repressor-mediated DNA loops. The J_{loop} values are consistent with previously published experimental results using DNA circularization assays and theoretical Gaussian polymer and Shimada and Yamakawa models. J_{loop} and repression decrease for the larger loops as expected. Comparison of simulations and TPM experiments enables fine tuning of the simulation. Instead *in vitro* titrations of DNA looping as a function of protein concentration suggest that loops *in vivo* operate in a narrow range of effective concentrations of just a few picomolar LacI.

896-Pos Board B665
Studies of T4 Primosome DNA Unwinding and DNA Breathing by Single-Molecule FRET and Linear Dichroism Experiments
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DNA 'breathing' is a thermally driven process in which nucleotide residues near single-strand (ss) - double-strand (ds) DNA forks and junctions temporarily adopt local conformations that depart from their most stable structures. It is thought that the transient occurrence of these 'open' conformations is centrally involved in the proper function of DNA-protein complexes responsible for replication, transcription, and many other reactions that involve the manipulation of the DNA genome. Such motions are difficult to observe in bulk measurements because they likely occur in the microsecond regime, which is an especially difficult timescale to access experimentally. In this work we report polarized light single-molecule fluorescence experiments in which microsecond rotational motions of the sugar-phosphate backbone are directly observed. These experiments simultaneously detect single molecule FRET and linear dichroism (FLD) signals from dsDNA replication fork constructs containing Cy3 - Cy5 FRET donor-acceptor probes strategically located near the replication fork junction on opposite strands. Our results show that significant local backbone motions are manifested on a ~100 microsecond timescale, which is a reasonable range for at least some types of DNA 'breathing' fluctuations. In addition we have shown that these motions can be perturbed by the presence of the T4 gp41 hexameric helicase.

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Single-Molecule Observation of IFT Turnover using a Photobleaching Gate
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Intraflagellar transport (IFT) is required for the assembly and maintenance of eukaryotic cilia and flagella. In *Chlamydomonas reinhardtii*, IFT trains are transported by kinesin-2 to the flagellar distal tip and by dynein-1b back to the basal body. Remodeling of IFT trains and motor protein regulation are believed to occur at the flagellar tip complex. Due to the complex and crowded nature of the flagellar turnaround zones, IFT train and motor behavior at the tip remains unclear. To investigate the turnover of a single IFT train or a motor cluster, we introduced a high-power laser gate that continuously photobleaches incoming IFT trains and selectively allows a single unbleached train to enter the flagellum. We observed that anterograde IFT trains pause at tip for ~4 s on average and split into multiple retrograde trains. In contrast, the KAP subunit of kinesin-2 releases from IFT trains at the tip after a shorter pausing period. Different from *C. elegans* IFT, in which KAP is recycled as a retrograde cargo, KAP in *Chlmaydomonas* returns to the basal body by diffusion with an average diffusion constant of 1.2 $\mu m^2/s$. We also observed a concentration gradient for the KAP-GFP background that increases towards the tips of flagella. We anticipate our photobleaching gate assays to be a starting point to address the detailed mechanism of IFT regulation at turnaround zones.

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Fully Streched Single DNA Molecules in a Nanofluidic Chip Show Large-Scale Structural Variation
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When stretching and imaging DNA molecules in nanofluidic devices, it is important to know the relation between the physical length as measured in the lab and the distance along the contour of the DNA. Here a single DNA molecule longer than 1 Mbp is loaded into a nanofluidic device consisting of two crossing nanoslits (85nm x 50 microns) connected to microchannels. An applied pressure creates a stagnation point at the crossing of the nanoslits. The drag force from the fluid stretches the DNA. We determine the degree of stretching of the molecule (i) without the use of markers, (ii) without knowing the contour length of the DNA, and (iii) without having the full DNA molecule inside the field-of-view. The analysis is based on the transverse motion of the DNA due its Brownian motion, i.e. the DNA's response to the thermal fluctuations of the liquid surrounding it. The parameter values obtained by fitting agree well with values we obtain from simplified modeling of the DNA as a cylinder in a parallel flow.
Secondly, DNA molecules stained with the intercalating dye YOYO-1 are de- and renatured locally following a modified version of the protocol used in Ref. 1. The result is a melting pattern which reflects the local AT/GC-content. Single molecules are loaded into the chip and imaged. Due to the almost complete stretching of the DNA, structural variations in the size range from kbp to Mbp can be detected and quantified from the melting pattern alone.
[1] W. Reisner, N. B. Larsen, A. Silahtaroglu, A. Kristensen, N. Tommerup, J. O. Tegenfeldt, and H. Flyvbjerg, PNAS, 107, 30 (2010) 13294.

