Single-Molecule Studies of Chromatin Fibers: A Personal Report

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Summary. With the advent of single-molecule techniques, macromolecular science has reached new horizons. Nowadays, we can observe, touch, stretch and twist biological macromolecules or their complexes, one-at-a time, in attempts to better understand their mechanical properties and to gain insights into their behavior in the living cell. Chromatin structure and function has been the focus of our research interests for many years. In the past decade, we have added some of the newly emerged single-molecule approaches to the more traditional biochemical and biophysical methods that we have been using throughout the years. This paper briefly summarizes our studies on individual chromatin fibers using the atomic force microscope (AFM), optical tweezers, and magnetic tweezers. We believe that our results so far have contributed significantly to our understanding of chromatin, but we also hope that this is only the beginning, and that more exciting times lie ahead.

Eukaryotic cells organize their DNA as chromatin, a nucleoprotein complex consisting of DNA, histones, and non-histone proteins (VAN HOLDE, 1988; TSANEV et al., 1992; WOLFFE, 1998; TURNER, 2002). The basic repeating unit of chromatin structure is the nucleosome, a particle in which ~146 bp of DNA are wrapped in a left-handed superhelix around an octamer of core histones, two molecules each of H2A, H2B, H3, and H4 (ARENTS and MOUDRIANAKIS, 1993; LUGER et al., 1997; HARP et al., 2000). Nucleo-

somes are connected with linker DNA, which is the binding site for linker histones (histones of the lysine-rich H1 family) and non-histone proteins. This array of nucleosomes appears under the electron microscope as beads-on-a-string structure at low ionic strength; further compaction occurs upon increasing the ionic strength to form the highly condensed chromatin fibers characteristic of the eukaryotic nucleus. The exact way in which these higher-order structures are organized and dynamically regulated remains a mystery (VAN HOLDE, 1988; VAN HOLDE and ZLATANOVA, 1996).

Chromatin has recently become a major focus of research effort, since we have come to realize that chromatin is not just a passive means to compact huge amounts of DNA into the confines of the eukaryotic nucleus, but is an active participant in the regulation of all activities that require access to the DNA: replication, transcription, and repair. Chromatin structure dynamically changes in space and time, to respond to the needs of the cell to differentially control specific genes in response to specific stimuli. Understanding the complexity of chromatin organization, dynamics and function requires a concerted effort on the part of the scientific community and the application of new, cutting-edge technology.

The last decade has witnessed the birth of a new field of interdisciplinary research in molecular biology that uses physics-based approaches to study the structure and dynamics of biological macromolecules, one molecule at-a-time (LEUBA and ZLATANOVA, 2001). The single-molecule biology research is based on looking at the structure and/or behavior of individual macromolecules — DNA, proteins, polysaccharides, or their complexes, one-at-a-time. The observation of a large number of such molecules constitutes the basis for obtaining statistically valid

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Fig. 1 a. Principle of operation of the AFM. The tip/cantilever raster-scans the biological sample on an atomically flat surface, and a topographic image is created from the changes in the laser signal caused by the deflections of the cantilever; these, in turn, are caused by atomic-level tip/sample interactions. **b.** Broadening effect of the AFM tip. The actual topographic AFM image created by the AFM tip is depicted by the solid curve drawn over the macromolecules on the substrate surface. The broadening effect affects only the end-to-end lateral dimensions of the feature; the heights of the sample and the distance between its internal features are not affected.

data. The unique feature of these methods is that they allow elucidation of the heterogeneity of the properties of individual molecules; such heterogeneity is often masked in bulk biochemical and biophysical experiments, which only evaluate properties averaged over the entire ensemble of the molecules under study. Here, we will briefly summarize the main results of our effort to study chromatin structure and dynamics using single-molecule approaches.

