Anisotropy-based assay of RT mobility on a didoexy-terminated Template/Primer (T/P) substrate, single-molecule fluorescence measurements of RT shuttling kinetics, and molecular dynamics simulations. We find that effi- enz, an NNRTI, does not significantly alter RT-T/P binding affinity but sub- stantially increases RT mobility on the T/P substrate, reducing the time spent in the polymerase-competent position. Furthermore, we show that the drug re- sistance K103N mutation in RT does not affect efavirenz binding affinity, but instead eliminates the increased shuttling we typically observe upon NNRTI addition. Taken together with our experiments probing the effect of the cog- nate dNTP on these dynamics, we provide compelling evidence that NNRTIs act in part by loosening the thumb and fingers clamp of RT on its T/P sub- strate, permitting highly dynamic T/P translocation and/or dissociation, and that NNRTI-resistant mutations in RT likely confer a structural resistance to this dynamic change.

2161-Pos Board B180
Single Molecule Analysis of Thermus Thermophilus SSB Protein Dynamics on Single-Stranded DNA
Jichuan Zhang, Ruobo Zhou, Taejik Ha.
University of Illinois at Urbana-Champaign, Urbana, IL, USA.
Single-stranded (ss) DNA, generated as a transient intermediate during various cellular procedures, is bound with and protected by single-stranded DNA bind- ing (SSB) proteins. Previous studies on Escherichia coli (Eco) SSB, a representa- tive homotrimeric SSB, revealed that SSB proteins are highly dynamic on ssDNA. Its spontaneous migration behavior along ssDNA and the interconversion between its different binding modes on ssDNA were believed to help reg- ulate many DNA processes. Here we used single-molecule fluorescence resonance energy transfer (FRET) to study TtSSB, an SSB homologue originated from Thermus thermophilus (Tt) (in collaboration with Professor Tsutomu Mikawa), an extremely thermophilic bacterium species. TtSSB protein is a homodimer that contains four DNA bind- ing motifs called OB folds. We obtained multiple lines of evidence that TtSSB proteins also diffuse on ssDNA, similar to EcoSSB. By migrating on ssDNA, TtSSB protein could transiently melt short DNA hairpins, similar to EcoSSB. Additionally, our data indicated that TtSSB proteins have multiple binding modes dependent on salt conditions. Low salt concentrations favored smaller binding site size and cooperative binding behavior of SSB proteins on ssDNA. Fluctuations between multiple binding modes were observed under certain salt conditions. Single molecule analysis also revealed a novel binding configuration which might be an intermediate state during the interconversion between two main binding modes.

2162-Pos Board B181
A Single-Molecule View of the Functional Helicase-Primase Complex of the Bacteriophage T4
Wonbue Lee
University of Oregon, Eugene, OR, USA.
We have studied the assembly mechanisms and DNA unwinding activity of the bacteriophage T4 helicase-primase (primosome) complex using single molecule Fluorescence Resonance Energy Transfer (sm-FRET) techniques. These experiments employed surface-immobilized DNA model replication forks labeled in the duplex region with donor/acceptor (Cy3/Cy5) chromo- phore pairs, and the time-dependent SM-FRET signal was monitored during the DNA unwinding process. We used these approaches to investigate the subunit stoichiometry of the primosome and the assembly pathway required to form a functional and fully active primosome-DNA complex. Our results confirm that the gp41 hexameric helicase binds only weakly to the DNA fork junction, but that the addition of a single subunit of gp61 primase sta- bilizes the primosome complex at the fork junction, resulting in the forma- tion of a fully active helicase with a gp41:gp61 subunit stoichiometry of 6:1. The functional primosome complex exhibited enhanced [GTP]- dependent processive activity, which was reflected in an increase in the number of sm-FRET conversion events. Moreover, we showed that the use of other assembly pathways resulted in incorrect subunit stoichiome- tries, the formation of metastable DNA-protein aggregates and a loss of helicase functionality. These single-molecule studies support the results of recent ensemble experiments and provide a direct ‘real time’ visualization of the assembly pathway and unwinding activity of the functional T4 primo- some complex.

