Controlled Modulation of Lipid Bilayer State by a Photosensitive Membrane Effector

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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 and organize 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 appeared to be primarily multilamellar structures, which may promote changes in absorbance in our spectrophotometric assays. We found that the presence of salt could alter the structural organization of these membranes, with increased absorbance at higher temperatures.

2746-Pos Board B176
Measurement of the Viscosity of E. coli Membranes using Molecular Rotors and Film
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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 and was sensitive to the phospholipid composition of these 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 osmolality 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.
Germany, 1CAT Catalytic Center, RWTH-Aachen University, Aachen, Germany, 1IEAP, 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 presented on a membrane model system that was inoculated with a photosensitive and thus active variety of cholesterol. Azobenzene-cholesterol (azo-chol) 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-chol. 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.

2751-Pos Board B180 Creating Fluid Supported Lipid Bilayers with High Amounts of Phosphatidyethanolamine
Anne Sendecki. 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 inter-actions, 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.

2752-Pos Board B182 Ion-Mobility Mass Spectrometry Assay for Incorporation of Phytanic Acid into Myscel Phospholipids
Glen Humphrey1, Peter S. Backlund1, Paul S. Blank1, Joshua Zimmerman1. 1PPB NICHD NIH, Bethesda, MD, USA, 2NICHD NIH, Bethesda, MD, USA. Human dysferlinopathies (Limb-Girdle Muscular Dystrophy 2b, Myoshi Myopathy) are muscle-wasting syndromes caused by mutations in the dysferlin protein. Membrane 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 phos- pholipids. Phytic acid-containing phospholipids may improve the resistance of the muscle fiber sarcolemma to stretch-induced damage. To measure phytic 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 profiled by LC/MS and correlated with LC/MS of standard, 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 phytic acid, the ion mobility of these species was compared with diphytanoyl 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 phytic acid chain. We estimate that these species represent 5% of PC. Using this methodology, we will identify phytic acid containing species in the other classes, in order to determine the total amount of phytic acid-containing phos- pholipid incorporated in the muscle.

2753-Pos Board B183 Fluctuation-Induced Interactions between Membrane-Bound Proteins
Kayla Sapp, Lutz Maibaum. Department of Chemistry, University of Washington, Seattle, WA, USA. 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.

2754-Pos Board B184 Effect of Phosphatidylinositol-Bisphosphate (PIP2) Lipids on Membrane Structure and Forces
Sourav Haldar, Paul S. Blank, Joshua Zimmerman, Donald C. Rau. Program on Physical Biology, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, USA. 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 prevented preventing fusion of bilayers. Our working hypothes is that the orientation of the hydrogen bond network on the bilayer sur face determines the amplitude of the hydration force. We are testing this hypothesis using the 1,2-dioleoyl-sn-glycerol-3-phospho-[1'-myo-inositol-4',5'-bisphosphate](PI(4,5)P2) lipid and its isoforms 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, they have utilized small angle X-ray diffraction (SAXS) and moni-tored the structural consequences of osmotic pressure in multimamellar suspensions of dioleoylphosphatidylcholine (DOPC) in the presence of PI(4,5)P2. Our preliminary data (powder X-ray diffraction and reconstructed electron den-sity 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.