Atomic force microscopy (AFM): static analysis of chromatin structural characteristics

Our involvement in single-molecule studies of chromatin dates back a decade ago when we began using atomic force microscopy (AFM) as a way to visualize chromatin fiber structure. In the AFM, the sample is deposited on atomically flat surfaces (mica, glass, gold), and raster-scanned by a sharp tip mounted on the back of a flexible cantilever (Fig. 1a). Interactions of the atoms on the apex of the tip and on the sample cause deflections of the cantilever, whose direction and magnitude are recorded by a laser-based photo-detection system. Since the tip is not infinitely thin (the radius of curvature at its apex is usually ~ 5 nm), it cannot follow the contours of the sample with high-fidelity, producing the so-called broadening effect of the tip (Fig. 1b). Due to this effect, all samples look somewhat larger than they actually are, and individual tips may produce somewhat different lateral measurements for the structural features observed. We have used the AFM to visualize a wide variety of chromatin samples: extracted from cells, biochemically manipulated, or reconstituted from well-characterized, purified individual components. In addition to visual inspection of the images, we have also performed quantitative measurements of center-to-center internucleosomal distances, angles between DNA entering and exiting nucleosomes, fiber heights, and, on occasion, have counted the number of nucleosomes per unit fiber length. The main results from our imaging studies are summarized in Table 1, and some representative images are shown in Figure 2. We will focus on only a couple of results that have a more general impact on our views of chromatin fiber structure.

Imaging chromatin fibers deposited on mica or glass from low ionic strength buffers, i. e. in their purportedly most extended state, revealed rather compact, irregular, three-dimensionally organized fibers (see example in Fig. 2). The fibers appeared as beads-on-a-string only after removal of linker histones, and a similar morphology was observed on nucleosomal arrays reconstituted from naked DNA and core histones only. The diameter of the native fiber was ~ 30 nm even at its most extended state, and was ~ 10 nm only in linker histone-lacking fibers. Thus, the accepted notion that the fiber forms a 10 nm structure at low ionic strength, and then compacts into a 30 nm structure upon salt addition, is flawed; this notion came from electron microscopy observations in which samples are highly dehydrated, and may thus lose their native structure. Thus, the term 30 nm fiber is a misnomer for the salt-

Table 1. Major results and conclusions from our AFM imaging of chromatin fibers

Imaged substrate	Major results	Reference
CE ^a chromatin fibers at different salt concentrations	At 10 mM NaCl the fiber condenses slightly; at 80 mM NaCl highly compacted, irregularly segmented fibers	ZLATANOVA et al., 1994
Unfixed or glutaraldehyde-fixed CE chromatin fibers	Loose, three-dimensional, 30 nm irregular structures even in the absence of salt; beads-on-a-string fibers seen only in H1/H5-depleted fibers	LEUBA et al., 1994
Native or LH-depleted fibers	Loose, three-dimensional, 30 nm irregular structures even in the absence of salt; beads-on-a-string fibers seen only in H1/H5-depleted fibers	YANG et al., 1994
Progressively trypsinized CE chromatin fibers	Cleavage of LH tails results in fiber lengthening whereas cleavage of H3 N-tails flattens the fiber; Zig-zag morphology persists at later stages of digestion and is attributed to retention of the globular domain of LH in fiber	LEUBA et al., 1998b
Reconstitution of CE chromatin fibers depleted of LH or of LH and the N-tails of H3 with either intact H5 or GH5	The three-dimensional organization of nucleosomes in extended (low ionic strength) chromatin fibers requires the globular domain of LHs and either the tails of LH or the N-terminal tails of H3	LEUBA et al., 1998a
Chromatin fibers isolated from cells with normal or elevated levels of m ⁵ C; Nucleosome arrays reconstituted from either unmethylated or <i>in vitro</i> methylated 208-12 ^b and core histones; additional reconstitution of LH	DNA methylation induces chromatin compaction only in the presence of bound LH; AFM results substantiated by MNase digestion patterns and sucrose gradient centrifugation; AFM imaging can visualize alternative nucleosome positioning on adjacent 208-bp repeats (the distribution of center-to-center distances on 208-12 is bimodal)	KARYMOV et al., 2001
Nucleosome arrays reconstituted from 208-18 and either histone octamers, H3/H4 tetramers or the histone-fold protein HMf from <i>Archaea</i>	The HMf-nucleoprotein complexes are <i>bona fide</i> chromatin structures; The HMf-containing mononucleosomes are less stable than the canonical octasomes	TOMSCIHK et al., 2001
Nucleosomal arrays reconstituted from a 5.4 kbp circular template and control or totally-tailless recombinant histones	Beads-on-a-string; Center-to-center distance frequency distributions indistinguishable for control and tailless reconstitutes	AN et al., 2002

^aCE: chicken erythrocyte^b208-12: a tandemly repeated DNA sequence that has a nucleosome positioning 208-bp-sequence repeated 12 times (SIMPSON et al., 1985).

compacted fiber, and its use should be dropped, as already suggested by VAN HOLDE and ZLATANOVA (1995, 1996).

