

occurs only after a force of  $\sim 65$  pN has been applied. This suggests that the DNA molecules interact at their point of contact via melting of base-pairs, possibly creating a 3- or 4-stranded complex.

The existence of chiral and force dependent interactions between bare DNA molecules has strong implications for the study of DNA in confined environments and raises interesting questions as to their potential importance in vivo.

#### 2223-Plat

##### Physical Modeling of Chromosome Segregation in *E. Coli* Reveals Impact of Force and DNA Relaxation

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The physical mechanism by which *Escherichia coli* segregates copies of its chromosome for partitioning into daughter cells is still a mystery, partly due to the difficulty in interpreting the complex dynamic behavior during segregation. In several other species of bacteria, a physical segregation mechanism consisting of filament depolymerization generates a force that pulls the newly replicated chromosome apart from the nascent strand. This suggests that a similar acting, yet undiscovered force-generating mechanism may exist for *E. coli*. In our previous work, we developed a model for a Rouse polymer in a viscoelastic medium that accurately describes the motion of bacterial chromosomal loci between segregation events. In this work, we adapt our model to examine the effects of a segregation force applied to a single monomer on the dynamic behavior of the polymer. Previous measurements of chromosome segregation in *E. coli* demonstrate that the origin of replication is among the first regions to be segregated and exhibits a mean displacement that scales as  $t^{\alpha/2}$  where  $\alpha/2=0.30$ . Our model demonstrates that the observed power-law scaling of the mean displacement and the behavior of the velocity autocorrelation function is a consequence of the relaxation of the DNA polymer and the viscoelastic environment. We show that the ratio of the mean displacement to its variance during segregation is a critical metric that eliminates the compounding effects of polymer and medium dynamics and provides the segregation force. Using this theory and accounting for additional ATP-dependent fluctuations, we predict the force of segregation in *E. coli* to be approximately 0.13 pN.

#### 2224-Plat

##### Multiple Dynamic DNA Rearrangements are Tightly Coupled to Distinct Stages of Human Telomerase Catalysis

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Telomerase is essential for telomere homeostasis in most rapidly dividing cells, including the majority of human cancers. Telomeres are specialized chromatin structures which distinguish the natural ends of eukaryotic chromosomes from sites of DNA damage. Catalytically active human telomerase is minimally comprised of the telomerase RNA (hTR) and the protein telomerase reverse transcriptase (hTERT). DNA binding by telomerase is mediated by both hTR and hTERT, which align the 3' end of a single-stranded DNA substrate with a region of hTR that templates the reverse transcription reaction. After synthesis of a single telomere DNA repeat (GGTTAG in humans), the nascent DNA strand must dissociate from the RNA template and translocate to realign with the template for the next round of DNA repeat synthesis. Here, we present a novel single molecule assay that permits direct detection of individual human telomerase enzymes bound to a DNA substrate. Conformational dynamics within the enzyme-substrate complex are directly monitored as a distance-dependent change in the energy transfer efficiency between two dye molecules - a phenomenon known as Förster Resonance Energy Transfer (FRET). We have found that human telomerase, like other telomerases, actively manipulates the DNA:RNA hybrid length during DNA synthesis. When template synthesis nears completion, we observe structural dynamics throughout the entire primer that suggest concurrent manipulation of the hybrid and reorientation distal protein-DNA contacts. Through both FRET and a nuclease protection assays, we have shown that this rearrangement induces motion of the entire DNA primer, threading the 5' DNA out of the telomerase complex. Threading of the DNA allows telomerase to maintain contact with the DNA for processive repeat addition while simultaneously providing potential interaction sites for other telomeric binding proteins.

#### 2225-Plat

##### Studies of T4 Primosome DNA Unwinding by Single-Molecule Fluorescence-Detected Linear Dichroism

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The replication machine (replisome) of the T4 bacteriophage is an important model system for studies of DNA synthesis in all higher organisms. The helicase-primase (primosome) complex is one of three major sub-assemblies of the replisome, whose function is to processively unwind double-stranded DNA to expose single-stranded daughter strands for template-directed DNA synthesis. The detailed mechanism by which the primosome complex assembles and performs duplex DNA unwinding is not understood. Recently, we developed and applied single-molecule fluorescence-detected linear dichroism (smFLD), which can detect fluctuations of the transition dipole orientation on microsecond-to-millisecond time scales. We have studied model DNA replication fork constructs with cyanine fluorophores rigidly incorporated at the fork junction to monitor 'breathing' of the backbone as it interacts with the weakly binding T4 hexameric helicase. Our recent results [1] indicate that the time scale and distribution of microsecond breathing fluctuations at the replication fork junction are heavily influenced by the presence of the weakly binding T4 helicase, and suggest that these motions are on the pathway to the formation of the fully assembled DNA-primosome complex. The possible significance of these findings for helicase mechanisms will be discussed.

[1] Phelps, C.; Lee, W.; Jose, D.; von Hippel, P. H.; Marcus, A. H. "Single-Molecule FRET and Linear Dichroism Studies of DNA 'Breathing' and Helicase Binding at Replication Fork Junctions," Proc. Nat. Acad. Sci. (in press, accepted July 26, 2013, doi/10.1073/pnas.1314862110).

#### 2226-Plat

##### Observing Tautomerization of a Deoxycytidine Analog Kp1212: Molecular Origin of Lethal Mutagenesis Against Hiv

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A novel class of antiviral drugs has been developed based on a principle termed "lethal mutagenesis" which aims at artificially increasing the mutation rates such that the viral population collapsed due to the intolerable amount of mutations. Specifically, KP1212 is a mutagenic deoxycytidine analog that elevates the G to A mutations in viral genome shown by clinical studies. It was hypothesized that the mutation originates from the tautomerization of KP1212 from the canonical amino-keto form to the imino-keto form, which results in favorable base pairing to A. We systematically characterized the tautomeric equilibria of KP1212 under physiological conditions using two-dimensional infrared (2D IR) spectroscopy. The intrinsic picosecond time-resolution of 2D IR enables us to detect various tautomers that are rapidly interconverting. Moreover, the unique 2D IR cross-peak patterns for each tautomer allow for unambiguous assignment which is not possible by traditional techniques. Strikingly, we observed significant amount of enol population which is regarded as the "rare" tautomer in DNA bases. Finally, we have performed temperature-jump (T-jump) 2D IR experiments to measure the tautomerization kinetics. We found that with a 10°C T-jump, the enol populations increase at the expense of the keto populations on the nanosecond timescale. The nanosecond tautomerization is much faster than the speed of polymerase (1 millisecond/base), therefore allowing sufficient time for the formation of mismatched base pairs.

#### 2227-Plat

##### DNA Sequencing with Protein Nanopores: A Story of Strands and Nucleotides

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Protein based nanopores have shown great potential as components of DNA sequencing devices. However for commercial exploitation it is imperative that the resolution of the method is improved upon. To do so we must understand the biophysics that underpins the movement of both DNA and single nucleotides through engineered protein nanopores.

Here we present a molecular modeling and simulation study of the widely studied pore-forming toxin; alpha hemolysin (aHL). We use atomistic molecular dynamics with applied electric fields to mimic the experimental conditions. Umbrella sampling has been utilized to calculate the free energy profiles of single nucleotides through the aHL pore, to pinpoint the location of the energetic barriers experienced by each of the 4 DNA bases.

Our results provide insights of direct relevance to the production of commercial nanopore-based DNA sequencing devices.