upon binding is crucial in elucidating the mechanism of protein function. Here, we use engineered DNA Holliday junction (HJ) as a single-molecule FRET reporter to study how CueR, a Cu(I)-responsive MerR-family metalloregulator, interacts with its DNA substrate for transcriptional regulation. By analyzing the single-molecule structural dynamics of the engineered HJ in the presence of varying concentrations of both apo- and holo-Cuer, we show how this metalloragulator interacts with and change the structures of the two HJ conformers, forming various protein-DNA complexes at different protein concentrations. We also show how apo- and holo-CueR differ in their interactions with DNA, as well as their similarities and differences with other members of the MerR-family of regulators, in particular in their mechanisms of switching off gene transcription after activation. This method of using engineered HJs to quantify changes in the structure and dynamics of DNA upon protein binding provides a new tool to elucidate the correlation of structure, dynamics, and function of DNA-binding proteins.

397-Pos

Complex Kinetics of the λ Repressor-Mediated DNA Loop

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λ repressor-mediated DNA loop formation and breakdown were monitored by Tethered Particle Microscopy (TPM) (1). The dwell times of the looped and unlooped DNA states, as revealed from TPM traces, were analyzed and revealed a complex kinetics for both loop formation and loop breakdown. A mechanism is proposed were λ repressor non-specific binding to DNA may play an important physiological role.

(1) C. Zurla, C. Manzo, D.D. Dunlap, D.E.A. Lewis, S. Adhya, L. Finzi, ''Direct demonstration and quantification of long-range DNA looping by the λ bacteriophage repressor.'', *NAR*, **37**, 2789-2795, 2009.

398-Pos

Free Energy Landscape of Nonspecific Protein-DNA Encounter

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Although structural, thermodynamic and kinetic studies of protein-DNA recognition have enhanced our understanding of both nonspecific and specific interaction mechanism, a few points are still in questioned, which are related to (i) how fast a protein can reach a given target on DNA and how long it will reside on DNA to perform its function, (ii) the energetic nature of protein-DNA interactions accompanied by conformational change, and (iii) the state of water in the DNA grooves and its role in the process of protein-DNA recognition. Here we have used the nuclease domain of colicin E7 (N-ColE7) from E. coli in complex with a 12-bp DNA as the model system to draw a picture of how a protein is encountering DNA. Brownian Dynamics (BD) coupled with Molecular Dynamics (MD) simulations are performed to provide the encoutering process in multiple timescales. Several encounter complexes, which have different positions and orientations of protein around DNA in the initial structures, are extracted from MD trajectories. Then those encounters are simulated using BD to estimate the association rates at different protein binding sites on DNA, characterize the reaction pathway based on the free energy landscape and determine the spatial and orientational aspects required for the association. The results facilitate better understanding of sequence-independent protein-DNA binding landscapes and suggest the favorable encounter states.

399-Pos

Single-Molecule Study on Microrna Machineries: Micrornaprocessing With Immunoprecipitates At the Single-Molecule Level

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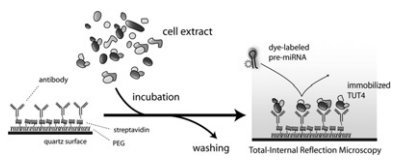
MicroRNAs (miRNAs) regulate gene expression via RNA silencing. Drosha initiates miRNA biogenesis by releasing a hairpin RNA (pre-miRNA) from a primary miRNA transcript. The pre-miRNA is processed into the mature miRNA by Dicer. It was also discovered that TUT4 (terminal uridylyl transferase 4) interferes with Dicer processing by uridylating pre-miRNAs. Discovery of these enzymes, however, was not accompanied with the study on the molecular mechanisms because of the lack of purified recombinant proteins.

