Looping efficiency depends on the length of the intervening sequence with preference for a 15 nucleotides spacer or longer between the pyrimidine-tracts. RRMs 3 and 4 bind the 5′ and the 3′ pyrimidine-tracts, respectively, in a specific directionality, and work synergistically for efficient RNA looping in vivo.

386-Pos

Single-Molecule Imaging of DNA Curtains Reveals Intrinsic Energy Landscapes For Nucleosome Deposition

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We employ nanofabricated diffusion barriers to organize DNA into molecular curtains allowing us to directly image thousands of aligned molecules and determine coarse-grained intrinsic energy landscapes for nucleosome deposition on model DNA substrates. Our results reveal distributions that are correlated with recent in silico predictions, reinforcing the hypothesis that DNA contains some intrinsic positioning information. We show that cis-regulatory sequences in human DNA coincide with peaks in the intrinsic landscape, whereas valleys correspond to non-regulatory regions, and we present evidence arguing that nucleosome deposition in vertebrates is influenced by factors not accounted for by current theory. We also demonstrate that intrinsic landscapes of nucleosomes containing the centromere-specific variant CenH3 are correlated with patterns observed for canonical nucleosomes, arguing that CenH3 does not alter sequence preferences of centromeric nucleosomes. However, the non-histone protein Scm3 alters the intrinsic landscape of CenH3-containing nucleosomes, enabling them to overcome the otherwise exclusionary effects of poly(dA-dT) tracts, which are enriched in centromeric DNA. In addition, these methods establish a platform that allows direct visualization of DNA binding proteins, DNA translocases and chromatin remodelers as they interact with single fluorescent nucleosomes and denser chromatin arrays.

387-Pos

Fluctuating Forces Facilitate Protein-Mediated DNA Looping

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Lac repressor-mediated DNA looping has become a paradigm for long-range genetic regulation. Our earlier experimental results have shown that forces on the order of a hundred femtonewtons can drastically disrupt the formation of DNA loops. This exquisitely high sensitivity to applied force implies that tension in the DNA, two orders of magnitude smaller than typical piconewton intracellular forces, may provide a mechanical pathway for transcriptional control.

We investigate how such mechanical switching is affected by fluctuating forces instead of static forces inside a cell. Our results show that by slightly increasing the magnitude of the fluctuations, which are on the order of tens of femtonewtons, the DNA loop formation rate can be significantly increased while the magnitude of the average tension in the DNA remains the same. This result contributes to our understanding of how protein-mediated DNA looping processes, which are extremely sensitive to force, can function in a noisy *in vivo* environment.

To study the effects of force fluctuations on DNA looping, a random series of optical forces displaying the statistics of Gaussian white noise is applied to a surface-tethered DNA molecule by axial optical tweezers. The lifetimes of the looped and unlooped states are measured under fluctuating forces that have the same average magnitude but different fluctuation strengths. Our results show that, as compared to the noise free case, the lifetime of the unlooped state decreases by about a factor of two when fluctuations on the scale of tens of femtonewtons are applied while the lifetime of the looped state remains constant.

388-Pos

On the Structure, Function and Metalloregulatory Properties of the Zinc-Activated Repressor *Streptococcus Pneumoniae* AdcR