2163-Pos Board B182
Probing Conformational Changes and Dynamics in eIF4A Helicase during RNA Unwinding by Single-Molecule FRET
Yingjie Sun, Amit Meller.
Boston University, Boston, MA, USA.
The translation initiation factor eIF4A is the prototypical DEAD-box RNA helicase and it has a “dumbbell” structure consisting of two domains connected by a linker. Our previous studies have shown that eIF4A/eIF4H complex can bind directly to loop structures and repetitively unwind the RNA hairpin. To further illuminate the conformation change of eIF4A during RNA unwinding, we label several well chosen mutant eIF4A with donor and acceptor and encap- sulate eIF4A and RNA substrate within lipid vesicles. The capping of eIF4A and RNA is detected by multicolor colocalization and the interdomain and in- tradomain distance change of eIF4A is investigated by single-molecule Fluo- rescence Resonance Energy Transfer (sm-FRET). First we show that the linker which connects the two domain is very flexible by showing broad donor-acceptor distance distribution. Second we demonstrate that eIF4A in the presence of eIF4H can repetitively unwind the RNA hairpin substrate by undergoing the open-and-closed conformation using the energy from ATP hy- drolysis. And the interdomain interaction is mainly responsible for the helicase activity. Furthermore, we studied how RNA aptamer and small molecule, hip- puristanol, interact with eIF4A and change its conformation. These studies can further illuminate the molecular mechanism how they inhibit the helicase activity of eIF4A.

2164-Pos Board B183
Sequence Specific pausing of RecQ Helicase
Yaze Sun1, Junghoon I1, Yeonee Seol1, Susanta Sarkar1, Marie-Paule Strub1, Kata Sarlós2, Mihály Kovács2, Keir Neuman1.
1National Institutes of Health, Bethesda, MD, USA, 2Éötvös University, Budapest, Hungary.
Escherichia coli RecQ helicase is a Super-Family 2 helicase that plays an es- sential role in the maintenance of genome stability via its participation in the repair and homologous recombination of DNA. The strand separation (unwinding) mechanism of RecQ has not been well characterized to date. Here, we report the study of RecQ unwinding using magnetic tweezers and focus on understanding the sequence specific pausing behavior observed in both dsDNA unwinding and ssDNA translocation. We compare the unwinding and pausing behavior of RecQ on different DNA sequences and in different pulling and unwinding geometries, which allow us to examine how the key unwinding properties (i.e., unwinding rate, processivity, pause location, pause duration, and their distributions) depend on DNA sequence, DNA geometry, and applied tension on the DNA. DNA unwinding by wild-type RecQ helicase is interrupted by strong sequence-dependent pauses. Pausing is significantly reduced for thrombin-cleaved RecQ and truncation constructs lacking the HRDC domain, and for point mutants that disrupt the single-stranded binding affinity of the HRDC domain. We propose a model for the sequence depend- ent pausing by RecQ and speculate on the in vivo ramifications of this behavior.

2165-Pos Board B184
Analyzing Unique Residues of E. Coli Dead-Box Protein DbpA via Molecular Dynamics Simulation
Vincent I. Park1, Yuan Zhang2, Jung-Chi Liao2.
1Great Neck South High School, Great Neck, NY, USA, 2Columbia University, New York, NY, USA.
Dead-box proteins are RNA helicases ubiquitous in RNA metabolism. E.coli DbpA is a bacterial DEAD-box protein activated by 23S rRNA, a special and unique task important for its functions in ribosome biogenesis. However, the mechanism of the coupling between RNA binding and activities at the ATP binding site is unknown. In this study, we compared the seed alignments of the DEAD-box protein family and DbpA to determine 11 key unique residues specific to DbpA. To analyze the impact of key unique residues, each unique residue was computationally mutated and modeled. In total, 12 molecular dy- namics (MD) simulations, including the one for the wild-type structure of DbpA and 11 for the single-mutation models, were conducted for 5 ns each. Re- sults of each mutant model were compared to those of the wild-type to observe structural changes caused by the mutation of each unique residue. Model compari- sons from 3 of the 11 unique residues, i.e. V170A, A333G, and V29I,
revealed observable and quantifiable conformational changes at the ATP and RNA binding sites. Trajectories of minimum distances among residues, and model-to-model ratios of RMSF were used as parameters to quantify the structural effects of the mutations. Atomic interactions propagated from the mutational residues to both ATP and RNA binding sites were observed due to the changes of side chains of these mutations. We hypothesize these three residues play structural roles in the unique functions of DpbA in RNA interactions. Future studies targeting key unique residues may reveal connected allosteric pathways within the protein structure and assist studying the diversity of function among DEAD-box proteins.

