(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.

2746-Pos Board B176

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 livee 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.

2748-Pos Board B178

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.

2749-Pos Board B179

Controlled Modulation of Lipid Bilayer State by a Photosensitive Membrane Effector

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