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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

Sania Ibragimova, Michelle Marincel, Manish Kumar, Helen Nguyen, Claus Helix-Nielsen, Julie Zilles.

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

Galya Staneva, Michel Seigneuret, Hélène Conjeaud, Nicolas Puff, **Miglena I. Angelova**.

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

Il Yun, Hye-Ock Jang, Hyang-Gun Shin, Dong-Keun Jeong, Hye-Won Park, Dong-Soo Jeong, Eun-Jong Choi, Won-Hyang Ryu, Won-Ick Park, In-Kyo Chung, Moon-Kyung Bae.

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

Radda Rusinova, Ashley Hobart, Olaf S. Andersen.

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

and single-channel gA assays to examine the effects of (diC8) phosphoinositides -PI, PI(4,5)P_2, PI(3,5)P_2, PI(3,4)P_2 PI(3,4,5)P_3 as well as long-chain PI(4,5) P_2 on the lipid bilayer. The diC8 phosphoinositides, except for PI(3,5)P_2, alter lipid bilayer properties with potency that depends on their logP with PI being the most potent and PIP3- the least. When comparing di-oleoyl-PI(4,5)P_2 to the naturally occurring 1-stearyl-2-arachidonyl-PI(4,5)P_2 the naturally occurring PIP_2 is the more potent bilayer modifier, being active at 10 μM nominal concentrations in the planar bilayer assay. Our results show that application of exogenous PIP_2 and its structural analogues (with changes in acyl chain length or phosphorylation state) alters lipid bilayer properties. We propose that these PIP_2 lipid bilayer effects may play be important for some of its many different effects on membrane protein function.

2715-Pos Board B701
Fenamates Alter Bilayer Properties
R. Lea Sanford, Subhi J. Al'Aref, Roger E. Koeppe II, Olaf S. Andersen.
Fenamates are a family of non-steroidal anti-inflammatory drugs (NSAIDs). They are widely prescribed to manage pain and inflammation and, like other NSAIDs, inhibit the cyclooxygenases; they have also been proposed to have anti-epileptic and neuroprotective effects. The fenamates modulate a variety of ion channels, with mechanism(s) of action that range from direct fenamate-protein interactions (binding) to non-specific membrane-mediated effects. We therefore examined whether fenamates alter bilayer properties at concentrations where they modify membrane protein function. To this end, we used a gramicidin-based fluorescence assay to investigate whether the fenamates could alter bilayer properties (as sensed by a bilayer-spanning channel), and to establish dose response curves for the fenamates bilayer-modifying effects. The fenamates we examined were flufenamic acid, meclofenamic acid, mefenamic acid, niflumic acid and tolfenamic acid. All of them increased the rate of fluorescence quenching, meaning that they shifted the gramicidin monomer-dimer equilibrium in favor of the monovalent cation permeable dimers. These results thus show that fenamates alter bilayer properties, most likely by softening the bilayer. Niflumic acid was the most potent modifier of bilayer properties and tolfenamic acid the least potent. All the fenamates have limited solubility, which limited the concentration range that could be studied to 300 μM (or less). To examine the alteration of bilayer properties in more detail, mefenamic acid was tested using single-channel electrophysiology in planar bilayers. The electrophysiological results support the data from the fluorescence assay. The fenamates alter bilayer properties at the concentrations where they have been reported to alter membrane protein function. This suggests that there may indeed be a membrane contribution to the fenamates' mechanism of action.

2716-Pos Board B702
Statins Modify Bilayer Mechanical Properties
Subhi J. Al'Aref, R. Lea Sanford, Roger E. Koeppe II, Olaf S. Andersen.
Statins exert their primary mechanism of action through inhibition of HMG-CoA reductase, thereby preventing cholesterol synthesis. Additionally, statins have many, so called ''pleotropic'', effects that are independent of HMG-CoA reductase inhibition. Because statins are amphiphiles that modulate the function of different, structurally unrelated membrane proteins, we explored whether statins could alter lipid bilayer mechanical properties at the concentrations where they alter membrane protein function. To this end, we used a gramicidin-based fluorescence quench method as well as single-channel electrophysiology. We found that atorvastatin, fluvastatin, lovastatin, mevastatin, pravastatin, and simvastatin increase the rate of fluorescence quenching, meaning that they shift the gA monomer $\leftrightarrow$ dimer equilibrium toward the conducting dimers. Statins thus alter bilayer mechanical properties, with fluvastatin being the most active and rosuvastatin the least active. When examined electrophysiologically, simvastatin, pravastatin, and fluvastatin increase the lifetime and appearance rate of channels formed by both short (13-residue) and long (15-residue) gramicidin analogues, with fluvastatin being the most active and pravastain being the least active. The changes in gA channel function depend on the channel-bilayer hydrophobic mismatch, as we observe the larger effects on the shorter channels; the channels with the larger hydrophobic mismatch. We conclude that statins alter lipid bilayer properties by a common mechanism, through an increase in bilayer elasticity, and that specific channel-statin interactions are not involved.

