1052-Plat
Dynamics of DNA Supercoil Relaxation by Type II Topoisomerases
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Dynamics of DNA supercoil relaxation by type II topoisomerases
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Type II topoisomerases are some of the main targets of anti-cancer drugs, since they catalyze DNA decatenation and unwinding which is crucial for cell division. A recent crystal structure shows that, during the catalytic cycle, a yeast type II topoisomerase can bend a 34 base pair DNA segment by up to 150 degrees. Bacterial gyrase, another type II topoisomerase, can wrap an approximately 100 bp DNA segment into a tight 180 degree turn. By substituting diaminopurine (DAP) deoxyribonucleotides for dATP in PCR reactions, completely triply hydrogen-bonded DNA fragments have been produced and found to be stiffer than normal DNA. These and normal DNA fragments were used as substrates for observations of topoisomerase II-mediated relaxation of plectonemes introduced in single molecules using magnetic tweezers. Observations at several ATP concentrations revealed bursts of stepwise events separated by pauses. Michaelis-Menten fitting of the data for both recombinant human topoisomerase II alpha and *E. coli* gyrase showed that Vmax and Km both decrease in DAP-substituted with respect to normal DNA. However, while the characteristic pause interval increased for human topoisomerase II alpha operating on DAP-substituted with respect to normal DNA, it was unchanged for *E. coli* gyrase. These dynamic measurements not only support the hypothesis that the strand passage reaction involves DNA bending but also suggest that DNA bending and subsequent steps in the catalytic cycle, perhaps involving ATP hydrolysis, are more efficiently coupled in gyrase than in human topoisomerase II alpha.

1053-Plat
Complex Kinetics of Apobec3g Interaction with Single-Stranded Nucleic Acids
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Human APOBEC3G (A3G) is a host cell cytidine deaminase capable of restricting replication of retroviruses by deaminating single-stranded (ss) viral DNA, and also by directly inhibiting RT-catalyzed polymerization reactions. We have studied the kinetics of A3G interaction with model 10-, 20-, 40- and 60-nt ssDNAs as well as with long polymeric DNA by three independent approaches: (i) surface plasmon resonance (SPR) analysis, (ii) fluorescence polarization spectroscopy (FP), and (iii) single-molecule (SM) DNA stretching. Our results suggest the following: (1) Binding kinetics of A3G protein to all ssDNA oligonucleotides are multi-step with the faster, weaker binding gradually converting to more stable binding with much slower on/off rates; (2) The typical A3G-ssDNA on/off times measured in our experiments range between ~10 and 10,000 sec, and the corresponding K_d values range between 0.1 and 10 μM; (3) Higher concentrations of A3G, longer A3G/ssDNA incubation times, as well as ssDNA length were found to correlate with slower ssDNA on/off kinetics, suggesting that A3G/A3G interactions in the bound state are responsible for slow protein kinetics.Taken together, these observations suggest that multimerization of A3G on ssDNA leads to a gradual decrease in its on/off kinetics, which in turn enhances this protein's ability to stall reverse transcription via formation of a ''roadblock'' for reverse transcriptase.

1054-Plat
Single-Molecule Studies of the Effects of Small Compounds on the Activity of Translation Initiation Factor eIF4A
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The translation initiation factor eIF4A is the prototypical DEAD-box RNA helicase as a subunit of eIF4F. It facilitates the binding and scanning of the ribosome via unwinding secondary structures in the 5' UTR of mRNAs during translation initiation. Here single-molecule Fluorescence Resonance Energy Transfer (sm-FRET) assay for both structured and unstructured substrates is performed and it shows that eIF4A unwinds the substrates in a discrete step manner although it is a highly nonprocessive motor. It also shows that eIF4A is a bidirectional helicase and the step size is about 6 base pair. Pateamine A and silvestrol are small-molecule modulators of eIF4A activity and they are identified as potent inhibitors of translation. It was demonstrated that Pateamine A and silvestrol act as a chemical inducer of dimerization and promote the interaction between eIF4A and RNA, however the molecular mechanisms by which they regulate eIF4A activities still remain elusive. Our sm-FRET assay shows that both pateamine A and silvestrol stimulate the helicase activity of eIF4A. Understanding how the processivity of eIF4A is influenced by these molecules will help regulating the translation at the initiation step.

