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Chromatin Imaging with Time-Lapse Atomic Force Microscopy

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Abstract

Time-lapse atomic force microscopy (AFM) is widely used for direct visualization of the nanoscale dynamics of various biological systems. The advent of high-speed AFM instrumentation made it possible to image the dynamics of proteins and protein-DNA complexes within millisecond time range. This chapter describes protocols for studies of structure and dynamics of nucleosomes with time-lapse AFM including the high-speed AFM instrument. The necessary specifics for the preparation of chromatin samples for imaging with AFM including the protocols for the surface preparation are provided.

Keywords

Chromatin dynamics; Nucleosome dynamics; Atomic force microscopy; AFM; Time-lapse AFM; Single-molecule techniques

1 Introduction

Chromatin dynamics is needed for executing all genetic function by the cell such as DNA replication and transcription. A fundamental unit of chromatin, the nucleosome core particle (NCP), is very compact and considered stable. A regular NCP includes 147 bp of DNA duplex that is tightly wrapped around an octameric core, comprising histones H2A, H2B, H3, and H4. The NCP structure is stabilized by electrostatic interactions, specific hydrogen bonds, and salt bridges, identified in the high-resolution crystal structure of NCPs [1]. The high stability of the NCPs leads to the question of how the DNA within the nucleosome can be accessed by regulatory proteins and polymerases for transcription and DNA replication. Chromatin remodeling proteins are capable of the DNA dissociation from the histone core [2–5] suggesting that nucleosome dynamics should be involved into the nucleosome dissociation process. Indeed, studies performed in the past decade with the use of various techniques, including single-molecule approaches, showed that the NCP is not a static structure. Rather, DNA can spontaneously and transiently dissociate, and single-molecule fluorescence and time-resolved techniques revealed that nucleosomes undergo local dissociation of DNA in the absence of remodeling proteins [6–10] and this process occurs on the subsecond timescale [8]. Atomic force microscopy (AFM) was instrumental in direct visualization of the nucleosome dynamics with the nanometer-range spatial resolution [11, 12]. Implementation of high-speed AFM (HS-AFM; (reviewed in refs. 13–15) capable of the nanometer resolution on the millisecond timescale made it possible to identify various pathways of the NCP dynamics [16, 17]. The AFM methodologies enabling direct

visualization the chromatin dynamics using regular and high-speed time-lapse AFM modes are outlined in this chapter.

A schematic illustrating the principles of the AFM operation is shown in Fig. 1. A sharp stylus (AFM tip shown as a triangle) reads the sample topography (shown as a bumpy profile) while it moves over sample in a raster pattern termed scanning. The tip cantilever works as a spring pressing the tip against the sample during scanning. The vertical movement of the tip is detected by the optical lever principle in which the tip displacement is measured by the changing of the laser spot location on the position-sensitive photodetector (PSD). Note that no special contrasting sample is needed for AFM imaging. Additionally, scanning can be performed in any media at ambient conditions, including physiological conditions enabling direct visualization of the sample dynamics as described in this chapter. AFM instruments include a number of important features that enable the production of high-resolution images. First, the position of the sample relative to the tip is controlled by the scanner with an accuracy of less than 1 nm. Second, the tip can be atomically sharp. Third, the displacement of the tip relative to the surface is determined with subnanometer accuracy. All these features are critical for the use of AFM for biomedical studies including the chromatin dynamics.

2 Materials

Prepare all solutions using deionized water; Aquamax Water System (Aquamax Laboratory, Van Nuys, CA) produces low-conductivity water (18.2 M Ω) with a required quality. Use analytical grade reagents when preparing the solutions.

