Platform L: Self-Assembled-Session: Sliding on DNA

124-Plat What Controls Intermolecular Sliding? The Dynamics-Function Relationship of a DNA Sliding Clamp

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DNA sliding clamp proteins attach to polymerases and slide along DNA to effect rapid, processive replication of DNA. The clamps also play an important role in the coordination and implementation of alternative polymerases. Little is known about the interactions between the clamps and DNA, but the presence of many positively charged residues inside the clamps may be a source of friction, i.e., may curtail the sliding due to attractive interactions with the negatively charged DNA. By single-molecule spectroscopy we found that the diffusion constant for the beta-clamp (the bacterial DNA sliding clamp) diffusing along DNA is at least three orders of magnitude less than that for diffusion through water alone. Here we present analysis of over 100 ns of molecular dynamics simulations of the beta-clamp interacting with DNA, showing the role of individual and groups of residues in the dynamics-function relationship of this protein-DNA interaction.

125-Plat How Does A Protein Find Its Site On DNA?

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Recognition and binding of specific sites on DNA by transcription factors is central to regulation of gene expression. To locate its specific site on DNA, not only must a transcription factor (TF) recognize it amongst the 1e6–1e9 of alternative sequences, it must do so in mere seconds within the crowded environment of cell, and further hampered by fluctuations, DNA compaction, and a myriad of other obstacles.

Here we propose a model that takes into account spatial (3D) diffusion and sliding (1D) diffusion on DNA. In contrast to previous studies our model takes into account disordered (sequence-specific) energy of protein-DNA interactions. We demonstrate that the disorder leads to a significant slowdown making the overall search process prohibitively slow. We propose that proteins can overcome this slowdown by being flexible and having (at least) two distinct conformations: one in which the disorder is diminished and sliding is efficient, and the other conformation that provides tight sequence-specific binding. We consider several mechanisms of coupling between the conformational transition and sliding. We propose kinetic pre-selection model that is able to provide rapid search. These observations explain widespread flexibility in DNA-binding proteins and are in good agreement with recent NMR studies of TFs.

126-Plat Thermodynamic Restrictions on Evolutionary Optimization of Transcription Factor Proteins

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Conformational fluctuations are believed to play an important role in the process by which transcription factor proteins locate and bind their target site on the genome of a bacterium. Using a simple model, we show that the binding time can be minimized, under selective pressure, by adjusting the spectrum of conformational states so that the fraction of time spent in more mobile conformations is matched with the target recognition rate. The associated optimal binding time is then within an order of magnitude of the limiting binding time imposed by thermodynamics, corresponding to an idealized protein with instant target recognition. Thus, we claim that it is possible for the overall binding rate of a transcription factor to approach the theoretical limiting value but only by a suitable choice of energy spectrum of conformational sub-states, and only if the dimensionless binding rate is of the order of one, or larger than one, where dimensionless binding rate is determined as the product of binding rate and the average time spent by the protein on one DNA base pair in one tour of 1D sliding along DNA. Numerical estimates suggest that typical bacteria operate in this regime of optimized conformational fluctuations.

127-Plat Single Molecule Imaging Of Protein-DNA Interactions: 1D Diffusion Of Lac Repressor Proteins Along DNA

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Single molecule imaging of protein-DNA interactions: 1D diffusion of Lac repressor proteins along DNA

A puzzle in protein-DNA interactions has been the faster-than-diffusion binding of some proteins to their specific DNA target sites. To unravel this puzzle, the mechanism by which a site-specific DNA-binding protein finds its target needs to be elucidated. We used single molecule imaging method to directly visualize the interactions of Lac repressor proteins (LacI) with DNA. LacI was observed to bind to nonspecific DNA and perform 1D Brownian motion along the DNA molecule. The 1D diffusion characteristics obtained indeed predict a LacI-target binding rate 90 times faster than the 3D diffusion limit, and thus resolve the 100-fold discrepancy between experimental data and theory. The diffusion mechanism of LacI on DNA (sliding versus hopping) is investigated and a new diffusion model is proposed. Effort in tracking LacI in vivo will be discussed.

