2652-Symp Exploring Chromatin Remodeling With Three-dimensional Electron Microscopy

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The extreme packaging of DNA into chromatin constitutes a physical barrier to all DNA transactions. A variety of mechanisms have evolved that take advantage of this packaging and make chromatin a highly dynamic and regulated entity. The ATP-dependent chromatin remodeling complexes are large (often $>$ 1 MDa), multi-subunit assemblies that utilize the energy from ATP hydrolysis to non-covalently alter the structure of nucleosomes. Despite a significant body of biochemical and genetic data we do not understand how these complexes remodel chromatin.

We are taking a structural approach to elucidating the mechanism of ATP-dependent chromatin remodeling and use as our main techniques three-dimensional electron microscopy (3D EM) and single-particle methods. Our model system is RSC, a remodeling complex from the yeast S.cerevisiae.

We obtained an initial low-resolution reconstruction of RSC using a novel reconstruction method we developed that addresses some of the difficulties encountered with the more traditional methods when attempting to obtain ab initio structures from a heterogeneous sample (as is the case with a flexible macromolecule). The structure of RSC suggests a potential binding pocket for its substrate, the nucleosome. Our structural analysis also revealed a significant degree of conformational flexibility in one of the large domains in the complex.

We are currently working towards obtaining a reconstruction of RSC bound to a nucleosome and are starting to map the location of a number of the RSC subunits as a first step in understanding the roles they play within the complex. We are also beginning to collect the large data sets required to move towards our goal of obtaining high-resolution structures.

2653-Symp Under the Hood of the Replisome: A Single-Molecule View of DNA Replication

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Advances in optical imaging and molecular manipulation techniques have made it possible to observe individual enzymes and record molecular movies that provide new insight into their dynamics and reaction mechanisms. In a biological context, most of these enzymes function in concert with other enzymes in multiprotein complexes, so an important future direction will be the utilization of single-molecule techniques to unravel the orchestration of large macromolecular assemblies. I will discuss results of single-molecule experiments on the replisome, the molecular machinery that is responsible for replication of DNA. We stretch individual DNA molecules and use their elastic properties to obtain dynamic information on the proteins that unwind the double helix and copy its genetic information. Furthermore, we use fluorescence microscopy to obtain detailed information about the spatial distribution of eukaryotic replication origins along DNA.

Minisymposium 4: The Physics of Protein Folding/ Unfolding

2654-Minisymp Mutations as Trapdoors: The Rop-dimer with two Competing Native Conformations

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Conformational transitions are the molecular basis of protein function. Structure-based models, based on the funneled energy landscape, typically accommodate a single basin for the native state. However, it is possible to accommodate multiple folding basins to represent different structures of the investigated protein. The presented study focuses on the homodimer Rop, which was the subject of extensive studies investigating the effect of mutations in the hydrophobic core. The mutants show strong changes in folding rates and Rop's ability to bind RNA. We investigate the possibility of two competing conformations representing a parallel (P) and the WT anti-parallel (AP) arrangement of the monomers. Given equivalent energetic bias towards each of the two states, P has a lower free-energy barrier and is therefore more accessible than AP. We suggest that the mutations trigger a trapdoor on the energy landscape. They undo the evolutionary bias towards the RNA-binding state AP, resulting in the competition of P and AP. This competition of two states with distinct kinetic behavior leads to the experimentally observed strong changes in kinetics. Apart from explaining Rop's mutational behavior, the general concept of competing states which are triggered by external factors might be applicable to explain allosteric control or signaling, for example.

2655-Minisymp Single Molecule Atomic Force Microscopy Reveals Kinetic Partitioning of the Mechanical Unfolding Pathway of T4-lysozyme

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Kinetic partitioning is believed to be a general mechanism for proteins to fold into their well-defined native three-dimensional structure from unfolded states following multiple folding pathways. Here we use single molecule atomic force microscopy (AFM) to present direct experimental evidence of the kinetic partitioning of the mechanical unfolding pathway of T4-lysozyme, a small protein composed of two sub-domains. We observed that upon stretching from its N- and C-termini, T4-lysozyme unfolds via multiple distinct unfolding pathways: the majority of T4-lysozymes unfold in an all-or-none fashion, and a small fraction of T4-lysozymes unfold in three-state fashion involving unfolding intermediate

states. The three-state unfolding pathways are not following well-defined routes; instead they display great variability and diversity in their individual unfolding pathways. The unfolding intermediate states are kinetic traps along the mechanical unfolding pathway and are likely to result from the residual structures present in the two sub-domains after crossing the main unfolding barrier. Investigation of the mechanical unfolding of the circular permutant T4 lysozyme revealed that the coupling between the two sub-domains plays critical roles in defining the complex mechanical unfolding behavior of T4-lysozyme. Our results provide direct evidence for the kinetic partitioning of the mechanical unfolding pathways of T4-lysozyme, and demonstrate that single molecule AFM is a powerful tool to investigate the folding/unfolding dynamics of complex proteins containing multiple sub-domains.

