Protein-Nucleic Acid Interaction II

1256-Pos Board B166
HU Induced Perturbation to the Structure and Dynamics of a Flexible DNA Substrate
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The structure and dynamics of the bacterial chromosome are maintained by many proteins. One of the most abundant proteins, a member of the DNABII family of DNA-binding proteins, is HU (Heat_Unstable). HU, an architectural protein introduces sharp bends of 120-160 degrees into DNA upon binding. The DNA bending induced by these proteins is important for transcriptional regulation, initiation of replication, Mu transposition, base excision repair, recombination, and negative supercoiling in bacteria. HU exhibits a strong preference for distorted or kinked DNA and binds with high affinity to nicks, gaps, cruciform, and sticky ends. Little structural or dynamic information is available regarding the perturbation of these DNA motifs upon HU binding; although they all contain a flexible junction. Increased understanding of HU binding to these motifs would illuminate the nature of HU regulation of DNA structure. This information also gives insight into the general mode of recognition and binding for all non-sequence-specific DNA binding proteins found in both prokaryotes and eukaryotes. We have employed several fluorescence spectroscopic techniques to address the nature of the HU-DNA binding interaction. Using fluorescence anisotropy measurements, we have determined the dissociation constant in solution for HU binding to 3'overhang DNA substrate to be 3.41E-09+/-1.2E-09 M, which is in good agreement with literature results. The stoichiometry for the specific binding of HU to these substrates is one to one, while non-specific binding leads to higher order complexes. Preliminary fluorescence resonance energy transfer (FRET) results suggest bending of the DNA substrate upon HU binding. Further information about the mechanism of HU-induced bending, structural recognition and binding is obtained through the use of FRET mapping, time-resolved fluorescence spectroscopy, and stopped-flow kinetic methods.

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Archaeal DNA Organization: The Mechanism of Alba I & II Revealed
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Throughout the kingdoms of life cells face a similar problem, namely the size of their genome is very large compared to the volume of the cell. Although each organism employs its own set of proteins to compact their genome, up to a factor of 10.000, the method of compaction seems highly conserved. Besides, DNA organization is known to be a key regulatory mechanism involved in many important processes such as gene regulation and DNA replication. The protein Alba, one of the most abundant proteins in Archaea, has been suggested to play an important role in DNA organization. Previous studies have shown that Alba binds as a dimer to non-specific DNA sequences and is able to condense DNA. However, little is known about its mechanism and structural role in DNA-organization. Here we show, using several single-molecule imaging and manipulation techniques such as AFM, double and quadruple optical tweezers, the condensing and possible regulation mechanism of Alba. From the surprising structural changes to the protein-bound DNA the binding constant, footprint and cooperativity factor are obtained by using a McGhee von Hippel analysis. We furthermore prove that the Archaea protein Alba II, which is thought to have a regulatory function, controls the binding efficiency of Alba I by changing the cooperativity of the Alba & Alba II protein mix.

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Molecular Dynamics Models of Two Proposed Protein Structures of Salmon Protamine
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Protamine is an arginine rich 32 residue DNA binding protein. It is hypothesized that protamine allows DNA to be densely packed in the later stages of spermogenesis, although no experimental structures of DNA-bound protamine have been solved. We present models of two possible bound structures for salmon protamine to DNA. The first structure has an extended conformation within the major groove (s1) and a second structure modelled with an alpha-helix between the 19th and 23rd residues (s2). These protamine structures were modeled into a 40mer of double stranded B-form DNA and simulated by molecular dynamics with explicit water using the Amber99SB force field. The structure of protamine bound DNA did not significantly differ from a control simulation of DNA. The positively charged protamine displaced sodium counter ions from the DNA backbone decreasing the density of sodium around the nucleotide. The arginines additionally displaced the water molecules within 20 Å from the phosphates on the DNA backbone. Calculated binding energies for the s1 and s2 protamine:DNA complexes were −680.6 kJ/mol and −692.7 kJ/mol, respectively. The protamine displacing the water and counter ions could allow DNA packing with greater density, while retaining a near-B-form DNA structure.