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A response to a change in transition metal ion concentration is mediated by metal-sensing transcriptional regulators that harbor metal-specific coordination sites. Zinc homeostasis in the gram positive human pathogen *Streptococcus pneumoniae* (*Spn*) is maintained by two novel zinc-regulated repressors, SczA and AdcR. *Spn* AdcR is the first putative metal-dependent member of the MarR family of transcriptional repressors. Expression profiling on BHI media under microaerobic conditions with a wild-type and isogenic Δ*adcR* strain reveals that AdcR regulates the expression of genes encoding the high affinity zinc uptake system *adcRCBA*, a group of zinc-binding pneumococcal histidine triad proteins (PhtA, PhtB, PhtD and PhtE) and an orphan AdcA homologue (AdcAII). Much of the *adcR* regulon is necessary for the virulence of *Spn*. Analytical ultracentrifugation experiments reveal that AdcR is a 32 kDa homodimer. X-ray absorption spectroscopy is consistent with a primary five-coordinate N/O regulatory site, a finding unprecedented for a zinc-sensing metalloregulatory protein. As expected, Zn(II) binding strongly activates *adc* operator DNA binding on the basis of quantitative fluorescence anisotropy assays (pH 6.0, 0.2 M NaCl, 25°C). Nearly complete backbone (H_N, ^{15}N, $^{13}C\alpha$, $^{13}C\beta$) resonance assignments of apo-AdcR (pH 6.0, 0.05 M NaCl, 35°C) reveal a highly α-helical two-fold symmetric homodimer, and that zinc binding perturbs resonances in the C-terminal regulatory domain, as well as the N-terminal winged helical DNA binding domain. Mutagenesis of at least two His in a highly conserved histidine-rich sequence in the regulatory domain (His108, His112), significantly modulates zinc regulation in vitro and in vivo. Progress on the solution structure and residue-specific dynamics of AdcR in the apo- and zinc activated states will be reported. Supported by NIH grants GM042569 (to D.P.G.), F32 AI084445 (to F.E.J.), GM042025 (to R.A.S.) and AI060744 (to M.E.M).

389-Pos

DNA Structure Specificity of Bacillus Stearothermophilus PcrA

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Helicases are molecular motors that play critical roles in nucleic acid transactions, including replication, repair, recombination, and transcription. As observed in a number of diseases resulting from mutations in helicase genes, helicases are important for maintenance of cellular functions. A feature of these diseases is an increase in malignancies as a result of genome instability resulting from unregulated DNA recombination. Gram-positive bacteria harbor a conserved helicase, PcrA, which is involved in UV-damage DNA repair, plasmid rolling-circle replication, and regulation of DNA recombination. PcrA has been shown to inhibit RecA-mediated DNA strand exchange reaction and displace RecA from the DNA. Homologs of PcrA, including Rep and UvrD helicases, have been shown to be 3′ to 5′ helicases. However, PcrA homologs from *Staphylococcus aureus* (*S. au.*), *Bacillus anthracis*and *Streptococcus pneumoniae*also exhibit 5′ to 3′ helicase activity. In these studies, we have explored the directionality and DNA structure specificity of *Bacillus stearothermophilus* (*B. st.*) PcrA. We have demonstrated that *B. st.* PcrA does not have 5′ to 3′ directionality on standard partially duplex DNA substrates containing a 5′ oligo dT tail. However, similar to *S. au.*PcrA, *B. st.* PcrA unwound DNA substrates with a hairpin structure found at the dsDNA replication origin in the rolling-circle replication plasmid pT181 with high efficiency. These included substrates with only a 5′ single-stranded region. These results indicate that though the *S. au.* and *B. st.* PcrAs are 60% identical, they have different activities. Our future work will explore the amino acid sequence differences in these helicases that lead to their differential biochemical activities.

390-Pos

Single Molecule Studies on Hcv RNA Polymerase Activity

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NS5B is an RNA-dependent RNA polymerase capable of initiating RNA synthesis de novo. However, the detailed underlying mechanism remains elusive. It is unclear how the enzyme locates the 3′-terminus of the RNA template. Previous studies suggested that the nucleic acid binding channel of NS5B accommodates approximately 10 residues of a single stranded RNA. Although the contacts between the polymerase and its nucleic acid substrate are maximized, the 3′-end of the primer is not properly positioned under these conditions and such complexes are therefore unproductive. Hence, it is conceivable that the NS5B-RNA interaction is highly dynamic. Of note, nonnucleoside inhibitors of NS5B were shown to inhibit formation of a competent complex. To address this problem, we have conducted single molecule FRET (SM-FRET) experiments. This approach allowed us to obtain a direct visualization of both the positioning and dynamics of NS5B in complex with its RNA template. We performed our experiments on single -donor (Cy3)/acceptor (Cy5) fluorophore labeled- RNA substrates, which were surface-immobilized to enable long observation times. Binding of NS5B caused a significant increase in FRET. SM-FRET studies on RNA-protein complexes revealed protein sliding dynamics occurring in the millisecond time scale. These dynamics change with the RNA template length, and with the presence of complementary DNA strands that restrict the motion of NS5B. A nonnucleoside inhibitor is observed to compromise binding of NS5B to the template. Taken together, our single molecule studies provide direct evidence for the ability of NS5B to slide along its RNA template. Sliding of NS5B provides a plausible mechanism that facilitates

