the effect of glycerol on the surfactant - lipid system in terms of surfactant self-association (critical micelle concentration, CMC), membrane partitioning (partition coefficient K and αHmic), and the onset of membrane solubilization (i.e., bilayer-to-micelle transition, characterized by a specific surfactant-to-lipid mole ratio in the membrane denoted Resat) by isothermal titration calorimetry (ITC). One effect expected for glycerol is its tendency to 'salt out' hydrophobic molecules. This promotes all aggregation phenomena (K increases, CMC decreases) but has little effect on the balance between the aggregates (Resat ~ const.). Furthermore, glycerol gradually dehydrates the polar head group, which renders the effective molecular shape more favorable for a bilayer (K increases) whereas micellization is much less affected (CMC ~ const) so that bilayers are stabilized compared to micelles (Resat increases). Our results indicate that the behavior of the sugar based surfactant octyl glucoside seems governed by the salting out effect, whereas for ethylene oxide surfactant C12EO8, headgroup dehydration seems to be the key effect explaining the effects of glycerol.

849-Pos Board B728

Synthetic and Mycobacterial Trehalose Glycolipids Confer Dehydration Resistance to Supported Phospholipid Monolayers

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We have recently demonstrated that the glycolipid trehalose dimycolate (TDM), a major outer membrane component of dehydration resistant Mycobacterium tuberculosis (MTb), can impart significant dehydration resistance to supported phospholipid membranes. We now report studies of other, related glycolipids both natural and synthetic that exhibit behavior similar to TDM, conferring protection from desiccation to membranes of which they are constituents. We examined solid-supported lipid monolayers, characterizing membrane integrity and two-dimensional fluidity before and after de- and re-hydration with fluorescence imaging and fluorescence recovery after photobleaching (FRAP). As with TDM, the degree of protection is dependent on the fraction of synthetic lipid in the monolayer and there is a distinct minimum fraction needed for protection by each glycolipid. We can control the synthetic lipid fluidity and minimum protecting fraction by designing and synthesizing lipids with particular hydrophobic chain lengths, saturation, and branching, thereby illuminating the role of molecular structure in biophysical function. The advent of these synthetic, protective glycolipids opens the door to the creation of lipid bilayers and liposomes since the relevant hydrophobic and hydrophilic domain sizes can be controlled. These new structures allow investigations of the physical origins of well-known mycobacterial properties beyond dehydration resistance in controlled experimental contexts, such as the inhibition of membrane fusion.

850-Pos Board B729

How Small Polar Molecules Protect Membrane Systems Against Osmotic Stress

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We investigate how small polar molecules, urea and glycerol, can act to protect a phospholipid bilayer system against osmotic stress. The osmotic stress can be caused by a dry environment, freezing, or through exposure to aqueous systems with high osmotic pressure due to solutes like in saline water. A large number of organisms regularly experience osmotic stress and it is a common response to produce small polar molecules intracellularly. We have selected two ternary systems of urea-water-dimyristoylphosphatidylcholine (DMPC) and glycerol-water-DMPC as model systems to investigate the molecular mechanism behind this protective effect, and we put a special emphasis on applications in skin care products. Using solid-state NMR, DSC, X-ray diffraction and sorption-microbalance measurements we study the phase behavior of the lipid system both exposed to an excess of solvent of varying composition and for systems exposed to water at reduced relative humidities. In this we have arrived at a rather detailed thermodynamic characterization. The basic findings are: i) In excess solvent the thermally induced lipid phase transitions are only marginally dependent on the addition of urea(glycerol). ii) For lipid systems with limited access to solvent the phase behavior is basically determined by the amount of solvent irrespective of the urea(glycerol) content. iii) The presence of urea (glycerol) have the effect to retain the lipid in liquid crystalline phase down to low relative humidities (64% for urea, 75% for glycerol at 27°C), whereas the transition to the gel phase occurs already at a relative humidity of 94% in pure water, demonstrating the protective effect of the polar molecules against osmotic stress. iv) In skin care products urea and glycerol are referred to as a moisturizer, which we find slightly misleading as it replaces the water while keeping the physical properties unaltered.