1259-Pos Board B169
The 186 Bacteriophage Repressor as a Model System to Study Nucleosome Repositioning by Single Molecule Microscopy
Haowei Wang, Ian Dodd, Keith Shearwin, David Dunlap, Laura Finzi.
Using AFM imaging, we showed that the 186 bacteriophage repressor, 186 CI, aggregates into a disc-shaped heptamer. Few specific binding sites (operators) for the repressor on the phage's DNA mediate its interaction with the disc. Nucleoprotein complexes were detected where DNA is wrapped around or looped by the repressor disc. Also, operator flanking sequences with different affinity for the protein were found. The striking resemblance in shape and size with the nucleosome and the sequence affinity range suggests that the 186 transcriptional regulatory system could serve as a simplified model to study nucleosome dynamics and repositioning. Besides AFM imaging of wt complexes, dynamic tethered particle microscopy (TPM) and various combinations of operator mutations have been used to characterize the equilibrium between the fully vs. partially wrapped and looped DNA-186 CI complexes.

1260-Pos Board B170
Unfolding of Non-Telomeric G-Quadruplexes of Varying Stabilities by Replication Protein A
Sujay Ray, Mohammad Haroon Qureshi, Abby L. Sewell, Soumitra Basu, **Hamza Balci**.
We studied the stability of various G-quadruplex (GQ) structures formed by sub-sections of a highly heterogeneous and guanine rich 45 nucleotide long DNA sequence, called TH-12 and located in the promoter region of tyrosine hydroxylase (TH) gene, using single-molecule FRET and conventional biochemistry methods. TH-12 has seven regions that contain two or more consecutive guanines capable of forming various GQs of potentially different stabilities. TH-12 is within a conserved region of TH promoter, is in the immediate vicinity of transcription start site, and is invariably included for maintaining the promoter activity. This work serves as the first step in identifying possible roles that these GQs, with potentially different stabilities, play in regulating TH expression. Abnormality in TH gene expression is associated with a variety of psychiatric problems, including schizophrenia, depression and bipolar disorder and is also linked to Parkinson's disease. We measured the UV-melting temperatures of these GQ structures formed by sub-sections of TH12 and established that some of these GQs are stable against Watson-Crick base pairing, in the presence of a complementary strand, at physiological potassium concentrations. Finally, we studied the viability of these structures against the DNA binding activity of human Replication Protein A (RPA), the most abundant single strand DNA binding protein in eukaryotes. Most GQs were unfolded at RPA concentrations that correlated with their melting temperatures, while a GQ formed by a certain sub-section of TH-12 remained folded even at micromolar RPA concentrations. We propose that this subsection of the DNA sequence is the most likely candidate for forming a physiologically relevant GQ that can be involved in TH regulation. These studies also demonstrate that it is possible to have stable enough GQs that can not be unfolded by RPA activity at physiological ionic strength.

1261-Pos Board B171
Biophysical Study of the G Quadruplex Formed by FMRP mRNA and of its Interactions with the FMRP RGG Box
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Fragile X syndrome, the most common form of inherited mental retardation in humans, affects about 1 of 3000 males and 1 of 5000 females. It is caused by the loss of expression of the fragile X mental retardation protein (FMRP), due to a CGG trinucleotide repeat expansion in the 5'-untranslated region (UTR) of the fragile x mental retardation-1 (fmr1) gene. FMRP has been shown by biophysical methods to use its arginine-glycine-glycine (RGG) box RNA binding domain to bind with high affinity and specificity to G quadruplex forming mRNA sequences. The binding of FMRP to a proposed G quadruplex structure in the coding region of its own mRNA (100 nucleotide fragment named FBS) has been proposed to affect mRNA splicing events for isoforms 1 through 3. In this study we truncated the original 100 nt FMRP mRNA region rich in guanines to 67 nt and used biophysical methods such as UV thermal denaturation, CD spectroscopy, and 1H-NMR spectroscopy to directly demonstrate its folding into a G-quadruplex structure. We have also analyzed the binding properties of the FMRP RGG box domain in the context of different protein isoforms to this truncated FMRP FBS mRNA.