2656-Minisymp Folding/Unfolding Trajectories of Protein G Measured by Single Molecule FRET Spectroscopy

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One of the goals of current studies of protein folding using single molecule FRET spectroscopy is to obtain information on the distribution of folding pathways that connect the myriad conformations of the unfolded state with the relatively unique conformation of the biologically-active folded state. These studies require immobilization of the protein in order to obtain sufficiently long trajectories for analysis. We have labeled the 56-residue immunoglobulin binding domain of protein GB1 (protein G) with the FRET pairs, Alexa Fluor 488 and Alexa Fluor 594, and attached the molecule via a 6-residue histidine tag to chelated copper ions embedded in a polyethylene glycol coated glass surface. Trajectories of emitted donor and acceptor photons at various urea concentration were recorded for periods between about 1 and 30 seconds (before bleaching) using a PicoQuant Micro Time 200 time-resolved fluorescence microscope. The majority of the trajectories ($> 80\%$) show simple behavior, i.e. a high FRET efficiency and short donor lifetime corresponding to the folded state, a low FRET efficiency and longer lifetime corresponding to the unfolded state, or trajectories with both levels and one or more unresolvable jumps between them corresponding to folding or unfolding barrier-crossing events. Histograms of residence times in the folded and unfolded states show approximately exponential distributions, indicative of a two-state system. Polarization measurements yield steady-state anisotropies < 0.1, consistent with the lifetime and re-orientational correlation time for the freely rotating donor. The donor-acceptor cross-correlation function for unfolded molecules is flat, indicating no dynamics in the time range 0.01–1 sec. All of these results indicate relatively little interference from artefacts due to sticking to the surface, dye blinking, or quenching from donor-acceptor complex formation, and therefore represent a significant advance in the investigation of single molecule protein folding.

2657-Minisymp Quantitative Characterization Of Hydration And Volumetric Changes In Protein Folding

Lally Mitra[1], Kazumi Hata[2], Ryohei Kono[2], Akihiro Maeno[2], Dan Isom[3], Jean-Baptiste Rouget[4], Roland Winter[1], Kazuyuki Akasaka[2], Bertrand Garcia-MorenoE [5], Catherine A. Royer[4]

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The exquisite specificity of protein structure, dynamics and function is intimately linked to the interactions of the protein with water. Differences in hydration that accompany conformational changes in proteins are known to give rise to differences in their volumetric properties. Hence, the study of pressure effects on proteins should, in principle, provide insight into these changes in hydration and the energetic and structural role of the solvent in determining the energy landscape of proteins. Despite these prospects, using volumetric properties to understand the role of solvent in protein conformational transitions has been hindered due to a lack of a fundamental and quantitative understanding of volume changes. The results presented here provide a quantitative characterization of the volumetric properties of a model protein (Snase) and demonstrate how subtle conformational changes can modulate these properties. Moreover these results underscore the complexity of volumetric properties of proteins and demonstrate that their interpretation in terms of solvation coupled with protein conformational transitions should be interpreted in the context of their complete temperature dependence. Finally, by testing the effects of insertion of ionisable residues at various positions throughout the Snase structure, we have mapped the structure of the transition state ensemble of Snase using pressure-jump kinetics techniques. Our results show that the β-barrel and abutting helix is well structured in the TSE, that the linker region between this subdomain and the terminal helix is partially structured, and that the C-terminal helix remains unordered in the Snase TSE.

