deviations from single exponential relaxation, indicating two distinct phases. Of particular interest is the observation that the rapid phase has a rate that is 10-20 times faster than the bending rate observed in the IHF-H' complex. Thus, reducing the energetic cost of bending/kinking DNA speeds up the bending rate by nearly the same factor as the increase in binding affinity, indicating that the free energy of the transition state is lowered by the same amount as the free energy of the complex. These results support our earlier conclusion, that spontaneous bending of DNA is the first step in the recognition mechanism.

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Mechanisms of the Type I Restriction Enzyme EcoKI: Characterizing weak interactions using AFM

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Common techniques for the characterization of biomolecular interactions are successful in detecting those of high affinity, but less effective at characterizing weak interactions. This limitation creates a methodological bias in investigations. Atomic force microscopy (AFM) provides a quick and gentle technique that allows the examination of biomolecules under near-physiological conditions. This means that direct observations of weak biomolecular interactions can be made, which in contrast with other techniques, prevents the necessity for averaging over a bulk number of molecules (typically $>10^9$).

Here we provide an example of how AFM can be used to characterize a biomolecular interaction, whose mechanism remains unclear after studies using other methods. The system studied is the DNA motor protein EcoKI. This is a bacterial type-I restriction enzyme which restricts the DNA of an invading virus. Restriction occurs between two sites and is preceded by the translocation of the intermediary DNA. The existing model for the enzyme was established over 30 years ago and involves two individual EcoKI monomers binding to two separate DNA sites. In this existing model the protein monomers would only meet after the DNA translocation. Using AFM we have shown that the enzyme monomers dimerize at one site, before any translocation, and that the dimerized complex then uses a diffusive looping mechanism to identify the secondary site. This demonstrates how AFM can be used to elucidate the mechanism of a well established macromolecular system. It also provides potential insight into the *in vivo* biology of type-I restriction-modification enzymes and other higher-order proteins. Such insights include: the kinetics and dynamics of site location; evolutionary implications; the protection of host DNA in restriction systems; space and volume considerations of large translocating complexes; and the positioning of sub-units in type-I systems.

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Transcriptional Activation by the Human Progesterone Receptor: Towards a Predictive Understanding

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A mechanistic and thus predictive understanding of transcriptional regulation in humans is highly lacking. For example, the current understanding for transcription factors such as progesterone receptor (PR) is that it binds to promoter regions of PR-regulated genes, then recruits coactivating proteins and RNA polymerase in order to activate transcription. However, this framework does not account for the ability of PR to differentially and simultaneously regulate multiple gene promoters. Differential regulation may hinge, at least in part, on the uniquely coded assembly of transcription factors at each promoter. In order to elucidate the mechanisms of promoter-specific binding, thermodynamic approaches were used to dissect PR interactions at natural and synthetic promoter sequences containing multiple binding sites. The results of experiments employing quantitative footprint titrations and statistical thermodynamic modeling show that PR-promoter interactions follow specific codes for assembly; that PR binding is highly cooperative; and that efficient coactivator recruitment is exclusively coupled to cooperative interactions. These results correlate with cellular measurements demonstrating that PR-regulated promoters containing multiple binding sites generate synergistic increases in transcriptional activity. Taken together, cooperativity may be key in the activation of transcription. As a means to assess the chemical forces responsible for cooperativity we examined the role of monovalent cations in regulating receptor-promoter interactions. Our findings suggest cooperative interactions are thermodynamically linked to Na^+ binding to PR. Noting that PR directly regulates the expression of ion channels and pumps, it is possible that Na^+ is not only an allosteric effector but also a physiologic regulator of PR-activated transcription in humans.

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Unravelling The Role Of Alba In The Organization Of The Archaeal Nucleoid

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Alba is one of the most abundant proteins in thermophilic and hyperthermophilic archaea and is believed to play an important role in DNA organization. It is a dimeric protein that binds DNA with no apparent sequence specificity. Earlier studies have shown that Alba is capable of bridging DNA duplexes, which may be key to its organizational role. However, a comprehensive understanding regarding the action of Alba in DNA organization is currently lacking. Using a combination of single-molecule imaging and micromanipulation techniques we now define mechanistic, structural and kinetic aspects of the Alba-DNA interaction. Thus, we demonstrate that Alba has two modes of action. Depending on its concentration and conformation, the protein either bridges two DNA duplexes or cooperatively binds to and stiffens a single DNA duplex. Based on these observations we put forward a structural model that describes the multi-modal behaviour of Alba in the context of the dynamic archaeal nucleoid.

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Regulation of the nucleic acid chaperone activity of HTLV-1 Nucleocapsid Protein

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Nucleocapsid proteins (NC) of retroviruses are nucleic acid chaperones that facilitate nucleic acid remodeling. This property of NC proteins is critical for their role in viral genome dimerization, maturation and reverse transcription. In contrast to all other NC proteins studied to date, the human T-cell leukemia virus type 1 (HTLV-1) NC protein was shown to be an extremely poor chaperone. In this work, we demonstrate that the anionic C-terminal domain (CTD) of this protein is responsible for its poor chaperone function. Single molecule DNA stretching studies suggest that HTLV-1 NC dissociates very slowly from single-stranded DNA, which may be a primary reason for its poor chaperone activity. In contrast, a truncation mutant that lacks the CTD is a more effective annealing agent and displays faster off-rate kinetics. Under conditions of high ionic strength, the properties of the WT and CTD-deletion variant are much more similar to each other. Taken together, our data suggest that an electrostatic attraction between the anionic CTD and cationic N-terminal domain of HTLV-1 NC leads to polymerization onto ssDNA resulting in a poor ability to aggregate nucleic acids or to promote their annealing. This property of HTLV-1 NC makes it similar to typical SSB proteins, and may be related to this NC's role in excluding the viral restriction factor APOBEC3G from HTLV particles.

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DNA Interaction Properties of Nucleic Acid Chaperone Proteins from Retrotransposons

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Nucleic acid chaperone activity is an essential component of reverse transcription in retroviruses and retrotransposons. Using DNA stretching with optical tweezers, we have developed a method for detailed characterization of nucleic acid chaperone proteins, which facilitate the rearrangement of nucleic acid secondary structure. The nucleic acid chaperone properties of the human immunodeficiency virus type-1 (HIV-1) nucleocapsid protein (NC) have been extensively studied, and duplex destabilization, nucleic acid aggregation, and rapid protein binding kinetics have been identified as major components of its activity. The chaperone properties of other nucleic acid chaperone proteins, such as those from the retrotransposons LINE-1 and Ty3, ORF1p and Ty3 NC, are not well understood. We used single molecule DNA stretching to characterize the activity of wild type and mutant ORF1p and Ty3 NC. ORF1p binds both double-stranded DNA (dsDNA) and single-stranded DNA (ssDNA) with high affinity, and strongly aggregates both forms. It is therefore an excellent chaperone, and altering certain residues has dramatic effects on chaperone activity. Wild type Ty3 also strongly aggregates both dsDNA and ssDNA, and melted DNA exhibits more rapid reannealing in the presence of Ty3 NC, relative to that observed in