1. A vacuum cabinet or desiccator for storing samples. A Gravity Convention Utility Oven (VWR) is recommended.
2. Plastic tubes, 15 mL.
3. Eppendorf tubes, 1.5 mL.
4. Plastic cuvettes.
5. Scissors.
6. Razor blades.
7. 2 L glass desiccators and vacuum line (50 mmHg is sufficient).
8. Pipettes with plastic tips for rinsing the samples.
9. Tweezers.
10. Gas tank with clean argon gas. Nitrogen gas can be used as well.
11. Mica substrate: Any type of commercially available mica sheets (green or ruby mica) can be used. Asheville-Schoonmaker Mica Co (Newport News, VA) supplies thick and large (more than 5 $\times$ 7 cm) sheets (Grade 1) suitable for making substrates of different thickness and size.

12. Deionized water filtered through 0.2 μm filter for mica functionalization and AFM sample preparation.
13. Apparatus for protein gel electrophoresis.
14. AFM instruments.

3 Methods

3.1 Preparation of Nucleosomal DNA

The 601 Widom sequence was used as a template for the nucleosome assembly which has the very high affinity for binding of the histone core compared to other sequences [18].

1. Use plasmid pGEM3Z-601 that contains Widom-601 motif and generate DNA by PCR.
2. Run the PCR reaction (33 cycles of 94 °C/30 s, 54 °C/30 s, 72 °C/30 s) in buffer containing 2.5 mM MgCl_2 , 0.15 mM dNTPs, and 0.016 U/ μL of Taq DNA polymerase with the following primers: forward primer 5'-GEMf CGGCCAGTGAATTGTAATACG-3'; reverse primer GEMr 5'-CGGGATCCTAATGACCAAGG-3'. These primers produce 353 bp DNA template that was used in the AFM time-lapse experiments in [12, 17, 19].

3.2 Histone Octamer Assembly and Purification

Histone octamers were assembled according to the protocol described in [20]. Commercially available histones H2A, H2B, H3, and H4 (New England Biolab, Ipswich, MA) are suitable.

1. Take 80 μL of 1 mg/mL solutions of each histone (H2A, H2B, H3, H4), load them on Microcon centrifugal filter devices (MWCO 3,000, Millipore, Billerica, MA), and spin at $12,700 \times g$ for 35 min. Make 15 μL total volume in the recovery spin.
2. Add 100 μL of unfolding buffer (UB) containing 6 M guanidine chloride, 20 mM Tris-HCl, pH 7.5, and 5 mM DTT.
3. Use UV spectrophotometer (280 nm) to measure the histone concentration with a buffer as a reference. 1 mg/mL is the desired histone concentration.
4. Combine the histones in equal molar amounts.
5. Dialyze the histone mixture at 4 °C using Slide-A-Lyzer dialysis cassette with molecular weight cutoff of 7,000 (Pierce) against three changes of 250 mL of refolding buffer containing 2 M NaCl, 10 mM Tris-HCl, pH 7.5, 1 mM Na-EDTA, and 5 mM 2-ME. Overnight dialysis at 4 °C is sufficient for the assembly process. Measure the protein concentration with UV spectrophotometer.
6. Concentrate the sample with Microcon centrifugal filter devices (MWCO 10,000) in ~20 μL volume. A typical concentration run is for 10 min at $12,700 \times g$ followed by the recovery spin.

7. Perform gel permeation chromatography to separate fully assembled histone octamers from tetramers, dimers, and monomers.
8. To accomplish this step, the sample is loaded on Superdex 200 PG 3.2/30 column (GE Healthcare), which was pre-equilibrated with RB at 4 °C and fractions are collected. Figure 2 shows one of such chromatograms.
9. Analyze selected fractions around the major peak 1 and peak 2 by 15 % PAGE-SDS gel electrophoresis. The running acrylamide gel contained 15 % acrylamide, 0.375 M Tris, 0.1 % SDS, 0.08 % ammonium persulfate, and 0.05 % TEMED. The stacking gel is recommended to use for improvement of the resolution of the protein bands. It contained 6 % acrylamide, 0.125 M Tris, 0.1 % SDS, 0.08 % APS, and 0.05 % TEMED. Figure 3 shows the results of such an analysis. The gel was stained using Coomassie Blue. Lanes containing octamer (left fractions of first peak), tetramer (fractions of the corresponding to the right segment of peak 1), and dimers appearing in peak 2 are indicated.
10. Fractions containing histones H2A, H2B, H3, and H4 in equal ratios were pooled and concentrated using Microcon concentration device MCWO 10,000. This preparation can be stored in 50 % (v/v) glycerol solution at –20 °C.

