2010-Pos A Biophysical Characterization Of The Mechanism Of The DExH Helicase Nph-II

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NPH-II is an essential RNA helicase involved in early transcriptional termination of Vaccinia virus. NPH-II tracks along the backbone of an RNA substrate in a processive, unidirectional 3′ → 5′ manner and efficiently strips away bound material from the tracking strand. Although a detailed kinetic framework for unwinding has been elucidated, a rigorous physical mechanistic understanding of NPH-II's helicase activity is still lacking. In particular the exact meaning of the "kinetic step size" obtained through traditional helicase unwinding assays remains ambiguous. Here we report the results of a modified approach to that recently developed by Serebrov and Pyle (*Nature* 2004, **430**, 476–480) that allows us to obtain site specific unwinding kinetics of product release and each translocation event. Implications for stepping behavior, initiation, translocation, duplex destabilization and product release are discussed.

2011-Pos Translocation of the NS3 Helicase from Hepatitis C Virus

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The NS3 helicase, which is essential for Hepatitis C viral replication, is a member of the DExH proteins that constitute a subgroup of the Superfamily 2 helicases. Proteins from this superfamily are involved in all aspects of RNA metabolism. The NS3 helicase is a processive molecular motor that can unwind both RNA and DNA duplexes. It does so by utilizing the energy of NTP hydrolysis to translocate along its nucleic acid substrate. Our previous single molecule FRET study was designed to observe the relative motion of nucleic acid strands during unwinding have shown how DNA duplex separation is achieved in regular 3bp steps within larger periodic top strand release steps[1]. However, the motion of NS3 relative to its substrate remains unclear. Using site specific fluorescence labeling of the NS3 protein we have been able to monitor NS3 translocation directly in bulk and at the single molecule level on DNA and RNA to better understand the motion of NS3 relative to its tracking strand as well as the top strand. By correlating the motion of NS3 relative to its substrate with strand separation, our understanding of the overall mechanism of unwinding has become clearer.

References

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Protein-Nucleic Acid Interactions - II

2012-Pos The ATPase Cycle Mechanism Of The DEAD-box rRNA Helicase Dbpa

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DEAD-box RNA helicases ATPase enzymes that couple the free energy of ATP binding, hydrolysis and product release to the unwinding of duplex RNA. However, limited quantitative information regarding the rate and equilibrium constants defining the ATPase cycle of RNA helicases is available, including the distribution and flux of populated biochemical intermediates, the catalytic step that limits the enzymatic reaction cycle and how ATP utilization and RNA interactions are linked. We present in this study a complete, quantitative kinetic and equilibrium characterization of the rRNA-activated ATPase cycle mechanism of DbpA, a DEAD-box rRNA helicase implicated in ribosome biogenesis. rRNA activates the ATPase activity of DbpA by promoting ATP hydrolysis. ATP hydrolysis by DbpA-rRNA occurs via a gamma phosphate attack mechanism and is readily reversible. The rRNA-activated steady-state ATPase cycle of DbpA is limited *both* by ATP hydrolysis (or a step preceding) and P_i release, which occur with comparable rates. Consequently, the predominantly populated biochemical states during steady-state cycling are the ATP- and ADP-P_i-bound intermediates. The ADP-bound states are favored under physiological nucleotide concentrations. The steady-state parameters (k_{cat}, $K_{m,RNA}$, $K_{m,ATP}$) predicted from the rate constants are comparable to the experimentally determined values. ATP or ADP and rRNA binding are weakly coupled. In contrast, ADP-P_i and RNA binding display strong thermodynamic coupling which causes DbpA-ADP-P_i to bind rRNA with $>$ 10-fold higher affinity than in the absence of nucleotide, suggesting that DbpA undergoes significant conformational change. The data are consistent with unwinding being coupled to strong rRNA binding. The quantitative knowledge of the DEAD-box helicase ATPase cycle is critical for developing mechanistic models of ATP utilization and helicase activity, and will help develop testable hypotheses regarding the cellular functions of these molecular motor enzymes.

