budding. Both findings point to an important function of lipid microdomains, the so called rafts, in the late virus lifecycle.
This work focuses on the detection of Förster Resonance Energy Transfer (FRET) between a raft marker, a GPI-anchored cyan fluorescent protein (CFP), and gp41 fusion proteins labeled with yellow fluorescent proteins (YFP)to elucidate raft clustering. Since energy transfer is highly dependent on the distance between the participating molecules, efficient FRET can be considered as a strong indication for close proximity of raft marker and fusion proteins and, therefore for colocalization in lipid microdomains. Fluorescence lifetime imaging microscopy (FLIM) was employed to accurately investigate FRET in living cells. In combination with acceptor fluorescence analysis the ascertained energy transfer efficiencies provide reliable information about clustering independent of expression level and fluorophore concentration. Several gp41 chimera were produced to address the role of different protein domains for raft association but also intracellular distribution and trafficking. The impact of truncations of the cytoplasmic tail as well as mutations of, the cholesterol recognition amino acid consensus (CRAC) domain, intrinsic trafficking signals and a palmitoylation site were studied in this context.

2710-Pos Board B696
Establishing Conditions for Compartmentalized and Lamellar Biomimetic Supported Biomimetic Membranes
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Lipid bilayers have typically been used as model systems of biological membranes to investigate for example protein reconstitution, endo and exocytosis, organelle fusion and membrane permeation induced by channel-forming proteins and toxins. However, lipid membranes suffer from low stability and technical application requires stabilization for example by crosslinking, deposition on a support or encapsulation with hydrogel. Recently, self-assembling amphiphilic block copolymers have shown promise as biomimetic membranes due to their stability, which makes it possible to work in a broader range of temperature, pH, pressure and time. However, polymers generally form films with higher hydrophobic thickness than lipid bilayers. Also, lipid membranes are able to form planar bilayers on a support surface by vesicle fusion with the surface, whereas block copolymers cannot. Here we combine lipids and block copolymers and investigate stability and deposition on a support surface. We have mixed PMOXA-PDMS-PMOXA triblock copolymer with zwitterionic or positively charged lipids. We have investigated mixed polymer/lipid vesicles by transmission electron microscopy, quartz crystal microbalance with dissipation monitoring, dynamic light scattering, stopped-flow measurements and atomic force microscopy. We show that mixing the triblock copolymer with lipids allowed us to form mixed polymer-lipid vesicles rather than segregated lipid vesicles and polymer vesicles. These vesicles had the stability of polymer vesicles, but were able to adhere well to quartz and mica surfaces like lipid vesicles. We demonstrated that planar supported membranes or tethered compartmentalized membranes could be formed on mica and quartz and showed that the transition depends on the lipid to polymer molar ratio. We concluded that mixed polymer/lipid membranes present an attractive environment for transmembrane proteins, because the addition of only 20 mol% polymer lead to vesicles with polymer-like stability and low water permeability while still providing a sufficiently lipidic protein environment.

2711-Pos Board B697
The use of Light-Induced lo Domains in Giant Unilamellar Vesicles to Mimick raft Dynamics: Application to the Effect of the Ganglioside GM1
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Cholesterol and sphingolipid-enriched raft lipid domains play important roles in numerous cellular processes. Recently, giant unilamellar vesicles containing lo domains have become valuable tools for the modeling of raft properties. One topic which has been adressed is the dynamics of micrometer-sized lo domains formation in GUVs. This is usually done by varying the lipid composition and temperature. However, these slow procedures cannot reproduce the very dynamics of raft formation and size modulation in biomembranes, that can occur in subsecond timescales. Here, we propose a simple method which allows one to monitor the dynamics of lo domain formation in GUVs on a faster timescale. The method is derived from the initial observation by several authors of a photosensitizing effect of the fluorescent probes used for lo domain detection in GUVs, which promotes lipid oxidation. Such oxidized lipids are able as such to induce raft formation. It has been relevantly emphasized that such photooxidation may lead to artifacts. Here, we show that such oxidation-induced lo domain formation can also be purposely used, since it provides a way to trigger raft-type microdomain growth in GUVs on a faster timescale, relevant to cellular processes, and to study the effect of any parameter on such dynamics. We illustrate the usefulness of this approach by studying the effect of the ganglioside GM1, an essential component of cellular rafts. We show that GM1 has a profound influence on lo domain formation, dynamics and stability in GUVs, which may bear relevance to physiological situations.