Here we report a novel method that combines single-molecule fluorescence with immunoprecipitation, which is useful for studying proteins that are difficult to purify. On quartz surface in a microfluidic chamber, where single-molecule observation is going to be made, TUT4 proteins in crude cell extract are immobilized with specific antibody. After effectively washing away unwanted other proteins from the chamber, the interaction between proteins and dye-labeled RNAs are observed in real time. The direct observation reveals the uridylation process at the molecular level and helps identify distinct modes of action. This newly developed method of immunoprecipitation in singulo may be applied in studying other proteins such as Drosha that cannot be obtained as purified.



Membrane Physical Chemistry I

400-Pos

Nanomechanics of Lipid Bilayers: Heads or Tails?

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Understanding the effect of mechanical stress on biological membranes is of fundamental importance in biology since cells are known to perform their function under the effect of a complex combination of forces. The chemical composition and the lateral organization of such membranes are the ultimate responsible for determining their cellular scaffold and function. Micrometer-scale assays have revealed a wealth of information regarding the overall membrane mechanical resistance. Nonetheless, they are restricted to the use of giant bilayers, thus providing a mesoscopic outlook on the bilayer mechanical stability. Here we use force spectroscopy to quantitatively characterize the nanomechanical stability of supported lipid bilayers as a function of their chemical composition thanks to a molecular fingerprint that reveals itself as a repetitive jump in the approaching force curve, hallmark of bilayer rupture. By systematically probing a set of bilayers exhibiting different chemical composition, we first show that both the headgroup and tail have a decisive effect on their mechanical properties. While the mechanical resistance dramatically changes for phospholipids composed of a 18:0 chain with varying headgroups within a wide range (3nN-66nN), the chain length increases the mechanical stability in ~ 6 nN for every extra pair of -CH_2 groups present in the chain along the series DMPC-DSPC. Furthermore, each unsaturation in the chain readily decreases the mechanical stability of the bilayer by ~1.5 nN. Finally, and contrary to previous belief, we demonstrate that upon introduction of cholesterol the mechanical stability of membranes not only increases in the liquid phase (DLPC) but also for phospholipids present in the gel phase (DPPC). This work highlights the compelling effects of subtle structural variations of the chemical structure of phospholipid molecules on the membrane behaviour when exposed to mechanical forces, a mechanism of common occurrence in nature.

401-Pos

The Phase Behavior of Supported Lipid Bilayer Mixtures and Cell Membranes Imaged By Secondary Ion Mass Spectrometry

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Supported lipid bilayers have been used as a tool to study the biophysical properties of model membranes with defined compositions. Special attention has been given to the role of cholesterol on the phase behavior of lipid membranes, in particular, to the formation of lipid rafts and complexes. Common techniques used to elucidate the phase behavior of binary and ternary lipid bilayers (i.e. fluorescence and atomic force microscopy) have been limited by their inability to provide direct information on the spatial composition of membranes. Secondary ion mass spectrometry (NanoSIMS) has proved to be a powerful tool for imaging the lateral organization of lipid bilayers with a spatial resolution in the order of tens of nanometers. In this study, a NanoSIMS is used to image lipid bilayers containing isotopically-labeled cholesterol. Additionally, human cell membranes are also imaged.

402-Pos

Direct Measurement of Time-Dependent Domain Coarsening in Giant Unilamellar Vesicles

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Liquid domains appear on giant unilamellar vesicles (GUVs) composed of a ternary mixture of saturated phospholipids, unsaturated phospholipids, and cholesterol when the temperature is quenched below the miscibility transition temperature. If the vesicle is taut, domains diffuse freely in the membrane and coalesce when they collide. This process is called coarsening. As the domains coarsen, the average radius of the domains increases with time as t^x, where x is the power law exponent of radius growth. The power law exponent has been

predicted to be 1/3 [Gomez et al., Saeki et al.] or 1/4 [Yanagisawa et al.]. The power law exponent has been measured previously for liquid domains in vesicle membranes as 0.15 [Saeki et al.] and 2/3 [Yanagisawa et al.]. Here we present an independent measurement of the power law exponent.

