

(big rectangle). This nuclear plasticity, measured as projected nuclear area fluctuations, showed a non-monotonous relation to actin polymerization state. Also, myosin contractility was determined to be necessary for such nucleus plasticity. The effect of cytoskeletal organization and their active forces on chromatin plasticity was further quantified by tracking the dynamics of condensed chromatin regions, which showed increased dynamics corresponding to enhanced nuclear plasticity. In summary, using cells of defined geometries to specify cytoskeletal organization, our work demonstrates the role of active cytoskeletal forces in regulating nuclear and chromatin plasticity.

Membrane Physical Chemistry III

2745-Pos Board B175

Amino Acids and Peptides Stabilize Fatty Acid Membranes against Salt-Induced Flocculation

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The prebiotic formation of biopolymers (specifically DNA, RNA, and proteins) has long been a mystery and is important for understanding the origin of life on earth. These bio-molecules are composed of building blocks that would have been dispersed in early oceans. Our previous work has shown that RNA bases and ribose bind to and stabilize fatty acid vesicles [Black et al. PNAS 110, 13272 (2013)]. Our results implied that the building blocks of a biological polymer could have spontaneously associated with components of the first membranes to form stable structures. We have now shown that protein building blocks, too, stabilize fatty acid vesicles against salt-induced flocculation. Using spectrophotometry, we measured the presence of flocs (and other structures) in fatty acid solutions, with and without amino acids and over a range of temperatures. Using fluorescence microscopy, we identified the structures that caused changes in absorbance in our spectrophotometric assays. We found that the two most hydrophobic prebiotic amino acids, leucine and isoleucine, prevent salt-induced flocculation. Moreover, although alanine and glycine, which are less hydrophobic, had little effect on flocculation, dipeptides composed of these amino acids preserved vesicles in the presence of salt even at 60 degrees C. These vesicles appeared to be primarily multilamellar structures, which may promote reactions between components of biopolymers more effectively than unilamellar vesicles. Thus prebiotic membranes could have facilitated the formation of peptides by bringing amino acids together, and peptides could have increased the formation of stable membranes. Such an auto-amplifying system, combined with selection for more effective peptides, could have led to the first cells.

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Measurement of the Viscosity of E. coli Membranes using Molecular Rotors and Flim

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We have employed molecular rotors, small organic molecules whose fluorescence lifetime is sensitive to the viscosity of the environment, to assess the viscosity of the E.coli plasma membrane. We used Fluorescence Lifetime Imaging Microscopy (FLIM) which allowed us to measure the fluorescence lifetimes (and thus viscosities) on the level of single cells. We probed the viscosity of membranes both in live cells and in spheroplasts, where the outer membrane was removed by lysozyme treatment. Viscosity values obtained for both environments were similar implying that the molecular rotor used indeed localized to the plasma membrane as shown previously for fluorophores of similar structures. Measurements on life cells show a rather broad spread of viscosities between individual cells in population; such heterogeneity of physical parameters of the cell has been reported previously for the diffusion of protein in the cytoplasm of bacteria. The viscosity of membranes was temperature dependent as we have observed a change in viscosity when cells grown at 37 degrees Celsius were measured at lower temperatures than the growth.

Subjecting the cells to a hyperosmotic shock by increasing the medium osmolarity by adding NaCl also elicited a change in viscosity and yet a larger spread of viscosity values between individual cells, which is consistent with previous observations that a fraction of cell within a population seems to respond to the osmotic shock more strongly than the others.

The values of viscosity measured for the plasma membrane of E.coli in this study are higher than those measured previously in E.coli lipid extracts or in the plasma membrane of life eukaryotic cells but slightly lower than what was reported previously for the Gram-positive bacterium *Bacillus subtilis*.

