlation spectroscopy (FCS) to characterize membrane domain nucleation in biomimetic planar supported bilayers. The dynamic HOT system allows for the creation of multiple traps from a single light source, each of which can be controlled individually in real time. Silica microspheres are being trapped into arbitrary patterns for system optimization. Receptor-bound microspheres associated with nano-domains in planar supported bilayers act as handles for dynamic HOT manipulation. Our hypothesis is that by trapping multiple microsphere-bound receptors, the associated heterogeneous lipid domains will nucleate a larger domain upon interaction in a manner that depends on the lipid type, cholesterol and protein content. Fluorescence imaging is used to visualize lipid domain formation, and subsequent lateral diffusion of lipid species will be measured with FCS as a function of trap-induced confinement. These results will ultimately lead to new insights into domain formation in membranes.

441-Pos Board B221
Lipopolymer Crowding in Polymer-Tethered Lipid Bilayers Alters Lipid Mixing Behavior and Protein Sequestration in the Presence of Raft-Mimicking Lipid Mixtures
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It is now well established that the cytoplasm and plasma membrane of cells are characterized by high concentrations of proteins. Consequently, macromolecular crowding and confinement effects are believed to play important roles in the organization, dynamics, and function of proteins in cellular environments. However, the functional significance of molecular crowding remains unclear and conclusions are still controversial, due to the complexity of cellular systems. To address this experimental challenge, here we introduce a model membrane system on the basis of a polymer-tethered lipid bilayer, in which lipopolymers act as crowding agents. Our experiments show that changes in lipopolymer concentration have a profound effect on the lipid mixing behavior of raft-mimicking lipid mixtures forming micron-size liquid-ordered (l_o) and liquid-disordered (l_d) phase separations. Complementary fluorescence correlation spectrometry (FCS), confocal fluorescence intensity analysis, and photon counting histogram (PCH) analysis experiments demonstrate that lipopolymer-mediated molecular crowding also influences the lateral diffusion of lipids and membrane proteins, as well as membrane protein sequestration in raft-mimicking lipid mixtures. Interestingly, our experiments confirm that elevated lipopolymer concentrations cause the formation of buckling structures, which can be seen as a stress relaxation phenomenon. Notably, the extent of membrane buckling is substantially different in l_o and l_d lipid regions.

442-Pos Board B222
Why Cholesterol should be Found Largely in the Cytoplasmic Leaf of the Plasma Membrane
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In the mammalian plasma membrane, cholesterol can translocate rapidly between the exoplasmic and cytoplasmic leaves, and has been found predominantly in the latter. We hypothesize that it is drawn to the inner leaf to reduce the bending free energy of the membrane caused by the presence there of phosphatidylethanolamine (PE). It does this in two ways: first by simply diluting the amount of PE in the inner leaf and second by ordering the tails of the PE so as to reduce its spontaneous curvature. Incorporating this mechanism into a model free energy for the bilayer, we find that between 50 and 60% of the total cholesterol should be in the inner leaf of human erythrocytes.

443-Pos Board B223
Do Gel Phase Lipid Bilayers Behave Like Euler Elastica?
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The elasticity of fluid phase lipid membranes can be characterized using the Helfrich Hamiltonian, which depends only on the square of the total curvature and the Gaussian curvature and the corresponding curvature moduli: the mean bending modulus and the Gaussian curvature modulus. Even at large curvatures approaching the inverse bilayer thickness, the effect of higher order terms of the elastic energy are minimal. Recently, a method has been developed to derive the bending modulus for fluid membranes from the stress-strain relationship of a buckled membrane [1,2]. The method also predicts the shape of the membrane, an *Euler Elastica*, and this serves as a check that the membrane indeed follows quadratic curvature elasticity. Using a coarse-grained lipid model, we analyze the shape of a buckled membrane in a gel phase and show that it does not behave like an *Euler Elastica*, even at low curvatures. The deviation from the theory suggests that higher order terms of the total curvature reduce the energy penalty for high curvatures. We present an extended version of the Helfrich Hamiltonian that captures this effect and show that it describes the shapes that we observe in simulations. We then calculate the bending modulus, as well as the modulus describing higher order corrections.
[1] Noguchi H., "Anisotropic surface tension of buckled fluid membranes", *Phys. Rev. E* **83**, 061919 (2011).
[2] Hu M., Diggins P., and Deserno M., "Determining the bending modulus of a lipid membrane by simulating buckling", *J. Chem. Phys.* **138**, 214110 (2013).

444-Pos Board B224
Revisiting the Link between Lipid Membrane Elasticity and Microscopic Continuum Models
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Fluid lipid membranes can be described by a continuum-elastic Hamiltonian that features two central parameters mean and Gaussian curvature modulus. Of those two, the Gaussian modulus is much less understood, since it affects the membrane energetics only through topology or boundaries and is thus difficult to measure experimentally. Moreover, recent work by Hu et al. [Biophys. J. 102, 1403 (2012)] to determine this modulus from computational studies revealed discrepancies with more microscopic expectations, according to which this modulus should be given by the second moment of the lateral trans-membrane stress profile. Our goal in the present study is to revisit the arguments linking the Gaussian modulus to properties of thin sheets, using thin plate theory as a starting point, but allowing for a number of generalizations. For instance, membranes are more generally described as anisotropic continua with in-plane fluidity and a nontrivial distribution of pre-stresses. It is easy to see that in this case linear elasticity, combined with the additional but common approximation of a linear strain tensor, will predict that the Gaussian curvature modulus vanishes. We therefore need to include the possibility of nonlinear strains (while still using linear constitutive equations), but this in turn leads to some subtle inconsistencies within Monge gauge. Another difficulty is that virtually all existing theories treat Young's modulus and the Poisson ratio as constant throughout the width of the membrane. Starting from fundamental linear elasticity theory, amended by a nonlinear strain tensor, and using consistent geometrical constructions, we