393-Pos Board B193
Time Dependence of Switching Between Polymerization and Editing Mode DNA Binding by Klenow Polymerase
Hiromi S. Brown, Vince J. LiCata.
Klenow polymerase is the large fragment of DNA polymerase I from *E. coli*. Klenow possesses 5'-3' polymerase and intrinsic 3'-5' exonuclease activities with two distinct active sites that are located ~30 angstroms apart. During DNA replication, proofreading activity enhances replication fidelity by excising misincorporated nucleotides from the 3' end of primer strand. The first step of the proofreading process is sending the 3'-primer terminus to the proofreading site, which requires separation of the primer terminus from the template strand. Here we examined the binding of Klenow to matched and mismatched primed-template DNA (pt-DNA) by monitoring the steady state fluorescence intensity change of a single 2-aminopurine base site-specifically substituted in the template strand within the duplex part of pt-DNA. The changes in fluorescence intensity allow us to follow shuttling of the primer terminus between the polymerization and proofreading sites. We have found that partitioning of the primer between the two active sites depends on: 1) the number of mismatched bases at the primer-template junction, 2) the presence or absence of divalent ions, 3) the type of divalent ions, and 4) the time in the presence of the divalent ions. A significant time dependence of the partitioning between the two sites was observed with the matched and single mismatched pt-DNA in the presence of Mg^{2+}. The slow kinetic dependence of the primer partitioning between the polymerization and proofreading sites in Klenow polymerase helps explain previous conflicting reports of the equilibrium partitioning between sites.

394-Pos Board B194
Developing a Synthetic Mimic of Promoter Searching by RNA Polymerase
Erika Cline, Ming-Hsin Li, Shi Yu, Seung-Ho Jung, Seok Ki Choi, Neha Kaul, Edgar Meyhofer, Nicholas A. Kotov, James R. Baker, Jr., L. Jay Guo, Ronald G. Larson, Nils G. Walter.
In order to initiate transcription, RNA polymerase first has to find its promoter sequence on a potentially very long, entangled, genomic DNA template. It is thought that RNA polymerase and other enzymes bind DNA randomly and then search for their specific recognition sites via one-dimensional diffusion, sliding or hopping along the DNA. For the enzyme to diffuse along the DNA efficiently, it must be associated only loosely and non-specifically, in part by electrostatic interactions between positive charges in its DNA binding cleft and the negative charges along the DNA backbone. To determine how physical and chemical properties such as surface charge density, shape, size, and hydrogen bonding capacity affect the one-dimensional diffusion of enzymes along a DNA template, we have replaced both components with various biological and non-biological substitutes possessing similar properties and are observing their interaction by total internal reflection fluorescence microscopy (TIRFM) at the single molecule level. In particular, we have used either positively charged dendrimers or organic nanoparticles and microtubules, highly negatively charged biological fibers, as electrostatic substitutes for the polymerase and DNA, respectively. The long-term goal of this project is to replace the enzyme, DNA, and recognition site with synthetic components possessing physical and chemical properties that may be systematically altered. By directly observing the interaction between the synthetic polymerase and DNA analogs in real-time, and characterizing the effect of various properties on their interaction, we will not only learn more about the process of promoter searching, but we will gain predictive power over whether two synthetic particles can interact in a biological fashion. Ultimately, our work will lead to insights into biomedically relevant processes like DNA transcription, replication, repair, and modification.

395-Pos Board B195
Quantitative Determination of Oct4-Sox2 Heterodimer Formation with Nanog Promoter Element
Tapan Kumar Mistri, Chen Sok Lam, Wibowo Arindrarto, David Rodda, Yong Hwee Foo, Wei Ping Ng, Sohail Ahmed, Paul Robson, Thorsten Wohland.
Oct4 and Sox2 are key transcription factors (TFs) essential for maintaining pluripotency as well as the self-renewal ability of embryonic stem cells (ESC). Oct4 and Sox2 have been previously described to synergistically control pluripotent-specific expression of a number of genes. It has already been reported that Oct4 and Sox2 cooperatively bind with an oct-sox cis-regulatory element and thereby regulates the transcription of a number of important target genes such as *Fgf4, Oct4, Nanog* and *Sox2.* Presently, there is no quantitative assay characterizing the cooperative regulation of the TF complex. In this study, we focus on a quantitative investigation regarding the binding pathway of ternary complex formation and how the cooperativity works. We are using a combination of biophysical (*eg.* single-wavelength fluorescence cross-correlation spectroscopy) and biochemical assays to confirm the functionality of our protein constructs and to demonstrate that Sox2 has a crucial role in helping Oct4 to bind to the oct-sox element whereas Oct4 does not promote Sox2 binding. We further establish that Oct4 and Sox2 are only able to form a heterodimer in the presence of the oct-sox element and do not interact without DNA. Such quantitative measurements will provide deeper insight into the Oc4-Sox2 regulatory network and move us towards a systems level understanding of the regulation of pluripotency.