851-Pos Board B730

How Bilayer Curvatures Modulate Molecular Reaction Efficiencies In A Membrane Junction

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Chemical reactions between ligand/receptors taking place in the intermembrane space between opposing lipid bilayers are crucial for maintaining processes vital for eukaryotes and multicellular organisms, e.g. vesicle trafficking and immune responses. The mechanism of chemical reactions taking place in bulk are understood in terms of molecular collective properties such as pressure, temperature and concentration, whereas a reaction taking place between bilayers proceed by more subtle pathways. The properties of the membranes into which the receptors are embedded modulate reaction efficiencies and should be understood in terms of contact area, receptor density and interbilayer forces.

We here present results from a single-vesicle based binding assay showing how the curvature of two lipid bilayers drastically alters binding efficiencies. We studied three biochemical binding reactions (i) trans-SNARE complexation, (ii) streptavidin/biotin recognition and (iii) calcium-mediated lipid chelation all taking place in the confined space of a membrane junction. We measured binding probabilities as a function of membrane curvatures and found that the probability of successfully completing the binding reaction could be changed by up to two orders of magnitude depending on the bilayer shape.

We built a simple model based on receptor membrane density, electrostatic bilayer repulsion and membrane shape to account for the observation. We believe the presented assay and model to have an impact on the understanding of biological recognition reactions taking place across a membrane junction, such as vesicle trafficking and fusion, as well as neuronal and immunological synapse formation.

852-Pos Board B731

Effect of Poloxamer 188 on the Osmotic Response of Cell Membranes

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Poloxamer 188 (P188), an amphiphilic triblock copolymer of poly(ethylene oxide)-block-poly(propylene oxide)-block-poly(ethylene oxide) has been shown to effectively interact with injured plasma membrane and restore its function both *in vitro* and *in vivo*. Elucidation of the mesoscopic and molecular mechanism that mediates the interaction between this triblock copolymer and damaged membranes will help to improve the current approach in development of Poloxamers for therapeutic purposes. Previous work done by our group examined the interaction between P188 with phospholipid monolayers and demonstrated that P188 inserts selectively into damaged portions of the membrane and corrals surrounding lipids. Upon restoration of membrane integrity, the inserted polymer is squeezed out. Here, the effect of P188 on membrane permeability under osmotic stress was investigated by using giant unilamellar phospholipid vesicles, the simplest biomimic membrane which retains the essential curved bilayer structure. Results will be presented detailing how the osmotic gradients and P188 concentration affect P188 in corralling membrane lipids.

853-Pos Board B732

Self-assembly Formation of Multiple Tethered Lipid Bilayers

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Tethered lipid bilayers have been proven powerful as experimental models in studies of membrane-spanning proteins, which are currently the most important targets in drug discovery. Tethering of planar lipid membranes reduces the influence from the solid support on the lateral mobility of the membrane constituents and provides a sufficiently large solvent reservoir underneath the membrane for studying molecular transport events. Inspired by cell-cell junctions, where membrane residing proteins control the separation between two or more membranes without interfering with their integrity, we developed a new self-assembly route for formation of multiple macroscopically homogenous and highly fluid tethered lipid bilayers (lipid diffusivity~5 μm2/s 1) with compartmentalized inter-membrane volumes geometrically confined by membrane-anchored DNA duplexes. The formation of multiple macroscopically homogeneous planar membrane-membrane junctions with sealed inter-membrane liquid reservoirs was accomplished using so called bicelles, which is a versatile class of model membranes generally composed of a mixture of the long-chained dimyristoyl phosphatidylcholine (DMPC) and the short-chained dihexanoyl PC. Quartz crystal microbalance with dissipation (QCM-D) was

used to monitor the formation of such architectures and to study of their ion permeability.