2013-Pos Comparative Kinetic Studies on Three Forms of TATA-binding Protein Reveal the Nature of the Structural and Energetic Changes Associated with DNA Binding

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The kinetics of fluorescently double-labeled DNA binding to yeast full-length TATA Binding Protein (yTBP), to the C-terminal core domain of yTBP (cTBP), and to full-length human TBP (hTBP) were studied as a function of temperature and protein concentration. The N-terminal domains of yTBP (60 amino acids) and hTBP (159 amino acids) have a low level of homology. The C-terminal domains (CTD) of yTBP and hTBP (80% homologous) are virtually identical throughout the DNA binding domain. In all three proteins, initial DNA binding is nearly the same (k_1), even though the NTD of yeast appears to occupy part of the DNA binding region. The kinetics require at least four states of the TBP_three corresponding to different conformers of TBP-DNA. Movement of the NTD in yTBP (see accompanying abstract) occurs in forming the first intermediate, I1, and is associated with a high E_a not found in either hTBP or cTBP. Constancy of k_1 requires a compensating large increase in the entropy of activation in yTBP for the conformational change. Common to all TBP forms is the high E_a associated with conversion of the second intermediate (I2) to the "final" state. The large enthalpic barrier for this transition must derive from the C-terminal domain, presumably involving motion at the hinge separating the two subdomains of the CTD. The pattern of step-wise energetic and entropic changes are remarkably similar in cTBP and hTBP, despite the very large NTD in the latter; for both of these species there are large differences from yTBP, showing that the NTD's in yTBP and hTBP modulate the kinetics and structural changes in quite different ways.

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2014-Pos Energetics of a Full-Length, Human Transcription Factor Binding to a Complex, Multi-Site Promoter

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Board B129

Transcription factor-promoter assembly is a key aspect of transcriptional activation, yet the mechanisms are largely undetermined. To understand the physical origins by which these proteins bind DNA we are rigorously studying full-length human progesterone receptor (PR) and its ability to assemble at multi-site promoters. Classical models of PR function state that the receptor binds DNA at palindromic progesterone response elements (PRE) located in promoter regions of genes. Upon binding, PR recruits coactivating proteins and activates transcription. Using a synthetic promoter containing two palindromic PREs, we have previously shown that the A-isoform of PR (PR-A) binds a promoter thermodynamically either as a monomer or as a preformed dimer with a mild amount of cooperativity between PREs. Additionally, cooperativity between PREs is key in PR-As ability to recruit coactivating proteins. However, as noted, these results were obtained with a synthetic promoter. To address the mechanisms of PR binding at a natural promoter, we have performed a similar dissection on the naturally occurring mouse mammary tumor virus (MMTV) promoter. Quantitative footprint titrations and statistical thermodynamics shows PR-A binds to the one palindromic PRE and three half-sites of the MMTV promoter with a large amount of cooperativity. In fact, resolution of the binding energetics reveals multiple cooperative interactions between half-sites and between the half-sites and the full-PRE. Furthermore, we found monomers, as opposed to dimers, bind at each of the three half-sites. These findings are in contrast to previous semi-quantitative work showing PR bound to the MMTV promoter with no cooperativity and dimers bound to each half-site. This work represents the first quantitative study on a natural promoter with a full-length nuclear receptor, thus providing the wherewithal to study human PR regulated promoters.

2015-Pos How Local Stiffness And Intrinsic Curvature Of DNA Can Modulate DNA-Protein Interactions

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Board B130

Mechanical deformation of DNA plays an essential role in modulating the activity of the genetic code. Especially the formation of DNA loops is a recurring motif in biology. We are concentrating on the refinement of experimental techniques to measure how the mechanical properties of DNA affect and define the kinetics of any DNA-protein interaction in the presence of external and intrinsic forces.

To this end, we present single-molecule data on the role of sequence-depend local stiffness and intrinsic bend of DNA on binding kinetics of transcription factors. Our model system is the bacterial lactose repressor (LacR from E. coli). It acts by inducing a loop in the substrate DNA, which in turn prevents transcription. We describe experimental results on the loop-formation kinetics of several different DNA substrates, which only differ in the intrinsic bend of the loop-forming region and compare them to predictions derived from numerical simulations which will be detailed in another poster at this conference.

2016-Pos Unfolding Upon Binding: Elucidation Of The Complex Binding Dynamics Of The Ets-1 Transcription Factor By Computer Simulations

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Board B131

The human Ets-1 transcription factor plays an important role in developmental processes and cancer. The protein is folded in solution, and unfolds the HI1 inhibitory helix upon binding its target sequence. To elucidate the interactions responsible for this

unusual behavior, we performed large scale molecular dynamics simulations of the protein with and without inhibitory regions, in solution and bound to high affinity DNA. Analyses of the variance-covariance matrices of protein fluctuations showed that the motion between the HI1 and H4 helices, and the motion between HI1 and HI2 helices are correlated in solution, and anti-correlated in the DNA-bound state. These motions demonstrate that in solution H1 is stabilized by a persistent macrodipolar interaction with H4 and sustained hydrophobic packing against H2, while in the DNA-bound state, these stabilizing interactions are disrupted. Our analyses identified the interactions responsible for these disruptions.