2712-Pos Board B698
Partitioning and Single-Molecule Diffusion Dynamics of Bodipy-FTY720 in Biomembranes
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FTY720, a synthetic analog of sphingosine that has immunosuppressive properties, is the first oral drug to be approved by the U.S. FDA for treatment of multiple sclerosis (under the trade name Gilenya). We have examined the partitioning and diffusion properties of a fluorescent (Bodipy) FTY720 analog (Bdp-FTY720) in the plasma membrane (PM) of epithelial cells (HTB126) and model membranes. Based on two-channel confocal co-localization imaging with DiI-C_{12}, a liquid-disordered phase marker, Bdp-FTY720 has an affinity for the liquid-disordered lipid phase in both binary and ternary phase GUVs. Bdp-FTY720 resides in both the cytoplasm and the plasma membrane of HTB126 cells. The translational diffusion of single Bdp-FTY720 molecules was investigated in the PM of intact cells and giant PM vesicles (GPMVs) using fluorescence correlation spectroscopy. In GPMVs, the two-dimensional anomalous (α=0.9) diffusion of Bdp-FTY720 is 3×10^{-8} cm^2/s. The corresponding cytosolic Bdp-FTY720 diffuses mostly as a fast species (3.5×10^{-7} cm^2/s); a small population (~2%) diffuses at a slow rate (1.5×10^{-8} cm^2/s). On the nanosecond time scale, the Bdp-FTY720 anisotropy in GPMVs decayed as a biexponential (f_1=40 $\pm$ 20 ns, β_1 = 0.10 $\pm$ 0.04, f_2=2.4 $\pm$ 0.9 ns, β_2=0.06 $\pm$ 0.02) with an estimated order parameter of 0.62 in the lipid bilayer. In the cytoplasm, the Bdp-FTY720 rotational motion is significantly slower (f_1=120 $\pm$ 50 ns, β_2 = 0.13 $\pm$ 0.01, f_2=4.4 $\pm$ 0.7 ns, and β_1 = 0.088 $\pm$ 0.009). These results indicate heterogeneous Bdp-FTY720 interactions in the cytoplasm compared with a hindered diffusion in the PM. These findings elucidate the biophysical characteristics of this new FTY720 analog and set the stage for its application in cell biology.

2713-Pos Board B699
Ethanol and Dimyristoylphosphatidylethanol Alter Transbilayer Lateral and Rotational Mobility of Neural Membranes from Brain Membranes
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Intramolecular excimerzation of 1,3-di(1-pyrenyl)propane (Py-3-Py) and fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene (DPH) were used to evaluate effects of ethanol and 1,2-dimyristoylphosphatidylethanol (DMPET) on the rate and range of lateral mobility and the range of the rotational mobility of bulk bilayer structures of neuronal membranes (SPMV) isolated from brain membranes. In a concentration-dependent manner, both ethanol and DMPET increased the rate and range of the lateral mobility and the range of the rotational mobility of bulk bilayer structures of SPMV. Selective quenching of Py-3-Py and DPH by trinitrophenyl groups were utilized to examine the transbilayer asymmetric lateral and rotational mobility of SPMV. Ethanol and DMPET had a greater increasing effect on the lateral and rotational mobility of the outer monolayer as compared to the inner monolayer of SPMV. It has been proven that both ethanol and DMPET exhibit a selective rather than non-selective fluidizing effect within the transbilayer domains of the SPMV. DMPET had a greater potency(about 5,000-times) on aforementioned structural parameters of SPMV than ethanol. The sensitivity of SPMV to ethanol and DMPET was remarkably higher than model membranes of total lipids and phospholipids fraction isolated from SPMV. However, its sensitivity came out to be slightly higher than that of model membranes.

2714-Pos Board B700
Phosphoinositides Alter Lipid Bilayer Properties
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Phosphoinositides are involved in cell-signaling pathways that regulate such vital cell functions as membrane excitability and trafficking, and cell metabolism, motility and proliferation. At the plasma membrane phosphatidylinositol-4,5-bisphosphate (PIP_2), which constitutes approximately 0.25% of cell phospholipid, is a key lipid messenger in membrane-delimited signaling. PIP_2 regulates structurally and functionally diverse membrane proteins representing voltage- and ligand-gated ion channels, inwardly rectifying ion channels, transporters and receptors. The mechanism(s) by which PIP_2 regulates many of its various receptors remain to be elucidated. Here we explore the notion that the amphiphilic phosphoinositides, by adsorbing to the bilayer/solution interface, alter bilayer properties such as curvature and elasticity. Such changes in bilayer properties can alter the equilibrium between membrane protein conformational states thereby altering function. Taking advantage of the gramicidin channels' sensitivity to changes in the lipid bilayer properties, we used fluorescence-based