Platform D: Protein Assemblies

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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.

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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.

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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.

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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