Smooth & Skeletal Muscle Electrophysiology

854-Pos Board B733

ATP Regulates Mammalian Neuromuscular Transmission by Dramatically Decreasing the Resting Muscle Chloride Conductance via P2Y1

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In skeletal muscle, extracellular ATP arises locally from released synaptic vesicles and broadly from active muscle fibers. We examined the direct effect of ATP on developed, innervated mammalian skeletal muscle (*ex vivo* rat *levator auris longus*) using measurements of individual muscle fibers with two intracellular microelectrodes. Near the neuromuscular junction (NMJ), 20μM ATP prolonged the decay of miniature endplate potentials (mEPP tau) by 31 ± 7.5% and extended the membrane potential responses induced by step current pulses ($\Delta E_{m(Ipulse)}$) by 59 ± 3.0%. These responses correlated with an increased input resistance (R_{in}) of 31 ± 2.0%. Analogous increases in non-synaptic regions reveal that ATP acts throughout the muscle fiber. In contrast, 50μM adenosine, a well-characterized metabolite of ATP, induced no apparent increase in mEPP tau, $\Delta E_{m(Ipulse)}$ or R_{in}. Applying established pharmacology showed that the ATP receptor mediating these effects is likely the G-protein-coupled P2Y1, since 20μM ATPγS and ADPβS (slowly hydrolysable analogs of ATP and ADP) and 10μM 2-methylthioadenosine-5′-O-diphosphate (2MeSADP) mirrored the effect of ATP. Furthermore, 20μM MRS2179 blocked activation by 20μM ADPβS. The significant effect of ATP on R_{in} was presumably achieved by closing chloride channels, which maintain the largest conductance of resting muscle. This was confirmed with the chloride channel blocker anthracene-9-carboxylic acid (200-500μM), which mimicked ATP and prevented additional increases by 20μM ATPγS. This ATP response appears unique to mammals, as 50μM ATP induced no analogous increase in the archetypical frog NMJ. Our work outlines a novel mechanism by which physiological levels of ATP regulate synaptic transmission and dramatically alter the resting membrane properties of mammalian skeletal muscle. This has potential implications for the physiology of muscle excitability and fatigue, and the pathophysiology of Thomsen and Becker myotonias.

855-Pos Board B734

Cycloxygenase-2 Inhibitor Celecoxib Is A Potent Activator Of Vascular KCNQ K^+ Channels And An Inhibitor Of L-type Ca^{2+} Channels

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Cyclooxygenase-2 (COX-2) inhibitors are important members of the family of non-steroidal anti-inflammatory drugs (NSAIDs). Celebrex® (celecoxib) and Vioxx® (rofecoxib) were introduced in 1999 and rapidly became the most frequently prescribed new drugs in the United States. Vioxx® was voluntarily withdrawn from the market because of a reported increased risk of myocardial infarction and stroke in patients taking the drug for prolonged periods of time. Celecoxib has been reported to inhibit several classes of ion channels, but its effects on vascular smooth muscle ion channels have not been described. Using whole-cell perforated patch clamp techniques we examined effects of celecoxib on K^+ and Ca^{2+} currents in A7r5 rat aortic smooth muscle cells. Application of 10μM celecoxib enhanced K^+ current by 2-3 fold and substantially inhibited Ca^{2+} currents with an apparent positive shift in the voltage-dependence of activation. Both effects were reversible on washout. Nether rofecoxib (10μM), another selective COX-2 inhibitor, nor diclofenac (10μM), a nonselective COX inhibitor, affected Ca^{2+} or K^+ currents in A7r5 cells. We previously reported that KCNQ5 channels are the predominant K^+ channels determining outward potassium current at negative membrane potentials in A7r5 cells. We estimated cumulative dose-response curve of celecoxib on isolated KCNQ5 currents. Celecoxib enhances KCNQ5 current in 3.5 fold with an EC_{50} of 6.9 ± 1.5 μM, without shifting the activation curve. Celecoxib (10μM) was unable to restore KCNQ current inhibited by 100 pM vasopressin (AVP, vasoconstrictor hormone) or 1nM PMA (PKC activator) but inhibition of L-type Ca^{2+} currents (with a positive shift of activation) persisted. The effects of celecoxib, but not rofecoxib, on vascular Ca^{2+} and K^+ channels may explain the differential risks of cardiovascular diseases in patients treated with Celebrex® or Vioxx®.

856-Pos Board B735

Basal Ca^{2+} Entry Controls NFAT Transcriptional Activity, Proliferation And Migration Of Human Vascular Smooth Muscle Cells

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We have previously shown that in the rat the sarco/endoplasmic reticulum calcium ATPase, SERCA2a, controls vascular smooth muscle cell (VSMC) proliferation through modulation of the activity of the transcription factor NFAT. Here we tested the hypothesis that SERCA2a, controls human VSMC proliferation by inhibiting voltage-independent Ca^{2+} entry and the NFAT transcription pathway.