The other equally important observation was that the fibers were in no way regular, contrary to the expectations of the solenoid model proposed in the mid 1970's (FINCH and KLUG, 1976; THOMA et al., 1979). The AFM images, taken together with images obtained by cryo-electron microscopy (e. g. BEDNAR et al., 1995, 1998; GRIGORYEV, 2001), served as a bases for reevaluation of the validity of the solenoid model

(VAN HOLDE and ZLATANOVA, 1995, 1996; WOODCOCK and HOROWITZ, 1995; see also ZLATANOVA et al., 1998, 1999). The new model of chromatin fiber structure suggests nucleosomal particles connected by stretches of straight linker DNA; salt-induced compaction occurs, at a first level, as a result of decreasing the entry-exit angle of the DNA into and out of the particle. Mathematical modeling based on known structural parameters of the core particles and on the assumption of straight linkers produced fibers of the morphology portrayed in Figure 2c (WOODCOCK et al.,

Fig. 2. Some example AFM images of chromatin fibers isolated from cells or reconstituted *in vitro*, and mathematical modeling. **a.** Unfixed chicken erythrocyte chromatin fibers imaged on glass in air (S. LEUBA and G. YANG, unpublished). **b.** Linker histone-depleted chicken erythrocyte chromatin fiber on mica in air (S. LEUBA, unpublished). **c.** Mathematical model of a chromatin fiber. See text. **d.** Nucleosomal array, reconstituted from 208-18 DNA and core histones by salt dialysis, and imaged on mica in air. The DNA substrate for reconstitution contains 18 tandem repeats of a 208 bp sequence; each repeat is known to position a single nucleosomal particle at a specific position. Thus, reconstitution on the 208-18 DNA fragment results in a string of ~ 18 nucleosomes. **e.** Circular plasmid reconstituted with recombinant assembly factors and intact recombinant core histones. **f.** the same plasmid reconstituted with core histones missing their N-terminal extensions. **g.** H3/H4 tetrasome array, reconstituted from 208-18 DNA and H3/H4 tetramers by salt dialysis, imaged on mica in air. **h.** Nucleosomal array, reconstituted from 208-18 DNA and archaeal-histone HMf (Histone from *Methanothermobacter fervidus*) by salt dialysis, imaged on mica in air.

1993; LEUBA et al., 1994; YANG et al., 1994; VAN HOLDE and ZLATANOVA, 1996).

AFM imaging can be successfully combined with biochemical manipulations to address important structural issues. We will illustrate this approach on the example of experiments aimed at determining the molecular determinants of fiber structure. In the approach presented in Figure 3a, chromatin fibers were first biochemically manipulated by salt dissociation and mild trypsinolysis, to controllably remove either the linker histones or the linker histones and 26 amino acids from the N-terminal of histone H3. These depleted fibers were then reconstituted with either intact linker histone or its isolated globular domain. AFM imaging of the resulting structures, and quantitative measurements of the parameters mentioned above revealed an interesting, and rather unexpected feature: the native three-dimensional fiber morphology that was lost upon removal of linker histones could be restored by the presence on the fibers of the globular domain of linker histones and either the tails of linker histones or the N-terminal tails of H3 (Fig. 3b). This structural redundancy of the tails of linker histones and those of H3 in three-dimensionally organizing linear arrays of nucleosomes can be explained on the basis of the crystal structure of the core particle (Fig. 3c). Of all histone tails that protrude from the particle, only the tails of H3 are in close proximity to the linker DNA, and can thus participate in the neutralization of its charges, to allow fiber compaction. The results from this reconstitution study (LEUBA et al., 1998a) were in agreement with the data from a study of partially trypsinized chromatin fibers (LEUBA et al., 1998b).

Atomic force microscopy (AFM): an attempt at a dynamic study of real-time nucleosome unraveling under force

AFM can be used for force spectroscopy of biological macromolecules (ZLATANOVA et al., 2000). In this application, the cantilever with the attached tip is moved in the z-direction only, upwards and downwards. If a molecule that is immobilized on the surface is also attached to the tip (either through specific linkages or through non-specific adhesion), then the upward movement of the cantilever will stretch the molecule. The behavior of various macromolecules — DNA, proteins, polysaccharides — under such mechanical load has been the subject of numerous recent studies (reviewed in ZLATANOVA et al., 2000).