formation of a productive complex. Conversely, interference with these dynamics provides a possible mechanism by which nonnucleoside analogue inhibitors of NS5B block de novo initiation of RNA synthesis.

391-Pos

Simulating the Relaxation of DNA Supercoils By Topoisomerase I

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Many cellular processes involving DNA, including replication and transcription, result in significant superhelical stresses. During transcription, for example, RNA polymerase locally untwists about a helical turn of the DNA double helix. Then to elongate the RNA transcript, it proceeds along the template strand of the DNA and thereby induces supercoiling. DNA topoisomerases play an important role in relieving these stresses. Here we focus on understanding the action of human DNA topoisomerase I (Topo1) which operates in three basic steps: (i) cleaving a single strand of the DNA double helix, (ii) allowing the DNA superhelical stresses to relax, and (iii) religating the DNA. Recently, the Dekker lab, at Delft University of Technology, performed single molecule experiments to probe the relaxation of supercoils by topo1. A significant molecular dynamics (MD) effort (>100 cpu years) by the Andricioaei lab, at the University of California Irvine, characterized the energetics and topological changes of topo1 in complex with only a short fragment of DNA (~20 bp). Including a longer length of DNA to represent a biologically relevant length-scale (greater than a persistence length), is computationally prohibitive for MD and was necessarily neglected. Here we introduce an elasto-dynamic rod model as a first approximation to provide a dynamic description of the DNA as it relaxes. The rod model describes bending and torsion of the DNA helical axis, electrostatic and self-contact interactions, and approximates the hydrodynamic drag on the molecule. For our simulations, we provide as initial conditions, a plectomemic supercoil. The MD simulations serve to provide boundary conditions to the rod model by characterizing the torque applied to the DNA by topo1 as it rotates. Here we present preliminary results for the relaxation rates of supercoils.

392-Pos

Nucleoprotein Complex Formation By *Bacillus Subtilis* Spo0J/ParB

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Although prokaryotes lack the machinery utilized by eukaryotes to achieve well organized chromosome segregation, all cells must faithfully segregate their chromosomes in every cell division cycle. In many bacteria, this process is dependent upon a partitioning locus composed of an ATPase called ParA, a DNA binding protein called ParB, and centromere-like binding sites (*parS*) that are present adjacent to the origin of replication. ParB binds the *parS*, sites and the ParA ATPase acts on the ParB-*parS* complex to facilitate segregation of replicated origins toward opposite cell poles. In *Bacillus subtilis*, the ParA protein is called Soj and the ParB protein is referred to as Spo0J. There are eight *parS* sites surrounding the origin of replication of the *B. subtilis* chromosome. Spo0J can bind to these sites and spread along the DNA up to 15 kilobases, forming a nucleoprotein complex. The Spo0J-*parS* complexes are not only a substrate for Soj/ParB, but they also serve to recruit the highly conserved structural maintenance of chromosomes (SMC) complex to the origin. The SMC condensin complex appears to function in both organizing the origin region and facilitating chromosome segregation. To investigate how Spo0J spreads along the DNA and ultimately how this complex recruits SMC, we have employed single molecule fluorescence imaging to directly observe the formation of the nucleoprotein complex of purified *B. subtilis* Spo0J and lambda DNA. We characterize the physical properties of this nucleoprotein complex and the kinetics of its formation.