Single-channel recording showed that proliferating VSMC possessed high spontaneous voltage-independent basal channel activity (nPo: 0.415 ± 0.050; n=31). SERCA2a gene transfer, using an adenoviral vector, to proliferating VSMCs abolished the activity of these channels (0.091 ± 0.096; n=26) compared to control cells infected with an adenovirus encoding GFP (0.315 ± 0.035; n=42). SERCA 2a gene transfer also down-regulated expression of transient receptor potential channels TRPC4, TRPC5 and stromal interacting molecule 1 (STIM1) suggesting their involvement in the channel activity.

SERCA2a gene transfer also inhibited VSMC proliferation and migration as well as the NFAT activity. Furthermore, NFAT activity was inhibited by depolarization-induced Ca^{2+} influx and by several calcium channel inhibitors such as nifedipine, mibefradil, carboxyamidotriazole and 2-aminoethoxydiphenyl, suggesting involvement of different types of Ca^{2+} channels in the control of NFAT and proliferation. Our data suggest that different types of Ca^{2+} channels are involved in the control of NFAT transcriptional activity and proliferation. By controlling submembrane Ca^{2+} concentration, SERCA2a finely regulated basal Ca^{2+} current *via* regulation of translocation and/or expression of STIM1 and TRPCs.

Acetylcholine Receptors

857-Pos Board B736

Cellular Basis Of Nicotine-induced nAChr Upregulation

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The upregulation and preferential assembly of high sensitivity $(\alpha4)_2(\beta2)_3$ neuronal nicotinic acetylcholine receptors (nAChRs) contribute to aspects of nicotine addiction such as sensitization and tolerance. The cellular mechanisms of these events, however, remain elusive. We employed fluorescently tagged nAChRs to study plasma membrane upregulation by total internal reflection fluorescence microscopy (TIRFM) as well as changes in intracellular receptor stoichiometry using pixel-based Förster's resonance energy transfer (FRET). To delineate the effect of β2 on α4 nAChR trafficking in the absence of nicotine, mouse neuroblastoma (N2a) cells were transiently transfected with either α4-meGFP/wildtype β2 or α4-meGFP/wildtype β4 subunits (m = monomeric; e = enhanced) and imaged at 48 h post-transfection by TIRFM. To set TIRFM parameters, cells were co-transfected with the pCS2-mcherry plasmid, which served as a reference probe. pCS2-mcherry expresses mcherry with a lyn kinase membrane localization signal, allowing visualization of the PM using red emission from mcherry. The α4-meGFP reporter was used to detect receptor expression at the PM. Results showed that the α4-meGFP/wildtype β2 receptors trafficked to the PM in ~10 % of the cells while ~90 % of imaged cells displayed α4-meGFP/wildtype β4 at the PM. In the presence of nicotine (0.1 μM for 48 h), α4-meGFP/wildtype β2 transfected N2a cells displayed a clear increase in receptor trafficking to the PM when visualized using TIRFM. Pixel-based sensitized emission FRET studies on N2a cells transiently transfected with an α4-mcherry and β2-meGFP FRET pair showed that chronic nicotine exposure (1 μM, 24 h) resulted in an increase in assembly of the high sensitivity $(\alpha4)_2(\beta2)_3$ population of receptors, a phenomenon that was blocked by co-incubation with the competitive nAChR antagonist, DhβE (1 μM). These preliminary results point to a modulatory role of β2 subunits as well as a possibly important role for activity-dependent receptor upregulation.

858-Pos Board B737

α-7 Nicotinic Receptor Positive Allosteric Modulators have Varying Kinetic Effects on Desensitization and Current Amplitude

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The α-7 nicotinic receptor is an important neuronal subtype of ligand gated ion channels. It forms a pentameric homomer that is activated by acetylcholine or nicotine to evoke rapidly activating and desensitizing currents. Activation of α-7 receptors has been implicated as a therapeutic strategy in schizophrenia and Alzheimer disease. Small molecule positive allosteric modulators (PAM's) have been shown to enhance α-7 currents, and are classified as type 1 or type 2 PAMs. The type 1 PAMs enhance the current amplitude but do not alter