Platform S: Protein Assemblies and Protein Nucleic Acid Complexes

1079-Plat

Ion Mobility-Mass Spectrometry Measurements Combined with Molecular Modeling Yields the Architecture of DNA Polymerase Complexes

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Understanding how DNA polymerases interact with the rest of the replication machinery is of primary importance in unraveling some long-standing questions surrounding the mechanisms of DNA replication. We have been developing a method that combines both molecular and coarse-grained modeling and ion mobility-mass spectrometry (IM-MS) measurements in order to determine the positions of the protein subunits within the complex. The method works by first ionizing and desolvating the protein assemblies using nano-electrospray ionization. We then estimate the sizes of the protein ions by measuring their transit time through an IM separator, and measure their masses to determine their composition. To construct a model of the complex, MS information is used to infer subunit connectivity and IM information is used to filter the size of the assembly against a field of computationally derived model structures. Central to our method is the use of these measurements on various subcomplexes generated either in the solution or gas phases. When available, we use crystal structures of proteins and subcomplexes to build up models of complexes of unknown architecture, calculating and comparing their sizes with what is being measured by IM. Here, we present our most recent models for three different polymerases (II, III, and IV) bound to the prokaryotic DNA sliding clamp, the clamp loader bound to the clamp, and the chi-psi dimer bound to the clamp loader. The presentation will be evenly balanced between the development of this hybrid approach and its application to DNA polymerase III.

1080-Plat

Direct Measurement of Inter-filament Forces in Neurofilament Networks: Synchrotron X-ray Diffraction Study under Osmotic Pressure

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Neurofilaments (NFs) are the major protein constituents in neuronal processes (axons and dendrites) that impart mechanical stability and act as structural scaffolds. The filaments assemble from 3 different subunit proteins (NF-L, NF-M, NF-H) to form a 10 nm diameter flexible polymer with radiating unstructured sidearms. Recent work, showed that at high protein concentration, the NFs form a nematic hydrogel network with a well-defined interfilament spacing as can be measured by synchrotron small angle x-ray scattering (SAXS) [1]. The x-ray/phase behavior study showed the role the different subunit protein compositions play in the interfilament interaction. In order to directly elucidate the interfilament forces responsible for the mechanical properties of NFs hydrogel, we conducted a SAXS-osmotic pressure study, which yielded pressure-distance curves at different subunit compositions and monovalent salts. We show that filaments composed with NF-L and NF-M strongly attract each other through their polyampholyte sidearms, in particularly at high monovalent salt. However, filaments comprised of NF-L and NF-H, at high NF-H grafting density, show a distinctly different pressure-distance dependency, with much larger interfilament spacing and weaker salt dependence. Supported by DOE DE-FG-02-06ER46314, NIH GM-59288, NSF DMR-0503347, and the Human Frontier Science Program organization.

[1] J.B. Jones, C.R. Safinya, Biophys. J. 95, 823 (2008).

1081-Plat

DNA Heats Up: Energetics of Genome Ejection from Phage Revealed by Isothermal Titration Calorimetry

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It has been experimentally shown that ejection of double-stranded DNA from phage is driven by internal pressure reaching tens of atmospheres. This internal pressure is partially responsible for delivery of viral genome into the host cell. While several theoretical models and simulations nicely describe the experimental data of internal forces either resisting active packaging or equivalently favoring spontaneous ejection, there are no direct energy measurements available that would help to verify how quantitative these theories are. We performed direct measurements of the enthalpy (which is essentially equivalent to the internal energy) responsible for DNA ejection from phage λ, using Isothermal Titration Calorimetry (ITC). The phage capsids were ''opened'' in vitro by titrating λ particles into a solution with purified LamB receptor and the enthalpy of DNA ejection process was measured. In this way, enthalpy stored in phage was determined as a function of packaged DNA length comparing wild-type phage λ (48.5 kb) with a shorter λ-DNA length mutant (37.7 kb). The temperature dependence of the ejection enthalpy was also investigated. The values obtained were in good agreement with existing models and provide a better understanding of double-stranded DNA packaging and release mechanisms in motor-packaged viruses (e.g., tailed bacteriophages, Herpes Simplex, and adenoviruses).

1082-Plat

Nucleotide Control of Replication Initiation: NTPase Mechanisms of *E.coli* DnaB-DnaC Complex

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In E. coli, interactions between the primary replicative helicase, DnaB protein and the replication factor, DnaC protein, are at the heart of the chromosomal DNA replication. To address the role of the DnaC in processes of the free energy transduction by the helicase, we have quantitatively examined DnaB-DnaC complex using fluorescence titration, analytical ultracentrifugation, and rapid chemical quench-flow techniques.

