The Y family of DNA polymerases is a group of DNA damage tolerance enzymes with the specialized ability to bypass DNA lesions by inserting nucleotides opposite damaged sites in DNA. Translesion synthesis (TLS) responsible for most of the mutagenesis induced by UV radiation requires the $UmuD'_2$ protein (the cleaved form of the $UmuD_2$), UmuC, and RecA. $UmuD'_2$ activates UmuC, the catalytic subunit of the Y family DNA polymerase V, for mutagenic DNA replication. $UmuD_2$ and $UmuD'_2$ make a remarkable number of specific protein-protein contacts to DNA polymerases. Despite the nearly identical primary structure of $UmuD_2$ and $UmuD'_2$, their interactions with the same partner can differ in affinity and functional significance. Analysis of the UmuD/UmuD'-pol III interactions by affinity chromatography indicated that UmuD has a lower affinity for alpha (α) subunit of *E. coli*'s replicative polymerase III than does UmuD'.

We aim to understand how binding of UmuD dimers is coordinated with the activity of α subunit. We are utilizing the biochemical and biophysical methods to look at the kinetics of α subunit activity and at the formation of α-$UmuD_2$ and α-$UmuD'_2$ complexes. We are characterizing the ability of the α subunit to copy both damaged and undamaged DNA in the presence of UmuD and UmuD'. In order to quantify the binding of UmuD and UmuD' to the α subunit, we are determining the K_d (equilibrium dissociation constant) for this interaction by measuring intrinsic tryptophan fluorescence of the α subunit.

1751-Pos Board B595

Single-Molecule Studies of Fork Dynamics in E. coli DNA Replication

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We present single-molecule studies of the Escherichia coli replication machinery. We visualize individual E. coli DNA polymerase III (Pol III) holoenzymes engaging in primer extension and leading-strand synthesis. When coupled to the replicative helicase DnaB, Pol III mediates leading-strand synthesis with a processivity of 10.5 kb, 8-fold higher than that of primer extension by Pol III alone. Addition of the primase DnaG causes a 3-fold reduction in the processivity of leading-strand synthesis, an effect dependent upon the DnaB-DnaG protein-protein interaction rather than primase activity. A single-molecule analysis of the replication kinetics with varying DnaG concentrations indicates that a cooperative binding of 2-3 DnaG monomers to DnaB halts synthesis. Modulation of DnaB helicase activity through the interaction with DnaG suggests a mechanism that prevents leading-strand synthesis from outpacing lagging-strand synthesis during slow primer synthesis on the lagging strand.

1752-Pos Board B596

Molecular Mechanism of the Acceleration of the Damaged Base Extrusion and its Recognition by Bacterial MutM DNA Glycosylase: Free Energy Simulation Studies

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8-Oxoguanine (8oxoG) is frequently generated endogenously through the attack of reactive oxygen species on the genome. In bacterial base excision repair system, the enzyme, 8-oxoguanine (8oxoG) DNA glycosylase (MutM), carries out the search of the damaged base 8oxoG in DNA. After having encountered the damaged base, the enzyme flips it out of DNA helix, and it enters into the active site where the catalytic cleavage occurs. The search of 8oxoG by MutM is a difficult task, because 8oxoG differs subtly from undamaged guanine (G), and compared to G, it is very rare under normal conditions in DNA (about 1 in 10^5). To determine the factors involved in the specific recognition function of MutM, free energy (potential of mean force) simulations and targeted molecular dynamics simulations are performed for a number of different systems. The simulations indicate that base extrusion and entrance into the active site is, essentially, a three-step process. We also analyzed the free energy contributions of different components, such as the effects of DNA-bending induced by the binding of MutM to DNA and several important residues, in the base extrusion of the damaged and undamaged base.