3.3 Nucleosome Reconstruction

1. Mix in equimolar concentrations histone octamer sample prepared as described above with the DNA template containing the Widom 601 sequence in the refolding buffer containing 2 M NaCl and keep for 30 min at room temperature.
2. Dialyze the mixture into buffers of decreasing ionic strength using Slide-A-Lyzer Mini Dialysis Unit MWCO 3500 (Pierce). A dilution series was prepared using 10 mM Tris–HCl with NaCl concentrations 1 M, 0.67 M, and 0.5 M. Keep diluted samples at 4 °C for 1 h before dialysis against one change of volume of 0.2 M NaCl overnight.
3. Concentrate nucleosomes to obtain ~300 nM using Microcon centrifugal filter device, MWCO 10,000 (7,000 × *g* for 10 min at 4 °C), and dialyze against one change of 200 mL of the buffer containing 10 mM Tris–HCl, pH 8.0, and 1 mM EDTA for 3 h at 4 °C. Take UV spectrum to measure the DNA concentration and run AFM to characterize the sample (*see protocol below*). Figure 4 shows AFM images of assembled nucleosomes. The sample at such conditions remains stable for several months.

3.4 Sample Preparation for AFM Imaging in Air

The sections below describe the procedure for the preparation of samples of nucleosomes for AFM imaging. This protocol utilizes the methodology of mica functionalization with 1-(3-aminopropyl) silatrane (APS-mica) that provides very reproducible and reliable results. The APS synthesis protocol is described in [21].

3.4.1 Preparation of the APS-Mica Substrates

1. Prepare a 50 mM APS stock solution in water and store it in refrigerator. The stock solution can be kept for more than a year at 4 °C.
2. Prepare the APS working solution from the stock in a 1:300 ratio in water by diluting 45 μ L of the stock in 15 mL H₂O to make the working APS solution for mica modification; it can be stored at 4 °C for several days.
3. Cut both sides of the mica sheets to make strips of the needed size (typically 1 cm $\times$ 3 cm) and cleave the strips with a razor blade, or scotch tape to make them as thin as $\sim$ 0.1 mm. Do not touch the cleaved mica surface.
4. Place the mica strips in appropriate plastic tubes (*see* Note 1 for more details).
5. Pour the working APS solution to cover the mica strip completely.
6. Leave the tubes/cuvettes on the bench for 30 min. After 30 min discard the APS solution.
7. Rinse both sides of the mica with deionized water and completely dry both sides of the mica strips under argon flow.
8. The strips are ready for the sample preparation. Additional storage in a vacuum for 1–2 h is recommended when the environment is humid. Dry APS mica strips can be stored under Ar gas in the clean dry cuvette or vacuum desiccator for 2 weeks without losing the DNA binding activity. *See* Notes 2 and 3 for the APS mica storage and the shelf life.

3.4.2 Preparation of the Nucleosome Samples for Imaging of Dried Samples in Air

1. Prepare the solution of the nucleosome in a buffer containing 10 mM HEPES (pH 7.5) and 4 mM MgCl₂. The nucleosome concentration between 1 nM and 0.3 nM is optimal.
2. Cut the APS mica substrates to a desired size (1 $\times$ 1 cm squares for the Multi Mode AFM instrument (Bruker-Nano, Santa Barbara, CA) are optimal) and place 5–10 μ L of the nucleosome solution in the middle of the substrate for 2 min.
3. Rinse the sample thoroughly with deionized water (1–3 mL per sample) to remove all buffer components. 5 mL or 10 mL plastic syringes are useful for rinsing. Attach an appropriate plastic tip instead of a metal needle.
4. Dry the sample with clean argon gas. Additional drying of samples for an hour or two prior to imaging is recommended to ensure low tip adhesion. The samples can be stored in vacuum cabinets or desiccators filled with argon. The

¹Depending on the size of the mica strip, the plastic disposable 3 mL cuvettes or plastic 15 mL tubes are suitable for these purposes.

