fibrillation, in support of our REMD simulations. An important finding from our REMD simulations is that fullerene C_{180}, albeit with the same number of carbon atoms as three C_{60} molecules (3C_{60}) and smaller surface area than 3C_{60}, displays an unexpected stronger inhibitory effect on the β-sheet formation. A detailed analysis of the CNP-peptide interaction reveals that strong inhibition of β-sheet formation by CNPs results from the strong hydrophobic and aromatic-stacking interactions between CNPs and Aβ peptides. These results demonstrate that CNPs can efficiently inhibit the aggregation of Aβ peptides and reveal the molecular insights into the inhibition mechanisms. Our study reveals the significant inhibitory role of fullerene hexagonal rings on the aggregation of Aβ(16-22) and full-length Aβ peptides and also provides novel insight into the development of drug candidates against Alzheimer’s disease.

DNA Replication, Recombination, and Repair

336-Pos  Board B116
Single Molecule Studies of RPA’s Sequential Binding to ssDNA Reveals a Highly Stiff and Stable State Induced by the Binding of Zinc
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337-Pos  Board B117
Studies of the FtsK DNA Translocase using Two-Color Tethered Fluorophore Motion
Peter F.J. May1, Pawel Zawadzki2, Lidia K. Arciszewska2, David Sherratt1, Achilles N. Kapanidis3.
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338-Pos  Board B118
Unraveling the Interplay between Single-Stranded DNA-Binding Protein, DNA Polymerase and Single-Stranded DNA
Jordi Cabanas Danes, Tjalle P. Hoekstra, Iddo Heller, Erwin J.G. Peterman, Gijs J.L. Wuite.
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Bacteriophage T7 gene 2.5 protein (gp2.5), a single-stranded DNA binding (SSB) protein contains two main structural elements which confer its essential functions. First, an oligosaccharide/oligonucleotide binding fold which can interact with ssDNA and thus provide a protective role while removing secondary structure impediments. Second, an acidic C-terminal tail capable to interact with other gp2.5 units and with other replisome proteins, such as the T7 DNA primase-helicase (gp4) and polymerase (gp5), allowing for organizational roles within the replisome. The impact of SSBs on the replisome dynamics is hard to study using conventional biochemistry tools. Here, we present data obtained from a unique combination of optical tweezers and confocal fluorescence microscopy, which offers real-time high spatial and temporal resolution to study protein-DNA interactions. Our results show that the SSB gp2.5 binds efficiently to ssDNA, forming highly static protein-DNA complexes. We also observed a decrease in the ssDNA end-to-end length indicating that the protein binds by bending or wrapping ssDNA, resulting in a highly tension-sensitive binding mode. Using Eu-DnaB, we show that the helicase with a wild-type (wt) gp2.5 or a mutant lacking the terminal phenylalanine (ΔF), the interaction motif with DNA polymerase, affects replication activity. We show that wt gp2.5 alters the rate, duration and probability of occurrence of replicative and proofreading polymerase events in contrast to the ΔF mutant or the absence of the protein. Thus, our findings indicate that the presence of gp2.5 strongly stimulates the function of DNA polymerase within the replisome.

340-Pos  Board B120
Effects of DNA Structural and Topological Constraints on HMGA2 Binding
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HMGA proteins belong to the super family of the high mobility group (HMG) proteins act as architectural transcription factors. They are known to modulate transcription of many genes by altering the chromatin structure. Recently, a new function of HMGA2 working as a replication fork chaperone has been uncovered and this finding implies the HMGA2 might recognize the particular

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fork structure during DNA replication. In order to gain insights of how HMGA2 interacts of DNA forks, we performed single-DNA manipulation studies of the binding of HMGA2 to DNA fork mimetic DNA constructs and topological constraints such as DNA fork with three DNA arms, supercoiled DNA, DNA hairpins, and ssDNA. Ours results show that HMGA2 preferentially binds to supercoiled and forked DNA comparing to torsion unconstrained DNA and it has the least binding affinity to single-stranded template. The AT-hook DNA-binding domains in HMGA2 are critical form binding - removal of two of the three AT-hooks completely abolishes the binding. In conclusion, our studies show that HMGA2 proteins recognize structure rather than specific nucleotide sequences for binding via their unique AT-hook binding domains.

341-Pos Board B121
Single-Molecule Analysis of Transcription-Coupled Repair
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Transcription-coupled repair (TCR), a subpathway of nucleotide excision repair (NER), has been known to lead to more efficient repair than the global NER repair (GGR). Here we use magnetic trapping of single DNA molecules to study the interactions of TCR proteins with stalled RNA polymerase (RNAP). Single RNAP molecules are stalled on a DNA transcript using either a CTP-less cassette or a thymine-thymine dimer located on the transcribed strand. Stalled RNAP is displaced by the Mfd translocase, and a long-lived intermediate is formed. We characterize the interaction between UvrA/UvrB proteins and the long-lived intermediate formed upon Mfd displacement of RNAP. This interaction leads to formation of a pre-incision complex that is catalytically competent for DNA incision, and the activity of this complex is tested for in the magnetic trap upon addition of UvrC.

