used to quantify the stoichiometry of protein association and to determine the number of proteins in a complex. In protein hetero-dimerization studies, it is highly desirable to be able to control protein co-expression fractions. The expression of each protein should be selected in order to maximize the likelihood of protein association. Yet protein expression is the output factor from a lipid-mediated transfection process in which only the plasmid DNA (input parameter) can be explicitly controlled. In this work we develop and test a working model which relates the protein expression fraction of two proteins to the plasmid DNA mixing fraction. Experimentally, we express two differently colored fluorescent proteins in CV-1 cells and split their emitted signal into two different detection channels based on color. The intensity fraction from the two channels is used to determine the relative amount of expressed protein in the cell. The experiments show that the DNA mixing fraction determines the average protein expression fraction. We extend this work by examining the cell-to-cell variation in protein expression and constructing a simple model which relates the expression variation to the average number of active plasmids. This study provides insight into the fundamentals of lipid-mediated transfection and demonstrates that we can control protein co-expression fractions through selection of plasmid DNA mixing fractions. This work is supported by NIH grant R01GM064589.

Molecular Mechanics & Force Spectroscopy II

3908-Pos
DNA Stretching Kinetics and Entropic Bottlenecks

Lorenzo Bongini[1], Pasquale bianco[2], Vincenzo Lombardi[2].
[1]University of Barcelona, Barcelona, Spain, [2]Università di Firenze, Sesto Fiorentino, Italy.

The use of a force clamp protocol during single molecule measurements of the DNA over-stretching transition by means of optical tweezers allow to experimentally access the kinetic of both DNA elongation and relaxation. The data are interpreted by means of a phenomenological two state model which allows to measure the cooperativity of the process. The comparison between experiments performed at different temperatures highlights the entropic nature of the free energy barrier that separates the compact and extended states of DNA. Insights on the structure of the intermediate state are provided.

3909-Pos
Multiple Binding Modes of Actinomycin D Reveal the Basis for its Potent HIV-1 and Cancer Activity

Thayaparan Paramanathan[1], Ioana D. Vladescu[2], Micah J. McCauley[1], Ioulia Rouzina[3], Mark C. Williams[1].
[1]Northeastern University, Boston, MA, USA, [2]Harvard University, Cambridge, MA, USA, [3]University of Minnesota, Minneapolis, MN, USA.

Actinomycin D (ActD) is a well studied anti-cancer agent that is used as a prototype for developing new generations of drugs. However, the biophysical basis of its activity is still unclear. Because ActD is known to intercalate double stranded DNA (dsDNA), it was assumed to block replication by stabilizing dsDNA in front of the replication fork. However, recent studies have shown that ActD binds with even higher affinity to imperfect duplexes and some sequences of single stranded DNA (ssDNA). These features suggest that ActD may alternatively destabilize complementary dsDNA. In this work we use optical tweezers to stretch and relax single dsDNA molecules in the presence of varying ActD concentrations. We observe that ActD binds with highest affinity to two separate DNA strands that are connected by ActD. This binding mode is ~1000-fold stronger than ActD's intercalation into dsDNA. We are able to characterize at least two classes of ActD-ssDNA binding sites that differ in dissociation times (~10% of sites with ~1000 sec off time, and the rest with ~10 sec off time). The much weaker ActD binding to dsDNA relative to ssDNA leads to duplex destabilization, in contrast to conventional intercalation. At saturation, the ActD-dsDNA complex becomes indistinguishable from the saturated ActD-ssDNA. These results suggest that two separate, anti-parallel DNA strands constitute the highest affinity natural substrate for ActD binding, with K_d ~10-100 nM and a relatively slow off rate. This finding supports the hypothesis that the primary characteristic of ActD that contributes to its biological activity is its ability to inhibit cellular replication by stabilizing DNA bubbles during RNA transcription, thereby stalling the transcription process.

3910-Pos
Atomistic Simulation of Estrogen Receptor-Coactivator Peptide Complexes to Identify Specific Binding Sites

Pradip K. Biswas[1], Rajendram Rajnarayanan[2].
[1]Tougaloo College, Tougaloo, MS, USA, [2]University at Buffalo, Buffalo, NY, USA.

