sequence chi (5′-GCTGGTGG-3′). The chi sequence occurs roughly once every five kb in the E. coli genome, suggesting that RecBCD must travel for long distances along genomic DNA.
In our assay, we observe the enzymatic activity of RecBCD on individual DNA molecules. Fluorescently labeled RNA polymerase and hydrolytically inactive EcoRI(E111Q) were selected as model roadblock proteins. By preparing a DNA substrate with these fluorescently labeled proteins, we directly observed the outcome of collisions with RecBCD. Our results indicate that RecBCD is able to push and eventually displace multiple proteins without reducing its rate of translocation. These results offer the first direct observation of collisions between a helicase and other proteins along the same DNA helix. We propose that the highly processive, dual motor structure of RecBCD is necessary for stimulating recombination many thousands of bp away from the initial dsDNA break. Our results provide additional evidence that an essential, if underappreciated, aspect of helicase function is the ability to clear dsDNA for further processing by other enzymes.

329-Pos
The DNA-Gate of Gyrase Is Predominantly in the Closed Conformation During DNA Supercoiling

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DNA topoisomerases catalyze the inter-conversion of DNA topoisomers and impact key cellular events such as replication, recombination, and transcription. Gyrase catalyzes the introduction of negative supercoils into DNA *via* a strand-passage mechanism. In the first step, a DNA-segment, the gate-DNA, binds to gyrase. The gate-DNA is cleaved, and a covalent DNA-gyrase complex is formed. A second DNA segment, the transfer-DNA, is passed through the gap, and the gate-DNA is re-ligated. Strand passage requires opening of a transient protein interface at the cleavage site, the so-called DNA-gate, by ~2 nm. The intermediate cleavage complex presents an inherent danger of double strand DNA breaks and thus genome instability, and cleavage complexes have consistently been detected in very low amounts. In contrast, a recent study predicted frequent opening of the topoisomerase II DNA-gate. Here, we present a single molecule FRET study that monitors both the conformation of DNA bound to the DNA-gate of gyrase, and the conformation of the DNA-gate itself. DNA bound to gyrase adopts two different conformations, one slightly, one severely distorted from B-DNA geometry. Distortion requires cleavage, but neither ATP nor a transfer-DNA. The DNA-gate of gyrase is predominantly in the closed conformation, in agreement with $<5\%$ of cleavage complexes in equilibrium. Importantly, gyrase with an open DNA-gate is also not significantly populated during the relaxation and supercoiling reactions. Presumably, distortion of the gate-DNA unlatches the DNA-gate, and prepares it for transient release by the transfer-DNA, thus providing a strict coupling of gate-opening to strand passage.

330-Pos
Investigating the Nucleation and Extension Rates of E.coli and Deinococcus RecA Along Duplex DNA

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RecA is a protein which promotes the exchange between two homologous DNA molecules in homologous recombination process. When individual RecA molecules assemble on DNA, the DNA is stretched and underwound to form a nucleoprotein filament with its rigidity and end-to-end length increased. We have developed single-molecule tethered particle motion (TPM) experiments to study the assembly dynamics of RecA proteins on individual duplex DNA molecules. The TPM method is capable of measuring the changes in DNA length by observing the bead's Brownian motion, thus allowing us to monitor RecA nucleation and extension in real-time. Using much shorter DNA (a few hundreds basepairs), TPM experiments offer improved sensitivity, since the DNA length change can be readily detected as soon as a few RecA bounded to duplex DNA molecules. Our experiments indicated a faster nucleation rate compared to the previous reports (Galletto et al., 2006). Moreover, we have compared the nucleation and extension rates of E. coli RecA with the RecA from Deinococcus radiodurans, UV-resistant bacteria, under different nucleotide states, ATP and ATPγS. Deinococcus radiodurans RecA are found to nucleate faster (~1.6×10-2 bp-1min-1) but extend slower (~0.3-1.5 RecA/sec) under ATP.This difference reflects the physiological role of Dr. RecA when extensive UV-damaged DNA molecules are present.