²As prepared, the APS mica sheets can be stored dry (plastic tubes or cuvettes) in the argon atmosphere for at least a week.

³Nitrogen gas can be used but it is recommended to use Ar gas as it is heavy and does evaporate from the tube.

samples, as prepared, can be imaged many times provided that after imaging they are stored as described. Their shelf life is several months [21].

3.5 AFM Imaging

This section describes the AFM imaging procedures for imaging dried and wet samples.

3.5.1 AFM Imaging in Air—For imaging in air, any type of tip with a spring constant of approximately 40 N/m and a resonant frequency between 300 and 340 kHz can be used. For example, Olympus silicon probes (Asylum Research, Santa Barbara, CA) with a spring constant of 40 N/m and a 300 kHz resonant frequency in air work reliably in the tapping/oscillating mode for imaging in air. Probes with similar characteristics are currently manufactured by a large number of other vendors.

1. Mount the sample prepared at **step 4** above onto the AFM stage using a double-stick tape.
2. Tune the AFM probe to find the resonance frequency corresponding to the AFM probe. Adjust the drive amplitude. For the Multi Mode AFM, 6–8 mV is typical. Set the image size to 100×100 nm and start approaching the surface.
3. Gradually reduce the set point until the surface of the sample is clearly seen. Increase the scan size and acquire the images.
4. Typical AFM images of mononucleosome sample obtained with the use of the functionalized procedures are shown in Fig. 4. Nucleosomes appear as white blobs with DNA filaments of different sizes on the flanks. The flank sizes correspond to the asymmetric position of 601 motif within the DNA template [12]. Note a number of features of the APS-mica procedure enabling the quantitative analysis of the samples [12]. First, the background is smooth, enabling unambiguous visualization of DNA. Second, the concentration of sample was adjusted in such a way that the molecules are spread over the surface with no overlap. Third, no glutaraldehyde fixation was used for obtaining these images.

3.5.2 Imaging in Aqueous Solutions—For imaging in liquid a regular AFM, Si_3N_4 , 100 μm long probes (SNL, Bruker-Nano/Veeco, Santa Barbara, CA) with a spring constant of approximately 0.06 N/m and a resonance frequency around 7–10 kHz are recommended. AFM probes with similar characteristics from other vendors are available. The protocols described below assume the use of Multi Mode AFM (Bruker-Nano/Veeco), but they can be adapted to any type of AFM. Protocol for imaging with HS AFM is given in a separate chapter.

1. Mount the tip on the tip holder.
2. Place the stage with the attached APS-mica substrate on the instrument stage. It is a scanner for the MM AFM instrument. Mica pieces 1×1 cm work well for MM AFM. Double-stick tape can be used to attach the modified mica

substrate to the metal discs. Do not glue APS-mica as the glue vapors react with the mica surface deteriorating the surface.

3. Use the video camera to find the tip and approach the surface manually, leaving a 500–100 μm gap between the tip and the surface.
4. Place a droplet of the prepared sample solution and readjust the spot position. The spot changes due to the difference in the refractive indexes of air and water. For MM AFM, 50 μL of the solution is sufficient to fill the gap. Note that due to the elevated hydrophobicity of APS-mica compared to bare mica, the spot does not spread far; therefore, no O-ring is required to keep the solution in place.
5. Find a resonance peak. Typically it is quite a broad peak, around 7–10 kHz, for the Multi Mode AFM instrument. Follow the recommendations given in the instrument manual on how to find the peak in fluid.
6. Start the computer-controlled approach. Operate with the set point voltage and drive amplitude parameters to improve the quality of images. Minimize the drive amplitude. The number varies from tip to tip, but the free amplitude as low as 10 nm or less and a scanning rate of approximately 2 Hz provide good-quality pictures.
7. The image acquisition starts with the area 1–2 μm , select the area of interest with ~ 500 nm, and start time-dependent acquisition. Scan rate 1–2 Hz is typical for MM AFM with 512×512 data acquisition density.

