Platform D: Protein Assemblies

46-Plat

Biophysical Characterization of the Complex Formed By Disf and RNA Polymerase II

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DISF is an RNA polymerase II (pol II) elongation factor. It can bind to pol II and regulates the synthesis of mRNA both positively and negatively. Biochemical analyses show that DISF can interfere the action of TFIIS, a mRNA cleaving enzyme at 3′ end but stabilize the ternary complex when mRNA is greater than 35 nt. To resolve these puzzle, we set out to use various biophysical means, including single molecule FRET (and electron microscopy reconstruction techniques, to characterize the complex formed by DISF and pol II. Here, we report a low resolution 3D structure of yeast pol II yeast pol II complexed with DISF and also smFRET constraints toward depicting the geometric relationship between mRNA and DISF.

47-Plat

Structure-Function Analysis of the HIV-1 Integrase in Complex With Two Cellular Proteins: Ledgf and INI1

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Integration of the human immunodeficiency virus type 1 (HIV-1) cDNA into the human genome is catalyzed by the viral integrase protein (IN) that requires cellular cofactors for viral infectivity. Recently, we solved a cryo-EM structure at 14 Å resolution of the HIV-1 integrase in complex with the lens epithelium-derived growth factor (LEDGF), a cellular transcriptional coactivator, in presence and absence of DNA (1). This structure revealed the molecular mechanism of DNA integration in the human genome. Another cellular co-factor, the integrase interactor 1 protein (INI1/SNF5) which is a part of the SWI/SNF complex, an ATP dependant chromatin remodeler, has been shown to binds directly to integrase. Its function in the viral DNA integration process is not well characterized, but its presence is critical for viral infectivity. We stably formed, in vitro, a complex comprising IN, LEDGF and a fragment of INI1. *In vitro* functional assays have been performed and a 15 Å resolution cryo-EM structure of the ternary complex has been solved. The structure function analysis and the effect of INI1 on the DNA binding, 3′ processing and integration reaction will be presented.

(1) Michel, F., Crucifix, C., Granger, F., Eiler, S., Mouscadet, J.F., Korolev, S., Agapkina, J., Ziganshin, R., Gottikh , M., Nazabal, A., Emiliani, S., Benarous, R., Moras, D., Schultz, P. and Ruff, M. (2009). Structural basis for HIV-1 DNA integration in the human genome, role of the LEDGF/P75 cofactor. EMBO J., 28, 980-991.

48-Plat

ZAPA Controls the Scaffolding Function of FtsZ Through Three Synergistic Activities

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A key event in the formation of the bacterial cytokinetic apparatus is the attachment of FtsZ polymers to the membrane and their concentration at midcell in a structure termed the Z ring. Z rings serve as mechanical scaffolds, which recruit other cell division proteins to establish functional divisomes. The scaffolding function of Z rings is essential for the persistence of divisomes as coherent structures throughout cytokinesis.

Here, we study ZapA, a positive regulator of the scaffolding activity of FtsZ. We estimate, for the first time, the physiological concentration of FtsZ inside Z rings. We then investigate the mechanics of FtsZ gels at these concentrations of FtsZ and in the presence of varying amounts of ZapA. By analyzing wild-type FtsZ as well as polymerization deficient FtsZ mutants using quantitative rheometry, electron microscopy and sedimentation assays, we find that ZapA greatly enhances the stiffness of FtsZ gels by promoting the nucleation of FtsZ polymerization, by promoting lateral interactions between FtsZ polymers, and by crosslinking FtsZ polymers. We also describe, for the first time, the phenotype of *Escherichia coli* cells deleted for the *zapA* gene. The *zapA* null mutant strain shows increased occurrence of filamentous cells. Fluorescence microscopy of FtsZ-GFP in these filaments reveals failures in cytokinesis resulting from mechanical instabilities of Z rings. The *zapA* null mutation also increases the temperature sensitivity of strains carrying ts alleles of *ftsA*, *zipA* and *ftsZ* genes, arguing that protein products of these genes act collectively in the scaffolding function of Z rings.

Taken together, our *in vitro* and *in vivo* data provide support for a model in which three synergistic activities of ZapA act as a kind of glue that stabilizes contacts between FtsZ molecules and thereby promotes the mechanical coherence of Z rings.

49-Plat

DNA Nanomachines Investigated By Non-Denaturing Mass Spectrometry

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We are using a novel approach for the investigation of noncovalent protein-DNA assemblies in vitro, non-denaturing nano-electrospray Q-TOF mass spectrometry combined with ion mobility spectroscopy (IMS-MS/MS), to study assembly, size and shape of protein nanomachines which act on DNA.

Specifically we highlight recent results on the BcgI restriction-modification system, which consists of two types of subunits: BcgIA contains both endonuclease and methyltransferase motifs; BcgIB is homologous to the HsdS subunits of Type I RM systems that mediate DNA sequence recognition. Together they form an A2B protomer which is active only when bound to two copies of its site, and then cuts eight phosphodiester bonds, those on both sides of both sites, before dissociating from the DNA.

