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Dynamic and Static Measurements of A Single and Double Phospholipid Bilayer System
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We investigate the surface height fluctuations of single and double bilayers of DPPE supported on silicon using x-ray photon correlation spectroscopy (XPCS). In this techique, x-rays are incident on the membrane in a grazing incidence geometry and diffusely scattered x-rays are measured using an area detector. Time fluctuations of the scattering pattern can then be analyzed to yield the relaxation rate of surface height fluctuations. Bilayer and double bilayer systems were prepared utilizing combination of Langmuir-Blodgett and Langmuir-Schaeffer depositions. Static structural measurements were also made on these systems as well as on more complicated systems consisting of triple and five-fold bilayers of DPPE. Relationships between structure and dynamics of these systems will be discussed.

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Coexistence of Immiscible Mixtures of Palmitoylsphingomyelin and Palmitoylceramide In Monolayers and Bilayers
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A combination of lipid monolayer- and bilayer-based model systems have been applied to explore the interaction and organization of palmitoylsphingomyelin (pSM) and palmitoylceramide (pCer). Langmuir balance measurements reveal favourable interactions between the lipid molecules. A thermodynamically stable point is observed in the range 30-40 mol % pCer. The pSM monolayer undergoes hyperpolarization and condensation with small pCer concentrations, narrowing the liquid-expanded (LE) to liquid-condensed (LC) pSM main phase transition by inducing intermolecular interactions and chain ordering. Beyond this point, the phase diagram no longer reveals the presence of the pSM-enriched phase. Differential scanning calorimetry (DSC) of multilamellar vesicles reveals a widening of the pSM gel-fluid phase transition (41°C) upon pCer incorporation, with formation of a further endotherm at higher temperatures that can be deconvoluted into two components. DSC data reflect the presence of pCer-enriched domains coexisting, at different proportions, with a pSM-enriched phase that is no longer detected in mixtures containing >30 mol% pCer. Epifluorescence microscopy of mixed monolayers at low pCer content shows concentration-dependent, morphologically different pCer-enriched LC domain formation over a pSM-enriched LE phase, in which pCer contents close to 5 and 30 mol% can be determined for the LE and LC phases respectively. Fluorescence confocal microscopy of giant vesicles further confirms formation of pCer-enriched lipid domains. Vesicles cannot form beyond 40 mol% pCer contents. Altogether, the presence of at least two immiscible phase-segregated pSM-pCer mixtures of different compositions is proposed at high pSM content. A condensed phase (with domains segregated from the liquid-expanded phase) showing enhanced thermodynamic stability occurs near a compositional ratio of 2:1 (pSM:pCer). These observations become significant on the basis of the ceramide-induced microdomain aggregation and platform formation upon sphingomyelinase activity on cellular membranes.

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Diffusion of Nano-Meter-Sized Domains on A Vesicle
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The lateral diffusion of nano-meter-sized constituents (proteins, lipid rafts and so on) in lipid bilayers is of biological interest because the biochemical functions may be diffusion controlled. Saffman and Delbrück (SD) dealt with this issue using a hydrodynamic theory and derived an expression of the diffusion coefficient D forsmall domain size limit as $D(r)=k_BT[\ln(\eta_m h/\eta_m r)-0.0773]/4\pi\eta_m h$, where η_m and η_w are the viscosities of the membrane and the aqueous phase, respectively, h is the membrane thickness, r is the radius of the diffusing object. In this study we addressed the SD model by direct measurement of the intermediate scattering function of the nano-meter-sized liquid ordered domains in the fluid membrane using a contrast matching technique of neutron spin echo. We prepared ternary small unilamellar vesicles (SUVs) composed of deuterated DPPC, hydrogenated DOPC, hydrogenated cholesterol. The obtained intermediate scattering functions for the phase separated SUVs were fitted by a double exponential function, $S(q,t)/S(q,0)=A\exp(-D_0q^2t)+(1-A)\exp(-(D_0+D_d)q^2t)$, where A is the numerical constant, $D_0$and D_d are the diffusion coefficients of whole SUV and nano-meter sized domain, respectively. The fitting of $S(q,t)/S(q,0)$ gave $D_d = 2.3 \times 10^{-12}$ m^2/s, where the domains have the mean radius of 7.5 nm. The obtained domain diffusion coefficient agrees well with the SD prediction of $D= 2.35 \times 10^{-12}$ m^2/s using Ns/m^2 and Ns/m^2. Furthermore, by combining present data with the diffusion coefficient of a DPPC single molecule in L_d phase of $D_{DPPC} \approx 3.5 \times 10^{-12}$ m^2/s, we clearly demonstrates that the SD model well describes the observed diffusion coefficients in nano-meter length scale.

Platform W: Protein-Nucleic Acid Interactions II

1145-Plat
Single Molecule Studies of the Recognition Sequence Finding Mechanism of Protelomerase Telk
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Protelomerase TelK is an enzyme responsible for forming DNA hairpins in linear prokaryotic DNA. The mechanism by which this protein recognizes its target sequence and both quickly and accurately catalyzes DNA hairpin formation is poorly understood. To investigate the target recognition process, we used TIRF microscopy to visualize quantum dot-labeled TelK interacting with both nonspecific DNA and DNA containing the TelK target sequence. While many sequence-specific DNA-binding proteins (SSDBP) have been shown to scan DNA in 1D as their primary method for locating their recognition sequence[1], we surprisingly find that TelK does not move laterally on either aforementioned DNA substrate and therefore does not search by 1D scanning. Measurements of a c-terminally truncated TelK mutant reveal the same behavior. Interestingly, this mutant forms DNA hairpins 50 times slower than wild type, and dissociates from nonspecific DNA at a comparably lower rate than full-length TelK. These results suggest that dissociation from nonspecific DNA is an essential step in the recognition sequence search. Complementary studies with high-resolution optical tweezers reveal that TelK binding to DNA is a highly tension dependent process and condenses the molecule by several nanometers, consistent with crystal structures of the protein-DNA complex[2]. Remarkably, this condensation is observed on nonspecific DNA as well, despite the fact that these DNA distortions are energetically expensive. These findings suggest that the TelK target sequence search may involve 3D hopping and intersegmental transfer in lieu of 1D scanning. This may represent a novel SSDBP recognition sequence search mechanism.
1. Halford, S. et al. Nucl. Ac. Res. 32 (2004)
2. Aihara, H. et al. Mol. Cell. 27, 901 (2007)

1146-Plat
Bacteriophage phi29 Translocates DNA Along A Left-Handed Helical Path During Packaging
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Bacteriophage phi29 employs a homomeric ring of RecA-like ATPases in order to package its dsDNA genome into the capsid at near-crystalline density. Previous single-molecule measurements of packaging have revealed the coordination of motor subunits, the step size of the motor, and the sensitivity of the motor to substrate modifications, thereby suggesting structural and kinetic models for the mechanism of translocation. However, traditional single-molecule experiments measure only the projection of the motor's motion onto the DNA longitudinal axis.
We directly observe that phi29 translocates DNA along a left-handed helical path by monitoring rotation of a bead attached to the side of the substrate DNA in a laser tweezers. Simultaneously, the response to applied torque is measured. This novel experiment probes the details of force and torque generation by the packaging motor. Combining these measurements with angstrom-scale laser tweezers observations of motor stepping suggests specific geometric models for the interaction of the motor and DNA during translocation.