3.5.3 Imaging in Aqueous Solutions with High-Speed AFM—This section describes the protocol for the preparation of nucleosome samples for imaging with high-speed AFM (HS AFM) instrument designed by T. Ando [22]. This paper also provides a protocol for the instrument operation. The instrument is designed for mica disks as small as 1 mm and 1.5 mm available from RIBM (Tsukuba, Japan).

1. Glue the glass rod to the scanner stage with a nail polish and leave it for 15 min to dry.
2. Glue mica disk to the glass rod with silylated urethane resin (Konishi bond ultra versatile SU, available on Amazon). Very small amount of the glue is needed. Use plastic pipette tip to place a small droplet of the glue to the glass rod. Press the mica firmly with a clean pipette tip and let the glue to harden for two hours.
3. Cleave mica by removing the top layers using a scotch tape. Check the scotch tape and the mica surface for a good cleavage; use magnifying glass for better inspection of the cleavage.
4. Apply 2 μL of freshly prepared APS solution in DI water (167 μM) to a freshly cleaved mica, place a plastic cap with a wet filter paper disk inside on the top of the scanner, and allow the modification for 30 min. A wet paper disk creates a humid atmosphere avoiding the evaporation of APS solution. Photographs in

Fig. 5 show the scanner with the mica mounted on the sample holder and the plastic cap with a wet paper disk inside (a). Photo in (b) shows the sample holder covered with the cap. While the modification process is going, mount the cantilever, so you will be able to start with imaging as the sample is prepared.

5. Place a cantilever in the cantilever holder using a microscope. BL-AC10DS-A2 cantilevers (Olympus, Japan) modified by the electron beam deposition method and sharpened by using plasma etcher as described in [22] are recommended. Place a cantilever using a Teflon-coated tweezer. Avoid any static discharge, which can also damage the tip. Tighten the screws half way. Tap a little to make sure that the cantilever is sitting in the groove. Fully tighten the screws without using extra force.
6. Place the cantilever holder on the AFM stage. The tip faces up.
7. Rinse the cantilever holder chamber with 120 μL DI water three to four times. Then rinse with the buffer two times. Finally fill the chamber with 110 μL of imaging buffer so that the tip is covered with buffer. Such procedure allows to get the tip fully immersed into the buffer avoiding potential spill to the scanner. Follow the protocol described in [22] for additional information on the instrument operation.
8. Adjust the position of the cantilever so that the laser hits it.
9. When the APS functionalization step is completed, rinse the mica with 40 μL of DI water total by applying ~ 4 μL for each wash. At the end of fifth wash, place a water droplet and let it sit for 5 min to desorb any leftover nonspecifically bound APS. Then complete the rest of the wash (sixth to tenth). Kim wipes are used to remove water. Caution: Touch the side and not the center of the mica and try to be quick between wash steps to avoid surface drying.
10. Rinse the APS mica five times with 3 μL of imaging buffer containing 10 mM HEPES (pH 7.5) and 4 mM MgCl_2 5 prior to depositing the nucleosome sample.
11. Apply 1.5 μL of freshly prepared nucleosome solution in the imaging buffer. The nucleosome concentration between 0.5 nM and 1 nM provides a good surface coverage.
12. Place the scanner with the sample on the top of the tip holder, so the mica with the sample deposited is faced down, and start the imaging. Follow the instruction described in [22]. A brief description is given below.
13. Auto-approach and use the set point A_s close to the free oscillation amplitude A_0 . $A_s = 0.95 A_0$ is the goal, although operating at even 83 % of the free amplitude can be used with precautions. The set point will be adjusted during the imaging. The cantilever amplitude is another parameter that should be kept at small as possible as the energy provided by the tip to the sample depends on

the square of the amplitude value [17]. The instrument operates stably at amplitudes as low as 1 nm [22]. A typical area of 150×150 nm contains several nucleosomal particles, the dynamics of which can be analyzed. Fig. 6a shows the set of images taken from [17]. Imaging rate can be as fast as 10–15 frames per second; however even smaller rate, ~300 ms per frame, was sufficient to uncover such details as transient unlooping of nucleosomes and such rare events as transient nucleosome translocations [17].

