Lac repressor (LacI) controls transcription of the genes involved in lactose metabolism. A key role in LacI function is played by its ability to bind simultane-ously to two operators, forming a loop in the intervening DNA. Recently, several lines of evidence (both theoretical and experimental) have suggested the possibility for the LacI tetramer to adopt different structural conformations by flexing about its C-terminal tetramerization domain. At present it remains unclear to what extent different loop geometries are due to DNA binding top-ologies rather than distinct protein conformations. We address these questions by employing single molecule tethered particle motion on LacI mutants with intramolecular cross-linking at different positions along the cleft between the two dimers. Measurements on wild-type LacI reveal the existence of three dis-tinct levels of effective tether length, most likely due to the presence of two differ-ent DNA looped structures. Restricting conformational flexibility with protein by cross-linking induces clear changes in the tether length distributions, indicating profound effects of tetramer opening (and its limitation due to cross-linking) on the looping conformations available to the system. Our data suggest an important role for large-scale conformational changes of LacI in the looping structures and dynamics.

322-Pos  Board B201
The Enfolding Arms of EcoRI Endonuclease as Probed by ESR Experiments
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Our research focuses on adding general principles applicable to site-specific protein-DNA interactions by linking function to structural, thermodynamic and dynamical properties. We present evidence of a model the interaction of EcoRI endonuclease with specific, miscognate (EcoRI*), and nonspecific DNA sequences. The crystal structure of the specific complex shows that the EcoRI "arms," invisible (disordered) in the structure of the apoenzyme, enfold cognate DNA upon bind-ing. We are using four pulse Double Electron-Electron Resonance (DEER) FT-ESR experiments to map distances and distance distributions between nitrotoxide spin labels placed on cysteine-substituted residues in the two "arms" of the EcoRI homodimer, between Cu²⁺ ions bound near the active sites, and between nitro-xide to Cu²⁺ positions. Our data show that the mean point-to-point distances between the "outer arms", between the "inner arms" and from the "outer arm" to the main domain are the same in specific, EcoRI*, and nonspecific complexes. This implies that the EcoRI arms must enfold the DNA in all three classes of complexes. However, an increase in the breadth of distance distributions is ob-served for noncognate complexes relative to that observed for the tightly com-plementary specific complex. These results are consistent with inferences from our thermodynamic analyses that the equilibrium ensemble of conforma-tional microstates is larger for noncognate than specific complexes. Our contin-uouwave (CW) ESR experiments probing the dynamics of the arms support this hypothesis. Nonspecific complexes have been shown to have an important func-tion in accelerating the location of correct recognition sites. It is striking that the EcoRI arms also embrace the DNA in the sliding nonspecific complex.

323-Pos  Board B202
Nuclear RISC Originates from Cytoplasmic Loaded RISC in Human Cells
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Studies of RNA interference (RNAi) provide evidence that in addition to the well characterized cytoplasmic mechanisms, nuclear mechanisms also exist. The mechanism by which the nuclear RNA-induced silencing complex (RISC) is formed in mammalian cells, as well as the relationship between the RNA silencing pathways in nuclear and cytoplasmic compartments is still unknown. Here we show by applying fluorescence correlation and cross-corre-lation spectroscopy (FCCS) in vivo that two distinct RISC exist: a large ~3 MDA complex in the cytoplasm and a 20-fold smaller complex of ~150 kDa in the nucleus. We further show that nuclear RISC, consisting only of Ago2 and a short RNA, is loaded in the cytoplasm and imported into the nucleus. The loaded RISC accumulates in the nucleus depending on the presence of a target, based on an mRNA-like interaction with impaired cleavage of the cognate RNA. Together, these results suggest a new RISC shuttling mechanism between nucleus and cytoplasm ensuring concomitant gene regulation by small RNAs in both compartments.

324-Pos  Board B203
A Dna Mimic Caught In The Act: 3D Electron Microscopy Shows EcoKI Methyltransferase In Complex With The T7 Antirestriction Protein Ocr.
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Type I DNA restriction-modification (R/M) systems are important agents in lim-iting the transmission of mobile genetic elements responsible for spreading bac-terial resistance to antibiotics. EcoKI, a Type I R/M enzyme from Escherichia coli, acts by methylat-ion- and sequence-specific recognition, leading to either methylation of the DNA target or translocation, followed by cutting at a random site, often hundreds of base pairs away. Consisting of one specificity subunit, two DNA translocase/endonuclease subunits, EcoKI is inhibited by the T7 phage antirestriction protein Ocr. Ocr mimics DNA with a pseudo-helical arrangement of charges, and is bent at a similar angle to that pre-dicted for target DNA. We present a 3D density map generated by negative stain electron microscopy of the central core of the restriction complex, M.EcoKI MS21 methyltransferase, bound to dimeric Ocr. Single particle analysis was car-ried out in IMAGIC and EMAN and resulted in a 3D reconstruction at ~18 Å res-olution. An atomic model of all 5 subunits was generated by automated docking and homology modelling. This was computationally fitted into the EM density, giving excellent agreement. Ocr binds through the center of the M.EcoKI complex, spanning the two DNA recognition sites and matching the path predicted for its substrate DNA. We also present a complete atomic model of M.EcoKI in complex with its cognate DNA giving a clear picture of the overall clamp-like operation of the enzyme. The model is consistent with a large body of publis-hed experimental data on EcoKI spanning 40 years.