2747-Pos Board B177

Polydopamine as an Efficient Polymer to Prepare Biologically Relevant Supported Lipid Bilayers

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Solid-supported lipid bilayers (s-SLBs) are widely used as versatile biological membrane mimics. However, lipid-solid surface interactions and ensuing frictions lead to a decrease in lipid mobility. To address this issue, we prepared polymer-supported lipid membranes using a soft polymer cushion as a lubricating layer. A bioinspired polymer, polydopamine was chosen due to its ability to easily form thin coatings onto a wide range of surfaces, with a control of the thickness depending on the immersion time of the substrate.

Polydopamine coated-mica was used to support zwitterionic phospholipid bilayers of dimyristoylphosphatidylcholine (DMPC) or dioleoylphosphatidylcholine (DOPC). Atomic Force Microscopy (AFM) was performed to verify the deposition of DMPC and DOPC bilayers, revealing the presence of lipid patches on the polymer surface. The addition of positively charged cholesterol (DC-Chol) in the membrane composition greatly improved the lipid surface coverage (up to 90%). Best results were obtained for 20 and 30% DC-Chol containing membranes. They were also further characterized with other surface-sensitive techniques such as fluorescence microscopy to assess the phospholipid mobility. Surface Plasmon Resonance (SPR) and Quartz Crystal Microbalance with Dissipation (QCM-D) results showed the irreversible deposition of the lipid bilayers on the polymer surface. The polydopamine polymer film proved to be efficient to maintain fluidity of the phospholipid bilayers, thus enhancing this class of membrane model. We will present practical application of our s-SLBs onto porous filters as an improved model of Parallel Artificial Membrane Permeability Assay (PAMPA) to better predict passive permeation of orally administered drugs.

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Polymer and Silica Supported Bilayer Formation Studied through Time Resolved Spatial Point Pattern Analysis of Vesicle Deposition

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Due to their optimal separation from the sensor-substrate, polymer supported bilayers (PSBs) provide biologically-relevant mimics of cellular membranes whilst staying amenable to numerous analytical, imaging and fluidic tools - a dual precondition for drug discovery through function-interaction analysis of cell-surface receptors and other membrane-interacting proteins. Although silica supported bilayers (SSBs) spontaneously form from silica-adsorbed vesicles, successful PSB formation via a similar method has thus far been limited by insufficient understanding of the underlying processes. Here, we generated a polymer support through incubation of poly-L-Lysine conjugated to alkyl chain terminated poly(ethylene)glycol on silica. This substrate yielded efficient vesicle deposition and spontaneous bilayer formation thereby providing a rare opportunity to address the mechanism of PSB formation.

Currently there is a lack of consensus about the mechanism of SSB formation, with putative mechanisms invoking (i) preferential vesicle adsorption at the edges of bilayer patches, (ii) vesicle dispersion through sequestration of substrate binding sites and (iii) complete spatial randomness, where each scenario is expected to show a unique point pattern for vesicle adsorption. In order to differentiate between these scenarios, we measured deposition of SUVs encapsulating sulforhodamine B (SRB) over silica and polymer substrates, with these surface bound SUVs imaged via TIRF microscopy. As the SUVs bind to the solid substrate, they yield a diffraction limited point spread function (PSF) that is fit with a two-dimensional ellipsoidal Gaussian distribution. Rapid dye photo-bleaching facilitates the observation of only the freshly deposited SUVs. The obtained pattern of vesicle locations is compared to a complete spatial random distribution using Ripley's K-function analysis. Preliminary results indicate random deposition during the early stages of bilayer formation (<4% lipid surface coverage), but the observed deviations at higher loading indicate exclusion from vesicle/bilayer regions.

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Controlled Modulation of Lipid Bilayer State by a Photosensitive Membrane Effector

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Germany, ⁴CAT Catalytic Center, RWTH-Aachen University, Aachen, Germany, ⁵IEAP, Christian-Albrechts-University of Kiel, Kiel, Germany. The lipid membrane matrix represents a 2-D liquid-crystal, the properties of which, at fixed other conditions, are locally modulated by the presence of effectors as e.g. cholesterol (passive) or proteins (passive and active). Not only does the incorporation of effectors into the host matrix locally or even globally modify the initial state of the host per se, most probably the state of the matrix in turn serves as a control tool for regulating the work of functional units (e.g. proteins).