331-Pos
Effect of DNA Supercoiling on DNA Decatenation and Unknotting Followed By Brownian Dynamics Simulations

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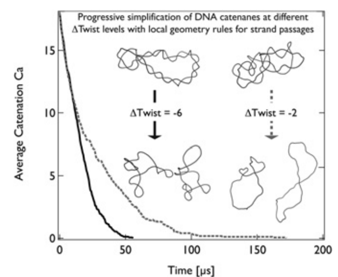

Replication of circular DNA proceeds through a stage of multiply interlinked catenanes that have to be rapidly spatially separated. In addition, knotting of circular DNA has also to be avoided. In bacteria, topology simplification requires participation of two type II topoisomerases: gyrase and topo IV. Several simulation approaches were applied to explain the very efficient topology simplification in that system. Mainly two strategies were explored: in the first one, the system follows its free energy gradient influenced by supercoiling, and in the second one, specific geometrical rules are defined for the selection of strand passages (hooking, chirality). The Monte-Carlo methods usually used to estimate the efficiency of these strategies do not allow to follow DNA topology simplification dynamically, to evaluate its speed, for example. To overcome this limitation, we simulated DNA unknotting and decatenation by Brownian dynamics, which allows for a natural integration of the strategies mentioned above. By following the topological state of the simulated DNA chains (see figure), we show that the combination of supercoiling and local geometrical selection rules provides an important drive for unknotting and decatenation, especially at low topological complexity.

332-Pos
Does T7 DNA Polymerase Backtrack During Proofreading?

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DNA replication is an essential cell process in which the genetic information is copied by replicative DNA polymerases (DNAp). The molecular basis of DNA replication is the addition of nucleotides by DNAp to a growing primer, using single-stranded DNA as a template. High fidelity of the processive T7 DNA polymerase comes from nucleotide selection at the polymerase active site, but is increased several orders of magnitude by an additional intrinsic proofreading ability. In this kinetic process, a partly melted primer shuttles to the exonuclease active site where incorporated mismatches are excised. After excision of erroneous nucleotides, the trimmed primer can shuttle back to the polymerase active site to resume replication. Elucidating the mechanism of the shuttling between these two activities of DNAp is essential for understanding the proofreading mechanism of DNA polymerases.
Transfer of the primer to the exonuclease active site is induced by disruption of the primer-template structure upon the incorporation of a mismatch. Application of tension to the DNA also destabilizes the primer-template structure and can therefore be used to shift the fine-tuned balance between polymerization and proofreading (Wuite *et al*, 2001; Ibarra *et al*, 2009).
Using optical tweezers, we study the kinetic coordination between exonuclease and polymerase activities, while applying different tensions. In these experiments we observe an additional waiting state between proofreading activities, during which the DNAp remains bound to the DNA. The force-dependent rate out of this state suggests that DNAp enters a state comparable to RNA polymerase backtracked state, which was shown to play a role in tuning the fidelity (Shaevitz *et al*, 2003). We speculate that our observed waiting state might play a similar role in the fidelity of DNA polymerase.

333-Pos
A Single Molecule View of the Rad51-ssDNA Interaction

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Homologous recombination (HR) represents an essential DNA repair mechanism in living cells. The central molecular complex of HR is the nucleoprotein filament, a DNA-protein complex in which recombinase protein Rad51 is bounded onto single-stranded DNA (ssDNA) in a helical form. Earlier studies have shown that efficient filament formation is critical for correct DNA repair, therefore a detailed characterization of the interaction between Rad51 and ssDNA is essential to understanding homologous recombination.
We use a combination of single-molecule fluorescence microscopy, optical tweezers and microfluidics to study the interaction of Rad51 with ssDNA. With this approach, we are able to directly visualize Rad51 filament assembly and disassembly on ssDNA at the single-molecule level.