The data is discussed in the context of functional assays and additional biophysical characterization by Analytical Ultracentrifugation.

We are also presenting preliminary results on the assembly of other DNA-protein complexes with particular emphasis on natively unstructured proteins which adopt a defined conformation only when interacting with specific DNA sequences.

50-Plat

Structures and Interactions in Neurofilament: Gel Expanded To Gel Condensed Transition

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Neurofilaments (NFs) - the major cytoskeletal constituent of myelinated axons in vertebrates - consist of three molecular-weight subunit proteins NF-L (low), NF-M (medium), and NF-H (high), assembled to form mature filaments with protruding unstructured C-terminus sidearms. Liquid crystal gel networks of sidearm-mediated NF assemblies play a key role in the mechanical stability of neuronal processes. Disruptions of the NF-network, due to NF over-accumulation or incorrect sidearm interactions, is a hallmark of motor neuron diseases including amyotrophic lateral sclerosis. Using synchrotron x-ray scattering [1,2], and various microscopy techniques [1,3] we report on the role of the subunit sidearms on the structure and interaction of NF. We will show a direct measurement of forces in reconstituted NF-gels under osmotic pressure (P). With increasing pressure near physiological salt, NF-LMH, comprised of the three subunits near in-vivo composition, or NF-LH gels, undergo for $P>Pc \approx 10$ kPa, an abrupt nonreversible gel expanded to gel condensed transition. The transition indicates sidearm-mediated attractions between NFs consistent with an electrostatic model of interpenetrating chains. In contrast, NF-LM gels, remain in a collapsed state for PPc. In addition, single filament AFM measurements show that bending modulus is also regulated via intra-filaments interactions [4]. These findings, which delineate the distinct roles of NF-M and NF-H in regulating neurofilament interactions, shed light on possible mechanisms for disruptions of optimal mechanical network properties. Supported by DOE DE-FG-02-06ER46314, NSF DMR-0803103, and the Human Frontier Science Program organization. [1] J.B. Jones, C.R. Safinya, Biophys. J. 95, 823 (2008); [2] R. Beck et al., Nature Mat. (2009) in press; [3] H. Hess et al. Langmuir 24, 8397 (2008) [4] R. Beck et al. to be published.

51-Plat

Protegrin-1 (PG-1), An Antimicrobial Peptide Forms Ion Channels: Atomic Force Microscopy, Channel Conductance, and Molecular Dynamics Simulation Study

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Antimicrobial peptides (AMP) are an emerging class of antibiotics being investigated in search for safer and effective means to manage emerging antibiotic-resistant microbial strains. An extensive body of work makes protegrin-1 (PG-1) a model antibiotic candidate among β-hairpin AMPs. 3D structural substrate and

the mechanism underlying AMPs' ability to disrupt cell membrane defense are not completely understood. We present computational and experimental evidence showing that the β-hairpin PG-1 aggregates and forms ion channels in target cell membranes. We used complementary approaches, including Molecular Dynamics (MD) simulations, Atomic Force Microscopy (AFM) imaging, Planar Lipid Bilayer (PLB) reconstitution and cellular toxicity measurements. MD simulations indicate that PG-1 does not form fibrillar structures on the surface of DOPS/POPE bilayers. However, PG-1 aggregates into channel-like structures with loosely attached subunits when inserted into anionic lipid bilayers. AFM images show no PG-1 fibril formations on the lipid bilayers. However, on a negative non permeable surface, PG-1 formed fibrils that bear some resemblance to amyloids fibers. On the other hand, AFM images show channel-like structures formed by PG-1 when reconstituted in DOPS/POPE bilayers. In PLB electrical conductance measurements, we observed multiple single channel conductances consistent with the heterogeneous oligomeric channel structures seen in AFM images. In addition, PG-1 channel formation seems to be lipid-dependent: PG-1 does not form channels in PC membranes, but forms channels in membranes rich in PE, PG or PS. Unlike amyloid channels, Zn^{2+} does not inhibit PG-1 channel conductance. Microbial cells treated with PG-1 showed antimicrobial activity consistent with ion leakage. The combined results support a model where the β-hairpin PG-1 antibiotic permeates membranes by forming ion conductive channel-like structures and cause cell injury.
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52-Plat
S-Layer Self-Assembly on Supported Lipid-Bilayers: The Importance of Amorphous Precursors and Folding Transitions
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The outermost membranes of many archaea and bacteria are comprised of highly-ordered 2D arrays of surface layer (S-layer) proteins. Their functions include selective transport, structural scaffolding, mineral templating and propagation of or protection from pathogenesis. Although the primary and secondary structures of the isolated proteins determine their governing interactions, their functions emerge from the tertiary and quaternary architecture that stems from S-layer self-assembly, a process that is poorly understood. Here we report results using *in situ* AFM to follow 2D self-assembly of monomeric SbpA of *Lysinibacillus sphaericus* on supported lipid bi-layers (SLBs) at the molecular-scale. We show that the assembly process begins with adsorption of unstructured monomers, which form a mobile phase on the SLBs. These then condense into amorphous clusters, which undergo a phase transition to ordered 2D clusters of 2 to 15 folded tetramers. The ordered clusters then enter a growth phase in which new tetramers form from unstructured monomers exclusively at unoccupied lattice sites along the cluster edges, implying that new tetramer formation is auto-catalytic. We show that the analysis of growth dynamics leads to a quantitative model in which the main rate limiting parameter is the probability of tetramer creation. The estimated energy barrier of 51 kJ/mole for this process is much less than expected form scaling laws for folding of isolated proteins. Finally we present preliminary results from dynamic Monte Carlo simulations that show how the combination of non-specific interactions and directional bonds characteristic of many proteins lead to non-classical assembly pathways, such as the one observed here involving formation of amorphous clusters followed by relaxation to the ordered state.