342-Pos Board B122
Investigation of the Tus-ter Blocking Efficacy during the Chromosome Replication of Live Escherichia Coli Cells
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In E. coli, a circular chromosome is replicated in a bi-directional manner by two replication complexes that assemble at a single origin of replication (oriC). The two replication forks are thought to terminate in the termination region, which is flanked by 10 ter sites. Each of these sites is bound by a Termination utilization substance (Tus) protein, thereby forming Tus-ter complexes. In vitro studies have shown that the replication forks are blocked when they approach a Tus-ter complex from the non-permissive side, but not from the permissive side. However, the blocking efficacy of the Tus-ter complex on a replication fork approaching from the non-permissive side, and the subsequent dynamics, have not been examined in live cells. To shed light on these processes in vivo, we utilize quantitative fluorescence microscopy combined with microfluidics to study four different E. coli strains that possess either a normal oriC or only an ectopic copy (oriZ) (inserted 344 kb in the E. coli genetic map along the chromosome), and have either wildtype Tus present or knockout (Δ tus). In the oriZ strain, the clockwise replication fork (labelled via its sliding clamps) encounters the Tus-ter complex (via its nascent replication fork) earlier than the counter-clockwise fork does. On tracking the progression of the replication forks in oriZ strain, we find that the presence of the Tus-ter complex reduces the rate of replication. By monitoring the fluorescence intensity of the fork as well as the duplication of a chromosomal locus, we can determine that a replication fork can nonetheless bypass the non-permissive side of a Tus-ter complex. The oriZ Δ tus strain exhibits replication dynamics similar to that of oriC strain, whereas the chromosome dynamics differ substantially where the oriZ locus moves mostly towards cell-pole.

343-Pos Board B123
Extremophile DNA Photolyses: DNA Repair under Extreme Conditions
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Adaptation to extreme environments is a seminal characteristic of life on Earth. Nowadays is this property more strongly evident than in single-celled organisms. Bacteria, archaea, and eukaryotes all have representatives that thrive in cold or hot environments. Many of these extremophile enzymes share significant amino acid sequence homology in spite of very different optimal growth temperatures (TG). Based on a simple amino acid sequence analysis it is unclear what adaptive changes are required to tailor homologous enzymes to function in extreme environments. A case in point is DNA photolase (PL), a monomeric flavoprotein that binds UV-damaged DNA and repairs it by blue light activated picosecond electron transfer from a conserved flavin adenine dinucleotide cofactor (FADH2) to the tightly bound cyclobutylpyrimidine dimer DNA lesion. These enzymes also interact with second light-harvesting cofactor. Here we present a comparative analysis of three recombinant CPD photolyses, hyperthermophilic Sulfolobus solfataricus (TG=353K, rSpPL), mesophilic E. coli (TG=310K, rEcPL), and psychrophilic Colwellia psychrerythraea (TG=281K, rCpPL). All PLs utilize FADH2 bound in a highly conserved site for repair. In addition, each PL demonstrates different properties for its 2nd cofactor. We used a variety of biochemical, physical, and molecular biological tools to compare these extremophile enzymes with regard to repair yield, cofactor reduction potential and excited state properties. The difference in the stability of the redox state of the purified protein suggests different structural adaptations of each PL to their respective thermal environments. Denaturation studies reveal that rSpPL has a very stable structure, whereas rCpPL is extremely sensitive to its thermal and aqueous environments. A comparison of cofactor absorption and emission spectra reveal significant differences in how the flavin cofactor is bound in the binding pocket, in spite of significant amino acid sequence homology for this catty across all proteins.

344-Pos Board B124
Structure and Nano-Mechanics of DNA during the Initial Stages of Methyl-Directed Mismatch Repair
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DNA undergoing replication is a mechanically complex system on which mismatch repair machinery must not only identify errors on a concurrently replicating DNA molecule but also signal the information along the crowded DNA molecule to excision / re-synthesis elements. In the methyl-directed (dam/mutH-dependent) mismatch repair pathway in E. coli, if errors in newly-replicated DNA are detected by MutS, MutH will nick transiently hemi-methylated d(GATC) sites on the daughter strand in response to activating signals from MutS and MutL to initiate repair. The nearest d(GATC) may be over 1000 base-pairs away from the error, and the mechanics of how MutH-activating signals are transmitted along DNA remain poorly understood– most models focus on efforts by MutS/MutL complexes to contact latent MutH waiting on the DNA. Using a suite of single-molecule tools (atomic force microscopy, single molecule force spectroscopy, and tethered particle motion), we directly investigate the structure, forces, and nano-mechanical behavior of individual DNA undergoing the initial stages of repair. To understand how d(GATC) sites are identified after a replication error is found, we observe and bio-historically characterize the looping of individual DNA molecules by MutS and the structure of MutS/MutL/MutH complexes on DNA, and record both the mechanical strength of interactions between complexes of MutS, MutL, and MutH and with DNA and the resulting structure of the DNA molecule. Potential application and extension of these biophysical techniques to MutH-independent and mammalian mismatch repair systems will also be discussed.