368-Pos Board B168
Novel Polarizable Empirical Code for Accurate Prediction of Protein-DNA Interactions
Carlos J. Camacho.
Traditional molecular dynamics techniques can compute free energy differences between well defined states, but currently they cannot consistently estimate the full contribution of the solvent. In parallel, considerable efforts are being devoted to the development of polarizable force fields. Despite their solid theoretical foundation, these methods have yet to reach their full potential and, equally important, justify their computational cost. On the other hand, there is an urgent need to improve the predictive power of physically based empirical potentials to better understand the molecular basis of protein interactions and function, and expedite molecular engineering and drug design. Here, we propose a comprehensive and scalable approach to include polarization/dielectric effects that uses a novel structure based method to estimate physical interactions in proteins. The main hypothesis is that varying dielectric environments can be approximated by a ''water factor'' derived from a structural analysis of molecular water at the binding interface. Our approach goes beyond solvent accessible surface area techniques: both the accessibility to solvent and the nature of the water network (''bulk'', ''trapped'', or ''crystal'') are taken into account. Applications to DNA interactions of C2H2 zinc fingers transcription have shown that such a water factor improves the R2 correlations between predicted and experimental changes in binding free energy from around 0.6 to better than 0.94.

369-Pos Board B169
Probing the Conformational Dynamics of Protein-DNA Complexes Through Hydration
Gregory M.K. Poon.
The DNA-binding domain of the ETS family of transcription factors interacts with DNA in a sequence-selective manner, yet it tolerates remarkable variation in the recognition sequence. The biophysical basis for this sequence selectivity is currently unknown. Previous investigations have found significant differences in the thermodynamics of strong and weak ETS-DNA complexes. However, the ETS domain is structurally similar in both DNA-bound *and* unbound states. In the absence of significant coupled folding by either protein or DNA, selectivity may be linked to differences in conformational dynamics in the protein-DNA complex. Specifically, sequence selectivity may arise from differential reductions in conformational dynamics upon complex formation. We hypothesize that time-averaged differences in conformation dynamics can be resolved through their equilibrium hydration properties. The rationale for our hypothesis is that folded but conformationally mobile segments should be more solvent-accessible than segments that are relatively immobilized. To test this hypothesis, we are probing the hydration of various ETS-DNA complexes by high-resolution DNA and protein footprinting. We expect that this approach will provide a quantitative measure of a complex's overall conformational dynamics as well as identify structural elements that become conformationally stabilized upon complex formation.

370-Pos Board B170
Target Site Localization Dynamics of DNA-Binding Proteins in Vivo
Elena F. Koslover, Mario A. Diaz de la Rose, Stephanie C. Weber, Andrew J. Spakowitz.
Genetic regulatory responses are limited by the dynamics of transcription-factors searching for specific binding sites on DNA. This target search process is thought to occur by facilitated diffusion, a combination of three-dimensional diffusion and one-dimensional sliding. While facilitated diffusion is capable of significantly speeding up the search in vitro, the importance of this process in vivo remains unclear. Furthermore, the scaling of transport processes within the cell is modified by crowding and biological fluctuations, which thereby play a role in modulating the speed of regulatory response. We present a set of simulations for modelling the target-search dynamics of DNA-binding proteins in conditions relevant to both in vitro and in vivo settings. The simulations are used to address the role of DNA concentration and packing, as well as the effect of subdiffusive transport in the viscoelastic medium of the cytoplasm. We develop an effective theory for localization time-scales of DNA-binding proteins in vivo and extend our results to study the dynamics of regulatory transcription cycles in the cell.

371-Pos Board B171
Measurements of Force-Driven Changes in Bound Protein Numbers on a Single DNA
Botao Xiao, Houyin Zhang, Reid C. Johnson, John F. Marko.
DNA compaction and chromosome organization involve the dynamic interaction of long DNA molecules and many copies of various proteins. Determining numbers of proteins bound to large DNAs is important to understand their chromosomal functions and protein numbers may be affected not only by chemical factors, but also by physical factors such as mechanical forces generated in DNA, *e.g.*, by transcription or replication [1]. We performed single-DNA stretching experiments with bacterial nucleoid proteins HU [2] and Fis, where we verified that the force-extension measurements were in thermodynamic (chemical-mechanical) equilibrium. Given thermal equilibrium of protein binding, we could use a thermodynamic Maxwell relation to deduce the change of protein number on a single stretched DNA due to varied applied force. For the binding of both HU and Fis under conditions where they compact DNA, the numbers of bound proteins decreased as force was increased from 0.03 to 12 pN. This effect saturated with force for HU, but did not for Fis, reflecting the tighter binding of the latter to DNA. The experimental results agree well with expectations of binding numbers based on electrophoretic mobility shift assay data, and the HU data agree well with results from a simple statistical-mechanical model of DNA-bending proteins. This thermodynamic approach may be applied to measure force-driven changes in numbers of a wide variety of molecules bound to DNA or to other polymers; in the case of proteins binding to DNA, force-dependent binding suggests mechano-chemical mechanisms for gene regulation.
1. C. Bustamante, Z. Bryant, S. B. Smith, *Nature,* (2003).
2. B. Xiao, R. C. Johnson, J. F. Marko, *Nucleic Acids Res*, (2010).