1055-Plat
Single Molecule Studies Reveal Sliding Dynamics of Hcv NS5B in Complex with RNA
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NS5B is an RNA-dependent RNA polymerase capable of initiating RNA synthesis de novo. However, the detailed underlying mechanism remains elusive. It is unclear how the enzyme locates the 3′-terminus of the RNA template. Previous studies suggested that the nucleic acid binding channel of NS5B accommodates approximately 10 residues of a single stranded RNA. Although the contacts between the polymerase and its nucleic acid substrate are maximized, the 3′-end of the primer is not properly positioned under these conditions and such complexes are therefore unproductive. Hence, it is conceivable that the NS5B-RNA interaction is highly dynamic. Of note, nonnucleoside inhibitors of NS5B were shown to inhibit formation of a competent complex. To address this problem, we have conducted single molecule FRET (SM-FRET) experiments. This approach allowed us to obtain a direct visualization of both the positioning and dynamics of NS5B in complex with its RNA template. We performed our experiments on single -donor (Cy3)/acceptor (Cy5) fluorophore labeled- RNA substrates, which were surface-immobilized to enable long observation times. Binding of NS5B caused a significant increase in FRET. SM-FRET studies on RNA-protein complexes revealed protein sliding dynamics occurring in the millisecond time scale. These dynamics change with the RNA template length, and with the presence of complementary DNA strands that restrict the motion of NS5B. A nonnucleoside inhibitor is observed to compromise binding of NS5B to the template. Taken together, our single molecule studies provide direct evidence for the ability of NS5B to slide along its RNA template. Sliding of NS5B provides a plausible mechanism that facilitates formation of a productive complex. Conversely, interference with these dynamics provides a possible mechanism by which nonnucleoside analogue inhibitors of NS5B block de novo initiation of RNA synthesis.

1056-Plat
Protein Sliding and Hopping Kinetics on DNA
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DNA-binding proteins' alternating diffusion kinetics on and off nonspecific DNA (also called sliding and hopping respectively) are important for quantifying their target binding mechanisms. Using Monte-Carlo simulations, we deconvolved the sliding and hopping kinetics of GFP-LacI proteins on elongated DNA from their experimentally observed seconds-long diffusion trajectories. Our simulation results suggest the following: (1) In each diffusion trajectory, a protein makes on average hundreds of alternating slides and hops with a mean sliding time of several tens of ms; (2) sliding dominates the root mean square displacement of fast diffusion trajectories, whereas hopping dominates slow ones; (3) flow and variations in salt concentration have limited effects on hopping kinetics, while in vivo DNA configuration is not expected to influence sliding kinetics; furthermore, (4) the rate of occurrence for hops longer than 200 nm agrees with experimental data for EcoRV proteins. Experimental investigations of sliding proteins on DNA using SIMA [1] measurements on the timescale of milliseconds will be presented.
[1]. DeCenzo, S. H., M. C. DeSantis, and Y. M. Wang. 2010 Optics Express 18(16):16628-39.