128-Plat Peeling and Sliding in Nucleosome Repositioning

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DNA accessibility must arise in the presence of nucleosomes in eukaryotes. Both nucleosome desorption and sliding have been proposed as mechanisms for kinetic accessibility of the genetic material. However, transcription often requires the presence of processing enzymes. We investigate the mechanisms of both histone sliding and detachment with a stochastic model that couples thermally-induced, passive histone sliding with active motor enzyme–driven histone unwrapping. Analysis of a passive loop or twist defect-mediated histone sliding mechanism shows that diffusional sliding is enhanced as larger portions of the DNA is peeled off the histone. The mean times to histone detachment and the mean distance traveled by the motor complex prior to histone detachment are computed as functions of the intrinsic speed of the motor. Fast motors preferentially induce detachment over sliding. However, for a fixed motor speed, increasing the histone-DNA affinity (and thereby decreasing the passive sliding rate) increases the mean distance traveled by the motor.

129-Plat Dynamic Basis for One-dimensional DNA Scanning by the Mismatch Repair Complex Msh2-Msh6

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The ability of proteins to locate specific sites or structures among a vast excess of nonspecific DNA is a fundamental theme in biology. Yet the basic principles that govern these mechanisms remain poorly understood. For example, mismatch repair proteins must scan millions of base pairs to find rare biosynthetic errors, and they then must probe the surrounding region to identify the strand discrimination signals necessary to distinguish the parental and daughter strands. To determine how these proteins might function we used single-molecule optical microscopy to answer the following question: How does the mismatch repair complex Msh2-Msh6 interrogate undamaged DNA? Here we show that Msh2-Msh6 slides along DNA via one-dimensional diffusion. These findings indicate that interactions between Msh2-Msh6 and DNA are dominated by lateral movement of the protein along the helical axis and have implications for how MutS-family members travel along DNA at different stages of the repair reaction.

130-Plat Kinetics and thermodynamics of salt-dependent T7 gene 2.5 protein binding to single- and double-stranded DNA

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Bacteriophage T7 gene 2.5 protein (gp2.5) is a single-stranded DNA (ssDNA) binding protein that has essential roles in DNA replication and recombination in phage-infected cells. By measuring the DNA melting force in the presence of this protein as a function of DNA pulling rate we were able to estimate the rate of protein association with ssDNA at the boundary with double-stranded DNA (dsDNA). This rate appears to be higher than that expected for a diffusion-limited process and is also strongly salt dependent. These features suggest that the gp2.5 search for ssDNA sites at the helix-coil boundary is facilitated by protein pre-binding to dsDNA followed by one-dimensional sliding on the dsDNA. We estimate the rate of sliding and find it comparable to the sliding rate of other dsDNA binding proteins. The salt dependence of dsDNA binding is similar to that previously observed for ssDNA binding, while the three to four order of magnitude salt-independent difference between ssDNA and dsDNA binding is attributed to nonelectrostatic interactions involved only in ssDNA binding. The results support a model in which salt-dependent dimerization interactions must be broken for DNA binding, and suggest that these gp2.5 monomers, formed upon breaking a gp2.5 dimer, search dsDNA by 1D diffusion to find available binding sites on ssDNA

131-Plat Real-time Measurement Of The Rate Of Release Of A Transcription Elongation Complex During RNA Termination

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The elongating RNA polymerase complex moves along the duplex DNA between initiation and termination signals encoded within the DNA 'track' in a biased random walk. At each template position along the track the elongation complex may enter reaction pathways offering alternatives to direct RNA elongation. These alternative pathways include pyrophosphorolysis (the direct reverse of elongation), entry into paused or arrested states, and termination (in which the polymerase and the nascent RNA are released from the template DNA). The elongation complex is extremely stable at most template positions, deriving much of its thermodynamic stability from the nucleic acid scaffold at its core, including the disfavored transcription bubble in the dsDNA that is compensated by the formation of an 8–9 bps long RNA-DNA hybrid within the bubble, together with favorable interactions with the polymerase. The protein components of the complex can slide freely along the duplex DNA track, 'zippering' the scaffold along at its core and allowing movement forward (during nucleotide addition) or backwards (during pyrophosphorolyisis or entry into backtracked pauses or arrest states). Sliding in either direction is prevented during intrinsic termination by structure elements that form within the nucleic acid scaffold during this process. We have developed a surface plasmon resonance assay that can monitor synthesis of the nascent RNA and dissociation of the elongation complex in real-

time, as well as a model with which to fit these data to determine the kinetic rate constants for pausing and release during termination under different conditions. The rates for two different terminators have been determined in the presence or absence of transcriptional co-factors, NusA and N, and further on-going experiments will be described.

