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REVIEW

Studying Structure and Functions of Nucleosomes with Atomic Force Microscopy

Alexander A. Ukraintsev[1#], Mikhail M. Kutuzov[1#], and Olga I. Lavrik[1,2,a*]

[1]*Institute of Chemical Biology and Fundamental Medicine, Siberian Branch of the Russian Academy of Sciences, 630090 Novosibirsk, Russia*

[2]*Novosibirsk State University, 630090 Novosibirsk, Russia*

[a]*e-mail: lavrik@niboch.nsc.ru*

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Abstract—Chromatin is an epigenetic platform for implementation of DNA-dependent processes. Nucleosome, as a basic level of chromatin compaction, largely determines its properties and structure. In the study of nucleosomes structure and functions physicochemical tools are actively used, such as magnetic and optical "tweezers", "DNA curtains", nuclear magnetic resonance, X-ray crystallography, and cryogenic electron microscopy, as well as optical methods based on Förster resonance energy transfer. Despite the fact that these approaches make it possible to determine a wide range of structural and functional characteristics of chromatin and nucleosomes with high spatial and time resolution, atomic force microscopy (AFM) complements the capabilities of these methods. The results of structural studies of nucleosome focusing on the AFM method development are presented in this review. The possibilities of AFM are considered in the context of application of other physicochemical approaches.

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INTRODUCTION

DNA molecules are used by cells for storage and implementation genetic information. In eukaryotic cells, DNA is predominantly present in a chromatin composition, and both the level of chromatin compaction and chromatin spatial organization are important [1]. The basic level of DNA compaction is nucleosome, a complex consisting of eight histones and 147-bp DNA with its structure defining properties of chromatin to a large extent. The first ideas about the nucleosome structure were formulated in the middle of the 1970s [2-4]. Schematic representation of the nucleosome structure is shown in Fig. 1.

To date, main characteristics of the composition and structure of nucleosome, as well as its molecular dynamics, have been studied in sufficient detail. This knowledge was obtained largely due to the use of modern physicochemical research methods, including single-molecule techniques such as optical and magnetic tweezers, DNA curtains, an optical approach based on Förster resonance energy transfer (FRET), etc. [5-7]. These methods enable to measure a wide range of behavior parameters of biomolecules and their complexes, including chromatin and nucleosomes, with high spatial and time resolution.

Along with the listed methods, the technique of atomic force microscopy (AFM) is actively used. AFM is based on monitoring of the probe interaction with the surface. A probe in the form of a needle at the end of the cantilever scans the sample. To detect change in the vertical coordinate of the probe, a laser directed

Abbreviations: AFM, atomic force microscopy; cryoEM, cryogenic electron microscopy; DREEM, dual resonance frequency enhanced electrostatic microscopy; FRET, Förster resonance energy transfer; HS-AFM, high-speed atomic force microscopy; PARP, poly (ADP-ribose) polymerase; ssNCP, nucleosome with a single-stranded DNA.

* To whom correspondence should be addressed.