Results are presented on a membrane model system that was inoculated with a photosensitive and thus active variety of cholesterol. Azobenzene-cholesterol (azo-chole) exhibits a reversible trans-cis transition (365nm: trans- to cis-; 455nm: cis- to trans-). In a membrane, the azobenzene group, covalently connected to the cholesterol by an ester bond, is confined into the headgroup region. The system was explored by a combination of spectroscopic (UV-vis, NMR, mass spectroscopy), thermodynamic (Langmuir compression, calorimetry) and structural studies (X-ray/neutron reflectometry, grazing incidence X-ray diffraction). The conformational change of the guest upon illumination is coupled into the host system, inducing a transition of the whole membrane. The increased demand of headgroup area for the cis-azo-group pushes the membrane into a more compressed state, and vice versa for trans-azo-chole. The switching process between the two final states exhibits first-order kinetics. The state of the host bilayer is modulated as a response to the conformational switching of the guest effector via external light illumination. In a more general context, similar behavior may be found upon the conformational changes of membrane proteins during work.

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Creating Fluid Supported Lipid Bilayers with High Amounts of Phosphatidylethanolamine

Anne Sendeck, Matthew F. Poyton, Tinglu Yang, Paul S. Cremer. Chemistry, Pennsylvania State University, University Park, PA, USA. Phosphatidylethanolamine (PE) comprises 20-50% of overall phospholipid content in human cell membranes and constitutes 70-80% of the membranes in gram-negative bacteria. Its presence is specifically required for the proper folding of numerous membrane proteins, the function of active transport systems, cell division, fusion, blood coagulation, and may play a role in neurodegenerative diseases. Unfortunately, it is hard to work with this lipid in model systems like supported lipid bilayers and there is correspondingly less information known about its basic physical properties in bilayers. Specifically, the role of PE in lipid raft formation, vesicle fusion, cholesterol interactions, ion binding, and lipid flip-flop needs to be elucidated to understand its part in cell membrane function and disease pathways. To this end, my research has two goals. First, I have developed supported lipid bilayer systems that can operate with high mole percentages of PE. Second, I have begun to exploit such systems to explore the properties and functions of PE in membranes.

2751-Pos Board B181

Biophysical Analysis of a Successful Protocol to Reconstitute Tetramers of the M2 Muscarinic Receptor

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The human G protein-coupled receptor M2 muscarinic receptor has been functionally reconstituted in its tetrameric state into mixed lipid bilayers (Redka et al. 2013). This is achieved by first solubilizing the receptor in mixed detergent micelles composed of digitonin and sodium cholate, then reconstituting it into vesicles composed of phosphatidylcholine, phosphatidylserine and cholesterol, followed by detergent removal. To understand how the individual detergent and lipid components used in this empirical protocol contribute to the stability and activity of the receptor, we used isothermal titration calorimetry (ITC) to study the self-assembly of the mixed surfactant system; differential scanning calorimetry and pressure perturbation calorimetry to probe the phase behavior of the membrane; ITC, fluorescence (time-resolved) leakage assays and dynamic light scattering to characterize detergent-lipid interactions. The results suggest ideal mixing between digitonin and sodium cholate in the formation of mixed micelles that stabilize the receptor. Differences in membrane-partitioning behavior between the two detergents and the presence of a significant fraction of gel phase at the temperature used in the protocol contribute to non-equilibration of the detergents in the bilayer. The insights gained from this biophysical approach will aid in the mechanistic selection of detergents and conditions that influence function, oligomeric state, orientation, and accessibility of membrane proteins in future studies.

