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

Laura Finzi, Carlo Manzo, Chiara Zurla, David D. Dunlap.
Emory University, Atlanta, GA, USA.

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

Chuanying Chen, B. Montgomery Pettitt.
University of Houston, Houston, TX, USA.

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.

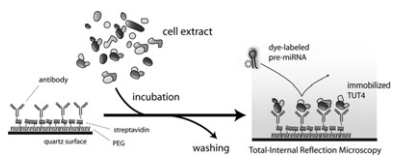
399-Pos

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

Kyu-Hyeon Yeom, V. Narry Kim, **Chirlmin Joo**.
Seoul National University, Seoul, Korea, Republic of.

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?

Sergi Garcia-Manyes[1], Gerard Oncins[2], Lorena Redondo[2], Fausto Sanz[2].
[1]Columbia University, New York, NY, USA, [2]Universitat de Barcelona, Barcelona, Spain.

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

Monica M. Lozano[1], Peter K. Weber[2], Ian D. Hutcheon[2], Steven G. Boxer[1].
[1]Stanford University, Stanford, CA, USA, [2]Glenn T. Seaborg Institute & BioSecurity and Nanosciences Laboratory, Lawrence Livermore National Laboratory, Livermore, CA, USA.

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

Cynthia A. Stanich, Aurelia R. Honerkamp-Smith, Sarah L. Keller.
University of Washington, Seattle, WA, USA.

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