provide a quantitative description of the positive and negative ions that surround the nucleosome. Results of these experiments will be presented. This work should have implications for nucleosome compaction, chromatin remodeling, and more generally electrostatics of highly charged biomolecules.

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Stress-Activated Sliding Motion: A Coupled-Potential Model
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In a notable study by Julicher & Bruinsma (BPJ 74, 1998), a model was presented describing the motion of an RNAP molecule during an elongation cycle. First, stepping motion of a catalytic (C)-site takes place. This generates stress in the molecule between this (C)-site and a front (F)-site. This stress activates forward sliding of the (F)-site by lowering the activation barrier hindering its motion.
Here we look at this RNAP model in terms of a coupled-potential paradigm, taken from an inchworm-like model of a polymer chain. This model describes how stress produced by forward motion of a (C)-like site in the polymer leads to activated sliding of an (F)-like site (Joseph, J Polymer Sci 16, 1978). The (F)-like site has two positions - (1) and (2) - and so possesses a double-well potential. This site is coupled in series to a linear spring - with a single-well harmonic potential. A (C)-like site occupies the spring free end.
We find that coupling (adding) these two potentials yields a net potential with the (F)-like site occupying position (1). Also, there is a large activation barrier hindering movement to position (2). However, when the (C)-like site at the spring free end is pulled forward, the stretching of the spring causes the equilibrium position of the harmonic potential minimum to be shifted forward. Coupling of this shifted potential reduces the net potential barrier, and this reduction activates forward sliding motion of the (F)-like site.
We conclude that this type of coupled-potential inchworm model yields insight into how stress can activate sliding motion during the RNAP elongation cycle.

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High Resolution Surface Plasmon Microscopy: From Nano-colloids To Single Nucleosome Imaging
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Surface plasmon resonance (SPR) has been widely recognized as a highly sensitive and non intrusive method for probing modifications of adsorbed layers, and in particular for its application to characterize biomolecular specific interactions such as antigen-antibody recognition.
We apply SPR to study nucleosomes, first level of DNA compaction around an octamer of proteins called histones. To maintain the DNA helix accessible to the transcription and replication machineries during the cell cycle, this complex is highly dynamical (formation, disassembly, or sliding of the nucleosome), leading to rapid modifications of the whole chromatin structure *in vivo*. We aim at understanding how the DNA sequence influences the structure and dynamics of the nucleosomes in chromosomes.
The scanning surface plasmon microscope (SSPM) set-up relies on the use of a high numerical aperture objective that confines the surface plasmon polaritons (SPPs) to an area of the interface much smaller (up to a few hundreds of nanometers) than their typical propagation length (few microns). Similarly to the Kretschmann configuration, SPPs are excited at the resonance angle θ_P. As an objective is used to focus the purely P-polarized (radial) light, the SPPs converge to the center of the illuminated area, leading to the creation of SPPs interferences that will reradiate in symmetrical rays and go through the objective to be detected. The presence of non-marked biological samples at the interface modifies the propagation conditions (i.e. the interferences) of the SPPs, leading to a change in the image contrast. The SSPM point spread function is about 150 nm in aqueous medium, providing high resolution images of biological samples *in vitro*.
We will present the SSPM study of the optical response of gold and latex nanoparticles and then the first images of non-marked single nucleosomes in liquid medium.