2017-Pos Restriction Endonuclease EcoRV-DNA Binding Measured By Self-Cleavage Assay

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Board B132

The novel self-cleavage assay we developed utilizing osmotic stress is broadly applicable to measuring DNA-protein interactions. We have become interested in type II restriction enzyme, EcoRV. Usually restriction endonucleases can distinguish between cognate and nonspecific DNA sequences quite efficiently in the absence of divalent cofactor that is required for cleavage. There are, however, many conflicting results in literature regarding ability of the EcoRV to distinguish between specific and nonspecific DNA sequences in the absence of divalent ions. One group only has demonstrated significant specificity. The majority of researchers do not see meaningful preferential binding, typically less than a 10-fold difference between the recognition sequence and nonspecific DNA in the absence of divalent metal ions. The x-ray structures for specific and non-cognate DNA-EcoRV complexes are, however, substantially different suggesting it is probable that EcoRV specific and nonspecific binding free energies also differ substantially. We have applied the self-cleavage assay to measure solution binding. This technique does not have the limitations of more commonly used assays as gel mobility shift, filter binding, and anisotropy of fluorescently labeled complexes. Our preliminary results are promising and indicate significant EcoRV binding specificity in the absence of divalent ions. We have also uncovered an unusual slow transition between specific binding modes that may account for the discrepancies seen in the literature. We do observe a strong dependence of the relative binding constant of EcoRV on osmotic pressure as would be expected from the x-ray structures.

2018-Pos Direct Observation of the Rates of Conformational Changes and DNA Bending in TATA Binding Protein upon Binding DNA

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Board B133

We have previously shown that four states describe the kinetics of TATAAAAG ($AdMLP_{dpx}$) binding to full length yeast TATA binding protein (yTBP), three of which describe the TBP-$AdMLP_{dpx}$ complexes. S. Khrapunov and M. Brenowitz have shown that the single Trp in the N-terminal domain (NTD) is less exposed in yTBP than in the yTBP-$AdMLP_{dpx}$ ensemble of complexes and is differently positioned with respect to the C-terminal domain (CTD) before and after binding $AdMLP_{dpx}$. Using rapid mixing and gated excitation, we have found that the first intermediate, I1, has the Trp emission more red-shifted than either the thermodynamically most stable conformer ("final" complex) or the initial TBP form. I1 then proceeds toward the equilibrium distribution of I1, I2, and "final". Kinetics tracking Trp fluorescence agree with the kinetics from FRET changes in the fluorescently labeled free and bound $AdMLP_{dpx}$. We acquired nsec fluorescence lifetime data in 6 second windows during the binding of a double-labeled fluorescent oligonucleotide to TBP (PicoQuant TCSPC). Analysis of these data allowed assignment of bend angles to the $AdMLP_{dpx}$ in the various conformational states. Movement of the NTD is coincident with formation of the first intermediate, I1, resulting in a bend angle in the $AdMLP_{dpx}$ that was some 10 degrees greater in I1 than that of the "final" complex. I1 thus represents a distinct observable third state of the NTD and of the DNA during the binding process. Owing to the low concentration of I2 (< 0.1 mole fraction), it was not possible to characterize either the bend angle of the $AdMLP_{dpx}$ or the Trp fluorescence of that conformer.

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2019-Pos Binding Studies between CUGBP1ab and (GTCT) Oligonucleotide Repeats

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CUGBP1/CELF (CUGBP1 and ETR-3 like factors) is a multifunctional RNA binding protein implicated to play a role in the genetic disease of Myotonic Dystrophy type 1 (DM1). Functional analysis suggests CUGBP1 may regulate the alternative splicing of certain mRNAs. It appears to have a broad specificity toward single stranded nucleic acids. Here, we report the characterization of binding interactions between CUGBP1ab; comprising of the RNA-Binding Domain (RBD) 1 and 2, and a group of single stranded DNA repeats using Isothermal Titration Calorimetry (ITC), fluorescence spectroscopy, and sizing chromatography. We used "GTCT" repeats as our model system because of its high specificity and vast *in vivo* applications. We have extensively measured the binding thermodynamics between CUGBP1ab and

(GTCT) n (n is the number of repeats). Our results demonstrate the complex with an average unit binding energies of −11kcal/mole Enthalpy (favorable), −25 cal/mol/K entropy (unfavorable), −8 Kcal/mol free energy and heat capacity changes close to zero to slightly positive. Stoichiometry was shown to be about one protein per GTCT repeat for n5. We have also performed fluorescence experiments to show the DNA binding induced quenching of intrinsic fluorescence of CUGBP1ab, which allowed us to validate the binding stoichiometry between the [(GTCT)n] and CUGBP1ab. We further carried out size exclusion chromatography analysis to characterize different bound complexes. Based on the results on the CUGBP1ab-oligonucleotide complexes we were able to derive a binding model of the interaction which suggests that there is a hierarchy order of binding of CUGBP1ab to the sites on a (GTCT)n. This model suggests that the trinucleotide-repeat-containing-polynucleotide CUGBP1 interaction is optimized for maximal sequestration of CUGBP1 *in vivo*.