DNA Structure & Conformation

396-Pos Board B196
Millisecond-Sub-Piconewton Force Steps Reveal the Kinetics of DNA Overstretching
Pasquale Bianco, Lorenzo Bongini, Luca Melli, Mario Dolfi, Vincenzo Lombardi.
Until now the structure, kinetics and energetics of the transition from the basic conformation of ds-DNA (B state) to the 1.7 times longer and partially unwound conformation (S state) have not been defined. The force-extension relation of the ds-DNA of λ-phage is measured here with unprecedented resolution using a dual laser optical tweezers that can impose millisecond steps in force of 0.5-2 pN via beads attached to opposite strands of the molecule (temperature 25 °C). This approach reveals the kinetics of the transition between intermediate states of ds-DNA and uncovers the load-dependence of the rate constant of the unitary reaction step. We show that (1) the elongation (ΔL) following the force step imposed on the molecule in the region of overstretching transition has an exponential time course with a rate constant (r) that depends solely on the force attained by the step (F) ; (2) r is related to the extent of elongation ΔL_0 through a power equation that is linearized by double log transformation. The size of the force step does not affect the slope (0.6) of the linearized relation, but shifts its vertical position. These results indicate that r is not related to viscous resistence to elongation-untwisting of the molecule, but only to the kinetics of a two-state reaction and explain the absence of hysteresis in the force-extension relation at equilibrium.
Supported by Ente Cassa di Risparmio di Firenze.

397-Pos Board B197
Overstretching DNA at 65 pN does Not Require Peeling from Free Ends or Nicks
Daniel H. Paik.
DNA exhibits a remarkable mechanical transition where its extension increases by 70% at 65 pN. Notwithstanding more than a decade of experimental and theoretical studies, there remains significant debate on the nature of overstretched DNA. We developed a topologically closed but rotationally unconstrained DNA assay, which contains no nicks or free ends. DNA in this assay exhibited the canonical overstretching transition at 65 pN but without hysteresis upon retraction (v_stage = 5 μm/s). Introduction of a controlled nick led to hysteresis in the force-extension curve. Moreover, the degree of hysteresis increased with the number of nicks. Hence, the generation of single stranded DNA from free ends or nicks is not an obligatory step in overstretching DNA, but rather a consequence.

398-Pos Board B198
Sequence Dependent Structural Transition of Short DNA by Tensile Force
Hongxia Fu, Hu Chen, Jie Yan.
B-S transition and DNA strand unpeeling transition, both occurring in a narrow force range near 65 piconewtons. Previous studies based on stretching DNA of a few micro-meters long (or longer) have revealed that the two transitions are distinct in transition kinetics, and the balance between them is sensitive to factors that affect DNA base pair stability. Further understanding of the transitions poses a challenge due to insufficient signal-to-noise ratio, difficulty in controlling the sequence, and the often presence of nicks when a long DNA is stretched. Here, by stretching short DNA of only a few hundred base pairs using a magnetic tweezers, a dramatic distinction in dynamics between the two transitions was revealed with a spatial resolution of a few nanometers and a temporal resolution of around 10 ms. In the unpeeling transition, stepwise extension change was observed, consistent with sequence-defined barriers in the strand unpeeling process. In the B-S transition, sequence-independent noise-like extension change was observed, suggesting that the transition does not require crossing significant energy barriers. Most importantly, we show that a nick-free DNA that undergoes the strand unpeeling transition can be switched to B-S transition under the same solution conditions when its two ends are blocked but the DNA remains torsion unconstrained.

399-Pos Board B199
Twist, Stretch and Melt: Quantifying How DNA Complies to Tension
Peter Gross, Niels Laurens, Lene Oddershede, Ulrich Bockelmann, Gijs J. Wuite, **Erwin J. Peterman**.
Central biological processes involve continuous mechanical manipulation of DNA. In cells, DNA is constantly twisted, bended and stretched by numerous proteins mediating genome compaction, gene regulation, expression, and DNA repair. Consequently, to understand these processes, it is imperative to have an in-depth understanding of how DNA complies to mechanical stress. The helical structure and the sequence of DNA, two physical features that have a strong impact on protein-DNA interactions, are not incorporated in the current