Nano-sized fullerene aggregates can enter cells and alter their functions, but the mechanisms of cell damage is unclear. In our previous work [1] we used coarse-grained molecular dynamics simulations to characterize the thermodynamics and kinetics of permeation of fullerene clusters through a model membrane. We also showed that high fullerene concentrations induce changes in the structural and elastic properties of the lipid bilayer, but these are not sufficient to cause a direct mechanical damage to the membrane. Now we explore the effect of fullerene on model membranes including an ion channel protein, Kv1.2, using computer simulations with both a coarse-grained and an atomistic representation. We also investigate the effects of a naturally abundant organic compound, gallic acid, on fullerene-membrane interactions. Recent work [2] has shown that gallic acid-coated fullerenes cause cell contraction. We use computer simulations to describe possible mechanisms of cell damage.

[1] Wong-ekkabut et al., Nature Nanotech (2008), 3, 363.
[2] Salonen et al., Small, in press.

1880-Plat

Force Calculations for DNA-PAMAM Dendrimer Interactions from Molecular Dynamics Simulations

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Polyamidoamine (PAMAM) dendrimers are known to bind and condense plasmid DNA (1,2,3). However, the nature of the interaction between dendrimers and DNA and the mechanism of compaction is not fully understood. Potentials of mean force and forces of interaction were calculated from all-atom umbrella sampling simulations of amine-terminated G3 dendrimers and a 24bp strand of double-stranded DNA. Our simulations show that dendrimers and DNA interact with each other over large distances. Simulations also reveal that even low-generation dendrimers can induce significant bending in DNA and that the dendrimer also deforms considerably upon interaction with the DNA. We compare forces calculated from these simulations with optical tweezer experiments on DNA condensation by dendrimers (3) and propose an explanation for the compaction of DNA by dendrimers observed at forces over 60pN.

1. A.U. Bielinska, J.F. Kukowska-Latallo, J.R. Baker, Gene Structure and Expression, 1353, 180 (1997).
2. W. Chen, N.J. Turro, D.A. Tomalia, Langmuir, 16, 15 (2000).
3. F. Ritort, S. Mihardja. S.B. Smith, C. Bustamante, Phys. Rev. Letters, 96, 118301 (2006).

Platform AC: Protein-Nucleic Acid Interactions

1881-Plat

The Impact of Bending and Twisting Rigidity of DNA on Protein Induced Looping Dynamics

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Protein induced DNA looping is a key regulatory mechanism involved in important processes such as gene regulation, DNA-transcription and -replication. The relation between the induced loop topology and DNA-protein dynamics is essential for understanding these processes. Bending and twisting rigidities of DNA are shown to have a profound influence on the formation and stability of these loops. We used FokI, a restriction enzyme that binds two asymmetrical recognition sites enhancing its specificity, as our model system. Controlling the orientation of both binding sites enabled us to explore the impact of the physical properties of DNA by inducing different loop topologies and measuring the resulting changes in DNA-protein dynamics.

The looping behavior is quantified using a tethered particle assay. With this assay we obtained the kinetics of protein induced loop formation with a single measurement by tracking up to 50 DNA tethers in parallel. The dwell times are extracted and compared using both a running average method and a hidden Markov analysis.

We used DNA substrates with a range of different spacing's between the two asymmetric recognition sequences. In addition we varied the orientation of these recognition sites and sampled how binding and loop formation is influenced by these different topologies. We show that both, the separation and orientation of the two recognition sites have a profound influence on the formation and stability of these looped DNA-protein structures. The results are understood and modeled in terms of the helical pitch and the bending energy involved in protein induced loop formation.

1882-Plat

Target Site Search Strategy Of Gene Regulatory Proteins

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Gene expression is orchestrated by a host of regulatory proteins that coordinate the transcription of DNA to RNA. Regulatory proteins function by locating specific binding sequences of DNA and binding to these sequences to form the transcription initiation complex. In many instances, these regulatory proteins only have several hundred copies that must efficiently locate target sequences on the genome-length DNA strand. The non-specific binding of regulatory proteins to random sequences of DNA is believed to permit the protein to slide along the DNA in a stochastic manner. Periodically, a thermal kick or an interaction with another bound protein will disengage the regulatory protein from the DNA surface, leading to three-dimensional diffusion. Eventually, the protein will reattach to the DNA at some new location that is dictated by both the diffusivity of the protein and the DNA configuration. Cycling through these random events constitutes a search strategy for the target site. We build a reaction-diffusion theory of this search process in order to predict the optimal strategy for target site localization. The statistical behavior of the DNA strand acts as a necessary input into the theory, and we consider several governing behaviors for the DNA strand. We explore the impact of DNA configuration on target site localization in order to predict how protein expression will vary under different experimental conditions.