We attempted to use AFM stretching as a way to

determine the forces that keep the integrity of nucleosomal particles. We expected that the mechanical stretching of a chromatin fiber will eventually lead to dissociation of the histones from the DNA, and we hoped to be able to measure the force required for this dissociation (Fig. 4a). Indeed, when we stretched native or linker histone-depleted chromatin fibers from chicken erythrocytes (LEUBA et al., 1999), or reconstituted nucleosomal arrays (LEUBA et al., 2000), we recorded force-extension curves with a saw-tooth appearance (Fig. 4b). Such curves have been reported in the literature for stretching of multi-domain proteins (RIEF et al., 1997; OBERHAUSER et al., 1998), and have been interpreted in terms of each peak representing the unfolding of one individually-folded domain. Careful inspection of the chromatin stretching curves, however, revealed that the peaks in the force curves did not represent opening of individual nucleosomes, but rather reflected stepwise lengthening of the suspended portion of the fiber upon detachment, popping-off of individual nucleosomes from the glass surface. AFM stretching of chromatin fibers will require manipulation of a fiber that is attached only at its ends to the surface and the tip, in order to avoid surface attachment artifacts. Such experiments have not been reported yet.

Mechanical stretching of a chromatin fiber with optical tweezers: unraveling of individual nucleosomal particles

The existence of surface attachment artifacts in the AFM stretching experiments made us turn to the use of optical tweezers. In collaboration with a group of Dutch physicists, we performed a series of experiments that allowed us, for the first time, to measure nucleosomal strength (defined here as the force that is needed to unwrap the DNA from around the histone octamer; BENNINK et al., 2001a).

The principle of operation of optical tweezers is based on the fact that light can exert pressure on a bead of certain optical properties in such a way that the bead can be kept suspended (levitated) at a point close to the waist of a laser beam shone through an objective (Fig. 5a) (BINNIG et al., 1986). If a trapped bead is forced out of this equilibrium position by some external force, it will be brought back to it by the action of light. As illustrated in the figure, the external force will be counterbalanced by the trapping force exerted on the bead by light. Thus, in the set-up of optical tweezers, one can measure the force applied to a macromolecule by attaching one of its ends to an optically-trapped bead, and its other end to

Fig. 3. Structural redundancy of the LH tails and the N-terminal tails of histone H3 in the three-dimensional organization of nucleosomes in the extended chromatin fiber. **a.** Experimental approach (for details see LEUBA et al., 1998a). **b.** Schematic of results: the three schematic drawings of nucleosomes show the three cases in which irregular, three-dimensionally organized chromatin fibers can be observed by AFM imaging and quantitations. The proper fiber structure requires the presence of the globular domain of LH, and either the tails of the LH, or the N-tails of histone N3. If both of these tails are missing, the fibers lose their three-dimensionality, flattening on the AFM surface. **c.** Possible explanation of the results based on the crystal structure of the nucleosome (LUGER et al., 1997). A two dimensional trace of the crystal structure is shown in black. The portions of the N-terminal core histone tails (and C-terminal tails of H2A) that are visible in the crystal structure are depicted in color and labeled appropriately. The location of the LH globular domain is indicated by the spherical object labeled GH5. A possible path for linker DNA is indicated by dashed blue lines. The N-terminal tail of core histone H3 protrudes from the particle at a location close to the dyad axis; such a location would allow this tail to interact with the incoming and exiting linker DNA helices, neutralizing their negative charges. Such a scenario would occur in cases when the LH tails are not strongly bound to the linker DNA, e. g. when they are highly phosphorylated.

Fig. 4. Schematics of the AFM approach to unravel single chromatin fibers by force. **a.** AFM-mediated unraveling of nucleosomes (concept leading to the experiments of LEUBA et al., 1999, 2000). An AFM tip is pushed into a chromatin fiber adhering to a glass surface in buffer. When the cantilever is raised, it deflects because of the chromatin tether between the tip and the surface. If a nucleosome unravels, the chromatin tether will lengthen abruptly leading to restoring the normal geometry of the cantilever. **b.** Example force extension curves of chicken erythrocyte chromatin fibers resulting from the experiment depicted in **a**.

another bead held by a pipette (through which force is applied to the molecule).