Gomez, J., Sagues, F., & Reigada, R. (2008). Use of an enhanced bulk diffusion-based algorithm for phase separation of ternary mixture. The Journal of Chemical Physics , 184115-1-184115-9.

Saeki, D., Hamada, T., & Yoshikawa, K. (2006). Domain-Growth Kinetics in a Cell-Sized Liposome. Journal of the Physical Society of Japan, 013602-1-013602-3.

Yanagisawa, M., Imai, M., Masui, T., Komura, S., & Ohta, T. (2007). Growth Dynamics of Domains in Ternary Fluid Vesicles. Biophysical Journal , 115-125.

403-Pos

Molecular Interactions in Phase Separation of DOPC/DSPC/cholesterol Ternary Mixtures

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With the aim of investigating molecular interactions between lipids involved in lipid-raft formation, the experimental phase diagram of a DOPC/DSPC/cholesterol ternary system was simulated using Monte Carlo simulation. Both pairwise (Ising-like) and multibody interactions were used to simulate the phase boundary of liquid-ordered phase and liquid-disordered (Lo-Ld) phase coexistence regions. The ''Composition Histogram Method'' (CHM) was specifically developed to quickly determine the compositions of coexisting phases as well as the thermodynamic tie-lines. The simulation demonstrated that the phase boundaries produced by pairwise (Ising-like) interactions alone generally do not agree with the experimental phase boundary. A much better fit for the experimental phase boundary was obtained by including a ''domain edge energy'' term, which is expressed in a form ofssmultibody interaction. Our result shows that the ''domain edge energy'' is essential for creating phase separation in lipid raft mixtures. The magnitude of this interaction energy determines the location of the critical point, the shape the phase boundary, and the size distribution of lipid domains in lipid raft mixtures. Any experimental condition that alters the domain edge energy, could significantly change the shape and location of the Lo-Ld phase boundary.

404-Pos

Molecular Dynamics Simulations of Ceramide Flip-Flop and Desorption in Lipid Rafts

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Ceramides are important signaling lipids, involved in processes such as apoptosis and cell differentiation. Upon stimulus acid sphingomyelinase hydrolyses sphingomyelin into ceramide on the extracellular leaflet. How ceramide generated on the extracellular leaflet induces an intracellular response remains unknown. Ceramide has been shown to associate and stabilize lipid rafts, which could create a signaling platform. We have undertaken molecular dynamics computer simulations (MD) of ceramide in both putative raft and non-raft bilayers. Using umbrella sampling we determined free energy profiles for moving ceramide and cholesterol from water to the center of raft and non-raft bilayers. The free energy barrier for ceramide flip-flop is 49 kJ/mol and 62 kJ/mol in the non-raft and raft bilayers. From these barriers, we estimate the rate of ceramide flip-flop is 0.3 s-1 and 0.003 s-1 in the non-raft and raft bilayers. The free energy for desorption can be equated to the excess chemical potential of ceramide in the bilayer compared to water. By comparing the chemical potentials, we can infer the relative affinity of ceramide and cholesterol for the raft and non-raft bilayers. Cholesterol has a large affinity for the raft bilayer compared to the non-raft bilayer, while ceramide has only a slight preference for the raft bilayer. These results provide a thermodynamic molecular-level description of the interactions of ceramide with lipid rafts, and the rate of translocation.