1883-Plat

Structural and Thermodynamic Means for Adaptable 3′ Splice Site Recognition

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The essential splicing factor U2 Auxiliary Factor ($U2AF^{65}$) identifies the 3′ splice sites of pre-mRNAs during the initial stages of spliceosome assembly. Short, poorly conserved pre-mRNA sequences mark human 3′ splice sites, including polypyrimidine (Py) tracts that are recognized by two consecutive RNA recognition motifs (RRMs) of $U2AF^{65}$. To understand how $U2AF^{65}$ adapts to divergent pre-mRNA splice sites, high resolution structures were determined of $U2AF^{65}$ complexes with a series of Py tracts. In parallel, the affinities, enthalpy and entropy changes associated with Py tract binding were analyzed using fluorescence anisotropy assays and isothermal titration calorimetry. The different Py tracts bind with optimized registers across the $U2AF^{65}$ surface, placing cytidines and uridines in preferred binding pockets. Small angle X-ray scattering (SAXS) analysis of wild-type and variant $U2AF^{65}$ proteins further demonstrated that the tandem RRMs adopt an extended, bilobal arrangement in solution (Fig. 1). The preferences for binding specific nucleotides at a subset of $U2AF^{65}$ sites, combined with the loose arrangement of RRM domains, altogether supports adjustable binding registers as a means for universal recognition of diverse 3′ splice sites.

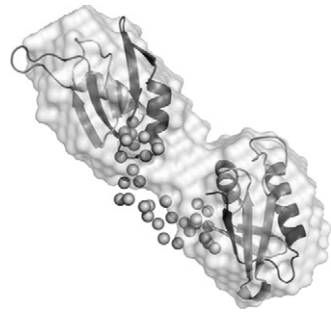

Fig. 1. Shape reconstruction of the $U2AF^{65}$ RNA binding domain.

1884-Plat

Single Molecule FRET Studies of Binding and Conformational Dynamics of HMGB -DNA Systems

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HMGB proteins are abundant non-histone proteins in eukaryotic chromatin. HMGB proteins are thought to act as architectural factors that enhance DNA bending and flexibility. The dynamic aspects of DNA bending by these proteins remain elusive. It has been proposed that these proteins associate and dissociate from DNA at fast rates. This highly dynamic behavior makes it difficult to study binding and conformational dynamics by traditional biochemical techniques. In this work we use single molecule fluorescence resonant energy transfer (smFRET) to study the binding dynamics of human HMGB2A and *S. cerevisiae* Nhp6A sequence non-specific single box proteins to DNA substrates. Studies were done using both total internal

reflection and confocal excitation. By using a FRET pair with dyes at the ends of the DNA substrates we are able to follow the bending dynamics of the substrates at the single molecule level. We carried on experiments with duplex DNA 15 bp and 18 bp long to determine binding affinity and potential binding of multiple units to the substrate. DNA substrates containing bulges were used for comparison to study the effect of pre-bent structures on binding affinity and conformational dynamics. Bending of the DNA substrates is observed by changes in FRET efficiency allowing determining the conformational dynamics of the system in real time with temporal resolution in the order of milliseconds.

1885-Plat
Slide into Action: Dynamic Shuttling of HIV Reverse Transcriptase on Nucleic Acid Substrates
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The reverse transcriptase (RT) of human immunodeficiency virus (HIV) catalyzes a series of reactions to convert single-stranded viral RNA into double-stranded DNA for host cell integration. This process requires a variety of enzymatic activities, including DNA polymerization, RNA cleavage, and strand displacement synthesis. As a major target for anti-HIV therapy, RT has been the subject of extensive research. Nonetheless, how the enzyme-substrate complex acquires specific functional configurations and switches between different functional modes remains unclear. Here, we used single-molecule fluorescence resonance energy transfer to probe the interactions between RT and nucleic acid substrates in real time (1). Surprisingly, RT was observed to slide on nucleic acid duplexes, rapidly shuttling between opposite termini of the duplex. Sliding kinetics were regulated by cognate nucleotides and non-nucleoside RT inhibitors, a major class of anti-HIV drugs, which stabilized and destabilized the polymerization mode, respectively. These long-range translocation activities facilitate multiple stages of the reverse transcription pathway. First, sliding allows RT to target the polymerization site by one-dimensional search. Remarkably, upon reaching the polymerization site, an RT molecule originally bound in the opposite orientation can spontaneously flip into the polymerization orientation without dissociation, enhancing the target search efficiency. Furthermore, sliding helps RT to actively disrupt secondary structures on the substrate and kinetically access the polymerization site, thereby facilitating strand displacement synthesis. It is remarkable that an enzyme could have such large-scale translational and orientational dynamics. This type of dynamic flexibility may be a general design principle for multi-functional enzymes, helping them to rapidly access different configurations required for different functions.
Reference:
1. S. Liu, E.A. Abbondanzieri, J.W. Rausch, S.F.J. Le Grice, X. Zhuang, ''Slide into action: dynamic shuttling of HIV reverse transcriptase on nucleic acid substrates'', Science (in press).

