which tethers α to the DNA template. This interaction releases α from β, which has been proposed to facilitate polymerase switching (*Silva et al.* in preparation). Furthermore, UmuD may bind α and disrupt its interaction with the single-stranded DNA (ssDNA) template in an effort to facilitate polymerase exchange. We use single molecule DNA stretching, a technique that was previously used to characterize the DNA binding properties of α (*McCauley et al.* ACS Chem. Biol. **3**, 2008), to investigate the possibility that UmuD disrupts the binding of α and ssDNA. Preliminary data suggests that, although UmuD alone does not bind DNA, its presence with α weakens the binding of α to ssDNA. Additionally, UmuD and α together appear to stabilize double-stranded DNA (dsDNA), which suggests that UmuD may facilitate α binding to dsDNA at the expense of ssDNA binding.

1429-Pos Board B199
Studying RecA Homology Search Mechanism using Single-Molecule Methods
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RecA recombinase plays an important role in repairing the double-strand DNA breaks in *E. coli*. To repair damaged DNA, RecA first forms a nucleoprotein filament on a single-stranded DNA molecule, this nucleoprotein filament then searches and pairs the homology duplex DNA, and finally carries out the strand exchange reaction to initiate the homologous recombinational repair. To ensure that the repair process is carried out within the limited timescale set by the cell cycle of *E. coli*, it is important that RecA nucleoprotein filament locates its homologous sequence efficiently. Several search mechanisms have been proposed: a one-dimensional sliding (1D), a three-dimensional hopping (3D), and a facilitated intersegment transfer model. We have used two single-molecule imaging techniques to investigate the homology search mechanism of RecA nucleoprotein filaments. By applying a lateral force of a few pN to a bead-tethered dsDNA, the dsDNA is extended horizontally, and the diffusion trajectories of the labeled RecA nucleoprotein filaments (labeled with a bead or quantum dot) can be visualized at the single-molecule level. From individual searching events, the diffusion coefficient of the RecA nucleoprotein filament is determined to be ~ 10^6 nm^2/s. The salt dependence of the diffusion coefficients from 0 to 200 mM NaCl allows us to distinguish its major search mechanism.

1430-Pos Board B200
Using a Single-Molecule Method to Visualize RecBCD Helicase Translocation along Single-Stranded DNA
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The *E. coli* RecBCD helicase initiates the repair of double strand DNA break in the homologous recombination pathway. RecBCD is a heterotrimeric enzyme composed of two helicase motors with different polarities: RecB is a 3'-to-5' helicase and RecD is a 5'-to-3' helicase. How RecBCD unwinds and translocates along duplex DNA is not clearly defined. Here we used a single-molecule tethered particle motion (TPM) experiment to visualize the RecBCD helicase translocation over long distance single-stranded (ss) DNA. We first prepared DNA substrates containing a $>$ 200 nt long, unstructured ssDNA gap flanked by double-stranded DNA for RecBCD loading. In the TPM experiments, the bead-labeled, biotinylated RecBCD helicases are found to recognize and bind to the blunt, double-stranded DNA end, and successfully translocate along the duplex/single-stranded/duplex DNA substrate, resulting in a gradual decrease in the bead Brownian motion amplitude. Successful observation of RecBCD translocation over long ssDNA gap in either 3'-to- 5' or 5'-to-3' direction indicates that wild-type RecBCD functions an ssDNA translocase.

1431-Pos Board B201
Single-Molecule Studies of the E. Coli Translesion Replisome
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Translesion polymerases (TLS pols) are enzymes that replicate over naturally occurring DNA lesions that slip past repair mechanisms, as part of the damage tolerance pathway. Found in all three domains of life, TLS pols have unique structural features that allow them to replicate over DNA adducts and modifications that would otherwise stall the replisome. This evolved ability comes at a sacrifice - the inability of TLS pols to incorporate opposite undamaged DNA with high fidelity - suggesting careful regulation of polymerase access to the replication fork.
We report a multiplexed, single-molecule assay to observe real-time DNA synthesis by a minimal *E. coli* replisome *in vitro* and have observed switching between Pol III, the replicative polymerase, and one of its translesion counterparts, Pol IV.