1057-Plat
Chromatin Distribution Guides Cell Division in *E. Coli*
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We recently discovered that *Escherichia coli* bacteria that are confined to narrow nanofabricated channels still divide unimpededly, despite the large size and irregular cell shapes that they adopt [1]. Previously, it was shown that

normal rod-shaped *E. coli* bacteria are able to place their cell division plane very accurately in the middle of the mother cell [2]. How are these bacteria able to robustly and accurately localize their cell division proteins? Here, we address this question using two-color fluorescent imaging of bacteria in narrow nanofabricated channels. The non-conventional shape of *E. coli* in this confinement provides new opportunities to study cell division in bacteria. Using our approach, we are able for the first time to observe the dynamical arrangement of bacterial DNA relative to some of the key cell division proteins. While Min proteins are effective in excluding cell division at the poles of rod-shaped bacteria, they do not generate a well-defined pattern for localization of cell division planes in more complicated cell shapes. Instead, we observe that localization of the divisome, i.e., the protein complex pertinent to cell division, correlates strongly with the pattern of DNA arrangement. As the bacterial chromosomes segregate and voids form in its distribution, the early arriving protein of bacterial divisome, FtsZ, localizes in these voids. Not all the voids are selected but only those which are near high circumferential curvature regions of the cell wall in these flattened cells. Our results show that localization of bacterial divisome is to a high degree coordinated with the chromosome distribution. The DNA forms a molecular scaffold which guides this important cellular process.

[1] J. Männik, R. Driessen, P. Galajda, J.E. Keymer and C. Dekker, Proc.Natl.Acad.Sci. U.S.A. 106 (2009) 14861.
[2] X.C.Yu and W. Margolin, Mol.Microbiol. 32 (1999) 315.

PLATFORM AC: Enzymes/Heme Proteins

1058-Plat

DNA Translocation Dynamics of HIV Reverse Transcriptase Revealed Through Single Molecule Kinetics of Nucleotide Binding

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Using single molecule methods we have investigated the rates of nucleotide binding and release by HIV Reverse Transcriptase in complex with DNA terminated by either ddT, d4T or AZT. The enzyme-DNA complex was absorbed to the surface of zero-mode wave guides and nucleotide binding was monitored using 5' fluorescently labeled nucleotides developed for single-molecule real-time DNA sequencing. Analysis of the pulse-width (PW) and inter-pulse duration (IPD) lifetimes corresponding to each nucleotide-binding event were used to determine nucleotide on- and off-rates for the enzyme-DNA complex terminated by each of the three different analogs. The PW distribution defined the kinetics of nucleotide dissociation but also revealed the dynamics of nucleotide-induced isomerization of the enzyme-DNA-nucleotide complex. Further, an analysis of the inter-pulse duration data, corresponding to the lifetime of the enzyme-DNA complex existing in unbound-nucleotide states, revealed biphasic kinetics. These data can be fit to a model where short-lived IPDs correspond to the second-order rate constant governing nucleotide binding to the enzyme-DNA complex when the DNA occupies the post-translocation site (P-site). The long lifetime IPDs are attributable to DNA translocation in that nucleotide cannot bind when DNA occupies the pre-translocation site (N-site). Thus, the long IPDs report the kinetics governing DNA translocation. We also show how these data can be quantitatively modeled using computer simulation/data fitting to achieve high precision kinetic parameters. Our findings support altered translocation dynamics of DNA terminated by d4T that results in increased pre-translocation site occupancy. This effect was most severe when the DNA was terminated with AZT. Retention of the DNA in the pre-translocated state provides a drug resistance mechanism by which increased ATP- or pyrophosphate-mediate excision can occur on the exposed phosphodiester bond of the analog terminator; resulting in rescued polymerization.

1059-Plat

TrbB from Conjugative Plasmid F: A Representative of a New Class of Dsbd-Dependent Disulfide Isomerases

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Bacterial conjugation is a process by which single-stranded DNA is unidirectionally transferred from donor to recipient bacterium. Conjugative plasmid transfer can accelerate the evolution of bacteria, facilitate various pathogenic processes, and disseminate genes encoding antibiotic resistance. For conjugative plasmid F, proteins involved in plasmid transfer are encoded in the transfer (*tra*) region. A subset of these proteins assembles to form the F plasmid pore complex, while others are thought to assist in pore complex assembly. One of these accessory proteins, TrbB, is proposed to mediate pore complex formation through disulfide isomerase activity.