372-Pos Board B172
Sequence Dependence of Binding and Exchange of Nonspecific Dna-Binding Proteins
John S. Graham, Reid C. Johnson, John F. Marko.
The multistep kinetics through which DNA-binding proteins bind their targets are heavily studied, but relatively little attention has been paid to mechanisms of how proteins leave the double helix. Using single-DNA stretching and fluorescence detection, we recently demonstrated that the sequence-neutral DNA-binding proteins HU, NHP6A and Fis, readily exchange with each other and that the rate and degree of exchange is dependent on the concentration of solution-phase protein, regardless of protein species. We now examine the sequence dependence of binding and the exchange reactions investigated previously. Our results indicate an apparent disparity between biochemically measured sequence dependence and the sequence dependence in our single molecule approach. Additionally, we demonstrate a coarse-grained sequence dependence of the exchange reactions and correlate those results with our observed binding specificity.

373-Pos Board B173
Stretching DNA to Quantify Non-Specific Binding by the Lambda Repressor (ci)
Sachin Goyal, Chandler Fountain, David Dunlap, Fereydoon Family, Laura Finzi.
Non-specific binding of regulatory proteins to DNA can be an important mechanism for target search and for storage. This seems to be the case for the λ repressor protein (CI), which maintains lysogeny after bacteriophage infection. CI binds specifically at two distant regions along the viral genome and induces the formation of a repressive DNA loop. However, single-molecule experiments and kinetic measurements show that CI also binds to DNA non-specifically, and that this mode of binding may play an important role in maintaining lysogeny. Therefore, we have quantified non-specific CI binding by stretching DNA at various CI concentrations using magnetic tweezers. We recorded the decrease in DNA extension caused by CI (see Fig. 1). Then, we used a novel theory extending work by Zhang & Marko [PRE 77, 031916, (2008)] to calculate the change in CI binding from the measured decrease in DNA extension. In this presentation, we will interpret new stretching data at various CI concentrations using our novel theory. Furthermore, we will discuss the versatility of our method for characterizing gene regulation via non-specific protein binding.

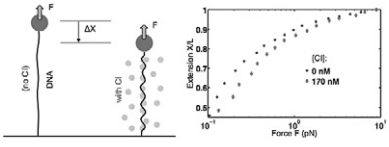

Fig. 1: (Left) A cartoon showing the decrease in DNA extension due to non-specific binding of CI proteins (green circles). (Right) DNA extension vs. force measured using magnetic tweezers in the absence (blue dots) and presence (red diamonds) of CI proteins [Data reported in JPCM (2010)].

374-Pos Board B174
FRET and Sm-FRET Characterization of a Lac Repressor-Dna Looping Landscape
Jason D. Kahn, Aaron R. Haeusler, Kathy A. Goodson, Douglas S. English.
DNA looping is fundamental to transcriptional regulation, and the archetypal loop is formed by the Lac repressor protein, LacI. Thermodynamic characterization of looping energetics with and without induction is a necessary input for quantitative modeling of gene expression, and the LacI system is also a test case for statistical mechanics and rod mechanics models of protein/DNA flexibility. We have systematically constructed a landscape of LacI-DNA looping variants patterned on previously developed molecules in which lac operators bracket a sequence-directed A-tract bend. The hyperstability of these loops enables study of LacI protein geometries that would otherwise be unstable with respect to unlooped single- or double-bound DNA. FRET donors and acceptors (Alexa