2020-Pos Molecular Electrostatic Properties of Human Telomeric Proteins TRF1 and TRF2

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Board B135

Telomeres are nucleoprotein complexes that protect the ends of linear eukaryotic chromosomes from degradation and fusion. Human telomeric DNA contains tandem arrays of double stranded TTAGGG repeats. This telomeric DNA forms specific complexes with many different proteins among which TRF1 and TRF2 are the most essential for maintenance of telomeres function. In particular, TRF1 is a negative regulator of telomere length, and TRF2 is involved in formation of a telomeric higher order structures (t-loops), and its function is more related to capping the DNA end. Both proteins bind to DNA as preformed homodimers. Although, cellular function of these proteins is different, their structure is similar. Both of them contain two conserved sequence motives which form specific domains, namely homodimerisation and Myb-DNA binding domain.

In order to reveal the molecular differences between TRF1 and TRF2 a more detail analysis of their molecular properties is necessary. Therefore, in our work we have carried out different theoretical chemistry calculations of both proteins and their complexes with DNA. Models of studied systems were based on X-ray structures of TRF1 and TRF2 Myb-DNA binding domains.

Both systems used in calculations contain the same sequence of DNA and include explicit structural water molecules [2]. In particular, molecular electrostatic potential for proteins and double stranded DNA was calculated and analyzed in terms of complementarities. Moreover, estimation of free energy of protein-DNA was calculated using PBSA methods in a similar way as for other TEBP telomeric protein from Oxytricha [2]. Obtained results were analyzed to characterize and compare molecular, particularly electrostatic, properties of Myb-DNA binding domains of both proteins.

References

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2021-Pos A Magnetic Tweezers Apparatus for Concurrent Force and Fluorescence Measurements of Protein-DNA Interactions

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Board B136

We have developed a magnetic tweezers device to perform concurrent force and fluorescence measurements of protein-DNA interactions. The design allows extension of a DNA molecule in the focal plane of an inverted microscope objective. This configuration exposes the full length of the DNA allowing direct length measurement as well as direct visual observation of protein-DNA interaction dynamics via fluorescence microscopy techniques.

2022-Pos The Effect of Femtonewton Forces on Protein-mediated DNA Looping

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Board B137

Inside a living cell, DNA is subject to a complex micromechanical environment which can give rise to mechanical tension in the DNA. To investigate the effect of such tension on the formation of regulatory DNA-protein complexes, we studied protein-mediated DNA loop formation and breakdown rates in an in-vitro model based on the lactose repressor in E. Coli using axial optical tweezers.

A 1298 bp DNA construct that carries two lacR-binding operators with an inter-operator spacing of 305 bp is attached with its ends to a microscope cover glass and a microsphere to serve as a handle for the optical tweezers. The microsphere is trapped in the approximately linear region of the optical potential in the axial direction, where the optical force is almost independent of the axial position of the microsphere. Loop formation and breakdown is observed as an apparent change in the length of the stretched DNA. The tension in the DNA is controlled by varying the laser power in the optical tweezers and quantitatively determined from the extension of the DNA molecule using the wormlike chain model.

We observe that when the tension increased by about 100 fN, the loop formation rate increased about sevenfold, whereas the loop breakdown rate remained virtually unchanged. This result will be discussed in the context of a theoretical model for protein-mediated DNA looping under tension that allows us to infer loop topology

from the force sensitivity data. In conclusion, we demonstrate that forces as low as hundred femtonewtons can indeed have a dramatic impact on the formation of regulatory protein-DNA complexes, suggesting possible mechanical pathways to gene regulation.