# These authors contributed equally to this work.

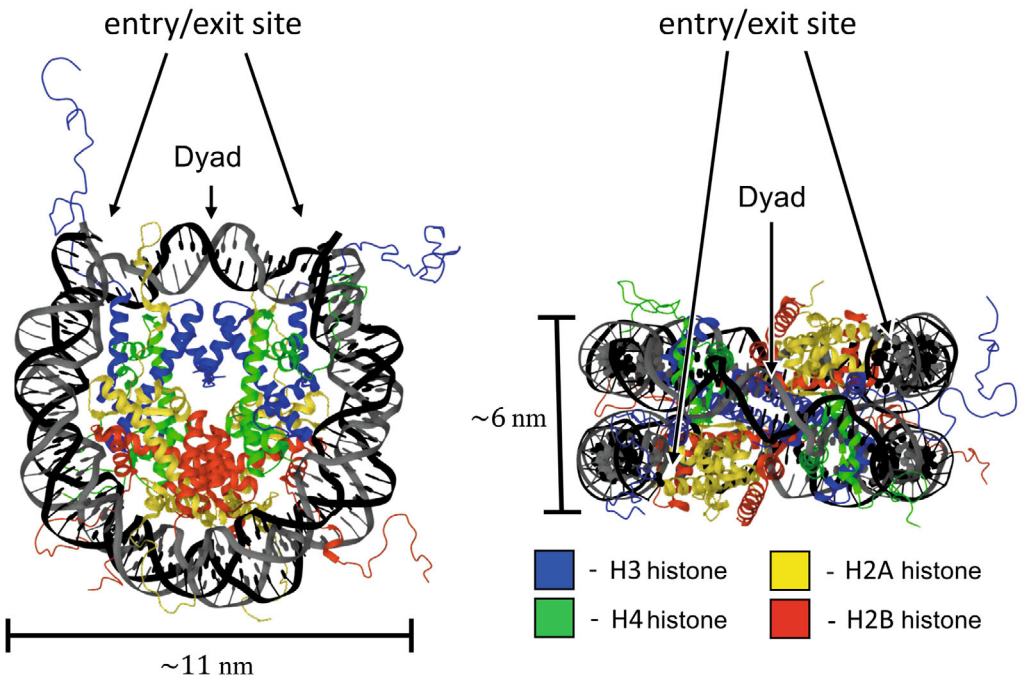

Fig. 1. Nucleosome core particle structure (PDB ID: 1KX5). Spatial structure of nucleosome is shown as a ribbon diagram in two projections.

at the cantilever and a photodetector recording the reflected beam are used. The beam reflected from the cantilever shifts according to the vertical displacement of the probe. Force of the cantilever interaction with the surface can be assessed based on the signal from the photodetector. Then, depending on the scanning mode, these data enable to obtain information on vertical coordinate of the sample surface directly or indirectly through the feedback mechanism. At present there is a wide variety of scanning modes and variants of scanning protocols developed.

CONTACT MODE AFM

The first nucleosome images were obtained using contact mode AFM, during which the probe-surface repulsive force regulated by the feedback loop [8, 9] is recorded (Fig. 2a). The authors of these works were able to show polynucleosomal structure in the form of "beads on a string" [8, 9]. In addition, main geometric parameters of the nucleosome disk were determined: its height and diameter, which were ~3 and ~32 nm, respectively, not including radius of the probe curvature, [9], as well as the number of DNA supercoil turns in the nucleosome [9].

The contact mode is characterized by a relatively large mechanical effect of the cantilever on the surface, which leads to severe deformation and damage to nucleosome samples during scanning. In addition, this "hard" scanning mode requires the use of probes with a curvature radius of at least 10 nm, which, as a whole, reduces sensitivity and accuracy of the mode. As a result, the first nucleosome images obtained by AFM contact mode are of a low quality, which largely limited the use of this method in studying nucleosome structure.

TAPPING MODE AFM

Introduction of the tapping mode AFM provided a way to reduce significantly deformation of the sample during scanning, which made it possible to use sharper probes. In this mode, the cantilever oscillates at its resonant frequency. The feedback loop records changes in the cantilever oscillation parameters and corrects the probe position relative to the surface, providing an equilibrium position around which the cantilever performs harmonic oscillations (Fig. 2b). Such changes dramatically increased resolution of AFM in examining biological molecules. In particular, Martin et al. [10] demonstrated advantages of this scanning mode over the contact mode in studying chromatin structure. In their work, the authors managed to determine the size of nucleosome more accurately than in the case of using contact mode by employing the tapping mode. By using the tapping mode of AFM, height and diameter of the nucleosome disk were determined as 4.3 ± 1.2 nm and 23.2 ± 4.5 nm, respectively, which, considering radius of the probe curvature, are close to the values obtained by X-ray crystallography [11].

