linearality and low resolution of long DNA substrates in conventional TPM experiments. The FTPM method offers the best resolution (56 bp at 433 bp long DNA) in the presence of only a small stretching force (0.20 pN). We have used the FTPM method to investigate the RecBCD helicase motion along 4.1 kb long chi-containing duplex DNA molecules, and observed that translocation rate of RecBCD changes after chi sequence under limited ATP concentrations. This suggests that chi recognition by RecBCD does not require saturating ATP conditions, contrary to what have been previously reported.

2136-Pos Board B106
Kinetic Mechanism for Single stranded DNA binding and Translocation by *S. cerevisiae* Isw2
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The chromatin remodeling complex Isw2 from *S. cerevisiae* (yIsw2) mobilizes nucleosomes through an ATP-dependent reaction that is coupled to the translocation of the enzyme along intranucleosomal DNA. In this study we demonstrate that yIsw2 is capable of translocating along single-stranded DNA in a reaction that is coupled to ATP hydrolysis. We find that single-stranded DNA translocation by yIsw2 occurs through a series of repeating uniform steps with an overall macroscopic processivity of $P = (0.92 \pm 0.01)$; this processivity corresponds to an average translocation distance of (24 ± 4) nucleotides before dissociation. This processivity corresponds well to the processivity of nucleosome sliding by yIsw2 thus arguing that single-stranded DNA translocation may be fundamental to the double-stranded DNA translocation required for effective nucleosome mobilization by the enzyme. Furthermore, we find that a slow initiation process, following DNA binding, is required to make yIsw2 competent for DNA translocation. We also provide both evidence that this slow initiation process likely corresponds to the second step of a two-step DNA binding mechanism by yIsw2 and a quantitative description of the kinetics of this DNA binding mechanism.

2137-Pos Board B107
Single-molecule Measurements Of DnaB
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The replicative helicase of E. coli, DnaB, is a ring-shaped hexameric motor protein capable of unwinding double-stranded DNA (dsDNA) at a fork. It is thought to do this through ''steric occlusion,'' in which DnaB encircles and translocates along one single strand of DNA (ssDNA), forcing the other single strand (the occluded strand) to pass outside the ring. Using magnetic tweezers, we have performed single-molecule measurements of the unwinding activity of DnaB in which the dsDNA is destabilized by force applied to either the occluded or encircled strand. Based on measurements of the velocity of the motor as a function of force applied to the occluded strand, we conclude that DnaB does not unwind dsDNA with a ''passive'' mechanism, i.e. it does not simply rely on thermal fluctuations to open proximal basepairs before stepping. We also present preliminary data of DnaB activity with force applied to the encircled strand, which probes DnaB's possible mechanisms for binding and translocation along ssDNA. Finally, we will report on the effects of the helicase loader DnaC and the primase DnaG on DnaB activity.

2138-Pos Board B108
Structural Transitions Of a Helicase-Partial Duplex DNA Complex during ATP Hydrolysis Cycle
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Helicases are ATP-dependent enzymes that use the structural changes induced by ATP binding, hydrolysis and release to unwind double stranded nucleic acids. We have studied the structural transitions of partial duplex DNA bound *E-coli* Rep helicase monomer by in vitro single molecule Förster resonance energy transfer (FRET) methodology. Constrained triangulation procedures were applied globally on FRET measurements from eight Rep mutants, donor labeled at different residues, and three DNA substrates, acceptor labeled at different nucleotides on duplex, to study these conformational states. A total of 96 different measurements were performed and used in the triangulation analysis. Such over-sampling reduces the likelihood of a single site with unusual photophysical properties to negatively impact the results. Our results show that binding of ATPγS to Rep induces a large conformational change which is then reversed in two approximately equal steps during ATP dephosphorylation (ATP to ADP.Pi transition) and ADP release. We do not observe a significant conformational change upon phosphate release (ADP.Pi to ADP transition). The large conformational change upon ATPγS binding is consistent with the rotation of the Rep domains in a direction that brings them closer to the duplex. In addition, we show that Rep has a preference to bind to ssDNA/dsDNA junction compared to the other sites along the ssDNA. Finally, we show that Rep remains in the closed conformation during all ATP hydrolysis intermediates when bound to the vicinity of ssDNA/dsDNA junction. Our studies not only reveal the structural transitions of Rep helicase-partial duplex DNA complex during ATP hydrolysis cycle but also demonstrate the potential of triangulation analysis as a versatile single molecule technique for probing structural information in physiological conditions.