2023-Pos The Impact Of Bending And Twisting Rigidity Of DNA On FokI Looping Dynamics

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Board B138

Many restriction enzymes cleave DNA by binding to multiple target sites, thereby forming a loop in the DNA. The bending and twisting rigidity of the DNA have a profound influence on the formation and stability of these loops. When a restriction enzyme has a preferred binding orientation looping dynamics will also be impacted by the imposed loop shape. We use a tethered particle assay to investigate the impact of the physical properties of DNA on the looping behavior of the asymmetric restriction enzyme FokI. In particular, by using DNA substrates with different spacing between the two asymmetric recognition sequences and by varying the orientation of these recognition sites, we sample how binding and loop formation is influenced. In the tethered particle motion experiments a bead is attached to a single DNA molecule that is immobilized to a glass surface. The position of the DNA-tethered bead undergoing Brownian motion is detected via CCD image analysis. The dynamics of formation and destruction of protein induced loops are observed by a change in the Brownian behavior of the bead. We show that FokI in the presence of Mg^{2+} cleaves DNA containing two recognition sites within a few seconds but no cleaving takes place in a buffer containing solely Ca^{2+}. In the total absence of divalent metal ions FokI is unable to form any (nonspecific) stable loops. We observed specific dynamic looping of FokI in Ca^{2+} and measured the dependence of the looping dynamics on the separation and orientation of the two recognition sites. The loop formation times and loop lifetimes are extracted and compared using both a running average method and a hidden Markov analysis. The results are discussed in relation to the physical properties of DNA.

2024-Pos Observation Of Oligonucleotide DynamicsBy Means Of Fluorescent Nucleoside Analog 6mi

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Board B139

To better understand the structural mechanism of recognition of architectural DNA binding proteins, such as HU and IHF, we are investigating the structure and dynamics of different DNA substrates. We are able to observe the structure and dynamics of DNA at the single residue level using fluorescent nucleoside analogs. The fluorescent analogs used in this study minimally distort the structure and functions of the nucleic acids targets. Specifically, the fluorescent guanosine nucleoside analog 6-methylisoxanthopterin (6MI), which H-bonds with cytosine similar to guanosine, is used to probe the global and local DNA dynamics. We have previously shown that this class of probes does not significantly perturb the structures of duplex DNA molecules. The 6MI was systematically incorporated into a 34 base pair oligonucleotide. By varying the complementary oligonucleotides length we were able to examine the effect of the probe located in either duplex or single strand regions in relation to the 6MI excited state. We also examined the affect of nucleic acid nearest neighbors upon the excited state of 6MI. The fluorescence lifetimes and anisotropy decays of the duplex oligomers were compared to 6MI monomer and single stranded DNA. When 6MI is incorporated into an oligomer, 6MI populates at least two different states which exhibit different lifetimes. We have calculated lifetimes for each component: the fast component exhibits a lifetime of 0.4ns, the medium component lifetime of 4ns, and the long-lived component exhibits a 6.5 lifetime similar to the monomer. The amount of quenching seems to be dependent on the location of the probe in the 34mer oligonucleotide. Future work will examine how the heterodimeric DNA binding protein, HU, will affect the structure and dynamics of the overhang oligonucleotides.

2026-Pos Interactions of the DNA Polymerase X of African Swine Fever Virus With Double-Stranded DNA. Functional Structure of the Complex

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Board B141

Interactions of the polymerase X of African Swine Fever Virus with the double-stranded DNA have been studied with fluorescent dsDNA oligomers, using quantitative fluorescence titrations, analytical ultracentrifugation, and fluorescence energy transfer techniques. Studies with unmodified dsDNAs were performed, using competition titration method. The ASV pol X binds the dsDNA with a site-size of $n = 10 \pm 2$ base pairs, which is significantly shorter than the total site-size of 16 ± 2 nucleotides of the enzyme - ssDNA complex. The small site-size indicates that the enzyme binds the dsDNA exclusively using the proper DNA-binding subsite. Fluorescence energy transfer studies between the tryptophan residue, W92, and the acceptor, located at the 5′ or 3′ end of the dsDNA, strongly suggest that the proper DNA-binding subsite is located on the non-catalytic C-terminus domain. Moreover, intrinsic interactions with the dsDNA 10- or 20-mer are accompanied by the same net number of ions released, independent of the length of the DNA, indicating the same length of the DNA engaged in the complex. The dsDNA intrinsic affinity is $\sim$2 orders of magnitude higher than the ssDNA affinity, indicating that the proper DNA-binding subsite is, in fact, the specific dsDNA-binding site. Surprisingly, the ASFV pol X binds the dsDNA with significant positive cooperativity, which results from protein - protein interactions. Cooperative interactions

are accompanied by the net ion release, with anions participating the ion exchange process.