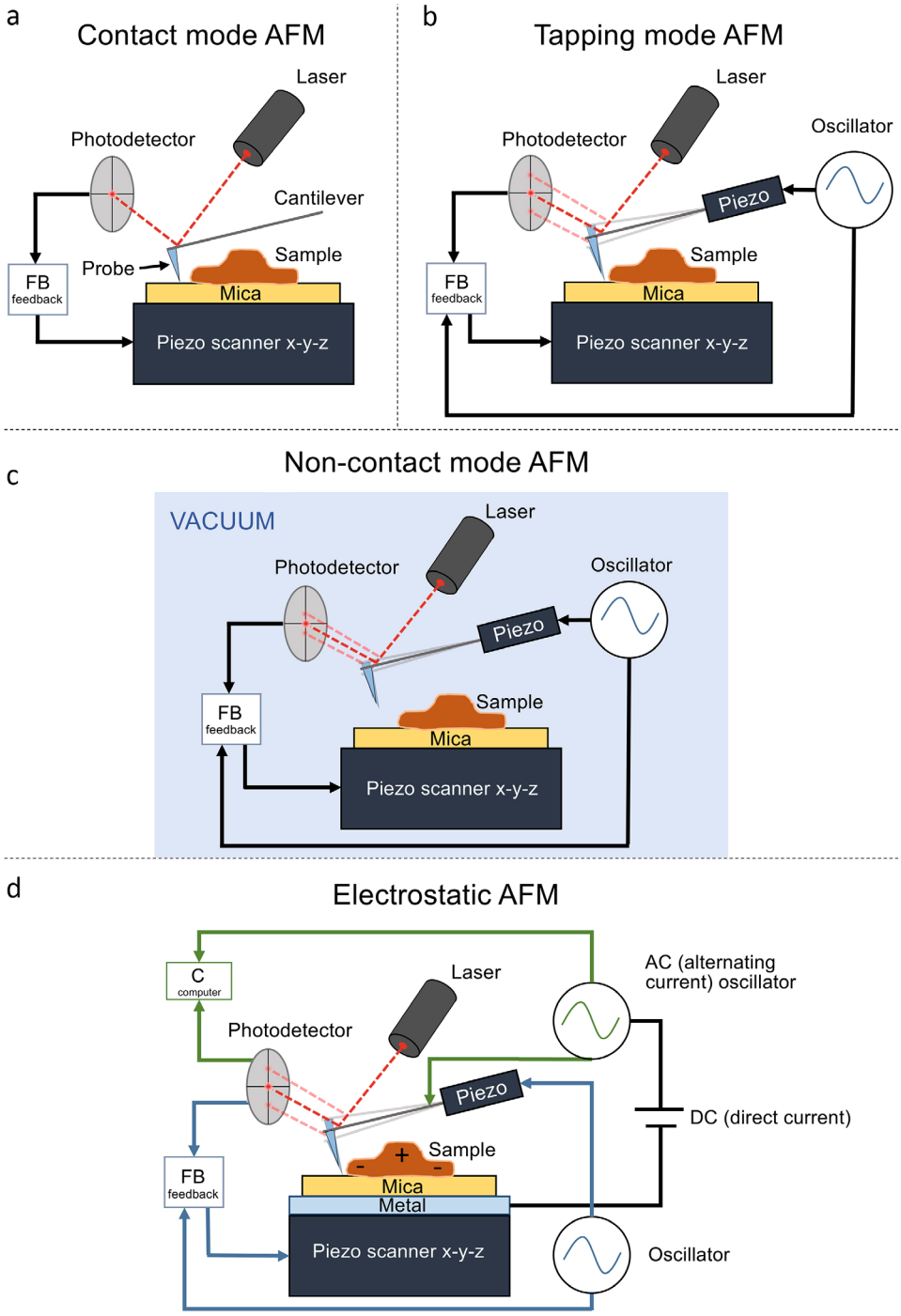

Fig. 2. Schematic representation of AFM scanning modes exemplified with a device with position of a piezo scanner at the bottom. a) Contact mode; b) tapping mode; c) non-contact mode; d) electrostatic AFM. Designations: FB, feedback; C, computer; AC, alternating current; DC, direct current.

Moreover, use of this approach enabled visualization of DNA in the linker region of chromatin (which was impossible with the contact mode scanning) as well as to confirm experimentally the nucleosome structure calculated by means of mathematical modeling [12].

The use of tapping mode AFM by Qian et al. [13] in their study of chromatin from the chicken red blood cells made it possible to visualize different levels of DNA compaction. Both individual nucleosome "beads" and their clusters on DNA strands were demonstrated on the obtained images. Presence of the sites with different DNA packing densities allowed the authors to suggest the role of compaction in the regulation of gene expression. In this work, geometric characteristics of fibrils that have diameter of 15-60 nm were determined, confirming the solenoid model of nucleo-

some packaging. In addition, it has been shown for the first time that the fibrils of lower compaction degree (~30 nm) are further folded to form chromatin structures of higher order (~60 nm), which, in turn, fold into even more highly organized chromatin structures (~90-110 nm or more).

Design of nucleosome-positioning DNA sequences [14], characterized by the unambiguous location of the nucleosome core on the DNA molecule and high stability of the formed complexes, enabled to create model structures with predicted location of nucleosomes. Use of such nucleosome-positioning DNA sequences made it possible to show stochasticity of nucleosome location in the natural chromatin using telomeric sites. These results confirmed the corresponding theoretical model of nucleosome positioning [15].