Our experimental approach was as follows: first we attached a naked λ -DNA molecule between the two beads, then we initiated chromatin assembly by introducing cell-free extracts from *Xenopus laevis* eggs into the flow cell. These extracts contain a pool of core histones and all protein factors necessary for chromatin assembly. The formation of the chromatin fiber was followed by monitoring the distance between the two beads: the shortening of the DNA tether that resulted from nucleosome formation was seen as a decrease in the distance between the bead

held in the pipette and the 'free' bead (for technical reasons the optical tweezers was turned off during this stage of the experiments). Once the fiber with ~ 240 nucleosomes assembled on the λ -DNA, the optical tweezers was turned on, and the fiber stretched by moving the pipette away from the trapped bead (Fig. 5b). An example force extension curve is presented in Figure 5c, with a blow-up of a small portion of this curve shown in Figure 5d. The sawtooth pattern of the force curve reflected discrete opening events, i. e., changes in the fiber structure that lengthened the stretched intermediates jumpwise. These abrupt changes in the fiber length were

Fig. 5. a. Schematic of the concept of optical trapping. For more explanation, see text. **b.** Schematic of our experimental approach (BENNINK et al., 2001a) to mechanically stretch chromatin fibers directly assembled in the flow cell from a single λ -DNA molecule and *Xenopus* cell free extracts. **c.** A representative force curve. **d.** A blow-up of a portion of the force-extension curve, with discrete opening events (unraveling of individual nucleosomes) clearly visible (BENNINK et al., 2001a). The length increments in the fiber accompanying each opening event are indicated as Δx_n . The different Δx_n distances correspond to unraveling of one nucleosome at a time, or of two or three nucleosomes simultaneously. These values are quantized with a unit step of ~ 65 nm which represents the lengthening of the fiber upon dissociation of one single nucleosome.

accompanied by sudden drops in the force; these drops gave the curve its saw-tooth appearance. Careful analysis of the data showed that the force dropped as the fiber lengthened in increments of ~ 65 nm (or multiples thereof). This is the expected fiber lengthening if individual (or small groups of) nucleosomes unraveled in jumps, releasing 65 nm of DNA that had been wrapped around the histone octamer. These opening events occurred at forces of 20–40 pN, in agreement with data from two additional studies (CUI and BUSTAMANTE, 2000; BROWER-TOLAND et al., 2002; for more detailed discussion on these two papers, see ZLATANOVA and LEUBA, 2002, 2003).

What is the physiological relevance of the data

from such *in vitro* pulling experiments? We believe that the strength of the nucleosome has direct consequences for transcription elongation (and other intranuclear processes that use DNA as a template). It has been recently shown that DNA and RNA polymerases from prokaryotic origin can develop forces as high as ~ 30 pN when threading the DNA molecule through their catalytic centers *in vitro* (YIN et al., 1995; WANG et al., 1998; WUITE et al., 2000). If, by inference, the eukaryotic enzymes develop forces in the same range, then the moving polymerases would be capable of clearing nucleosomes out of the way by themselves, without the need for auxiliary factors (e.g. chromatin remodeling factors). Such a proposition

Fig. 6. Schematic of the magnetic tweezers experimental approach used in our chromatin assembly experiments (LEUBA et al., 2003). **a.** Concept of magnetic tweezers. **b.** Schematic of the magnetic tweezers setup (box above is an enlargement of the square glass cuvette with the DNA-tethered superparamagnetic bead and the external permanent magnet). **c.** Schematic of stopped-flow chromatin assembly experiments. See text.

seems to be highly plausible in view of the numerous structural and functional similarities among the RNA polymerases from a variety of sources (UPTAIN et al., 1997; VON HIPPEL, 1998; MYERS and KORNBERG, 2000; EBRIGHT, 2000).

Following chromatin assembly under force with magnetic tweezers

Attempts to manipulate magnetic objects in solution for biologically-relevant research date back more than half a century (CRICK and HUGHES, 1949; YAGI, 1960) [for further historic notes, see GOSSE and CROQUETTE (2002)]. The prototype of the instrument we are using was built in the laboratories of D.