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Ion Exchange in the Nonspecific Bimolecular Association and the Unimolecular DNA Bending in Specific Binding of IHF to DNA
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Protein-DNA interactions are strongly modulated by salt. Previous studies on the equilibrium binding of a cognate DNA sequence H′ to integration host factor (IHF), an architectural protein from *E. coli* that bends its cognate site by nearly 180°, have shown that the slope SK_D of ~8 on a log(K_D) versus log([KCl]) plot depends on the anion type, suggesting that both release of counterions from the DNA and the uptake and release of ions from the protein must be playing a role. Here, we probe the effect of [KCl] on the bimolecular association/dissociation as well as the unimolecular bending/unbending rates, by monitoring the relaxation kinetics of the complex between IHF and ~35-bp long H' substrate end-labeled with a FRET pair, in response to a laser temperature-jump. Our results and analysis reveal two notable results. First, that the unimolecular bending step is nearly independent of [KCl]. Second, that the bulk of the salt-dependence appears in the nonspecific association/dissociation step, with the equilibrium constant for that step accounting for more than half of the total SK_D observed. The latter result is in contrast to what one might expect if counterion release from the DNA was the dominant contribution to the salt-dependence, since the extent to which the H' substrate makes contact with the protein in the fully wrapped specific complex is significantly greater than in the nonspecific complex. One possible scenario is that counterion release is the dominant term in the formation of the nonspecific complex, whereas in the transition from the nonspecific to the specific complex, the extent of the counterion release is masked by the uptake and release of ions by the protein, as a result of conformational rearrangements in the protein.

310-Pos Board B189
Analysis of RPA70N Involvement in RPA ssDNA Binding Activity
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RPA is the primary single-stranded DNA (ssDNA) binding protein in eukaryotes. It plays a central role in chromosomal DNA replication, repair and recombination pathways, protecting ssDNA from degradation by nucleases. RPA also mediates interactions with specific proteins active in these various DNA processing events. RPA has three subunits, each named after its molecular weight: RPA70 (domains N, A, B and C), RPA32 (domains N, D and C) and RPA14 (single domain). The N-terminal domain of RPA70 (70N) is flexibly linked by an 80 amino acid linker to the rest of RPA 70. It has long been established that RPA binds ssDNA with nM affinity through the action of domains 70A, 70B, 70C and 32D using 3 modes of binding. The first mode involves 70A and 70B spanning 8 nucleotides, the second mode adds 70C and spans 18 to 20 nucleotides, and the third mode adds 32D and spans 28-30 nucleotides. Tandem DNA binding by domains 70A and 70B is required for high affinity. Recently, a proposal has been made that 70N contributes to DNA binding function. However, 70N binding affinity is more than 1000-fold weaker than RPA70AB and all evidence shows 70N is primarily a protein-protein interaction domain targeting transcription factors and checkpoint proteins. This study aims to resolve this controversy by analyzing the effect of 70N on the ssDNA binding activity of the high affinity RPA DNA binding domains, 70A and 70B. We propose the use of size exclusion chromatography and isothermal titration calorimetry (ITC) to do a systematic comparison of the DNA binding properties of 70AB versus the 70NAB construct. We expect to show that 70AB DNA binding affinity is the same as 70NAB. This will provide conclusive evidence that the 70N domain is not involved in binding ssDNA.

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The Role of DNA "Bendability" in the Indirect Read-Out Mechanism of Protein-DNA Interactions
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Integration host factor (IHF) from *E. coli* is a DNA-bending protein that recognizes and binds to its specific sites primarily by the indirect read-out mechanism, in which sequence-dependent DNA dynamics and flexibility play an important role. The crystal structure of IHF bound to a 35-bp long cognate site H' indicates that the DNA is kinked at two sites separated by ~9 bp, resulting in a ''U-turn'' bend of the DNA. We use laser temperature-jump to perturb the IHF-DNA complex, and time-resolved FRET on end-labeled DNA substrates to monitor the bending/unbending dynamics. In our previous studies, we suggested that spontaneous DNA bending from transient disruption of base-pairing and/or stacking interactions at the site of the kinks may be the rate-limiting step in the transition from the nonspecific to the specific complex. Here, we investigate DNA bending kinetics for substrates with mismatched pairs introduced at the site of the kinks. These internal ''loops'' are expected to decrease the energetic cost of bending the DNA, which is reflected in the >10-fold increase in the binding affinity. Kinetics measurements on IHF bound to such DNA reveal

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