325-Pos  Board B204
Investigation of Dnmt1-DNA Interaction Using Fluorescence Fluctuation Spectroscopy
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DNA methyltransferase 1 (dnmt1) is an important factor in the epigenetic pro cess of DNA methylation. It is responsible for the regulation of tissue-specific pat terns of methylated cytosine residues. Pathological changes in these methyl ation patterns are connected with various diseases, for example certain types of cancer.
We investigated the functional nature of the interaction between dnmt1 and DNA. A construct was formed, consisting of a synthetic DNA strand, labeled with a synthetic fluorescent dye, and dnmt1, labeled with Green Fluorescent Protein (GFP).
To determine whether the functional form of dnmt1 is monomeric, dimeric or consists of even larger complexes, we measured the ratio of GFP to synthetic dye molecules using Fluorescence Fluotration methods such as Fluorescence Cross Correlation spectroscopy (FCCS), stoichiometry determination from a Burst Analysis experiment as well as Photon Counting Histograms (PCH) analysis.

326-Pos  Board B205
Thermodynamic Characteristics of pre-mRNA Splice Site Recognition
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Here, we reveal that an unusually large enthalpy-entropy compensation underlies recognition of polypyrimidine (Py) splice site signals. Competitive binding to Py tract splice site signals represents a prevalent means for alternative pre-mRNA splicing. The thermodynamic forces driving association of splicing factors with single-stranded (ss) pre-mRNAs represents a gap in the current under-standing of splice site selection. We compared Py tract interactions among three splicing factors: (1) U2AF55, an essential pre-mRNA splicing factor that recognizes constitutive 3' splice site signals; (2) Sex-Lethal, a prototypical alternative splicing factor that antagonizes U2AF65; and (3) TIA-1, an alternative splicing factor that promotes use of specific 3' splice sites. All three pro-teins bound polyuridine (U20) sequences with comparable or higher affinity than natural splice site sequences in fluorescence anisotropy assays. Consistent with the ability of Sex-Lethal to outcompete U2AF55 during splice site selec-tion, U2AF55 displayed the lowest and Sex-Lethal the highest affinities for the RNA sites. The enthalpic and entropic contributions were investigated in detail using ITC, initially using the homogeneous U20 site to avoid sequence-dependent compli-cations of the binding isothers. All three splicing factors exhibited an unusu-ally large enthalpy-entropy compensation underlying U20 binding, with magnitudes ~10-fold greater than those of typical protein-protein or protein-ligand complexes. Given that full thermodynamic characterizations of protein associ-ation with single-stranded RNAs are rare, this raised the question of the source of this unusual thermodynamic signature: Is a large enthalpy-entropy
compensation a general characteristic of ssRNA binding, an inherent property of Py tracts, or a signature of sequence-specific ssRNA recognition? These possibilities are clarified by thermodynamic comparison of purine-tract association by these Py tract splicing factors, contrasted with the purine-specific protein PAB.

327-Pos Board B206

The DNA Binding Protein H-NS and the SsrB Transcription Factor Counteract One Another to Silence and Activate Pathogenicity Island Genes in Salmonella

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The transcription factor SsrB activates transcription of genes located on Pathogenicity Island 2 (SPI-2) in Salmonella enterica. These gene products are responsible for forming a type-three secretion system that secretes effectors that facilitate invasion of the host cell. The gene is located outside of SPI-2 and encodes a product required for maintenance of the Salmonella-containing vacuole, providing an intracellular niche conducive to Salmonella replication and survival. Part of this process involves formation of Salmonella-induced filaments (Sifs). We set out to determine whether SsrB directly activates expression of the sifs4 gene, and whether H-NS could counter or prevent this interaction. In vitro transcription assays indicate that SsrB directly activates sifs4 transcription and this stimulation is prevented in the presence of H-NS. SsrB activation requires supercoiled templates; in the presence of linear DNA, no sifs4 transcripts are observed. Using atomic force microscopy, we show that H-NS forms multiple bridging complexes on supercoiled DNA. Additional experiments are underway to examine the effect of SsrB on these complexes. Supported by NIH GM-58746 and NSF MCB-0613014 to LJK and NUS R14400017112 to YJ.


328-Pos Board B207

TBP Carries Out Specific DNA Binding Involving Information From Both Grooves

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The TATA binding protein (TBP) is a basal transcription factor that binds specifically to the minor groove of TATA boxes. TBP is required for efficient recruitment of the transcription machinery, as it binds DNA, generating binding sites for TFIIH and RNA polymerase II. Only 10% of human protein coding genes possess a TATA box, and TBP is required for the transcription of the more common TATA-less genes also. We explored the energy contributions to TBP binding of a collection of 16 repeating DNA sequences, in a productive bent conformation. Binding energy is dominated by the cost of deforming DNA from the straight, B-DNA reference, to the conformation found in the complex. Interestingly, there is a positive correlation between the deformation energy of DNA and the interaction energy of DNA with TBP: poor interaction energies, and demonstrate that our method performs satisfactorily on protein models up to 5 Å from their native structures.

331-Pos Board B210

Understanding DNA- and RNA-binding Proteins Using Sequence and Structural Features

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The complex interactions between nucleic acid molecules and NA-binding proteins are an integral component of the gene regulation processes. Understanding which residues on these proteins bind nucleic acids is necessary in order to characterize these interactions. Due to the complicated nature of this problem, machine learning is often used to predict which residues are involved in the act of binding. The prediction of NA-binding residues can provide assistance in the functional annotation of NA-binding proteins. These predictions can also be used to expedite mutagenesis experiments for the study of NA-binding proteins, guiding researchers to the correct binding residues in these proteins. In this work we focus on three goals. First, we use SVM and various ensemble methods based on the C4.5 decision tree algorithm to predict DNA- and RNA-binding residues within proteins with high, balanced accuracy by analyzing sequence and structural characteristics. Second, we show that our classifiers can achieve similar results on several data sets which were used in previous works to identify DNA- and RNA-binding residues. Thirdly, we show that we are able to distinguish DNA-binding residues from RNA-binding residues using structure- and sequence-based features and sequence-based features only.