393-Pos

Theoretical Analysis of the Molecular Mechanism of Stabilization of Nova-RNA Complex System: Fragment Molecular Orbital Method Based Quantum Chemical Calculation For the Effect of the Complex Formation on the Electronic State of Biomacromolecular System

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The RNA-binding proteins (RBPs) specifically recognize the shape and/or sequence of RNA molecules for binding the target. The relationship between three-dimensional structure and RNA-binding mechanism of RBPs can be analyzed by performing computer simulations to provide deeper insight into this issue. In the present study, we performed Fragment Molecular Orbital (FMO) based quantum chemical calculations for neuro-oncological ventral antigen third KH domain (NOVA)-RNA complex system to study the molecular mechanism from the viewpoint of electronic state of biomacromolecules. We investigated the effect of the complex formation on an electronic state of NOVA. We found and that the charge redistributes all over the structure and that the secondary structure of NOVA is remarkably associated with the change of electronic state in the complex formation. The results indicate that the whole protein structure participates in realization of the best energetic stabilization in the complex formation and we speculate that secondary structure could play an important role to obtain the optimum inter-molecular interaction energy by associating with charge redistribution. Further, we employ molecular dynamics simulation method to consider structures fluctuating around the equilibrium state. We perform FMO calculations for the obtained snapshots and examine the change of electronic state. The results will provide deeper insight into the relationship between electronic state and structural fluctuation. The details will be reported at the meeting.

394-Pos

Investigating Classic Lac Repressor-Dna Looping Experiments Using a Computational Rod Model

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Protein mediated DNA looping is a well known gene regulatory mechanism. A commonly studied system that controls gene expression is lactose repressor (LacI) induced DNA looping. In two in vitro studies, the Müller-Hill group investigates how the lac repressor protein in E. coli forms loops with linear and cyclized DNA. Their experiments analyzed LacI induced looping on linear DNA over a wide range of interoperator lengths (6-21 helical turns) and on supercoiled DNA minicircles of 452 base pairs. In these experiments, electron microscopy, non-denaturing polyacrylamide gel elecrophoresis, and DNase I protection experiments were used to detect loop formation, estimate loop size, quantify loop stability, and for supercoiled DNA to detect loop topology (ΔLk). In our study, we exercise our computational rod model to make side-by-side comparisons of our predictions with their experimental observations. By making comparisons, we look to understand the energetic cost of loop formation and the resulting topology of the looped complex.

395-Pos

High Throughput Screening of Aptamers For Human Thrombin and Factor IXa

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The traditional method for discovery of DNA/RNA aptamers is *in vitro* evolution (SELEX), where multiple cycles of partitioning and amplification enrich aptamer candidates from a pool containing randomized segments of length, m. Previous to our work the consensus sequence for alpha-thrombin aptamers (ThbA) was found via five rounds of SELEX in a DNA pool with m=60, while factor IXa aptamers (FIXaA) were discovered in an RNA library of m=40 after 8 rounds. We isolated the same ThbA, FIXaA and a novel carbohydrate aptamer (CA) to validate our new method, High Throughput Screening of Aptamers. HTSA uses a single partitioning step, PCR, and counts survivors by massively parallel sequencing. We found the minimal ThbA in a library of DNA hairpins loops (m=15) containing 56,000 copies of each of the 1.1 billion possible sequences. We distinguished two sequence motifs well above the background. The ThbA motif contains the consensus (counted 46,000 times) and dozens of related sequences. The leading candidate in the CA family (29,000 counts) is a novel aptamer that binds glucose (Kd=1,400 nM) and alpha-methyl mannoside (Kd=500 nM). A known FIXaA was counted 52,000 times from a library of RNA hairpins (m=16 with 14,000 copies of each sequence). HTSA simplifies and shortens the discovery process, exhaustively searches the space of sequences within a library, simplifies characterization of the core binding domain, reduces the quantity of the target required, eliminates cycling artifacts, allows multiplexing of targets, and requires no complex automation.

396-Pos

Engineered Holliday Junctions As Single-Molecule Reporters For Protein-Dna Interactions

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Protein-DNA interactions are essential for gene replication and expression. Characterizing how proteins interact with and change the structure of DNA