2139-Pos Board B109
Deciphering the Mechanism of RNA helicase eIF4A in Translation Initiation
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Eukaryotic initiation factor eIF4A is a prototype protein of the DEAD box family of RNA helicases, and is part of the translation initiation complex eIF4F [1].eIF4A binds to the 5′ cap of mRNA and unwinds structures in the 5′-untranslated regions of mRNAs in ATP dependent manner. Although eIF4A has been studied extensively by classical bulk biochemical methods, a direct, unambiguous measurement of its helicase activity and its processivity has not been reported. Here, we use single molecule fluorescence assays to visualize its binding to RNA and melting secondary structures in RNA. Specifically, FRET efficiency dynamics is used to explore the binding location of eIF4A and its unwinding function. Our single molecule studies show that eIF4A has higher binding affinity towards the duplex site. We seek to elucidate any elementary steps and kinetic mechanisms involved with eIF4A unwinding of RNA. Furthermore, we selectively target eIF4A activity with small-molecule inhibitors acting in opposite manners[2]. The dynamics of stimulation and inhibition of eIF4A activity by *pateamine* and *hippuristanol* are measured at the single molecule level. The results will provide insight into the eIF4A's helicase activity and will distinguish between passive versus active unwinding mechanism. Our long-term goal in this project is to decipher the role of the initiation complex eIF4F in ribosomal recruitment, and develop methods to control this process. Then, we will probe eIF4A activity in the presence of other initiation (co)factors.
1. Rogers, G. W., Jr., Komar, A. A. & Merrick, W. C. eIF4A: The godfather of the DEAD box helicases. Prog Nucleic Acid Res Mol Biol 72, 307-331 (2002).
2. Bordeleau, M. E. et al. Stimulation of mammalian translation initiation factor eIF4A activity by a small molecule inhibitor of eukaryotic translation. Proc Natl Acad Sci. 102, 10460-5 (2005).

2140-Pos Board B110
PcrA Helicase ATPase Mechanism: RepD Modulation During Unwinding
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Helicases catalyse the unwinding of double stranded DNA or RNA for a variety of functions through various mechanisms. The monomeric, bacterial helicase PcrA is well characterized: it translocates ssDNA with discrete steps of one base per ATP. The ATPase activity is enhanced by DNA and the cycle includes a rate limiting cleavage step, followed by rapid phosphate release.
PcrA and the plasmid encoded replication initiator protein, RepD, act together during replication of some plasmids, containing antibiotic resistance. RepD is a dimer and binds to a specific origin sequence (*ori*) containing inverted complementary repeat (ICR) elements. ICRIII provides affinity and plasmid specificity, whereas ICRII is conserved amongst *ori* family members and contains the nick site for initiation. RepD binds to ICRIII and then nicks at a specific site in ICRII exposing a single-stranded region. PcrA helicase then binds to the ssDNA and begins unwinding. RepD has been shown to increase PcrA helicase activity; in the absence of RepD PcrA is a poor helicase. Measurements have shown that unwinding occurs at a reduced rate but the coupling ratio is unaltered.
We have analyzed the mechanism of the PcrA ATPase cycle with ssDNA and extended this to include probing how DNA junctions and RepD affect this cycle. The individual rate constants were determined to see how RepD modulates the ATPase rate. This included utilizing the fluorescent ATP analogue mantATP to monitor fluorescence intensity in rapid-reaction, kinetic experiments, allowing the initial binding and release kinetics to be explored. The analogue was further used for analysis of the hydrolysis step using quenched-flow measurements. Additionally, the fluorescent phosphate binding protein (MDCC-PBP) independently measures the phosphate release step.