2027-Pos Multiple Global Conformational States of the Hexameric RepA Helicase of Plasmid RSF1010 With Different ssDNA-Binding Capabilities Are Induced By Different Numbers of Bound Nucleotides

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Board B142

Global conformational transitions of the hexameric RepA helicase of plasmid RSF1010, induced by the nucleoside tri- and di-phosphate binding, have been examined using analytical ultracentrifugation and dynamic light scattering techniques. The global structure of the RepA hexamer in solution, modeled as an oblate ellipsoid of revolution, is very different from its crystal structure, with the axial ratio of the ellipsoid being ∼4.5 as compared to only ∼2.4 of the crystal structure. The large axial ratio and the experimentally determined partial specific volume strongly suggest that, in solution, the diameter of the cross-channel of the hexamer is larger than ∼17Å seen in the crystal. The global conformation of the helicase is modulated by a specific number of bound nucleotides. The enzyme exists in at least four conformational states, occurring sequentially as a function of the number of bound cofactors. These conformational states are different for ADP, as compared to AMP-PNP. Modulation of the global structure is separated into two phases, different for complexes with up to three bound nucleotides, from the effect observed at the saturating level of cofactors. This heterogeneity indicates different functional roles of the two modulation processes. Nucleotide control of helicase - ssDNA interactions occurs through affecting the enzyme structure and the ssDNA affinity *prior* to DNA binding. Only one conformational state of the helicase, with two AMP-PNP molecules bound, has dramatically higher ssDNA-affinities than the complexes with ADP. Moreover the same state also has an increased site-size of the enzyme - ssDNA complexes.

2028-Pos Combinatorial Control at Class III CytR Promoters

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Board B143

CRP and CytR mediate positive and negative control of the nine cistrons comprising the *E. coli* CytR regulon. Five Class III promoters share a common architecture in which tandem CRP sites flank an intervening CytR operator (CytO). CytR and CRP bind cooperatively to form a three-protein DNA-bound complex in which CytR represses CRP mediated transcriptional activation. Cytidine induces transcription when it binds to CytR by reducing CytR-CRP cooperativity. In addition, we have found that satellite CytR binding sites overlapping and occluding either or both the distal (CRP2) and proximal (CRP1) CRP sites is a general feature of these promoters. DNAse I footprinting has been used to generate individual site isotherms for each site of protein-DNA interaction. Global analysis of both wild type and reduced valence mutant promoters has allowed us to obtain the intrinsic affinities of each transcription factor for its respective sites and the specific patterns of cooperativity, competition and induction underlying the molecular interactions at each of these promoters. The results reveal major differences in the patterns of interaction. With respect to CRP binding, CRP2 fills first at *deoP2*, *udpP* and *nupG*, CRP1 fills first at *cdd* and only CRP1 fills at *tsx-p*$_2$. Patterns of induction differ, with both CRP2-CytR and CytR-CRP1 cooperative interactions eliminated in the presence of cytidine-liganded CytR at *deoP2*, whereas only CRP2-CytR and only CytR-CRP1 interactions are disrupted at *cddP* and *udpP,* respectively. Competition for CRP binding by CytR differs significantly between promoters, with respect to both the location and affinities of satellite sites. At the *cdd* promoter, CytR binds to the -10 region with sufficient affinity to occlude RNAP binding. In these ways, two transcription factors generate wide-ranging differences in molecular interactions underlying complex patterns of gene regulation.

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2029-Pos Structural Characterization Of CytR, A Bacterial Gene Repressor, Using NMR

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Board B144

CytR and CRP (cyclic-AMP receptor protein), mediate negative and positive control of the nine cistrons that comprise the E. coli CytR regulon. The promoters share a common architecture in which tandem CRP sites flank an intervening CytR operator. CytR and CRP bind cooperatively to form a three-protein DNA-bound complex in which CytR represses CRP mediated transcriptional activation. Cytidine induces transcription when it binds to CytR by reducing CytR-CRP cooperativity. CytR contains a unique DNA-binding feature; the protein dimer can bind to DNA sites comprised of palindromic recognition motifs that vary in proximity with spacing ranging from 0 to 9 basepairs. This spacer variation not only changes distance but also groove register of the recognition elements. These differences modulate the effect of cytidine binding on CytR-CRP cooperativity, hence on induction of transcription. Consequently, this protein's previously unexplored variations in specificity could further elucidate our understanding of protein-DNA interaction and transcriptional control. Nuclear magnetic resonance (NMR) spectroscopy of the DNA binding domain (DBD) provides both structure and dynamics information. From the structural analysis of CytR DBD alone and in the presence of DNA we are able to determine that the protein establishes one conformation upon

nucleic acid binding out of a previous equilibrium of multiple distinct forms. Comparison of the binding dynamics to different DNA sequences provides us with insight into the modes of specificity alterations arising from the structural conformations. This information allows us to describe a novel method of protein-DNA recognition and thus new pathways through which gene expression is regulated.