In absence of nucleic acid, DnaC reduces intrinsic affinity and increases negative cooperativity in nucleotide binding to DnaB helicase. The ground-state effects are accompanied by the reduced rate of ATP hydrolysis by the helicase. In presence of DNA, DnaB in the DnaB-DnaC complex recovers its nucleotide binding capabilities and ATPase activity. These data suggest that recognition of the oriC by the DnaB - DnaC complex and/or its entry into the pre-primosome requires diminished NTPase activity of the helicase. Analysis of nucleotide binding to the DnaC protein, engaged in the DnaB - DnaC complex, indicates that *prior* to the recognition of the oriC sequence and/or pre-primosome assembly, the DnaC protein in the complex is in a conformational state, which does not bind ATP or ADP. So, the formation of the replisome and the pre-primosome seems to preferentially require the presence of DnaC in a state free of cofactors. Significant positive cooperativity of the binding process indicates that small fluctuations in ATP and/or ADP concentrations can induce an all-or-none allosteric transition of bound DnaC molecules into the conformation, which has an increased intrinsic affinity for the nucleotides. The presence of such an all-or-none allosteric transition, encompassing all bound DnaC molecules, indicates that recognition of the oriC and the pre-primosome assembly includes complex interplay between different conformations of the DnaB - DnaC complex. The physiological importance of the obtained results will be discussed.

1083-Plat

Oligomerization And Interaction Of DDR1 With Collagen: An AFM And FRET Microscopy Study

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Activation of Discoidin Domain Receptor 1 (DDR1) by collagen is reported to regulate cell migration and survival processes. While the oligomeric state of DDR1 is reported to play a significant role in collagen binding, not much is known about the effect of collagen binding on DDR1 oligomerization and cellular distribution. Using fluorescence resonance energy transfer (FRET) microscopy we monitored the interaction between DDR1 tagged with either cyan (CFP) or yellow fluorescent protein (YFP) on live cells. Significant FRET signal, indicative of receptor dimerization was found even in the absence of collagen stimulation. Collagen stimulation induced aggregation of DDR1 followed by a sharp increase in FRET signal, localized in the regions of aggregated receptor. Further analysis of DDR1 aggregation revealed that DDR1 undergoes cytoplasmic internalization and incorporation into the early endosome. We found the kinetics of DDR1 internalization to be fast, with a significant percentage of the receptor population being internalized in the first few minutes of collagen stimulation. Our results indicate that collagen stimulation induces aggregation and internalization of DDR1 dimers at timescales much before receptor activation. These findings provide new insights in the cellular redistribution of DDR1 following its interaction with collagen type I.

1084-Plat

The Measured Electrostatic Charge on IgGs

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Blood plasma is a high-concentration fluid containing ~70 mg/ml protein. A major component of plasma (~10 mg/ml) is a changing, heterogeneous mixture of IgGs. At plasma concentrations electrostatics (charge, dipole, induced

dipoles, etc.) dominate chemical activity, hence solution properties. Protein charge can be determined accurately using a combination of electrophoretic and hydrodynamic measurements. It is essential to measure charge since calculated values (e.g., from isoelectric point determinations) may be in serious error. Monoclonal IgGs (mAbs) provide an important example where charge must be measured. Charge determinations for 11 different mAbs in 100 mM KCl at pH 6.0 show that calculated values overestimate the charge by ~17, with the discrepancy increasing to ~50 at pH 5.0. The mechanisms underlying charge suppression are unclear. There is nothing obvious in IgG structure (e.g., buried side chains, H-bonding, clustered charged side chains) that would account for the suppressed charge. It seems likely that weak ion binding (either site or territorial) by IgGs may occur since changing the solvent ionic strength and ion composition influence charge suppression beyond their Debye-Hückel effects. The unusual charge properties of IgGs may have both in vivo and in vitro significance. In vivo, charge suppression may provide a ''buffer'' that allows high plasma concentrations of IgGs with different amino acid compositions. Charge also may be important in Fc receptor binding of IgGs. Analysis of isolated Fc and Fab fragments reveals that the Fc fragment charge is less than +1 at pH 6.0, where the calculated charge is +9. In vitro, IgG charge correlates with increased solubility and reduced solution viscosity, properties that are important in drug formulation.

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1085-Plat

A Near Atomic Resolution Model of the Microvillus and the Organization of the Brush Border

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Microvilli are ~1-μm long finger-like projections present on the apical surfaces of certain, specialized absorptive epithelial cells. A highly symmetric hexagonal array of thousands of these structures form the brush border, which in addition to providing a significant increase in surface area also serves a barrier function against invading pathogens. Here, for the first time, we present an atomic model of the protein cytoskeleton responsible for this dramatic cellular morphology. This model incorporates spectroscopic, crystallographic, and microscopic data reported by several groups over the last 30 years into a single cohesive macromolecular complex composed of actin, fimbrin, villin, brush border myosin (Myo1A), calmodulin, and brush border spectrin. The biological, biochemical, and biophysical implications stemming from this model will be discussed.