1432-Pos Board B202
Impact of Macromolecular Crowding on DNA Replication
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Enzymatic activities in vivo occur in a highly crowded and confined environment mainly composed of protein and nucleic acid macromolecules. A crowded environment: 1) enhances the apparent concentration of the measured macromolecule, 2) leads to desolvation of water molecules around the proteins, 3) decrease degrees of freedom for diffusion and molecular hopping of proteins on their DNA substrates, and 4) enhances binding equilibria and catalytic activities of those macromolecules. However, the effect of macromolecular crowding on the structure of the proteins involved is poorly understood. We have characterized the effect of macromolecular crowding on the DNA replication machinery of bacteriophage T7. The structural effects that are involved in a crowded environment were probed using small angle X-ray scattering. Our results show the consequences of macromolecular crowding on the structure and the function of the bacteriophage T7 replisome. Under macromolecular crowding conditions the increased readout of the replisome activity is accompanied by a more compact structure of the replisome.

1433-Pos Board B203
Mathematical Model of Telomere Length Maintenance in Mitochondrial DNA of Yeast
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The terminal structures of linear mitochondrial DNA in yeast consist of repetitive long tandem units of different lengths (telomeres). Besides these linear telomeres other cyclic configurations as telomeric circles and telomeric loops were observed experimentally and are suspected to play an important role in the alternative mechanism of the telomere length maintenance in the absence of telomerase (ALT).
In this work we construct a mathematical model that captures biophysical interactions of various telomeric structures on a short time scale and that is able to reproduce experimental measurements in C. parapsilosis, P. philodendra and C. salmanticensis. We identify the key factors influencing the length distribution of telomeric circles, linear telomeres and telomeric loops using numerical simulations for the model we have constructed. Moreover, the model opens up a couple of interesting open mathematical problems in quasi-steady state approximation and discrete coagulation-fragmentation dynamical systems.

1434-Pos Board B204
Nucleoid Morphology in E. Coli is Cell Shape Dependent
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In a typical E.coli cell, nucleoid morphology, as determined from 3D fluorescence imaging of an Hu-mCherry fusion protein, has been shown to be a dynamic helicoidal ellipsoid . Using the replication of the origin as a marker to align data sets temporally, we have investigated the morphology of this structure with 5sec resolution throughout the cell cycle. Here we show that number and pitch of the helical turns is depend upon the portion of the genome that has been replicated and the size of the cells. Additionally, we show that longitudinal sub-structures vary as a function of cell cycle as well.

1435-Pos Board B205
A Multi-State Mechanism of Nucleotide Selection in DNA Polymerase I Revealed by Single-Molecule FRET
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To carry out accurate and rapid DNA synthesis, DNA polymerases must quickly sample among incoming nucleotides and discriminate against incorrect ones (not complementary to the template base). Precise and finely tuned molecular mechanisms regulating this process remain to be fully explained. An existing model based on crystallographic data suggests that a mobile segment of the protein known as the fingers domain can rapidly switch between an open and closed conformation, thereby fitting a complementary incoming nucleotide and rejecting non-fits before the chemical step of nucleotide incorporation. Exactly how the nascent base pair is sensed by the polymerase remains nebulous. We were able to gain insights into the workings of E. coli DNA polymerase I (Klenow fragment, Pol I KF) during the process of the nucleotide selection using a single-molecule FRET technique. To detect the conformational rearrangements around the nascent base pair, we specifically labeled a surface-immobilized primer-template with Alexa 488 (donor) and the introduced cysteine residue 744 on the mobile segment of the fingers domain of Pol I KF with Alexa 594 (acceptor). TIRF microscopy with FRET detection was used to monitor the conformational dynamics of individual Pol-DNA complexes in real-time, revealing that the protein rapidly samples three key states during