1753-Pos Board B597

Molecular Mechanisms in the Repair of the Cyclobutane Dimer

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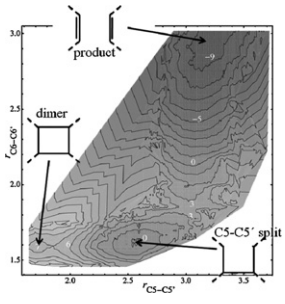
We investigate the molecular mechanism of the repair of the cyclobutane dimer radical anion in aqueous solution using ab initio MD simulations. Umbrella sampling is used to determine a two-dimensional free energy surface as a function of the C5-C5́ and C6-C6́ distances. The neutral dimer is unable to surmount a large free energy barrier for repair. Upon addition of an electron, the splitting of the C5-C5́ coordinate is virtually barrier less. Transition state theory predicts that the splitting of the C6-C6́ bond is complete on a ps timescale. The free energy surface suggests that the splitting of the two bonds is asynchronously concerted. Our work is the first to explicitly include the electronic degrees of freedom for both the cyclobutane dimer and the surrounding water pocket. The ab initio simulations show that at least 30% of the electron density is delocalized onto the surrounding solvent during the splitting process. Simulations on the neutral surface show that back electron transfer from the dimer is critical for the completion of splitting. To maximize splitting yield, the back electron transfer should occur beyond the transition state along the splitting coordinate.



1754-Pos Board B598

Regulation Of The DNA Damage Response By The DNA Polymerase Manager Protein UmuD

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Organisms experience DNA damage from environmental as well as endogenous sources. When bacterial cells experience DNA damage DNA and other stresses, the SOS response is induced, leading to upregulation of at least 50 genes in *E. coli*. Many of the genes whose expression is induced as part of the SOS response are responsible for DNA repair and cell cycle regulation. Another group of genes, specialized Y family DNA polymerases with the ability to replicate damaged DNA, play a role in tolerance to DNA damage at a potentially mutagenic cost. Multiple layers of regulation control the activity of these potentially mutagenic proteins. The function of Y family DNA polymerases is regulated by UmuD, a manager protein, and its cleaved form, UmuD'. The *umuD* gene products directly interact with both Y family polymerases as well as the beta processivity clamp. The goal of this study is to determine the conformation and dynamics of the *umuD* gene products in order to understand how they regulate the cellular response to DNA damage. We are using fluorescence resonance energy transfer (FRET) and hydrogen-deuterium exchange mass spectrometry (HXMS) to probe the conformations of UmuD and UmuD'. In HXMS experiments, backbone amide hydrogens that are solvent-accessible become labeled with deuterium over time, whereas those are are not accessible do not become labeled. Our HXMS results reveal that the N-terminal arm of UmuD, which is not present in the cleaved form UmuD', exhibits local partial unfolding. Residues that contact the N-terminal arm show large differences between UmuD and UmuD'. Additionally, there are substantial regions of stable conformation in both proteins. Complete characterization of UmuD and UmuD' dynamics is currently in progress.

1755-Pos Board B599

Mismatch Recognition Cycle in MutS and MSH2-MSH6 from Normal Mode Analysis and Simulations

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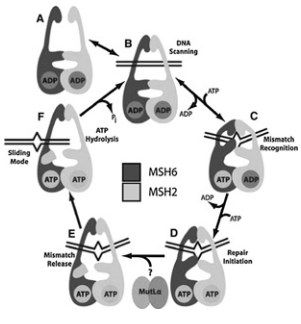
Post-replication DNA mismatch repair (MMR) is crucial in ensuring genetic fidelity in prokaryots and eukaryots. The initial step of MMR is recognition of defective DNA by MutS or its eukaryotic homologs. Binding of MutS to mismatched DNA, the subsequent initiation of repair, and eventual recovery to a mismatch scanning mode is coupled to ATPase activity in MutS. Crystal structures of MutS and the eukaryotic MSH2:MSH6 system place the ATPase domain far away from the DNA binding domains, implicating a complex allosteric mechanism.