We have quantitatively assessed each individual step of the Rad51-ssDNA interaction (i.e. nucleation, filament extension and disassembly). Moreover, we investigate the mechanical coupling between the ssDNA template and the reaction kinetics of filament by varying the tension on the DNA molecule. Hence, we have obtained new insight into the reaction pathway of this essential biological system.

334-Pos
Isothermal Amplification and Quantification of Nucleic Acids Using Intrinsic Fluorescence of Primers
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Real-time polymerase chain reaction (RT-PCR) is widely used to amplify, detect and quantify nucleic acids. Current RT-PCR specific probes (Molecular Beacons, TaqMan, Scorpions) use complicated mechanisms based on fluorescence resonance energy transfer, and require costly synthesis and considerable effort to achieve optimal sensitivity. Typically, a fluorophore-quencher pair is attached to the ends of a probe oligonucleotide, which doesn't fluoresce when free in solution. Upon probe hybridization to a target sequence, the fluorophore is separated from the quencher and a signal is released. Temperature cycling is another limitation of PCR since it requires expensive instrumentation for thermocycling and complicates rapid detection of pathogens in the field and at point-of-care.
We developed a new method, quadruplex priming amplification (QPA), which uses intrinsic fluorescence of primers for quantification of DNA products and can proceed under isothermal conditions. A key feature of QPA is that after polymerase elongation, the specifically designed guanine-rich primers are capable of forming a quadruplex structure with significantly more favorable thermodynamics than the corresponding DNA duplexes. As a result, target sequences are accessible for the next round of priming since their complementary strands are trapped in a quadruplex conformation and DNA amplification proceeds under isothermal conditions. In addition, 2-aminopurine (2Ap), which is part of the primers and quenched before polymerase elongation, regains its maximum emission upon quadruplex formation, which allows simple and accurate detection of product DNA. The advantages of QPA over traditional quantification methods and its thermodynamic bases will be discussed.

335-Pos
Binding Affinity and Displacement Synthesis Activity of Pol I DNA Polymerase on Different Gapped DNAs
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Gapped DNAs, intermediates in excision repair, can be filled by DNA polymerase I (Pol I) and sealed by ligase. Understanding the binding preferences of Pol I for different gaps and how their binding affinity correlates with displacement synthesis is helpful for investigating repair mechanisms *in vivo*. The roles of the 5′- or 3′-phosphate, and of magnesium, in the binding of Klenow and Klentaq polymerases to gapped DNAs, differing in the size of the ssDNA gap (0, 2, and 10 nt), were examined using a fluorescence anisotropy binding assay. 5 mM Mg^{2+} does not significantly alter the binding of gapped DNAs to Klenow, but Mg^{2+} weakens the binding of gapped DNAs to Klentaq. For Klentaq, but not Klenow, a 5′-terminal phosphate increasingly weakens the binding as the gap size increases. Under the same conditions, $Mg^{2+}$and 5′-phosphate do not alter the displacement synthesis ability of Klenow with gap2 and gap10 substrates, but do alter the activity of Klentaq. Conversely, a 3′-phosphate in the gap significantly weakens the binding of Klenow, but not Klentaq. We hypothesize that Klentaq can bind either the 5′- or 3′- end of the gap, while Klenow binds preferentially at 3′- end of the gap due to the 3′-end being pulled into the editing site. The binding affinity of Klenow, but not Klentaq, to different gaps increases as the size of the gap increases, and this correlates with the displacement synthesis ability of Klenow on gaps versus nicks. Klenow binds primed-template DNA substrates with 2-3 kcal/mol tighter affinity than gap0 or gap2 substrates, while Klentaq shows only a slight preference (0.7 kcal/mol) for primed-template over gapped DNAs, indicating that Klenow more significantly prefers replication over repair substrates.