2717-Pos Board B703
Effects of Fluorinated Alcohols on Lipid Bilayers Properties
Mike Zhang, Helgi I. Ingólfsson, Olaf S. Andersen.
Fluorinated alcohols are unique alcohols that, while sharing a common overall structure with ''normal'' alcohols, have fluorines bonded to the carbons instead of the familiar hydrogens. The high electronegativity of fluorine shifts the charge distribution in the molecules, which changes their pK and together the larger bulk of fluorine relative to hydrogen alters their physico-chemical properties. Fluorinated alcohols are extensively used as organic solvents for solubilizing polymers and proteins especially in organic synthesis and NMR studies. In addition, the fluorinated form of 2-propanol (hexafluoroisopropanol (HFIP)) is used in Alzheimer's disease research to solubilize the amyloid-beta peptide. The fluorinated alcohols modify the function of various membrane proteins and are know to interact with lipid bilayers, altering bilayer properties, structure and stability. Additionally, HFIP has been shown to increase the conductance of planar lipid bilayers (Capone et al, NR 16:1, 2009). The question then becomes to what extent do the fluorinated alcohols alter bilayer properties and at what concentrations? We probed the membrane-modifying potential of the fluorinated alcohols: trifluoroethanol (TFE), HFIP, and nonafluoro-tert-butyl alcohol (PFTB) using a gramicidin-based fluorescence assay. All the fluorinated alcohols tested alter bilayer properties in the low (PFTB) to high (TFE) mM range. Not surprisingly, the largest alcohol, PFTB, is the most potent and the smallest, TFE, the least. In addition, above their bilayer modifying concentration PFTB and HFIP break down lipid bilayer structures and solubilize lipid vesicles.

2718-Pos Board B704
Bilayer-Modifying Potential of Limonene and its Metabolites
Will R. Fletcher, R. Lea Sanford, Ruchi Kapoor, Olaf S. Andersen.
Membrane protein function depends on lipid bilayer properties, which can be modified by small molecules. This may account for some of the undesired clinical effects of amphiphatic drugs that modify bilayer properties. In this study, we assessed whether D-limonene and its metabolites (perillyl alcohol, perillaldehyde, and perillic acid) alter lipid bilayer properties, as sensed by bilayer-spanning gramicidin (gA) channels, using a fluorescence assay and single-channel electrophysiology. D-limonene and its metabolites are terpenes that are found in a variety of foods, particularly citrus oils. They have been shown as potential cancer prevention and treatment agents and as antimicrobials. These terpenes are also used as solvents and flavor and fragrance agents. Given the wide range of biological functions of these hydrophobic/amphiphilic compounds, it becomes important to determine to what extent they alter bilayer properties. Using the fluorescence assay, we find that at micromolar concentrations (nominal concentrations): D-limonene decreases gA channel activity, perillyl alcohol and perillaldehyde increase activity, and perillic acid has no apparent effect. When examined using single-channel electrophysiology, each terpene increased gA channel lifetime and appearance rate. In the case of perillaldehyde and perillyl alcohol there is agreement between the two assays but not for D-limonene and perillic acid. We are exploring the basis for these differences. The changes in gA channel function (bilayer properties) were observed at low membrane concentrations (mole fraction ~0.02-0.03), indicating that these terpenes are potent bilayer modifiers.

2719-Pos Board B705
Antidepressants Modify Lipid Bilayer Properties
Ruchi Kapoor, Helgi I. Ingólfsson, Roger E. Koeppe, Olaf S. Andersen.
Antidepressants canonically inhibit neurotransmitter re-uptake at synapses. In addition, the two major classes of antidepressants - tricyclic antidepressants (TCAs) and selective serotonin reuptake inhibitors (SSRIs) - have off-target effects that include inhibition of membrane proteins other than neurotransmitter transporters. The biological significance of these effects remains unclear, but this polypharmacy may contribute to the desired changes in brain function - including altered neuronal circuitry and connectivity. It may also contribute to these compounds' off-target and side effects. A common feature of many proteins modulated by antidepressants is that they span lipid bilayers. Thus, it may be important that antidepressants are amphiphiles that adsorb at the membrane-solution interface. Membrane proteins are coupled to the bilayer through hydrophobic interactions - meaning that conformational changes underlying normal protein function may involve local reorganization of the surrounding lipids. Because bilayer deformations incur energetic costs, which vary with bilayer properties, membrane protein function may be sensitive to changes in bilayer mechanical properties caused by amphiphile adsorption. That is, amphiphiles may affect membrane protein function by altering the bilayer contribution to the free energy difference between protein conformations. Using gramicidin (gA) channels as probes, we examined whether 19 different TCAs and SSRIs alter lipid bilayer properties and thus may be able to alter membrane protein function through bilayer-mediated mechanisms. All of the examined antidepressants alter gA channel activity in a dose-dependant manner, with citalopram (Celexa, Lexapro) being the least, and fluoxetine (Prozac), paroxetine (Paxil) and sertraline (Zoloft) the most bilayer-modifying. These effects are not enantiomer-specific, and are observed with gramicidins of varying lengths and with different bilayer thicknesses, demonstrating that antidepressants increase bilayer elasticity. The calculated octanol-water partition coefficient, as a measure of drug hydrophobicity, is insufficient to predict the relative bilayer-modifying potential of different compounds.