Normal mode calculations and molecular dynamics simulations of MutS and MSH2:MSH6 structures were carried out to explore the coupling between DNA binding and ATPase activity. The mode analysis reveals conserved dynamics between the bacterial and eukaryotic complexes. Individual modes correlate ATPase activity with the probing of DNA kinking that is characteristic of mismatched DNA. Furthermore, differential ATPase activity between the MutS dimer moieties as observed experimentally is coupled to release of MutS from the mismatch during repair. Based on the calculations and consistent with available experimental



data, a detailed mechanistic model of the allosteric conformational changes during DNA mismatch recognition by MutS is proposed.

1756-Pos Board B600
Working Mechanism of the Human Bloom's Syndrome Helicase
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Genome integrity is indispensable for unperturbed cell functioning. RecQ helicases play essential roles in genome maintenance. Mutations in three of the human RecQ isoforms (BLM, WRN or RECQL4) lead to severe diseases as the Bloom's, the Werner's and the Rothmund-Thomson syndromes, respectively, characterized by increased cancer predisposition and premature aging. Behind the serious genetic disorders stands the lack of repair mechanisms. BLM plays a crucial role in HR-based pathways by dissolving double Holliday-junctions and D-loops. The detailed working mechanism by which these ''roadblock remover'' functions are achieved is still unclear. We performed extensive kinetic, fluorescence spectroscopic and electrophoretic analyses to investigate the enzymatic cycle of BLM. In these studies wild-type and single tryptophan-containing BLM mutants were used. We demonstrate that BLM randomly and structure specifically binds DNA in the absence of nucleotide. ATP binds to DNA-bound BLM and induces a conformational change. ATP binding, hydrolysis and phosphate release occur rapidly and are followed by the rate limiting step of the cycle. This step is possibly a conformational change induced by DNA during translocation. BLM performs multiple ATPase cycles without dissociating from the DNA track. This results in the processive translocation activity of BLM. In contrast to other helicases (e.g. PcrA), BLM dissociates from the DNA strand at its 5′-end, thereby avoiding futile ATPase cycling. Our results emphasize the importance of investigating the basic working mechanism of different DNA helicases because these mechanisms may differ significantly. Moreover, understanding the basic working mechanism will greatly aid in understanding the complex functions of RecQ helicases.

1757-Pos Board B601
Direct Simulation Of Electron Transfer Reactions In DNA Radical Cations
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The electron transfer properties of DNA radical cations are important in DNA damage and repair processes. Fast long-range charge transfer has been demonstrated experimentally, but the subtle influences that experimental conditions as well as DNA sequences and geometries have on the details of electron transfer parameters are still poorly understood.
In this work, we employ an atomistic QM/MM approach, based on a one-electron tight binding Hamiltonian and a classical molecular mechanics forcefield, to conduct nanosecond length MD simulations of electron holes in DNA oligomers. Multiple spontaneous electron transfer events were observed in 100 ns simulations with neighbouring adenine or guanine bases. Marcus parameters of charge transfer could be extracted directly from the simulations. The reorganisation energy lambda for hopping between neighbouring bases was found to be ca. 25 kcal/mol and charge transfer rates of $4.1\times10\hat{}9\ s\hat{}-1$ for AA hopping and $1.3\times10\hat{}9\ s\hat{}-1$ for GG hopping were obtained.

1758-Pos Board B602
Studies of the Translocation Mechanism of Hepatitis C Virus NS3 Helicase with Computationally Mutant Constructs
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Hepatitis C virus (HCV) NS3 helicase unwinds double-stranded polynucleotide for HCV genome replication. Biochemical and single molecule studies have examined its enzymatic activity in depth, while the detailed translocation mechanism is still unclear. Our previous work has identified a list of hot-spot residues for its dynamic couplings and translocation by using an elastic network model (ENM). To further pinpoint key residues important for the polynucleotide movement, we used molecular dynamic (MD) simulation to study the conformational dynamics of NS3 helicase with computationally mutant constructs H293A, T324A, V432A and R461A. These mutations have been shown critical to the function of NS3 helicase by both experimental studies and ENM. We also simulated mutant constructs, T448A and P230A, which have only been predicted by ENM without experimental tests. Our results were consistent with experimental observations and suggested other important residues for polynucleotide translocation. Moreover, we have identified key hydrogen bond interactions between NS3 helicase and the polynucleotide for future experimental verification.