AFM is actively used to study effects of various histone variants, their post-translational modifications, and epigenetic DNA modifications on the nucleosome structure. Histone tail modifications are the key factor in regulation of both chromatin and cellular homeostasis dynamics. A hallmark of an active chromatin is hyperacetylation of histones, which likely results in a more open chromatin structure. It was shown in the work of Hizume et al. [16] by AFM that histone hyperacetylation causes decrease in the thickness of chromatin fibrils from HeLa cells. This result allowed to confirm a model of chromatin decondensation based on electrostatic interactions. Biotinylation, on the contrary, promotes chromatin condensation. In the works by Filenko et al. [17] and Singh et al. [18] the use of AFM made it possible to demonstrate that biotinylation of H4 histone at lysines 12 or 16 leads to compaction of the nucleosome structure, which is manifested by the increase in the number of DNA turns around the histone octamer.

Histone replacement with variants, as well as the nucleosome movement along the DNA molecule, are the basic mechanisms for regulating chromatin compaction, which, as a rule, are due to the work of remodeler proteins. RSC ("remodeling the structure of chromatin") is an ATP-dependent protein complex of chromatin structure remodelers that is homologous to the human SWI/SNF ("switch/sucrose non-fermentable" family of chromatin remodeling complexes) and mediates the nucleosome shift. Some details of its functioning were shown using the tapping mode AFM. In particular, it was found out that RSC moves nucleosome along the DNA until another nucleosome is reached or the break in the DNA chain is encountered [19].

Comparative analysis of the results of studies on the structure of nucleosome complex with the H3 histone variant (CENP-A) performed using AFM allowed to propose an alternative model of DNA folding in the centromeric region of chromosomes. This model involves DNA folding in the centromeric region as an array of parallel sites in the form of a "snake" instead of the characteristic canonical nucleosome zigzag folding [20]. The study of nucleosome structural features by AFM involving replacement of H2A core histone with its recently discovered H2AL2 version of the mouse histone, expression of which is associated with spermatogenesis, showed, that the DNA length in the nucleosome decreases from 147 to 130 bp [21]. Shortening of the DNA in the nucleosome composition is due to relaxation of its structure by partial unwrapping. In the same work it was also shown using cryogenic electron microscopy (cryoEM) that nucleosomes with H2AL2 have a more relaxed structure with a larger angle value between the DNA linker regions, which is in agreement with the data obtained by the AFM method. It is worth paying attention to the fact that the nucleosome becomes resistant to the action of such remodeler proteins as RSC and SWI/SNF in the process. Biological significance of this modulation of nucleosome properties remains the subject of research.

Nucleosome structure may be modulated by binding of a protein or a protein complex. One of the most illustrative examples is the study investigating the effect of the H1 linker histone on a nucleosome compaction. Using the capabilities of AFM, it has been shown

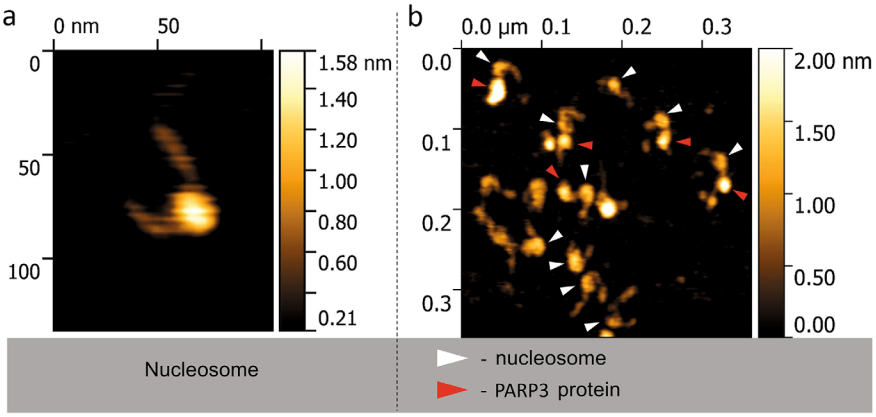

Fig. 3. Images obtained by using the tapping mode AFM. a) Nucleosome; b) nucleosomes with PARP3 protein.

