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3059-Pos
Application of Shotgun DNA Mapping to Yeast Genomic DNA Shotgun Clones
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Shotgun DNA mapping (SDM) is the ability to identify the genomic location of a random DNA fragment based on its naked DNA unzipping forces compared with simulated unzipping forces of a published genome. We have previously demonstrated proof of principle for shotgun DNA mapping by using plasmid pBR322 unzipping data amongst yeast genome background[cite preprint]. Currently we are validating the technique using unzipping data from yeast genomic DNA. Genomic DNA from yeast (S. cerevisiae) has been digested with restriction endonucleases to produce a library of random fragments, which we used to create a limited library of shotgun clones. Single-molecule unzipping constructs derived from these clones will be unzipping with optical tweezers (OT). In parallel, we have created a library of simulated possible unzipping force profiles, based on the known yeast genome sequence. The OT data and the library will be used in our existing SDM algorithms to identify each shotgun clone, and success rate will be determined via DNA sequencing of the clones. A major application of SDM we are working towards is mapping of nucleosomes and RNA Polymerase II molecules on native chromatin. We will report our progress towards this goal and also discuss other applications of SDM, including splice variant and telomere analysis.

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Separating Static and Dynamic Heterogeneity in Single-Molecule FRET Experiments with Burst Variance Analysis (BVA)
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Histograms of single-molecule FRET efficiency (E) are frequently employed to characterize macromolecular dynamics, and have been been used to better understand the structure and function of proteins such as E.coli RNA Polymerase, Calmodulin and LacY. While such plots are useful for characterizing simple distance changes represented by shifts in mean E value, substantial static heterogeneity, dynamic heterogeneity or both may exist within a single sample, making interpretation of the resulting E histogram difficult. To address this problem we introduce Burst Variance Analysis (BVA), in which we generate a 2D histogram of the mean FRET of a given molecule (E) versus its standard deviation (SD). We use simple theoretical considerations to predict the expected SD, and produce confidence intervals rigorously defining the boundaries beyond which the SD is consistent with dynamics. To validate the method, we performed both numerical simulations and experiments on well-characterized dynamic DNA hairpins. We found that BVA can detect dynamics near the diffusion timescale and over several orders of magnitude; additionally, we used it to distinguish between static and dynamic subpopulations, and separate them for analysis. Using BVA, we analyzed conformational dynamics in the Klenow fragment of E.coli DNA polymerase I, and found evidence for both static and dynamic subpopulations indistinguishable from one another in a simple E histogram. We expect this method to be broadly applicable to single-molecule FRET analyses of macromolecules, and to aid in identifying hidden static or dynamic heterogeneities in their behaviour.

3061-Pos
Visualizing the 1D Diffusion of Eukaryotic DNA Repair Factors Along a Chromatin Lattice
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The ability of DNA binding proteins to efficiently locate a target sequence or structure amongst a vast excess of nonspecific genomic DNA is a critical function affecting a variety of processes such as DNA repair, gene expression and DNA replication. This genomic search is further complicated in eukaryotes where DNA is organized into chromatin and it remains unclear whether nucleosomes act as obstructions which disrupt the scanning process, or whether eukaryotic DNA-binding factors are capable of bypassing these obstacles. Through the use of a single-molecule optical microscopy assay that aligns arrays of DNA molecules in an extended configuration we are able to visualize the facilitated diffusion of the mismatch repair factors Msh2-Msh6 and Mlh1-Pms1 along naked DNA as well as chromatin. Under physiological salt conditions Mlh1-Pms1 moved along DNA by a one-dimensional random walk and exhibited characteristics consistent with a model where the protein moved via a hopping mechanism while wrapped around DNA in a ring-like configuration. Notably, the average diffusion coefficient obtained for Mlh1-Pms1 is approximately an order of magnitude greater than that of its mismatch repair partner Msh2-Msh6 suggesting that these proteins may travel along DNA by distinct mechanisms. Additionally, application of hydrodynamic flow strongly biased the motion of Mlh1-Pms1, highlighting the importance of our technique that maintains DNA in an extended conformation in the absence of perturbing forces. Mlh1-Pms1 was able to freely bypass nucleosomes as it diffused along DNA whereas Msh2-Msh6 was partially confined by nucleosome barriers, passing much less frequently. This work demonstrates that Mlh1-Pms1 is capable of rapidly traveling along naked DNA as well as chromatin whereas the movement of Msh2-Msh6 is significantly hindered by the presence nucleosomes and suggests possible roles that facilitated diffusion may play in mismatch repair.

3062-Pos
Antiviral Signaling Mediated By RIG-I Translocation Activity
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RIG-I is a cytosolic multi-domain protein that detects viral RNA and elicits an antiviral immune response. Two N-terminal caspase activation and recruitment domains (CARDs) transmit the signal and the regulatory domain prevents signaling in the absence of viral RNA. 5'-triphosphate and double stranded (ds) RNA are two well known viral PAMPs (pathogen associated molecular patterns) that enable RIG-I to discriminate pathogenic from self-RNA. However, the function of the ATPase domain that is also required for activity is less clear. Using PIFE (protein induced fluorescence enhancement), a newly developed single-molecule fluorescence assay we discovered a robust, ATP-powered dsRNA translocation activity of RIG-I. The CARDs dramatically suppress translocation in the absence of 5'-triphosphate and the activation by 5'-triphosphate triggers RIG-I to translocate preferentially on dsRNA *in cis*. This functional integration of two RNA molecular patterns may provide a means to specifically sense and counteract replicating viruses.

3063-Pos
Following the Motions of a DNA Helicase on DNA in Real Time
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Using several experimental approaches, we are investigating the dynamics of individual PcrA DNA helicase interactions on DNA templates. Bulk biophysical and biochemical measurements are done in parallel to ascertain the viability and integrity of the protein and DNA substrates. For "Pacman", we are investigating the relationship between the hydrolysis of ATP and the mechanical motion of the enzyme along a DNA substrate. We have designed a mutant PcrA with two cysteines for attachment of a pair of dyes to follow internal protein motions using single-pair fluorescence resonance energy transfer (spFRET). For "Ring-a-bell" we are following the interaction of PcrA with replication protein RepC. Alone, PcrA is typically capable of translocating only ~80 bp before dissociating from a DNA template. With RepC, PcrA can achieve rolling circle replication of thousands of bp on a plasmid. Should the two proteins be bound together, they should create specific low and high spFRET signals as they translocate along a specifically labeled DNA substrate. And finally we are studying the interaction of PcrA and Holiday Junction DNA. Does PcrA separate the DNA from the center or from the ends of the DNA junction? We have upgraded our evanescent field fluorescence microscope to use alternating red (648 nm) and green (532 nm) lasers to illuminate the sample. We have added multiple syringes operating under computer control to follow experiments in real-time whereupon ATP is introduced into the liquid flow chamber. We have also upgraded our scanning confocal microscope to improve the alignment of the laser and the ease of use of the instrument.

3064-Pos
Quantum Dot Probes for Single-Molecule Rotation of Cell Surface Proteins
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Rotation of membrane proteins is a sensitive measure of their aggregation state and interactions. We have investigated the use of asymmetric quantum dots (QD) as non-bleaching imaging probes providing orientation-dependent optical signals from individual cell surface proteins. A commercial QD emitting at ~605 nm measures 10.9 x 5.3 nm and exhibits an initial fluorescence anisotropy of 0.11. Calculated rotational correlation times (RCT) for rotations about the particle short and long axes, 0.18 μs and 0.12 μs respectively, suggest that this nanoparticle can probe μs timescale molecular rotation. These QDs, and