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.

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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.

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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.

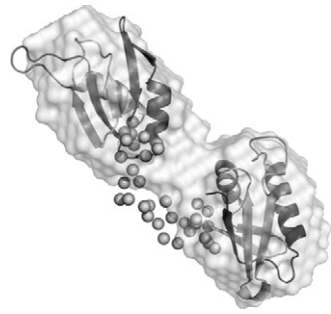

Fig. 1. Shape reconstruction of the $U2AF^{65}$ RNA binding domain.

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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