Platform M: Protein Aggregates

132-Plat AFM Imaging Of Recombinant Human Prion Aggregation Reveals Multi-step Formation Process Involving Stable Oligomeric Intermediates

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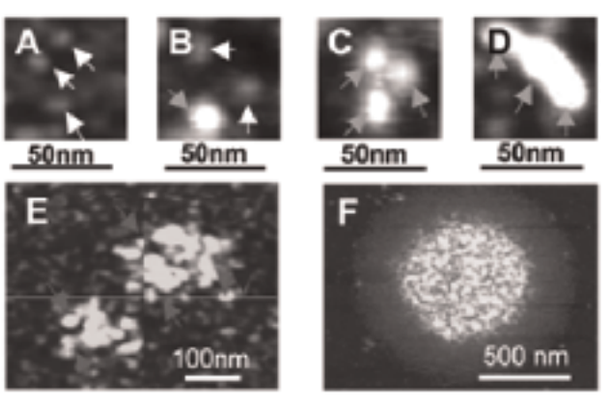
The key event in the prion disease is oligomerization of cellular prion protein (PrPc) into scrapie prion protein (PrPsc) with high beta sheet content and subsequent aggregation of PrPsc. To explore physical controls on this process, we used AFM to study aggregation pathways of recombinant human prion proteins, specifically comparing differences between normal (PrPcwt) and a mutant (PrPc10or) protein. Experiments were carried out in partly denaturing acid solutions that trigger oligomerization and aggregation. The results showed that both PrPcwt and PrPc10or followed the same pathway to formation of nonfibrillar aggregates, but did not follow a conventional growth process (Fig. 1): First, trimeric-to-tetrameric units formed from the monomers. These then combined to form larger oligomeric units of 8 and 10 monomers. These further aggregated to form micron-scale globular structures, which were clearly still comprised of the oligomers. The difference between PrPcwt and PrPc10or was in the rates of oligomerization and oligomers aggregation. PrPc10or exhibited higher oligomerization and aggregation rates and larger aggregates. The results support a hypothesis of multi-step aggregation in which mutant proteins are unstable and spontaneously form PrPsc oligomers, which slowly accumulates over time.

Fig. 1 AFM images showing prion aggregatation. A–E show evolution from monmers to 8–10mer units. The large aggregate in F is comprised of the same oligomeric units as in E. White, yellow and red arrows point to monomers, trimers and 8–10mers respectively.



133-Plat Sequential Conformational Changes Occurring During Aggregation of Amyloidogenic Proteins

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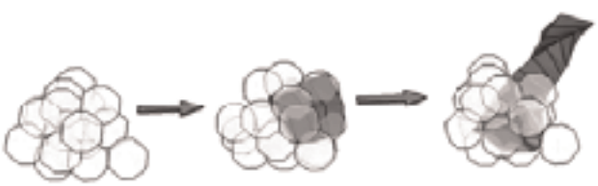
Many proteins or protein fragments can aggregate into cross ß-amyloid fibrils. These fibrils accumulate into insoluble plaques in vivo and are associated with the progression of neurodegeneration in Alzheimer's, Parkinson's and Creutzfeldt-Jakob's diseases. Transmissible spongiform encephalopathies in cows (mad cow), deer and elk (chronic wasting syndrome), sheep and hamsters (scrapie) also show accumulation of ß-amyloid during their progression.

Many mechanistic intermediates have been proposed for the assembly of amyloid fibrils from soluble precursor proteins and peptides. However few of these proposed intermediates have been observed directly. We will discuss the aggregation of ß-lactoglobulin and alpha-synuclein into ß-amyloid fibrils and our efforts to characterize the structural and size distributions through all stages of the assembly using a combination of bulk and single molecule methods.

We have observed that upon aggregation into a non-fibrillar aggregate, a conformational change occurs in the amyloidogenic proteins we have studied. Once in the colloidal aggregate, another conformational change occurs that is nearly irreversible and leads to the growth phase of amyloid formation.



134-Plat Mechanism of Lipid Bilayer Disruption by Oligomeric Alpha-Synuclein

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Parkinson's disease is characterized by the deposition of aggregated fibrillar α-synuclein in Lewy bodies within the brain. The nature of the pathogenic species in Parkinson's disease is not unequivocally established, but current thinking points towards early oligomeric species rather than the mature fibrillar forms of the protein. A possible mechanism by which cytotoxicity occurs is through permeabilization and disruption of lipid membranes by these early intermediates. A pore-like mode of action has been suggested for the increase in membrane permeability upon interaction with oligomeric α-synuclein. However such a mechanism has never been unequivocally proven. We have characterized lipid bilayer disruption by monomeric, oligomeric and fibrillar α-synuclein using a dequenching assay on liposomes. We found that vesicle leakage was