2752-Pos Board B182

Ion-Mobility Mass Spectrometry Assay for Incorporation of Phytanic Acid into Muscle Phospholipids

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Human dysferlinopathies (Limb-Girdle Muscular Dystrophy 2b, Myoshi Myopathy) are muscle-wasting syndromes caused by mutations in the dysferlin protein. Dysferlin loss impairs sarcolemmal repair that may contribute to disease progression. One therapeutic approach is to treat subjects with compounds that fortify the natural membrane tendency to reseal after damage, and minimize the inflammation that impedes regeneration and amplifies tissue destruction. Phytanic acid is a saturated branched chain fatty acid comprised of a 16 carbon aliphatic chain with 4 methyl groups (4ME 16:0); it is incorporated into phospholipids and triglycerides. Model phospholipid bilayers containing phytanic acid exhibit greater electrical stability than bilayers composed of straight-chain phospholipids. Phytanic acid-containing phospholipids may improve the resistance of the muscle fiber sarcolemma to stretch-induced damage. To measure phytanic acid incorporation into muscle phospholipids, dysferlin-deficient A/J mice were maintained on a defined diet supplemented with 2% phytol for three weeks; control animals received the defined diet without phytol. Muscle tissue lipids were analyzed by mass spectrometry. The muscle phosphatidylcholine species profiles were similar between phytol and control diet, except that some additional species were detected in the phytol diet muscle. The two most abundant novel species (m/z 790.7 and 862.7) are tentatively identified as PC 20:0-16:0 and PC 20:0-22:6. To confirm the presence of phytanic acid, the ion mobility of these species was compared with diphtanoyl PC standard and endogenous straight chain PC phospholipids. The ion mobility is intermediate between PC species containing either zero or two phytanic acid chains, consistent with having one phytanic acid chain. We estimate that these species represent 5% of PC. Using this methodology, we will identify phytanic acid containing species in the other classes, in order to determine the total amount of phytanic acid-containing phospholipid incorporated in the muscle.

2753-Pos Board B183

Fluctuation-Induced Interactions between Membrane-Bound Proteins

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The spatial organization of membrane-bound proteins is in part determined by interactions that originate from long-range correlations due to the membrane's elastic behavior. Even basic geometric mechanisms, such as the suppression of membrane height fluctuations near protein binding sites, can lead to nontrivial interactions between proteins that might result in their aggregation. To study the effect of such membrane-induced interactions, we devise a simple model that captures (a) a nonspecific repulsion between proteins, (b) elastic properties of the membrane, and (c) a local harmonic coupling between proteins and membrane shape. The model's dynamics is governed by Langevin equations to faithfully capture entropic effects and the importance of rare fluctuations. We find that the membrane induces an attractive interaction between the proteins, which aggregate to mitigate the entropic cost of suppressing membrane fluctuations. This generic mechanism might help explain the spatial patterns induced by membrane sculpting proteins.

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Effect of Phosphatidylinositol-Bisphosphate (PIP₂) Lipids on Membrane Structure and Forces

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It is widely accepted that the interaction between lipid bilayers at closest separation is dominated by a repulsive hydration force arising from the structuring of water molecules on the lipid bilayer surface. This force has been recognized as a major activation energy barrier preventing fusion of bilayers. Our working hypothesis is that the orientation of the hydrogen bond network on the bilayer surface determines the amplitude of the hydration force. We are testing this hypothesis using the 1,2-dioleoyl-*sn*-glycero-3-phospho-[1'-myo-inositol-4',5'-bisphosphate](PI(4,5)P₂) lipid and its isomers which differ in the position of phosphate groups on the inositol ring. Phosphatidylinositol-bisphosphate (PIP₂) lipids are pivotal in signaling and play an important role in exocytosis. Specifically, we have utilized small angle X-ray diffraction (SAXS) and monitored the structural consequences of osmotic pressure in multilamellar suspensions of dioleoylphosphatidylcholine (DOPC) in the presence of PI(4,5)P₂. Our preliminary data (powder X-ray diffraction and reconstructed electron density profiles) show that there are notable changes in bilayer structure, particularly the lamellar repeat distance, thickness, and forces in the presence of PI(4,5)P₂.