Fig. 7. Example experimental data on chromatin assembly with magnetic tweezers (LEUBA et al., 2003). **a.** Raw assembly curve recorded at 0.6 pN in a flow-stopped experiment. **b.** A rheostat experiment, with step-wise changes of the distance between the external magnet and the cuvette, hence of the force applied to the magnetic bead. **c.** Data from 18 individual assembly experiments performed at different forces: each point is the initial assembly rate at a given force. The dotted curve is an exponential fit to the data. **d.** An assembly curve recorded at 7.6 pN. Note the upward steps on the otherwise descending curve. These steps appear as a result of the dynamic equilibrium between nucleosome assembly and disassembly (for further details, see text, and LEUBA et al., 2003).

BENSIMON and V. CROQUETTE in Paris, and used in a series of high-impact studies of the properties of superhelical DNA (STRICK et al., 1996, 1998a, b; ALLEMAND et al., 1998), and the activity of various enzymes, including DNA polymerase (MAIER et al., 2000) and various topoisomerases (STRICK et al., 2000; CRISONA et al., 2000; DEKKER et al., 2002).

In magnetic tweezers, the macromolecule is attached between a surface and a magnetic bead (Fig. 6a). Manipulation of the external magnetic field can be used to apply stretching forces onto the tethered molecule, and/or to induce precisely known levels of superhelicity: stretching is achieved by changing the distance between the magnets and the cuvette, while superhelicity is introduced by rotating the external magnets either clockwise or counterclockwise. A schematic of our instrumental set-up is presented in Figure 6b (a blow-up of the cuvette with the DNA tether between the inner surface and the magnetic bead is also drawn).

We set up to investigate the dependence of chromatin assembly on the force applied to the DNA tether. This was an important issue to study, since it might give us an idea of how fast, and under what conditions nucleosomes form in the wake of passing polymerases. At least some polymerases seem to displace, temporarily, the nucleosomes out of their way; thus, nucleosomes need to reform on the already transcribed portion of the DNA, in order to restore chromatin function in DNA packaging and regulation of gene expression. The DNA molecule exiting the enzyme is still under the tension, as the polymerase pulls on the DNA to thread it through its active center.

In our experiments, we first attached a single λ -DNA molecule between the inner wall of the cuvette and the magnetic bead. We then introduced into the cuvette buffer solution containing preformed histone octamers and a recombinant nucleosome assembly factor (NAP-1); as soon as we saw initial chromatin formation (shortening of the distance between the wall and the bead), we stopped the flow and allowed assembly only against the magnetic force (Fig. 6c). The initial assembly rate was measured from the linear portion of the assembly curve at each given force (an example curve is presented in Figure 7a). It became clear that there was a strong dependence of the assembly rate on the force applied to the DNA tether (Fig. 7c), in agreement with some earlier, more preliminary single-molecule data (LADOUX et al., 2000; BENNINK et al., 2001b).

In a more sophisticated set of experiments, we changed the applied force rheostatically, in the course of a single assembly experiment (see data in

Fig. 7b). The data revealed a very interesting, and potentially important characteristic feature of the assembly process: the assembly rate changed instantaneously upon changing the tension applied to the DNA tether. This may mean that *in vivo* the assembly rate is dictated, to a large degree, by the DNA tension, which in turn is a function of the rate of transcription.

Finally, Figure 7d illustrates another interesting characteristic of the assembly process. The monotonous downward assembly curve (change of DNA tether length with time) is sometimes interrupted by upward-going stretches. As detailed in LEUBA et al. (2003) these abrupt changes in the curve reflect the dynamic equilibrium between nucleosome assembly and disassembly. This is the first real-time demonstration of this phenomenon that has previously been observed in mononucleosome bulk experiments (YAGER and VAN HOLDE, 1984), and theoretically predicted (WIDOM, 1999).

The next step in studying chromatin assembly under force will be on topologically constrained DNA tethers attached at the four ends of the DNA double-helix to the bead and the surface. Such an attachment will preclude the swivel of the tether around the attachment points, and will thus mimic more closely the situation *in vivo*, where DNA is anchored to insoluble components of the nuclear matrix in a topologically constrained manner (e. g. ZLATANOVA and VAN HOLDE, 1992).

Concluding remarks

The work described above illustrates the first steps in applying single-molecule static and dynamic approaches to the study of chromatin. It is our belief that the chromatin community will recognize the potential of and will embrace the new methodology, so that our understanding of chromatin structure, dynamics, and function reaches new levels.

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