555 and 647) positioned on each side of both operators allow detection of all possible looping topologies (two parallel and two antiparallel trajectories). Comparisons among these fluorophore position variants allow measurement of the population of each loop state. Finally, we have studied the effect of IPTG on loop geometry and stability. Bulk and single-molecule FRET studies show that: (1) Remarkably, all the constructs on the landscape form stable loops. (2) Parallel and antiparallel topologies have different LacI geometries detected by different FRET efficiencies, probably due to differential effects of DNA stiffness in the two contexts. (3) Some constructs form an extended loop that can be identified by loss of FRET. (4) Induction affords a mixture of the initial loop, a state with decreased FRET, and additional FRET-silent states that we tentatively ascribe to specific-nonspecific loops. (5) States on the borders of the landscape regions comprising uniform loops are maximally sensitive to IPTG. The overall results do not agree with rod mechanics models that consider only DNA bending and flexibility, confirming that protein flexibility must be considered in modeling DNA loops.

375-Pos Board B175

Kinetic Studies of Lambda Repressor-Mediated DNA Looping Suggest Physiological Role for Non-Specific Binding

Laura Finzi, Carlo Manzo, Chiara Zurla, Sankar Adhya, David D. Dunlap.

The kinetics of DNA loop formation and breakdown by the λ repressor or CI protein was characterized using the tether particle motion technique (TPM) and a novel method of analysis with increased time resolution. The kinetics of loop formation was described by a stretch exponential, while the kinetics of loop breakdown was found to be more complex and only the distribution of the long dwell times could be described by a power law. Comparison with the kinetics of loop formation and breakdown induced in DNA with mutated operators, shows that repressor bound at these sites may act as a nucleation site for further binding and loop stabilization, and may contribute to broaden the rate constants distribution. A model is suggested by which nonspecific binding of CI along the inter-operator distance may shorten the effective separation between the specific sites which mediate looping thereby lowering the potential energy necessary for loop formation. Finally, CI-mediated looping kinetics also shows that the frequency of transition between the looped and unlooped DNA conformation does not vary with CI concentration, despite the fact that the loop becomes thermodynamically more stable. The relevance of this feature to the robustness of the system is discussed.

376-Pos Board B176

The Role of Architectural Proteins in Lac-Mediated DNA Looping

Luke Czapla, David Swigon, Wilma K. Olson.

The widely abundant nucleoid protein HU contributes to both the spatial organization and biological processing of bacterial DNA. One such contribution of HU to biological processing is tied to gene regulation mediated by the looping of DNA induced by proteins, such as the tetrameric Lac Repressor protein, which simultaneously binds two distant operator DNA sites in the bacterial genome. Current understanding of how HU might contribute to the transcription of the *lac* genes derives from indirect theoretical and computational analysis of the effects of chain length on gene expression in *E. coli* cells with and without HU. We take a more direct approach to the question of how architectural proteins mediate the structure and looping of DNA *in vivo*, incorporating the structural effects of both HU and Lac Repressor on DNA in Monte Carlo simulations, taking advantage of new methods that we have developed to understand the properties of protein-bound DNA. We present our predictions from models for understanding Lac-mediated gene expression *in vivo,* and also demonstrate state-of-the-art calculations that incorporate our structure-based models in order to obtain more detailed insights into the phenomena observed in ring-closure experiments of DNA in the presence of architectural proteins.

377-Pos Board B177

Divide a Plasmid DNA Molecule into Two Independent Superhelical Domains by Sequence-Specific DNA-Binding Proteins: A DNA Superhelical Barrier Model

Fenfei Leng, Bo Chen.

Both prokaryotic and eukaryotic chromosome are organized into many independent topological domains. These topological domains are presumably formed through constraining each DNA end from rotating by the interaction with nuclear proteins, *i.e.,* DNA-binding proteins. However, so far, there is no direct evidence to support this hypothesis. In this study, we utilized two new *in vitro* methods, developed in our laboratory to examine whether certain sequence-specific DNA-binding proteins can separate a plasmid DNA molecule into different DNA superhelical domains. Our new methods are based on the successful construction of several plasmid DNA templates that contain many tandem copies of one DNA-binding sites in two different locations (, B., Xiao, Y., Liu, C., Li, C., and Leng, F. (2010) *Nucleic Acids Resesearch,* 38, 3643-3654). Using these new methods we discovered that several sequence-specific DNA-binding proteins, *i.e.,* LacI, GalR, AraC, λ O protein, can divide a plasmid DNA molecule into two independent superhelical domains. These independent superhelical domains are thermodynamically stable. Interestingly, CRP (E. coli cAMP receptor protein), a DNA-bind and -bending protein, cannot divide the plasmid DNA molecule into different DNA topological domains. Our results can be explained by a superhelical barrier model of nucleoprotein complexes in which DNA supercoils may be confined in localized regions. We propose that the DNA superhelical barriers are certain nucleoprotein complexes that contain stable toroidal supercoils assembled from DNA looping or tightly wrapping DNA around DNA-binding proteins. The biological significance of the new superhelical barrier model will be discussed. This work is supported by a NIH grant 5SC1HD063059-02.