2141-Pos Board B111
Single-Molecule Studies Of ATP-Dependent Restriction Enzymes
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Restriction enzymes (REs) are the central part of the defence system of bacteria against invading viruses. The protein complexes recognize viral DNA by the methylation state of their target sequence and destroy it by cleaving it into pieces. For this, the majority of REs need to interact with two distant target sites. This long-range inter-site communication can be accomplished either by passive 3D diffusive looping or by 1D motion along the DNA contour. Among the different classes of REs, Type I and Type III REs play a special role due to their helicase domains, which are key to the inter-site communication.
For Type I REs it is well established that the helicase domain acts as a dsDNA translocating motor. Cleavage is triggered after a pure 1D communication process, when two translocating motors from distant target sites collide.
In comparison, the communication mechanism for Type III REs has not been accurately defined and conflicting models including 3D diffusion and 1D translocation have been proposed. Using single-molecule DNA stretching based on magnetic tweezers, we provide evidence for a pure 1D communication mechanism in the absence of any 3D diffusive looping. Furthermore, we exclude translocation for inter-site communication due to the low ATPase rates and the observation that the enzymes move bidirectionally along DNA. From this we conclude that Type III REs use 1D diffusion to communicate between their distant target sites.
In order to test the diffusion hypothesis we have started to track the movement of Type I and III REs along DNA using a setup combining magnetic tweezers with single-molecule fluorescence (total-internal reflection fluorescence microscopy).

2142-Pos Board B112
Bacteriophage Phi29 Negatively Twists DNA During Packaging
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Bacteriophage phi29 employs a homomeric ring of RecA-like ATPases in order to package its dsDNA genome into the capsid at near-crystalline density. Previous single-molecule measurements of packaging have revealed the coordination of motor subunits, the step size of the motor, and the sensitivity of the motor to substrate modifications, thereby suggesting structural and kinetic models for the mechanism of translocation.
We directly observe that phi29 negatively supercoils DNA during packaging against applied force by monitoring rotation of a bead attached to the side of the substrate DNA in a laser tweezers. Simultaneously, torque generation and response to applied torque are measured. In this way, we probe the details of force and torque generation by the packaging motor. Combining these measurements with angstrom-scale laser tweezers observations of motor stepping suggests specific geometric models for the interaction of the motor and DNA during translocation.
The magnetic rotor bead technique introduced here allows simple application of torque and straightforward measurement of twist in a laser tweezers apparatus at arbitrary forces. We propose that this method can be applied with ease to a number of existing single-molecule experiments.

2143-Pos Board B113
Sequence-Dependent Kinetics of One-Dimensional Diffusion of p53 on DNA
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Proteins such as transcription factors that must locate and bind to one or a small number of cognate sites on the genome have been suggested to undergo alternating rounds of one-dimensional (1D) and three-dimensional (3D) diffusion. It has been argued that a single protein-DNA energy landscape as a function of the position of the protein on DNA cannot be both smooth enough for a rapid search process and rugged enough so that the protein binds stably to its cognate site (Slutsky and Mirny, Biophys. J. 87 (2004) 4021). We have offered instead a model of kinetic pre-selection whereby proteins diffuse with low friction along a search landscape and pause on cognate sites before adopting transitioning to a recognition landscape which accounts for the rapid localization process observed experimentally (submitted).
The transcription factor p53's role in tumor suppression gives it a biological need to locate and bind its promoters quickly. We developed a single-molecule fluorescence imaging approach to directly observe the protein diffusing in 1D on stretched DNA in vitro (Tafvizi et al., Biophys. J. 95 (2008) L01). By using total internal reflection microscopy to image fluorescently labeled p53 diffusing on stretched duplex DNA, we obtain information on the protein's diffusional properties as a function of its position on the DNA. Using a bioinformatics approach, we have identified a number of sites on our substrate DNA that closely resemble known p53 promoters. We present initial results that demonstrate the feasibility of this approach to correlate high-resolution information on diffusional properties with the positions of these binding sites.