3.6 Nucleosome Parameters Calculated from AFM Images

Schematics in Fig. 7 explain how the structural parameters of nucleosomes can be obtained from the AFM images.

The DNA template (353 bp; panel A) contains the sequence with a high specificity for nucleosome binding (147 bp, Widom 601 motif; blue color) located at different distances from the ends. Such an asymmetry was beneficial for structural analysis of nucleosome allowing us to distinguish the dynamics of the left and right flanks during the time-lapse imaging. DNA wraps around the histone core with 1 and $\frac{3}{4}$ turns to make a crystallographic structure [1]. However the dynamics of this structure can lead to nucleosomes with variable number of DNA turns around the histone octamer and schematically this dynamics is shown in Fig. 7b. The starting position in this set is the nucleosome with one turn around the core. Rotation of the arm by a quarter of turn leads to the nucleosome with 90° angle between the flanks. Further wrapping produces the structure with the parallel orientation of the flanks and additional one-quarter wrapping step leads to the crystallographic structure with 1.75 DNA turns with 147 bp around the core. One more rotation leads to the structure that is geometrically similar to the starting one, but the lengths of flanks are twice shorter. Table c summarizes the structural parameters of the structures described above.

3.6.1 Measuring of the Number of DNA Turns on AFM Images—Two major parameters measured in assigning the number of turns on the AFM images of nucleosomes are the lengths of the free DNA flanks and angles between the flanks. DNA contributes substantially to the nucleosome volume; therefore this parameter can also be used for additional validation of the DNA wrapping values.

1. Select a nucleosome and measure the lengths of both free DNA flanks. Use the table in Fig. 7c to calculate the number of turns.
2. Measure the angles between flanks. Select DNA segments ~10 nm from the attachment point and draw a line. DNA persistence length is ~45 nm, so a 10 nm DNA segment is typically straight.
3. The volume measurements: These are not direct measurements of the number of turns due to the tip convolution effect and the model used for the volume measurements. For the volume measurements, the blob was approximated as a segment of a sphere with a diameter measured at half-maximal height of the protein [12]. *See Note 4* for additional information. Numbers in Fig. 4 were obtained using the procedure described above.

3.6.2 Analysis of the Nucleosome Dynamics with Time-Lapse AFM Images

1. Acquire a necessary set of frames for the data analysis.
2. Select the areas containing the nucleosome of interest on all frames and analyze each nucleosome for each frame. It is instructive to make the frame assembly for each nucleosome as it is shown in Fig. 8a.
3. Measure the lengths of the flanks for each frame and plot the flank length values against time. *See* Fig. 8b for the reference.
4. Calculate the number of turns based on the length measurements by calculating the length of DNA incorporated into the nucleosome after subtracting the lengths of flanks from the free DNA size (*see* Note 4).
5. Measure the inter flank angles and calculate the number of the DNA turns using the table in Fig. 7c.
6. Measure the volumes and make the estimates for the nucleosome size based on these measurements as described in Note 5.
7. Generate the plot on the time dependence of the nucleosome dynamics as shown in Fig. 8c (*see* Note 6).

3.6.3 Analysis of the Nucleosome Dynamics with HS AFM—The analysis is essentially similar to the one described above and is performed over considerably larger dataset. The length and angle measurements are typically sufficient for the analysis.

1. Select the nucleosome of interest in the dataset and assemble a subset of images to identify the major effect.
2. Perform the length measurements and plot both measured values depending on time.
3. Identify the major events for the analysis. Figure 6 illustrates the unwrapping of the nucleosome accompanied by the formation of a small loop that transiently associates with the core. Note that only one flank changes during this process. Another flank remains unchanged.