DNA, RNA Structure & Conformation I

1759-Pos Board B603
Elucidation of the Mechanism of an Epigenetic Switch by Single-molecule Assays
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The lambda bacteriophage epigenetic switch determines the growth lifestyle of the virus after infection of its host (*E. coli*). It is now clear that the switch consists of a ~2.3 kbp-long DNA loop mediated by the lambda repressor protein. Using tethered particle microscopy (TPM), magnetic tweezers and AFM, our laboratory has novel, direct evidence of loop formation and breakdown by the repressor, the first characterization of the thermodynamics and kinetics of the looping reaction and its dependence on DNA supercoiling and repressor non-specific binding. These *in vitro* data provide insight into the different possible nucleoprotein complexes and into the lambda repressor-mediated looping mechanism which leads to predictions for that *in vivo*. The significance of this work consists not only of the new insight into a paradigmatic epigenetic switch that governs lysogeny vs. lysis, but also the detailed mechanics of regulatory DNA loops mediated by proteins bound to multipartite operators and capable of different levels of oligomerization.

1760-Pos Board B604
Elasticity of Sub-micron DNA Molecules Studied with Axial Optical Tweezers
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Understanding the elasticity of sub-micron DNA molecules is important because many crucial biological structures and processes occur on this length scale. Using optical tweezers to manipulate DNA molecules, however, is difficult when molecules are shorter than about one micron. The reason is that conventional optical tweezers stretch molecules laterally in the focal plane of the microscope objective, a mode in which steric hindrances from the coverslip and other surface effects are substantial. To overcome the problem, we developed and calibrated an axial optical tweezers that makes this length scale accessible by stretching the molecule in the axial direction of the laser beam. By varying the laser intensity, different stretching forces were applied to the DNA molecule, and the axial position of the tethered microsphere was obtained from its diffraction pattern.
We measured the force-extension relationships of four short ds-DNA molecules, which are 1298 bp-, 662 bp-, 390 bp-, and 247 bp-long, using the axial optical tweezers. Using a modified worm-like chain (WLC) model for the extended DNA molecule that incorporates excluded-volume entropic effects from the coverslip and microsphere are taken into account, we obtained effective persistence lengths and excluded-volume forces for these molecules. The fitted values for the persistence length decrease with the contour length of the DNA, which is qualitatively consistent with observations by Seol et al. on longer, micron- and sub-micron sized constructs (Seol 2007). Moreover, the excluded-volume forces are close to the theoretical predictions by Segall et al (Segall 2006).

1761-Pos Board B605
Structure Conversion Of Human Telomeric Sequence Studied By Single-molecule Tethered Particle Motion
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Telomeres contain G-rich tandem repeats of single-stranded DNA sequences at 3′ tail. The G-rich sequences can be folded into a secondary structure named G-quadruplexes by Hoogsteen base pairing in the presence of monovalent cations (such as Na+, K+). The folding of telomeric DNA into the G-quadruplexes may inhibit telomerase activity for the proliferation of cancer cells. Moreover, the change of a quadruplex conformation may play an important role in biological effect. Thus, understanding structure conversion between the folded and unfolded G-quadruplex structures, and how the structure conversion is mediated by ions, its anti-sense sequence and its stabilizers are important to telomere biology. Here, we have directly monitored the conversion between the folded and unfolded structures in human telomeric AGGG(TTAGGG)3 sequence by the single-molecule tethered particle motion (TPM) method. TPM method monitors the DNA length change caused by the G-quadruplex formation, and allows us to monitor the conversion mechanism