378-Pos Board B178

Single-Molecule Observation of Dynamic Bending and Cleavage of a Gate DNA by Human Topoisomerase IIα

Sanghwa Lee, Seung-Ryoung Jung, Joseph E. Deweese, Jo Ann Byl, Neil Osheroff, Sungchul Hohng.

Type II topoisomerases are an essential enzyme that resolves intrinsic topological problems encountered during DNA replication and RNA transcription. They have been a major target of antibacterial and anticancer drugs. Decades of research established that these ATP-dependent molecular machines operate by transporting a DNA duplex (the transport or T-segment) through a transient break in another DNA duplex (the gate or G-segment). However, mechanistic steps of the enzyme's catalytic cycle and their dynamics remain largely uncharacterized, and it is still an intriguing question how the complex series of conformational changes in DNA substrates and the enzyme are communicated, and integrated into a coordinated overall reaction cycle. Here we describe single-molecule fluorescence experiments to monitor the association/dissociation dynamics of human topoisomerase IIα, and accompanying bending/cleavage events of G-segment DNA. Our observation reveals that 1) In the presence of divalent ions, dynamic bending of G-segment, an intermediate step to the cleavage reaction, occurs in cleavage-competent sequences, and 2) The sequence specificity and efficiency of the cleavage reaction is determined by the deformability of the sequence, rather than by the chemical information stored in the sequence. 3) DNA cleavage reaction, which is tightly down-regulated to a minimum level, is greatly accelerated by the clamping motion of N-gate induced by nucleotide binding.

379-Pos Board B179

Single Molecule Rupture Force Measurements of TOPOII-DNA Binding

Yii-Lih Lin, Yi-Ren Chang, Tzu-Ming Ou, Chia-Shen Chang, Ting-Fang Wang, **Chia-Fu Chou**.

Using AFM and magnetic tweezers, we performed single molecule rupture force measurements on the binding of Topoisomerase (TOPO) II to double-stranded DNA. With a few hundred pulling events, using DNA and disulfide bond cross-linked mutant TOPOII as controls, we found the rupture force between wild type TOPOII-DNA complex is ~45 pN at a 50% accumulative probability.

380-Pos Board B180

Investigating the Interaction of Single UvrA Dimers with DNA Using a Combination of Fluorescent Microscopy and Optical Tweezers

Andreas Biebricher, Koen Wagner, Geri F. Moolenaar, Nora Goosen, Remus T. Dame, Erwin J.G. Peterman, Gijs J.L. Wuite.

Nucleotide excision repair (NER) is a DNA repair mechanism responsible for replacement of base pairs damaged e.g. by exposure to UV light. In *E. coli*, NER is initiated by the UvrA-dimer, which searches for and localizes damaged nucleotides to which then the UvrB-dimer is recruited. We study the damage search of UvrA by visualizing single, fluorescently labeled UvrA-dimers interacting with a DNA molecule manipulated by double optical tweezers. This approach enables us to observe UvrA binding in real time to DNA under different tensions.

We find that, in the presence of ATP, the UvrA-dimer displays static, non-specific DNA binding of long duration (>10s), in accord with a recent publication [1]. In addition, our results reveal that non-specific UvrA binding is tension dependent: Binding events are prolonged on DNA under tension, indicating that the dissociation rate decreases while the association rate remains rather constant, thus supporting a model in which UvrA binding unwinds DNA [2].

Finally, we observe that the duration of binding events decreases by several orders of magnitude if ADP instead of ATP is present in the buffer (<<1s). Binding is sensitive to tension, even more so than in the ATP experiment, resulting in a more than tenfold increase of the duration of binding events when DNA tension is increased from 5 to 65pN. In addition, our results demonstrate that UvrA has a higher binding affinity for ADP than for ATP. We are currently investigating whether ADP binding plays an important role in accelerating the DNA damage search *in vivo*.

[1] Kad, N. M., Wang, H., Kennedey, G. G., Warshaw, D. M., van Houten, B. (2010). Mol. Cell *37*, 702–713.

[2] Oh, E., Grossman, L. (1986). Nucleic Acids Res. *14*, 8557–8571.