Estrogen Receptors (ER) can lead to gene transcriptions that are found responsible for certain type of cancers including 70% of breast cancers. However, the process of gene transcription is preceded by the binding of an estrogenic ligand to the ER and then binding of this liganded ER with an activator protein which mediates the signal to the DNA. There are antiestrogenic compounds that can bind to the ER and block the binding of any coactivator protein to the liganded ER. However, no single antiestrogenic compound is found to work in all tissues - that means it cannot always block the binding of the coactivator protein. A successful drug molecule need to inhibit the liganded ER in all tissues. This needs a detail atomic level understanding of the interaction pattern of the liganded ER with coactivator protein. We investigate liganded ER interaction with small peptides to identify specific binding sites. Details of our investigation will be reported at the meeting.
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3911-Pos
Sources of Heterogeneity in the Forced Unfolding Pathway of Streptokinase Beta Revealed through High-Temperature Steered MD Simulations

K. Maria Mills[1], Dora Guzman[2], Zhibin Guan[2], Ioan Andricioaei[2].
[1]University of Michigan, Ann Arbor, MI, USA, [2]University of California Irvine, Irvine, CA, USA.

Force-pulling experiments on the unfolding of mechanical and non-mechanical protein domains have greatly increased our understanding of the structural stability of proteins. Because these experiments are done on the single molecule level, they also enable experimentalists to observe differences in the unfolding behavior of individual molecules. However, it is difficult to determine the source of unfolding heterogeneity through experiments alone. We present here evidence from experiments and simulations that the ß domain of Streptokinase, a non-mechanical protein, unfolds under force via three distinct pathways. High temperature SMD simulations were used to determine the source of the velocity-dependent heterogeneity observed in AFM force puling experiments. We show that hydrophobic interactions in the core of the protein underlie the differences observed in experiments and contribute significantly to the structural stability of the protein under force. Using an expansion of the Jarzynski equality[12], we calculate free energy surfaces to describe the energetics of the different pathways.

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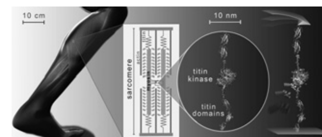
3912-Pos
Exploring the Function of Titin Kinase by Mechanical Single-Molecule Pump-And-Probe

Elias M. Puchner, Hermann E. Gaub.
Ludwig-Maximilians-Universität München, Munich, Germany.

Protein function like catalytic activity or molecular recognition is tightly coupled to conformation and its dynamics. Since protein conformation can be controlled by forces, diverse mechanisms evolved allowing biological systems to respond to mechanical strain. Recently, it was shown in combination with single-molecule force spectroscopy, MD-simulations and enzymatics that titin kinase acts as a force sensor regulating muscle gene expression and protein turnover [1]. However, there is no experimental access to investigate which force-induced conformation during the activation pathway is competent for ATP-binding. Here we develop a new AFM-based single-molecule pump-and-probe protocol to mechanically prepare a predefined conformation and to read out afterwards whether ATP bound. We show that ATP only binds to the conformation prepared after barrier two. Therefore, titin kinase exhibits a dual mechanical autoinhibition, which cannot be overcome by thermal fluctuations but by physiological forces acting on the M-band structure of the muscle sarcomere. This single-molecule approach might also become useful for the investigation of other conformation controlled processes such as hidden binding pockets, catch bonds or motor proteins.



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3913-Pos
Single Molecule Study of the Motion of Matrix Metalloprotease MMP1 on Type I Collagen Fiber Shows Proteolysis Driven Hindered Biased Diffusion

Susanta K. Sarkar[1], Barry Marmer[2], Gregory Goldberg[2], Keir C. Neuman[1].
[1]Laboratory of Molecular Biophysics, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD, USA, [2]Department of Biochemistry and Molecular Biophysics and Division of Dermatology, Washington University School of Medicine, St. Louis, MO, USA.

Diffusion plays an important role in many biological processes. Using single molecule fluorescence techniques, we have studied the diffusive motion of