1086-Plat

Structural Study And Modeling Of The Influenza Viral Ns1 Protein

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The influenza NS1 protein is an intriguing molecule that performs a large number of functions and interacts with different types of molecules including proteins and nucleic acids. The N-terminal domain and the C-terminal domain structures of the molecule have been solved and both domain structures exist in a dimeric arrangement. Based on docking studies and using a loop-modeling algorithm developed in our laboratory, we have developed structural models for the NS1 dimeric complex. These models are compared to each other and to the Bornholdt & Prasad model. The bindings of both the double-stranded nucleic acid molecule and the single-stranded poly(A) mRNA to the NS1 protein are investigated. The relative small interface surface areas for the dimeric complex are consistent with the conservation of the domain structure during the dimerization process. Structurally, considering the NS1 protein as a two-domains signaling molecule is discussed. While there is a lack of sequence homology, there exist similarities between the domain structures of the NS1 protein and other signaling molecules.

Platform T: Ion Motive ATPases

1087-Plat

Cryoelectron Microscopy of an ATP-dependent Cu pump from Archaeoglobus fulgidis

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CopA is an ATP-dependent Cu pump that belongs to the P1 subfamily of P-type ATPases. This subfamily shares core catalytic domains with other members of the family, such as Ca-ATPase and Na,K-ATPase, for which the X-ray crystal structures have been determined. Members of the P1 family are characterized by extended N-termini, which contain tandem repeats of metal binding domains (MBD). Compared to other P-type ATPases, P1 pumps have two extra transmembrane helices near the N-terminus and truncation of four C-terminal transmembrane helices. CopA from Archaeoglobus fulgidis is an unusual P1 pump because, in addition to the N-terminal MBD, it also has a C-terminal MBD. Although there is some uncertainty about their precise function, MBDs have been proposed to participate in regulation, in targeting, and in transfer of Cu to the transport sites. To study the structural disposition of the MBDs, we have expressed constructs of CopA with truncation of the N-terminus and the C-terminus either individually or together. We have used cryoelectron microscopy and helical reconstruction to determine structures of these constructs. Comparison of the double truncation with the C-terminal truncation revealed the location of the N-terminal domain. We constructed an atomic model by fitting X-ray crystal structures of relevant fragments into our map, which suggests a regulatory role for the N-terminal domain. By imaging somewhat wider tubular crystals with better order, we have been able to determine the structure of the C-terminal truncation at higher resolution. This new structure reveals the architecture of the transmembrane domain and allows us to place the extra two transmembrane helices with greater precision. Additionally, we are working on a structure of the N-terminal truncation, which should reveal the location of the C-terminal domain and help us determine its role in Cu transport.

1088-Plat

Molecular Insights Into The Modulation Of Sodium Binding Affinity And Voltage Sensitivity Of The Sodium-Potassium Pump From Molecular Dynamics Simulations, Electrophysiology And Structure

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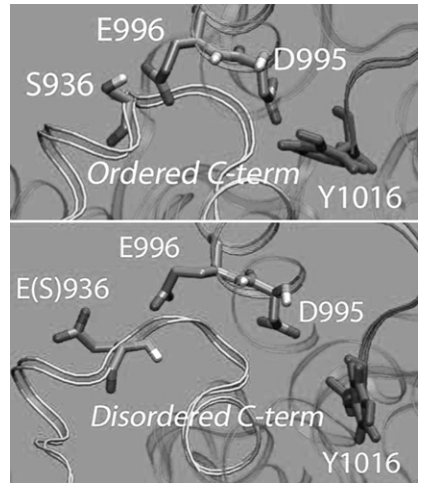
The Na^+-K^+ ATPase couples ATP hydrolysis to the export of three Na^+ ions and the import of two K^+ ions into the cell. It has been proposed that the C-terminus controls the pump's Na^+-binding affinity, but the molecular details of the pump's voltage sensitivity and regulation remain unknown. Combining data from Molecular Dynamics simulations, Electrophysiology and Crystallography, we propose a novel molecular mechanism of voltage sensitivity and regulation of the pump by the C-terminus.

When transmembrane electrical potentials are applied in simulations, a controversial PKA phosphorylation site: Ser936 becomes more accessible to cytoplasmic kinases than in the crystal structure. Phosphorylation of Ser936 has a disordering effect on the C-terminus, which is linked to Ser936 by a hydrogen-bonding bridge involving Asp995 and Glu996. Electrophysiological studies in *Xenopus* oocytes confirm the predictions and show that Ser936Glu, Asp995His and other related mutants distinctly alter the Na^+-binding affinity and voltage sensitivity. The study addresses a long-debated possible regulatory role of residue Ser936, and shows how the regulatory C-terminus is linked to Ser936 and Asp995, mutations in which cause Familial Hemiplegic Migraine.



1089-Plat

Cross-Linkable, Gain-of-Function Phospholamban (PLB) Mutant Reveals the Molecular Mechanism of SERCA2a Inhibition

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The PLB monomer inhibits the Ca2+ pump of cardiac sarcoplasmic reticulum (SERCA2a) by decreasing the apparent Ca2+ affinity of the enzyme. Here we