TrbB is a periplasmic protein encoded by plasmid F. This protein has a predicted thioredoxin-like fold and possesses a C-X-X-C redox active site motif. TrbB is proposed to function in the conjugative process by serving as a disulfide bond isomerase, facilitating proper folding of a subset of F-plasmid-encoded proteins in the periplasm. Previous studies have illustrated that a *trbB*$^-$ F plasmid in *Escherichia coli* (*E.coli*)lacking its native disulfide bond isomerase, $DsbC_{E.coli}$, experiences a 10-fold decrease in mating efficiency, but have not provided direct evidence for disulfide bond isomerase activity. We have demonstrated that TrbB can partially restore transfer of a variant of the distantly related R27 plasmid in which both chromosomal and plasmid genes encoding disulfide bond isomerases are disrupted. Additionally, TrbB displays both disulfide bond isomerase and reductase activities on substrates not involved in conjugation. Unlike canonical members of the disulfide bond isomerase family, however, TrbB lacks both an N-terminal dimerization domain and an alpha-helical linker. Although TrbB diverges structurally from other disulfide bond isomerases, like those isomerases, it relies on a chromosomally encoded protein in *E. coli* for maintenance of its redox active site.

1060-Plat

Single-Molecule Enzymology: Studies on Glutathione-S-Transferases

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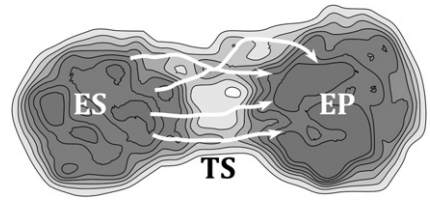
An emerging concept in enzymology is the catalytic transition state ensemble: instead of the single, well-defined transition state (TS) of classical theory, enzymatic reactions progress through multiple 'valleys' in the energy landscape, each with its own TS. Long-lived conformations of the enzyme-substrate (ES) complex would take different pathways through the TS ensemble, and would therefore result in functional heterogeneity (i.e., a distribution of catalytic rate constants). The functional properties of enzymes are therefore completely controlled by the nature of the energetic landscape for the ES complex and the TS ensemble. We have developed TIRFM (total internal reflection fluorescence microscopy) and FCS (fluorescence correlation spectroscopy) methods to measure turnover by single immobilized enzyme molecules. We applied these methods, complemented by molecular dynamics simulations and ab initio calculations, to two isoforms of glutathione-S-transferase (GST) to examine the validity of the TS ensemble model. GST isoform A1-1 is an abundant hepatic enzyme involved in detoxification, and is extremely promiscuous in its substrate range. GST A4-4 is the predominant isoform in the heart and brain, and is specific towards lipid peroxidation products. Contrasting the behavior of these two enzymes will address fundamental questions about the mechanisms of substrate promiscuity.



1061-Plat

Selenium NMR Spectroscopy as Versatile Probe of Selenoproteins

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Nuclear magnetic resonance spectroscopy is an essential biophysical technique due to its unsurpassed sensitivity to molecule's electronic and chemical structure. However, while NMR detection of carbon, nitrogen and protons is routine, biological 77Se NMR is not common. We develop 77Se solid and solution state NMR as a versatile spectroscopic method for examining the unique group of selenoproteins, thus allowing for an unprecedented record of the electronic structure of reactive enzymatic centers.

77Se is a spin 1/2 nucleus with a pronounced chemical shielding response. It exhibits a high sensitivity to the local environment and molecular motion, thus rendering it an excellent spectroscopic reporter on bonding, geometry and electronic structure. It has great potential for characterization of enzymatic reactions in which selenium plays a role. For example, it enables direct detection of diselenide and selenenylsulfide bonds as well as ionization states. By utilizing recent advances in selenoproteins' expression to uniquely insert 77Se in genetically engineered proteins, we demonstrate the detection of different chemical forms of 77selenocysteine in selenoproteins at a high magnetic field (14.1 T).