2144-Pos Board B114
Caught in the Act: Single Molecule Structure-Function Studies of Telomerase
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Telomerase is a cellular ribonucleoprotein reverse transcriptase catalyzing the addition of telomeric repeats onto chromosome ends. Given its role in maintaining genomic stability and replicative capacity of cells, telomerase is implicated in cancer development and ageing. Mechanistically, telomerase negotiates a complex set of catalytic substeps, likely requiring exquisite structural rearrangements, to promote processive elongation of chromosome ends. Due to the inherent asynchronicity in enzymatic reactions, traditional biochemical tools are ill-suited for studying how telomerase structure and conformational dynamics enable function. To circumvent this shortcoming, we have developed a single molecule FRET based structure-function assay for telomerase activity and processivity. This assay allows us to capture enzyme-primer interaction (i.e. binding events) and the relevant structural dynamics in real time and to evaluate the catalytic outcome of such interaction. To visualize binding, FRET dye pairs are site-specifically placed at key positions within telomerase to report structural dynamics within the enzyme when bound to surface-immobilized DNA primer. Subsequently, a novel FRET based hybridization approach utilizing a partially complementary detection oligonucleotide (DO) is used to determine the length of the primer previously bound by the labeled enzyme. For an appropriately designed DO, the primer length is revealed through the binding energetics between primer and DO. Proof of principle experiments performed with primers of known lengths show that this detection scheme has single nucleotide sensitivity and low error rates. Since the activity and processivity of telomerase is manifested in its ability to increase the length of a given primer, such scheme allows the determination of enzyme activity one binding event at a time. Using this assay, we have characterized how the structure and conformational dynamics of various Tetrahymena thermophila telomerase RNA motifs such as the pseudoknot and stemloop IV contribute to telomerase function.

2145-Pos Board B115
Conformation of Telomerase RNP Established through Footprinting and Single-Molecule FRET
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Telomerase, a ribonucleoprotein (RNP) expressed in all highly proliferating cells, is comprised of telomerase RNA, telomerase reverse transcriptase (TERT) and other protein cofactors. The complex compensates for the chromosomal shortening that arises with each round of DNA replication. Since mutations altering telomerase expression, assembly and regulation cause multiple human diseases, it is of utmost importance to understand telomerase at the structural level. However, while the structure of TERT in the absence of RNA has been recently published, the full RNP structure remains unknown.
Using a combination of single-molecule FRET and ensemble footprinting, we are probing the functional structure of telomerase RNP. In particular, we have determined the active conformation adopted by two conserved regions of telomerase RNA - the stemloop IV and the pseudoknot - upon assembly with TERT and other protein cofactors.
The pseudoknot, unformed in naked telomerase RNA, was folded in active RNPs. Proper pseudoknot folding was required for catalysis, as demonstrated by mutations that abolished telomerase activity when formation of either pseudoknot stem was disallowed. Conversely, compensatory mutations that reinstated basepairing in the pseudoknot region also restored telomerase activity.
Binding of TERT, in addition to allowing pseudoknot formation, brought loop IV into the proximity of the pseudoknot without affecting its conformation. Mutations in the loop IV region that abolished TERT binding and telomerase activity resulted in an extended RNA conformation with a substantially larger loop IV-pseudoknot distance, as well as protection of the pseudoknot by RNP proteins. Interestingly, the telomerase holoenzyme protein p65 could compensate for these effects of loop IV mutations, restoring the loop IV-TERT interaction, the folded conformation of stemloop IV and telomerase activity.
These results suggest that proper conformations of the pseudoknot and stemloop IV of telomerase RNA are critical for enzyme activity.