336-Pos
Conformational Dynamics of a DNA Polymerase At the Single-Molecule Level
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DNA polymerases are essential components of the cellular machinery necessary for survival of an organism through the accurate replication of the genetic material. The replication pathway of DNA polymerases has been extensively studied to elucidate the structures and dynamics of the polymerizing and proofreading modes, but currently only a static collection of individual states has been extracted. With the advancements in modern single-molecule fluorescence technology, the potential now exists to examine all enzymatic processes and transitions during real time dynamic measurements. By employing the E. coli DNA polymerase I Klenow fragment (KF) as a model system, along with single-pair FRET labeling, we designed an experimental system to examine conformational dynamics during both nucleotide selection and proofreading steps. In the absence of nucleotides, the bound KF complex was observed to cycle repeatedly between two distinct conformations (open and closed). In contrast, just a single conformation (closed) was populated in the presence of a correct incoming nucleotide. In addition, with the presence of mismatches at the primer-template junction, the previously hypothesized intermolecular and intramolecular pathways were directly observed for transfer of a DNA substrate between the polymerase and exonuclease sites of KF. The evolution and continuous advancement of single-molecule FRET methodology has provided the opportunity to witness events and intermediates previously unobservable in standard bulk studies, leading to a more complete view of the enzymatic pathway. Supported by NIH grant GM44060.

337-Pos
Topoisomerase IB Activity Investigated By Single Molecule Magnetic Tweezers: Mechanisms of Cytotoxicity
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Magnetic Tweezers (MT) are a powerful tool to investigate how single topoisomerase IB (topIB) molecules relax DNA supercoils [1,2]. MT studies have revealed that topIB activity is dramatically affected by the presence of camptothecin-class (CPT) inhibitors, used clinically as anti-cancer drugs [3]. In the presence of CPT, topIB remains covalently bound to DNA for much longer than in the absence of the drug (>100 s vs. ~ 2 s) and the rate of supercoil removal is significantly reduced, in particular for positive supercoils. The CPT-induced asymmetry in the rate of supercoil removal between positive and negative supercoils leads to an accumulation of positive supercoils in the G1 and S-phases in yeast cells in vivo [3].
Here, we present results on the G365C topIB point mutant, which exhibits CPT resistance and shows no accumulation of positive supercoils in vivo. In the MT assay in the absence of CPT, the G365C mutant shows activity similar to wt topIB. In the presence of CPT, G365C exhibits long-lived DNA-topIB complexes and slow supercoil removal for positive supercoils, similar to the wt enzyme. Surprisingly, for negative supercoils we found similarly long-lived complexes and slow supercoil removal for the G365C mutant. In contrast to the wt enzyme, the G365C mutant removes positive and negative supercoils with similar (slow) velocities in the presence of CPT. These results suggest that CPT cytotoxicity might be more strongly dependent on the asymmetry of the rate of positive vs. negative positive supercoil removal and the corresponding accumulation of positive supercoils than on the lifetime of the covalent DNA-topIB complex.
[1] Koster, et al. Nature 2005
[2] Lipfert, et al. Meth. Mol. Biol. 2009
[3] Koster, et al. Nature 2007

338-Pos
Dynamics of An Archael DNA Polymerase Revealed By Single Molecule Fret
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In the archaeon *Methanosarcina acetivorans*, DNA replication is done by DNA polymerase BI. The processivity of this enzyme is greatly enhanced by a conserved cofactor known as PCNA, which plays a crucial role in orchestrating many replication-related processes. To understand the dynamics of these proteins, we have used single molecule FRET to examine the behavior of PolBI labeled with FRET donor on various DNA structures labeled with FRET acceptor and the effect of PCNA on the dynamics of PolBI. The binding of PolBI to DNA was observed in the low nanomolar concentration range as expected. Interestingly, this polymerase is highly mobile on the DNA structures with two nonadjacent primer strands that are complementary to two different regions in the template strand, 20 nucleotides apart, as evidenced by the frequent transitions between two long-lived FRET states exhibited in single molecule trajectories. To explore the nature of this spontaneous motion, we considered several possible mechanisms including translocations along single- or double-stranded DNA, polymerase binding orientation flipping, and polymerase active site switching. We observed that changes made downstream of the primer/template