1886-Plat
Single Molecule Study Of the RNA Degradation and Polyadenylation Activities of the Archaeal Exosome
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RNA biosynthesis involves a certain frequency of errors due to inherent inaccuracies of enzymes involved. Although DNA damage is repaired, RNA errors are rapidly eliminated. In particular, the degradation of 3' poly A tail is very important in RNA metabolism to maintain the fidelity of mRNA synthesis in the cell. A key enzyme involved in both RNA processing and RNA degradation is the exosome complex. The archaeal exosome can function in reverse reactions as either an RNA polymerase or an RNA exoribonuclease. In the presence of free inorganic phosphate (P_i), the exosome phosphorolytically degrades RNA substrates from the 3' end, whereas in the presence of ADP, it polymerizes and extends the 3' end. The dynamics of the RNA degradation and polymerization reactions of the exosome are not well characterized and thus warrant further investigation in order to better understand the exosome function. Here, we measured the real time activities of the archaeal exosome using single molecule Fluorescence Resonance Energy Transfer (smFRET). Both degradation and polyadenylation activities were highly processive and followed the Michaelis-Menten kinetic parameters over a range of substrate concentrations. Using the combination of smFRET and gel-based assays, we examined these reactions in order to understand the reversibility between 3'→5' nuclease activity and 5'→3' polyadenylation under an equilibrium condition of ADP and P_i. We found that the enzyme can switch the direction rapidly between both reactions back and forth at the condition where both speeds of polymerization and degradation are equivalent.

1887-Plat
Direct Observation of NS3 Substeps at Single Base Pair Resolution
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Helicases are a ubiquitous class of nucleic acid motor proteins. They utilize ATP to catalyze the opening of double-stranded nucleic acids. Previous crystallographic and single molecule fluorescence studies have implied that a single ATP molecule would be used for every base pair (bp) being opened by a helicase. However, this has not been directly observed. The hepatitis C virus encodes NS3, an RNA helicase that is essential for viral RNA replication. Previous single molecule optical tweezers studies on NS3 unwinding have revealed an 11-bp periodicity with 3.6 bp substeps on average. By building an optical tweezers instrument with high spatial resolution and low drift, we were able to monitor the discrete unwinding substeps of NS3 on RNA at single base pair resolution. We show that under conditions where ATP binding limits the motor dwell time between successive substeps, single base pair substeps are clearly visible. We present a distribution of these substep sizes and the implication of this result on mechanisms of NS3 catalyzed RNA base pair opening and strand release. The distribution of motor dwell times between substeps gave us a direct measure of the coupling ratio between ATP and base pair opening, and we propose an integrated model to rationalize all these observations.

1888-Plat
Visualizing an RNA-dependent RNA Polymerase at Work: Polymerization And Strand-switching
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RNA-dependent RNA polymerases (RdRP) are responsible for the transcription and replication of the genomes of many RNA viruses. These enzymes have also emerged as an integral part of the RNA interference mechanism in plants. RdRPs share many of the structural features with other types of polymerases, However, the ability of certain viral RdRPs to perform both replication and transcription sets them apart from more commonly known polymerases such as DNA-dependent RNA polymerases.
We have applied magnetic tweezers to study the transcription kinetics of a model RdRP from Bacteriophage Phi6 (Phi6 RdRP), a double-stranded RNA (dsRNA) virus. During *in vivo* transcription, Phi6 RdRP binds the 3'-end of the antisense RNA strand within the dsRNA genome and polymerases a new sense RNA strand. Concurrently, the original sense RNA strand is displaced. In magnetic tweezers, we measure the transcription kinetics by following the length of the displaced sense RNA strand. As a result, we measure a rate of transcription of ~20 nt/s, which is comparable with our bulk experiments. However, in approximately 30% of transcription events in the magnetic tweezers, we also observe for the first time a conversion of the displaced sense RNA strand back to double-stranded form. By elimination of other possibilities, we attributable this to strand-switching of the RdRP. This is surprising given the 'closed' structure of the RdRP predicted from crystallography. However, it is a phenomenon that has also been observed for DNA helicases and provides a potential mechanism for RNA recombination observed in RNA viruses.

Platform AD: Membrane Receptors & Signal Transduction

1889-Plat
Coarse-grained Modeling And Simulation Of ErbB Receptors: Intramolecular Factors That Govern Outside-in Signaling And Activation
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Epidermal growth factor receptors (ErbB receptors) mediate a number of critical cellular processes including cell proliferation, differentiation, migration and apoptosis. These receptors have received a lot of attention because they have been implicated in a number of human cancers including breast, colo-rectal and prostate cancer. ErbB receptors are also important targets in cancer therapy. The activation and regulation of ErbB receptor function is known to be a highly regulated and multilayered process. A number of elegant structural