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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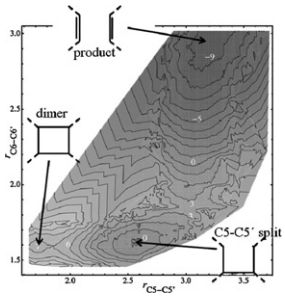
We investigate the molecular mechanism of the repair of the cyclobutane dimer radical anion in aqueous solution using ab initio MD simulations. Umbrella sampling is used to determine a two-dimensional free energy surface as a function of the C5-C5́ and C6-C6́ distances. The neutral dimer is unable to 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