53-Plat
A Predictive Theoretical Model For Clathrin Self-Assembly
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Clathrin is a protein that plays a major role in the creation of membrane-bound transport vesicles in cells. Clathrin forms soccer-ball-shaped lattices that coat a new vesicle as it forms. The clathrin molecule is known to take the shape of a triskelion, a figure with three bent legs. In vitro assembly of clathrin within a solution results in closed, nanoscale assemblies with various shapes and sizes. To understand how clathrin functions, particularly how it forms the lattice, we develop a theoretical model for the thermodynamics and kinetics of clathrin assembly in order to guide experiments toward the design of targeted nanoscale structures. Our model addresses the behavior in 2 and 3 dimensions, relevant to membrane/surface and bulk assembly, respectively. The clathrin triskelions are modeled as effective flexible pinwheels that form leg-leg associations and resist elastic bending and stretching deformations. Thus, the pinwheels are capable of forming a range of ring structures including 5-, 6-, and 7-member rings that are observed experimentally. Our theoretical model employs Brownian dynamics to track the motion of clathrin pinwheels at sufficiently long time scales to achieve complete assembly. With this theoretical model, we predict the phase diagram for clathrin assembly incorporating binding interactions, elastic deformation, and phonon modes. To verify the phase diagram, we perform dynamic simulations for a range of quenches into the phase diagram and compare phase separation across the binodal curve. We show that resulting Brownian dynamics simulations exhibit the hallmark behavior of spinodal decomposition with subsequent coarsening of ordered domains. These simulations demonstrate the effect of quench rate and leg elasticity on the final configurations of the lattice network and cluster-size distribution. We then proceed to discuss the assembly of specific nanoscale structures.

Platform E: Computational Methods

54-Plat
Molecular Dynamics Simulation of Phospholipid Bilayers and Monolayers Using a Polarizable Force Field
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The assumptions that underlie empirical force field models based on fixed molecular charge densities become questionable in the strongly heterogeneous electrostatic environment of bilayer membranes. Membranes contain regions that are polar (bulk water) highly charged (zwitterionic lipid head groups) and decidedly non-polar (hydrocarbon core). Using a recently developed polarizable Drude oscillator force field for lipids and water we present a study that illustrates the significant role played by electronic polarization effects in the electrostatic modeling of a phospholipid membrane. Specifically, we show that the inclusion of such many-body polarization effects can bring macroscopic electrostatic properties into quantitative precision with experimental observation.

55-Plat
The Small Angle Scattering Toolbox
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Small Angle Scattering (SAS) is a technique used to investigate structure and dynamics of macromolecules in solution. Proteins in buffer conditions close to their physiological environment, are subject to Xray or Neutron scattering experiments. The resulting one-dimensional scattering curves are directly related to their three-dimensional structure. The SAS technique is routinely used to determining the low resolution shape of protein and map specific large scale conformation changes in protein structures.
We present a recently developed computational platform for SAS data analysis and model construction/refinement.The Small Angle Scattering Toolbox (SASTBX) has tools four major modules: (1) Raw data reduction; (2) theoretical scattering profile calculation based on PDB structures; (3) Pair distance distribution function (PDDF) estimation; and (4) 3D model construction and structure refinement.
The toolbox can be utilized to read raw scattering images obtained from the detector to generate an intensity profile. The basic analyses, such as Guiner and Kratky plots can be carried out in real time to assess the sample and data quality while collecting data. The PDDF estimation is a fully automated procedure, linked with a database a known PDDF's allowing for a rough initial classification of the shape of the protein. Model data can be calculated on the basis of a spherical harmonics expansions. Initial structures can be further refined with normal mode movements or rigid-body motions.
The sastbx is built on the open source Computational Crystallography Toolbox (CCTBX). The toolbox is implemented by using Python/C++ hybrid approach: the computing intensive jobs are handled in C++, and the python allows easy integration between other components. The source code will be distributed as open source project.

56-Plat
Large-Scale Simulations of Fluctuating Biological Membranes
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We present a new computational model for lipid bilayers that allows the simulation of membrane systems on the micrometer scale. In our model, each ~25 nm^2 patch of bilayer is represented by a spherical particle. Mimicking the forces of hydrophobic association, many-body interactions suppress the exposure of each sphere's equator to the implicit solvent. This driving force towards high equatorial density stabilizes two-dimensional aggregates without necessitating crystalline order. This allows us to match both the surface