2658-Minisymp Direct Observation of Chaperone-induced Changes in a Protein Folding Pathway

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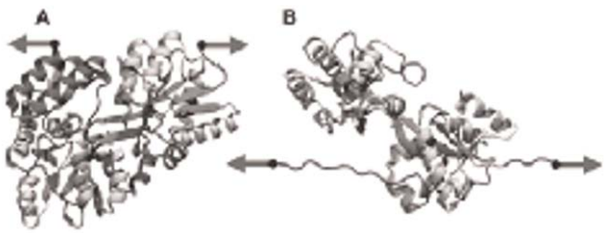
How chaperone interactions affect protein folding pathways is a central problem in biology. Using optical tweezers and all-atom molecular dynamics simulations, we studied the effect of chaperone SecB on the folding and unfolding pathways of maltose binding protein (A) at the single-molecule level. In the absence of SecB, we find that the MBP polypeptide first collapses into a molten globule-like compacted state and then folds into a stable core structure (B),

onto which several α-helices are finally wrapped. Interactions with SecB completely prevent stable tertiary contacts in the core structure, but have no detectable effect on the folding of the external α-helices. It appears that SecB only binds to the extended or molten globule-like structure and retains MBP in this latter state. Thus during MBP translocation, no energy is required to disrupt stable tertiary interactions.



2659-Minisymp The Conformational Diffusion Rate of Unfolded Protein Chains: Evidence from Cytochrome *c* Supports the Generality of k_{diff} as a Prefactor for Transition State Expressions for Folding Rates

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The nature of the prefactor in Arrhenius or transition state theory expressions for protein folding rates and the speed with which the conformers of unfolded protein chains interconvert are fundamentally related questions. We have measured the time constant for interconversion of disparate unfolded chain conformations of a small globular protein, cytochrome *c* (Abel et al., 2007, *Biochemistry*, *46*:4090–4099). The axial binding reactions of histidine and methionine residues with the Fe(II) heme cofactor were monitored with time-resolved magnetic circular dichroism (TRMCD) spectroscopy after photodissociation of the CO complexes of denaturant-unfolded protein obtained from horse and tuna, as well as from several histidine mutants of the horse protein. Using a kinetic model that fit both the reaction rate constants and spectra of the intermediates, we obtained an estimate of the conformational diffusion time (approximated as a first-order time constant for exchange between conformational subensembles presenting either a methionine or a histidine residue to the heme iron for facile binding) of 3 μs for these sequences, very close to the ~1 μs value often cited as the folding "speed limit". Expanding on the proposal that the conformational diffusion rate constant is the prefactor for folding (W. Y. Yang & M. Gruebele, 2003, *Nature*, *423*:193–197), the convergence of our result with similar values for other folding and nonfolding sequences of similar length suggests that k_{diff} may provide a general, approximately sequence-identity-independent prefactor for the transition state theory of folding.

Platform BD: Imaging and Optical Miscroscopy

2660-Plat Simultaneous Transport of Different Localized mRNA Species Revealed by 3D Single-Particle Tracking

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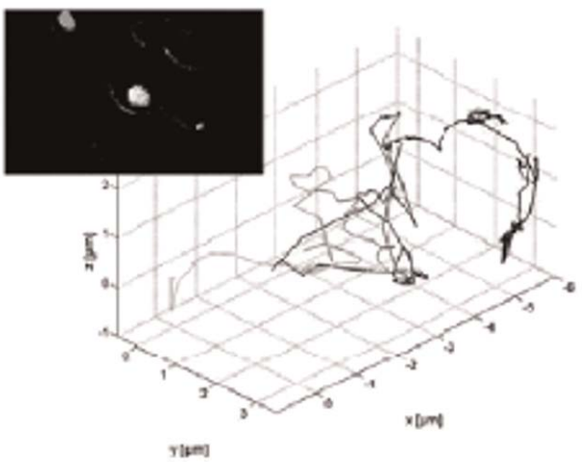
Intracellular mRNA localization is a common mechanism for creating asymmetric distributions of proteins in live cells. Previous studies have revealed that, in a number of cell types, different mRNA species are localized by the same transport machinery. However, it has been unclear if these individual mRNA species are specifically sorted into different or common ribonucleoprotein particles before or during transport. Using yeast as a model system, we analyzed the intracellular movement of individual pairs of localized mRNA in live cells using 3D particle tracking with dual-color detection. For this purpose, mRNAs were tagged with tandem repeats of either bacteriophage MS2 or lambda boxB RNA sequences and fluorescently labeled by fusion protein constructs that bind to the RNA tag sequences. We have tracked the transport of two different localized mRNA species in live cells. Our observations demonstrate that different localized mRNAs are co-assembled into common ribonucleoprotein particles and co-transported in a directional manner to the target site. Non-localized mRNAs or mutant mRNAs that lack functional localization signals form separate particles that are not transported to the bud.



2661-Plat High-Contrast Single-Molecule Microscopy by Selective Focal Plane Illumination

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