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2030-Pos Salt Dependence of the Affinity of the HIV-1 nucleocapsid protein for SL3 RNA

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Board B145

The nucleocapsid protein (NC) of HIV-1 plays critical roles in a number of viral processes, making it a target for anti-AIDS therapies. The NC domains of the *gag* and *gag-pol* precursor proteins bind to the major packaging determinant, stem-loop 3 (SL3), in the 5′-untranslated region of the viral RNA. The NC domains of the precursors and the mature NCp7 protein have two zinc fingers, with a single tryptophan (W37) in the second finger. W37 stacks on a guanine in the SL3 loop and in two other known NMR-based structures, quenching the tryptophan fluorescence. An earlier study from our lab determined the dependence of SL3-NCp7 stability on [NaCl], where SL3 was added to NCp7, and Kd determined from the decrease in W37 fluorescence [Shubsda et al. (2002) *Biochemistry 41*, 5276]. These "forward" titrations gave irreproducible results when [NaCl] $<$ 0.2 M but were well-behaved at 0.2 M NaCl or above, giving results consistent with 1:1 P:R (protein:RNA) complexes. Here we report "reverse" titrations where protein is added to RNA. These give reproducible values for Kd from 0.05 M $<$ [Na+] $<$ 0.8 M. The formal charges on SL3 RNA and NCp7 are -19 and +8, respectively. Thus, a complex with 2:1 P:R is almost electrically neutral and may have low solubility. Such complexes are favored in forward titrations where [P]$>>$[R] for early points, whereas complexes with more protein than RNA are not as likely for reverse titrations, which end at [P] = 3[R].

2031-Pos Observations of HIV-integrase in Complex with DNA

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Board B146

Integration of HIV-1 viral DNA into the host chromosomal DNA is accomplished by the viral integrase protein (IN). IN first cleaves two bases at the 3′ end (3′-processing) of the viral DNA made by reverse transcription. Next, integrase splices the viral DNA ends into chromosomal DNA. Although high-resolution structures of the individual domains of IN have been obtained, possible intermediate steps in binding of the two viral DNA ends to the protein and the structure of the active complex have yet to be determined. Complexes containing a pair of viral DNA ends bound to a tetramer of integrase can be assembled in vitro and these stable synaptic complexes (SSCs) are intermediates on the integration reaction pathway. While, in biochemical experiments, less than 20 base pairs appear to be involved in protein binding, much longer DNA seems to be required for the formation of SSCs. This may be pointing to intermediate complexes involving loci on the DNA molecules distal from their 3′-proccessed ends. We have used atomic force microscopy (AFM) to visualize SSCs and possible intermediates. IN complexes with two end-bound DNA molecules were abundantly observed in agreement with the biochemical data. Besides, one-DNA and rarely three-DNA-containing complexes were found. Bound protein volume estimates, and biochemical analysis, show IN tetramers and less likely dimers being the active units for complex formation. The complexes tended to aggregate with time after DNA binding suggesting that the protein undergoes conformational changes upon SSC assembly. We observe non-specific binding mostly of protein monomers and dimers at random positions along DNA chains but we do not observe IN-mediated bridging between DNA molecules. Therefore, if integrase protomers were bound to internal DNA regions during the assembly reaction, they must be more weakly associated and lost during sample preparation.

2032-Pos Direct Observation of DNA Untangling Magic by a Type-II Topoisomerase

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Board B147

Type-II topoisomerases(TopoII) are ubiquitous enzymes that play key roles in the maintenance of DNA topology in cells. They control the degree of supercoiling of DNA and untangle the catenanes that arise during replication or recombination. Lack of their activities during cell division ultimately causes cell death. TopoII untangles DNA catenanes in an ATP-dependent manner, by catalyzing the transport of one DNA segment to the other side of a second DNA segment through a transient double-stranded break in the second segment. The work of topoII would seem like that of a magician who fascinates the audience by solving a knot of rope without touching the knot. Here, we show movies of this unlinking magic taken in real time under an optical microscope.