899-Pos Board B668
Single Molecule Dynamic Transduction by Carbon Nanotube Circuits
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Nanoscale electronic devices like field-effect transistors (FETs) have long promised to provide sensitive, label-free detection of biomolecules. In particular, single-walled carbon nanotubes (SWNTs) have the requisite sensitivity to detect single molecule events, and have sufficient bandwidth to directly monitor single molecule dynamics in real time. Recent measurements have demonstrated this premise by monitoring the dynamic, single-molecule processivity of three different enzymes: lysozyme, protein kinase A, and the Klenow Fragment of polymerase I. Initial successes in each case indicated the generality and attractiveness of SWNT FETs as a new tool to complement other single molecule techniques. However, further generalization of the SWNT FET technique demands reliable design rules that can predict the success and applicability of these devices. Here, we address this need by focusing on the transduction mechanisms that link enzyme processivity to electrical signal generation in a SWNT FET. Using ten different lysozyme variants synthesized by mutagenesis, we systematically dissect the enzyme-SWNT interaction in order to understand this transduction. The data prove that mechanical displacements of charged functionalities near the SWNT attachment site are the primary sources of transduction, and that the resulting devices are sensitive enough to track the motions of just one charged amino acid. The purposeful incorporation of a charged group at a particular location allows the device to be designed to have sensitivity to particular chemical activities or allosterically-driven mechanical motions. The findings provide rules for the creation of similarly effective nanocircuits using a wide range of enzymes or proteins.

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Direct Observation of Recbcd Helicase as ssDNA Translocases
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The *E. coli* RecBCD is a heterotrimeric enzyme composed of two helicase motors with different polarities: RecB (3'-to-5') and RecD (5'-to-3'). This Superfamily I helicase is responsible for the initiation of DNA double-strand-break (DSB) repair in the homologous recombination pathway. We used single-molecule tethered particle motion (TPM) experiments to visualize the RecBCD helicase translocation over long single-stranded (ss) DNA (> 200 nt) without secondary structure. The bead-labeled RecBCD helicases were found to bind to the surface-immobilized blunt duplex DNA, and translocate along the duplex/single-stranded/duplex DNA substrate, resulting in a gradual decrease in the bead Brownian motion. Successful observation of RecBCD translocation over long ssDNA gap in either 3'-to-5' or 5'-to-3' direction indicates that both RecB and RecD are ssDNA translocases. We also applied continuous force (~ 0.2 pN) to stretch DNA substrates and to remove any potentially transient looped ssDNA structure, and observed continuous translocation of RecBCD. It confirms the ssDNA translocase activities of RecBCD helicase. About 78 % of active tethers showed full translocation across the ssDNA to the dsDNA region, and the other 22 % enzymes dissociated from the ss/dsDNA junction after translocating across the ssDNA region. We also prepared a double-gapped substrate containing two regions of ssDNA with opposite polarities (5'-to-3' and 3'-to-5') intermitted by duplex DNA. RecBCD was able to translocate across both ssDNA regions in either ssDNA orientation orders, with 20 - 40 % of tethers dissociating while entering the second ssDNA region. These results suggest a mechanism that RecBCD is able to switch motors and rethread into the other strand after translocating along an ssDNA gapped region.