further investigate the local nature of these bends, AFM images of HMO1-DNA complexes are imaged to probe the behavior of these complexes as a function of protein concentration. The results show that at lower concentrations, HMO1 preferentially binds to the ends of the double helix and links separate DNA strands. At higher concentrations HMO1 induces formation of a complex network that reorganizes DNA. Although nucleoid associated proteins are under intense investigation, little is known about HMO1. Our studies suggest that HMO1 proteins may facilitate interactions between multiple DNA molecules.

2156-Pos Board B175

Rationally Designed Coiled-Coil DNA Looping Peptides Control DNA Topology

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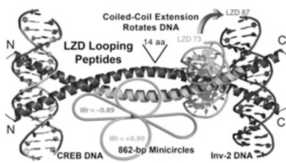
Artificial DNA looping peptides have been engineered to study the roles of protein and DNA flexibility in controlling the geometry and stability of protein-mediated DNA loops. The LZD (Leucine Zipper Dual-binding) peptides were derived by fusing a second basic DNA binding region onto the C-terminus of GCN4-bZip, inspired by Hollenbeck and Oakley. Two variants with different coiled-coil lengths were designed to control the orientations of DNA bound at each end. EMSA verified binding of two DNAs to one peptide. Ring closure experiments demonstrated that looping requires 310 bp, much longer than the length needed for natural loops. Variation of DNA binding site separation over a series of constructs that all cyclize to form 862 bp minicircles yielded positive and negative supercoils from two possible writhed geometries anchored by loops. Periodic variation in topoisomer distributions was modeled using canonical values for DNA persistence length and torsional modulus. These results suggest that the LZD proteins are much less flexible than natural looping proteins and that short DNA loops require protein flexibility. Small, stable, and tunable looping peptides may act as synthetic transcriptional regulators, bridges for targeting recombination, and components of protein-DNA nanostructures.



2157-Pos Board B176

Bringing PKR Monomers together: DSRNA-Induced Activation across an Inert Barrier

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Protein Kinase R (PKR) is a key component of the interferon-induced immune pathway and is activated upon binding to viral dsRNA via sequential dimerization and autophosphorylation. PKR contain a C-terminal kinase domain that acts as the catalytic center and two tandem dsRNA binding motifs. The minimal perfect duplex RNA required to activate PKR is approximately 30 bp, but 15 bp is sufficient for the binding of one PKR monomer. PKR is also activated by certain RNAs that contain internal loops, bulges and tertiary structure elements. However, the rules that distinguish RNA activators from those that fail to activate are not yet understood. We have developed a model molecular ruler to directly measure the RNA length requirements for PKR dimerization and activation across a barrier. The ruler consists of two duplex 15 bp regions separated by a variable length region of 2'-O-methyl modified RNA that acts as a rigid, inert barrier. Control experiments demonstrate that PKR does not bind to 2'-O-methyl dsRNA. Two PKR monomers bind to the ruler, independent of the length of the barrier from 15 to 5 bp. However, activation is favored at shorter barrier lengths and PKR binding affinity is enhanced as the barrier length is reduced. We propose that PKR kinase domains are able to interact across this barrier by extension of the unstructured linker lying between the kinase domain and the dsRNA binding motifs.

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Hydrational Control of ETS-Family Transcription Factors: A Possible Resolution of the "Specificity Conundrum"

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The ETS family of transcription factors is widely distributed among the metazoan phyla and regulates the expression of a wide range of genes. Despite their functional diversity, all ETS proteins share a structurally conserved DNA-binding (or ETS) domain. Given the highly overlapping sequence preferences among ETS members, it is as yet unclear how ETS proteins achieve functional specificity, a problem known as the "specificity conundrum." Compounding this problem is a current lack of understanding of the biophysical mechanism of sequence selectivity among ETS binding sites. We hypothesize that the structural conservation among ETS domains disguises physicochemical heterogeneity in their mechanisms of sequence recognition. We have previously demonstrated that the ETS-family member PU.1 (Spi-1) recruits a cooperative network of water-mediated contacts along the protein-DNA interface for high-affinity binding. We have now compared the thermodynamics and kinetics of sequence recognition between the ETS domains of PU.1 and ETS1 which represent extremes of sequence divergence (~30% homology) in the ETS family. We found that the thermodynamics and kinetics between the two structurally conserved ETS domains are highly differentiated under physiological conditions. More precisely, whereas high-affinity PU.1 ETS-DNA binding is enthalpically driven against an entropic penalty, ETS1 ETS-DNA binding is entropically driven. Kinetically, whereas ETS1 ETS associates rapidly with a high-affinity cognate site ($k_a > 10^7\ M^{-1}\ s^{-1}$), PU.1 ETS is strikingly slow ($k_a \sim 10^4\ M^{-1}\ s^{-1}$). This profound difference in association rate constants means that the high-affinity PU.1 ETS-DNA complex, despite being somewhat thermodynamically less stable than the corresponding complex with ETS1, is significantly longer-lived. If these differences that underlie the intrinsic heterogeneity in site recognition by ETS proteins extend to protein-protein and domain-domain interactions, they offer one potential biophysical resolution to the specificity conundrum.

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Kinetic Mechanism for Viral DSRNA Length Discrimination by MDA5 Filaments

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The viral sensor MDA5 has a characteristic ability to discriminate dsRNA length and it enables immune system to recognize long viral dsRNAs in the range of ~0.5 to 7 kb. Previous study showed that MDA5 forms filament along dsRNA and disassemble upon ATP, but it is insufficient to explain its length specificity as the intrinsic affinity of MDA5 for dsRNA only moderately depends on dsRNA length. We observed the details of filament formation and ATP induced disassembling procedure with single molecule fluorescence imaging and found that MDA5 utilizes a combination of end-disassembly and slow nucleation kinetics to rapidly "discard" short dsRNA and suppress rebinding through single molecule fluorescence experiments. In contrast, filaments on long dsRNA cycle between partial end-disassembly and elongation, by passing slow nucleation steps. Longer and more stable filament is generated through this dynamic rebuilding cycle to repair filament discontinuities, which are often present due to multiple, internal nucleation events. As the length of the continuous filament determines the stability of the MDA5:dsRNA interaction, the mechanism proposed here provides an explanation for how MDA5 utilizes filament assembly and disassembly dynamics to discriminate between self vs. non-self dsRNA.

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Measurements of the Functional Dynamics of Wild Type and K103N HIV-1 Reverse Transcriptase Reveal the Mechanism of Efavirenz Resistance

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HIV-1 reverse transcriptase (RT) is a primary target for drug development since it converts viral RNA into double stranded DNA that is subsequently integrated into the human genome. Nonnucleoside RT Inhibitors (NNRTIs) are routinely included in many first-line combination antiretroviral therapies and are also used to prevent mother-to-child transmission of HIV-1. Of note, the mechanisms by which NNRTI inhibit HIV-1 RT remain unclear despite the wealth of available structural and biochemical data. Furthermore, the structural mechanisms by which mutations in HIV-1 RT confer NNRTI resistance are also inadequately understood. To elucidate these mechanisms, we combined several complementary tools that included a novel fluorescence