2033-Pos New Insights into the Transition Pathway from Nonspecific to Specific Complex of DNA with Escherichia coli Integration Host Factor

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Board B148

Thermodynamics studies of the binding of a cognate DNA sequence to IHF, a prokaryotic DNA-bending protein, have revealed that, despite the severe distortion of the DNA, which is bent in a U-turn in the complex, the binding is enthalpically driven and entropically unfavored (Holbrook et al., *J. Mol. Biol.* 2001, *310*, 379). These results are in contrast to what is expected if the protein-DNA interactions are driven by the entropic release of ions associated with the free DNA and protein. To gain insight into the nature of the transition state ensemble separating the nonspecific and the specific complex, we have started a detailed investigation of the DNA bending/unbending kinetics, using time-resolved FRET on end-labeled DNA, in response to a ~10 ns laser T-jump perturbation of the IHF-DNA complex. We find that the activation enthalpy for the DNA bending rate (k_{bend}) is ~14 kcal/mol, in accord with a significant enthalpic penalty for kinking DNA. Furthermore, kinetics measurements at different [KCl] indicate that, for [KCl] < 300 mM, k_{bend} is independent of salt, while for [KCl] > 300 mM, k_{bend} decreases with increasing salt. To explain the positive activation enthalpy and the nonlinear salt-dependence of k_{bend}, we propose that in the uphill climb to the transition state ensemble, spontaneous bending/kinking of DNA is rate-limiting at low salt, whereas further bending of the DNA, accompanied by release of ions, becomes rate-limiting at high salt. Thus, the nature of the transition state ensemble depends on the ionic environment. Subsequent conformational changes in the protein that facilitate favorable interactions in the protein-DNA complex, and that contribute to the negative enthalpy change, must occur as the system leaves the transition state, downhill to the final complex.

2034-Pos Thermodynamic Characterization of E. coli Manganese Superoxide Dismutase Binding to Single- and Double-Stranded Polynucleic Acids

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Board B149

Bacterial manganese superoxide dismutase (MnSOD) has been shown to localize to the chromosomal portion of the cell and impart protection from ionizing radiation to DNA. The binding affinity of bacterial MnSOD to non-sequence specific double stranded oligomeric DNA has been quantitated previously by nitrocellulose filter binding and gel shift assays. We have examined the equilibrium binding of E. coli MnSOD containing tryptophan to poly(U), poly (A), poly(C), poly(dU) and double-stranded (ds) DNA. Equilibrium association constants, Kobs, measured by monitoring tryptophan fluorescence quenching, were examined as functions of monovalent salt (MX) concentration and type, as well as temperature, from which ΔG°obs and ΔH°obs were determined. The polynucleotides bind to MnSOD in the following affinity hierarchy, poly(dU)>poly (U)>dsDNA>poly(A)>poly(C). For each polynucleotide, Kobs decreases with increasing [K+]. For polyU, polyA and polyC the values of ΔHobs become less favorable with increasing [K+]; therefore, the salt concentration dependence of ΔG°obs has contributions from entropic and enthalpic origins such that δlogKobs/δ log[K+] is less negative than if it were a simple electrostatic binding event.

2035-Pos Interaction of the Adenoviral IVa2 Protein with the Viral DNA Packaging Sequence

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Board B150

Human adenovirus (Ad) is a non-enveloped dsDNA virus. Ad DNA packaging is the process whereby the Ad genome becomes encapsidated by the viral capsid. Specific packaging is dependent upon the packaging sequence (PS), which is composed of seven repeated elements, called A repeats, and is located near the left end of the viral genome. Two viral proteins, that have been shown to bind to synthetic DNA probes containing A repeats I and II (A-I-II), are also required for DNA packaging. These proteins are called IVa2 and L4-22kDa. Furthermore, in the absence of IVa2, L4-22kDa binding to the A-I-II probe is not detected by a gel-shift assay. These data suggest that both IVa2 and L4-22kDa proteins cooperatively interact with the PS to ensure specific recognition and packaging of the viral genome. In order to begin to define the molecular events that are responsible for initiating DNA packaging, the PS DNA binding properties of the IVa2 and L4-22kDa proteins must be studied quantitatively, in a well defined biochemical system. To this end, we have overexpressed the IVa2 protein in *E. coli*, purified the protein to homogeneity, and have begun to study its interaction with the PS DNA.

Interfacial Protein-Lipid Interactions

2036-Pos Membrane Binding Studies of the GM2 Activator Protein with Phosphatidylcholine Bilayers using EPR and Tryptophan Fluorescence

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Board B151

The GM2 Activator Protein (GM2AP) is a non-enzymatic accessory protein involved in the catabolism of ganglioside GM2. GM2AP is thought to bind GM2 in intralysosomal vesicles at acidic pH, thereby presenting the oligosaccharide head group for hydrolytic cleavage by β-Hexosamidase A (Hex A). A goal of our research is to determine the membrane bound orientation of GM2AP on phosphatidylcholine bilayer surfaces. We currently utilize both site-directed spin labeling (SDSL) electron paramagnetic resonance spectroscopy (EPR) and intrinsic tryptophan fluorescence to ac-