DNA and RNA Structure II

2168-Pos Board B187
Catch-Slip Transition in DNA Duplex
Tung T. Le, Harold D. Kim. Georgia Institute of Technology, Atlanta, GA, USA.
DNA experiences a wide range of mechanical stress in cells. Structural transitions of DNA under mechanical stresses have thus been a subject of intensive study. We recently used a single-molecule FRET assay to study sequence-dependent looping of double-stranded DNA (dsDNA). In this method, a 100-200 bp long dsDNA is terminated with single-stranded overhangs (~10-bp) that are complementary to each other. When DNA is completely unzipped or unwound, these two sticky ends can base pair and stabilize the DNA in the looped state. The stiffness of the DNA then exerts a restoring force on the duplex and induces unzipping. We found an unexpected relationship between the loop size and the unzipping rate: the duplex formed between the sticky ends ruptured more slowly with decreasing loop size. This phenomenon is reminiscent of a catch bond which becomes stronger under tension. We also observed a transition from a catch bond-like to a normal slip bond-like behavior for an 8-bp duplex. We present a catch mechanism based on the coupling between stretching and twisting of dsDNA to explain this counterintuitive phenomenon.

2169-Pos Board B188
Unfolding and Targeting Thermodynamics of DNA Stem-Loop Motifs
Irine Khutshivilli, Iztok Prislan, Hui-Ting Lee, Cynthia Lee, Luis A. Marكي.
University of Nebraska Medical Center, Omaha, NE, USA.
The repair action of the protein is initiated by a supposedly un-specific helical filament formation of its monomers around single stranded DNA overhangs at double strand breaks. We study the conditions that regulate the structure and thus the biological activity of this presynaptic filament. Now we report results of pressure tuning fluorescence spectroscopy (Schay et al. 2006 JBC, 281, 25972) concerning the strength of interaction at the monomer-monomer interfaces in the filament structures and results of electron microscopy concerning their topology and structural parameters. The fluorescence signal is based on the dye ANS that we show being bound near the monomer interfaces. The measurements yield new data concerning the role of ATP, Mg, and Ca as cofactors of the presynaptic filament formation and show the unique effect of bound K-ions significantly influencing both the stability and the topology of the presynaptic as well as of the self-aggregate filaments.

Acknowledgements: the authors are grateful to Prof. Kovács of EÖtvös University for help and valuable advices in electron microscopy. The work was supported by the Hungarian grant OTKA K 84271.

2170-Pos Board B189
Conformational Switching of Human G-Quadruplexed DNA with Ligand Binding under Molecular Crowding Conditions
Yasemin Kopkalli, Aaron Hope, Lesley Davenport.
Brooklyn College of CUNY, Brooklyn, NY, USA.
Formation of quadruplexed DNA (qDNA) within telomeric DNA has the potential to effectively inhibit the activity of telomerase, a key enzyme in tumorigenesis. The effects of molecular crowding (MC) within the cell can affect the folding of qDNA and modulate telomerase activity. Furthermore, MC can affect the ability of ligand binding to qDNA in promotion of the folded quadruplex conformation. Thus it is important that quadruplex-ligand binding be evaluated under more physiologically relevant conditions. We have examined the effects of MC on the association of N-methyl mesoporphyrin IX (NMM), a ligand which selectively binds to G-quadruplexed DNA (qDNA) and forms a hybrid conformation, whereas the HT8 sequence forms two

2166-Pos Board B185
Structural Stability of Rad51 Filaments of Self-Aggregates and of Presynaptic Complexes Studied by Electron Microscopy and Pressure Tuning Fluorescence Spectroscopy
Judit Fidy1, Melinda Fekete1, Guszti Schay1, Jozsef Kardos2.
1Semmelweis University Budapest, Budapest, Hungary, 2Eötvös Loránd University, Budapest, Hungary.
The maintenance and stability of the genetic material of cells is of vital significance. This task is fulfilled by complex mechanisms involving the associates of specific proteins that through several steps recognize and repair DNA damages. In this work we focus our attention on an early step in the process of homologous recombination of human cells that is related to the function of the protein Rad51. The repair action of the protein is initiated by a supposedly un-specific helical filament formation of its monomers around single stranded DNA overhangs at double strand breaks. We study the conditions that regulate the structure and thus the biological activity of this "presynaptic" filament. Now we report results of pressure tuning fluorescence spectroscopy (Schay et al. 2006 JBC, 281, 25972) concerning the strength of interaction at the monomer-monomer interfaces in the filament structures and results of electron microscopy concerning their topology and structural parameters. The fluorescence signal is based on the dye ANS that we show being bound near the monomer interfaces. The measurements yield new data concerning the role of ATP, Mg, and Ca as cofactors of the presynaptic filament formation and show the unique effect of bound K-ions significantly influencing both the stability and the topology of the presynaptic as well as of the self-aggregate filaments.

Acknowledgements: the authors are grateful to Prof. Kovács of EÖtvös University for help and valuable advices in electron microscopy. The work was supported by the Hungarian grant OTKA K 84271.