405-Pos

Dna Lipoplexes: Prediction of Phase Architectures Using Cg Simulations and Experimental Validation

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DNA lipoplexes are important mediators of transfection that offer a safer, although less efficient alternative to their viral counterparts. Thus improving the efficacy of DNA lipoplexes is essential for their exploitation in nanomedicine. Experimentally, it has been shown that the architecture of DNA lipoplexes is linked to their biological efficacy. Therefore the ability to predict the architectures of compositions of DNA lipoplexes would be highly desirable. However, prediction of the phase behaviour of such systems is difficult, largely owing to a complex interplay of intermolecular forces. Molecular dynamics simulations provide a potential strategy for predicting phase behaviour, but traditional, atomistic methods are not applicable to large DNA-lipid systems. Here, we present coarse-grained simulations of the lyotropic phase transitions of DNA lipoplexes as a function of lipid composition and water content. Our coarse-grained model of DNA uses a ~ 4 to 1 mapping of atoms to particles and is compatible with existing coarse-grained models of biomolecules. With the appropriate balance of water content and lipid composition, we are able to capture the transition from the originally lamellar phase to the inverse hexagonal phase. Our simulation results show an inverse hexagonal phase with a calculated d-spacing of 6.2 nm for a DOPE-DNA system. Together with the disorder of the hexagonal phase, this d-spacing increases with increasing cationic lipid (DOTAP) content, in agreement with experimental data obtained by SAXS and polarizing light microscopy. Our simulations have provide insights into the rearrangements that occur to effect the transition to the inverse hexagonal phase; this level of detail is difficult to obtain using experimental methods alone. Furthermore, our simulations have highlighted the increasingly important role of coarse-grained simulation methods for the design of novel DNA lipoplexes and applications in synthetic biology, in general.

406-Pos

Microrheology of Freestanding Lipid Bilayers

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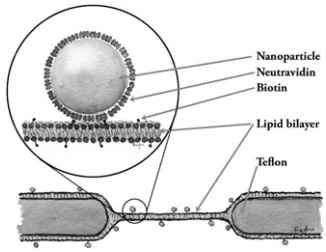
The macroscopic material properties of cellular membranes, determined by the composition and interactions of their constituent lipids, are important factors in the structure and function of all living cells. Fluidity is a key material property of membranes, yet the underlying lipid bilayer viscosity and other rheological parameters remain poorly quantified.

We adopt recently developed microrheological methods to study multiple composite freestanding ''black'' lipid membranes. Using high speed video particle tracking, we monitor dynamics of membrane-anchored nano- and micro-particles across a range of temperatures that span bilayer phase transitions. Two particle spatial correlation functions and the complex shear modulus are extracted from such measurements and provide information about fundamental membrane material properties. We find striking and previously unreported signatures of viscoelasticity in these lipid bilayers whose properties are sensitive to the bilayers' temperature dependent liquid ordered to liquid disordered phase transitions.



407-Pos

Coping With the Cold: Effect of Hibernation on Pulmonary Surfactant in the Thirteen-Lined Ground Squirrel

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Pulmonary surfactant, a mixture of phospholipids, cholesterol, and proteins, stabilizes the lung by reducing surface tension at the air-water interface of the alveoli. We hypothesized that lowering body temperature to approximately 5°C during hibernation would require compositional changes in surfactant lipids and perhaps proteins in order to maintain lung function. Large aggregate (LA) fractions were obtained by centrifuging lung lavage at 40,000 g for 15 min, with small aggregates (SA) remaining in the supernatant. Because hibernating animals have lower body masses, surfactant levels per animal were compared. Hibernation resulted in an increase in total surfactant due to increased LA with little change in SA. Cholesterol at 8 wt% PL was not altered. Hibernation was accompanied by a small (~15%) decrease in disaturated phosphatidylcholine and phosphatidylglycerol. Decreases were observed in the mRNAs for the surfactant proteins (SP-). Western analysis revealed levels of all SP-s decreased to approximately 10% (SP-A), 50% (SP-B), 90% (SP-C), and 50% (SP-D) of warm active levels. We speculate that the changes in surfactant LA levels might reflect the much lower breathing rate during hibernation. Furthermore, the compositional changes could arise, in part, from a slowing of metabolism. The decrease in disaturated phospholipid levels could lead to enhanced adsorption due to increased fluidity, but this suggestion will have to be confirmed experimentally.