⁴The length measurements can be performed in nanometers. These values can be converted in the number of base pairs. The conversion coefficient can be obtained from the length measurements of free DNA. Generate the histogram from multiple measurements (100 molecules is sufficient) and divide 353 bp by the mean length number. For APS mica procedure this value is very close to 0.34 nm for B-DNA base pair spacing.

⁵The volume measurements can be used, but these are not direct measurements of the number of turns due to the tip convolution effect and the model used for the volume measurements. We recommend to make a calibration plot using the volume measurements for a set of nucleosomes with the number of turns unambiguously determined by the angle and length measurements. Make a graph as a dependence of the volume on the number of the DNA turns. Use the same model of nucleosome for all measurements. The effect of the tip convolution effect can be incorporated by the measurements of the DNA heights and width. Similar parameters justify the measurements.

⁶The estimates of the DNA turns obtained by different methods should not exactly be the same, but typically they are close. Each type of measurements has limitation. The length measurements are limited by the identification of the DNA detaching point on the nucleosome image. The nucleosome size on the AFM images is enlarged due to the tip convolution effect and therefore the measured DNA length is shorter as it should be. Therefore, the length of wrapped DNA is increased leading to the elevated values for the DNA turns as it is seen in Fig. 8c. However, the dependence of the values over time should be similar and this is illustrated in Fig. 8c.

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Position-sensitive photodetector (PSD)

Fig. 1.

Schematic explaining the principles of AFM. The position of the tip relative to the sample is controlled by a piezoelectric scanner. The vertical displacement of the tip during scanning is detected using the optical lever principle, in which the position of the light spot on the PSD is measured. Figure was reproduced from [23] with permission. Copyright (2011) Elsevier

Fig. 2.
Size-exclusion chromatography of the octamer sample reconstitution. Three major peaks analyzed with gel electrophoresis are indicated

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Fig. 4. AFM images of reconstituted mononucleosome sample. The figures near each nucleosome correspond to the number of DNA turns calculated as described in the text. Figure was reproduced from [12] with permission. Copyright (2009) American Chemical Society

Fig. 5.
Photographs of the scanner with the sample holder and the cap (**a**); the same assembly with the cap covered the sample holder (**b**)

Fig. 6.

Loop formation and unfolding. (a) A set of images corresponding to 8.7 s (i), 14.7 s (ii), and 17.1 s (iii). In the image (ii), the position of the DNA dissociation and unlooping events are indicated with *white arrow*. (b) The length measurements for the looping and the loop unfolding process. The lengths of the left (arm 1) and right (arm 2) arms and the total DNA length are shown with *black squares*, *red diamonds*, and *green triangles*, respectively. In the graph (b), the *dashed lines* correspond to the image-acquired times shown in (a). The scan

rate was one frame per 301 ms. Figure was reproduced from [17] with permission.
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Fig. 7. Schematic for calculation of nucleosome parameters from the AFM images. **(a)** DNA construct used; the nucleosome-specific Widom 601 segment is shown in *blue color*. **(b)** The arrangement of the nucleosome DNA flanks depending on the number of DNA turns within the nucleosome. Table **c** shows the lengths of wrapped DNA depending on the number of the turns and angle between the flanks

Fig. 8.

Time-lapse AFM visualization of the nucleosome unwrapping process. (a) Consecutive AFM images of nucleosomes taken during continuous scanning in the buffer. Each frame size is 200 nm. (b) Dependence of arm lengths on the frame number. (c) The dependence of number of DNA turns around the core calculated from arm lengths (*black*) and from the angle between DNA arms (*red*) on the frame number. The dependence of nucleosome volume on the frame number is shown in *blue* (right *Y*-axis). Each frame takes about 170 s to scan. Figure was reproduced from [12] with permission. Copyright (2009) American Chemical Society