that H1 does not affect the degree of nucleosome compaction, but significantly stabilizes it, reducing structure fluctuations [22]. In our work on the influence of nuclear proteins poly(ADP-ribose) polymerases 1, 2, and 3 (PARP1, PARP2, and PARP3) on the degree of nucleosome compaction, it was found by AFM that PARP3 contributes to compaction of the structure of mononucleosomes [23] (Fig. 3, a and b). PARP1, PARP2, and PARP3 take part in the most DNA-dependent processes including regulation of the DNA transcription and DNA repair. At the same time, they catalyze the reaction of ADP-ribose moieties transfer to the target proteins using NAD^+ as a substrate in response to genomic DNA damage. PARP3 catalyzes the mono(ADP-ribosyl)ation reaction, while PARP1 and PARP2 catalyze synthesis of the poly(ADP-ribose). In addition, unlike PARP1 and PARP2, PARP3 does not interact with the HPF1 histone ADP-ribosylation factor [24]. Using AFM, we were able to establish a previously unknown feature of the PARP3 functioning, in particular, its influence on the nucleosome structure, which may indicate the role of this protein in regulating chromatin structure. In this work, catalytic reaction of the PARP enzymes proteins on nucleosomes has not been investigated, but other researches have observed synthesis of poly(ADP-ribose) catalyzed by the PARP1 and PARP2 proteins on the extended DNA substrates by the AFM method [25, 26], which indicated the possibility of similar studies in the case of nucleosomes.

NON-CONTACT MODE AFM

Another modification of the AFM is the non-contact mode AFM. It is based on the detection of changes in attractive forces caused by van der Waals interactions between the probe and the surface, which allows scanning without the need for direct contact of the cantilever with the sample. While realizing this mode, the cantilever oscillates with low amplitude at a frequency exceeding its resonant frequency, which leads to the increase in sensitivity compared to the tapping mode AFM. Scanning nucleosomes using this mode enabled to obtain an image with a resolution that even exceeded capabilities of cryoEM at the time of this work publication [27]. However, despite the impressive advantage, this scanning mode has not been widely adopted due to technical limitations. For example, scanning in the above-mentioned work of Davies et al. [27] was performed under a deep vacuum. It should be noted, that analysis of biomolecules and their complexes is preferable to be carried out in a solution to facilitate interpretation of the results, which is incompatible with operations under vacuum. In addition, vacuum conditions limit the study of dynamics of the investigated complexes.

Taking into account the limitations arising during implementation of the non-contact mode and low quality of the images that can be obtained by the contact mode, today the tapping mode AFM has become the most widespread in the study of biomolecules. Moreover, such AFM variants as MAC mode, PeakForce mode, high-speed mode, as well as electrostatic AFM are also based on the tapping mode AFM.

MAC MODE AFM

An alternative to the classic tapping mode is the MAC mode (magnetic alternating current mode AFM), the principles of which are based on excitation of the cantilever oscillations by magnetic field of the coil located under the sample [28]. The use of MAC mode provides lower force impact on the sample than the classic tapping mode of AFM, which contributes to maintaining sharpness of the cantilever tip and improves quality of the resulting image. For example, methylation of the nitrogenous base C in the CpG sequences has been shown to result in a tighter chromatin packaging in the presence of the H1 linker histone precisely using the MAC mode [29]. These results suggested a mechanism for a transcription regulation in large chromatin domains through methylation.

Further development of this scanning mode involved modification of the cantilever with an affinity agent; for example, an antibody, which allowed to combine a topographic study of the surface with molecular recognition of the objects in real time (PicoTREC AFM) [30, 31]. When the probe contacts the sample, the surface relief is recorded, and presence of the antigen at the scanning point could be detected from the change of the amplitude of cantilever oscillations. The use of this approach made it possible [32, 33] to demonstrate the mechanism of action of the SWI/SNF chromatin remodeler. In particular, SWI/SNF has been shown to promote removal of the H2A histone from nucleosome, resulting in relaxation of the nucleosome structure and accompanied by the release of ~80 bp DNA. These results enabled characterization of the changes in geometric parameters of the nucleosome in the case of H2A histone deletion, although, there are data obtained earlier by immunoprecipitation, quantitative PCR, and gel electrophoresis, indicating that the H2A/H2B histones are deleted from nucleosome under the action of SWI/SNF chromatin remodeler [34, 35].

PEAKFORCE TAPPING MODE AFM

A non-resonant scanning mode developed by Bruker (USA), PeakForce Tapping mode AFM, is one of alternative modifications among the scanning probe

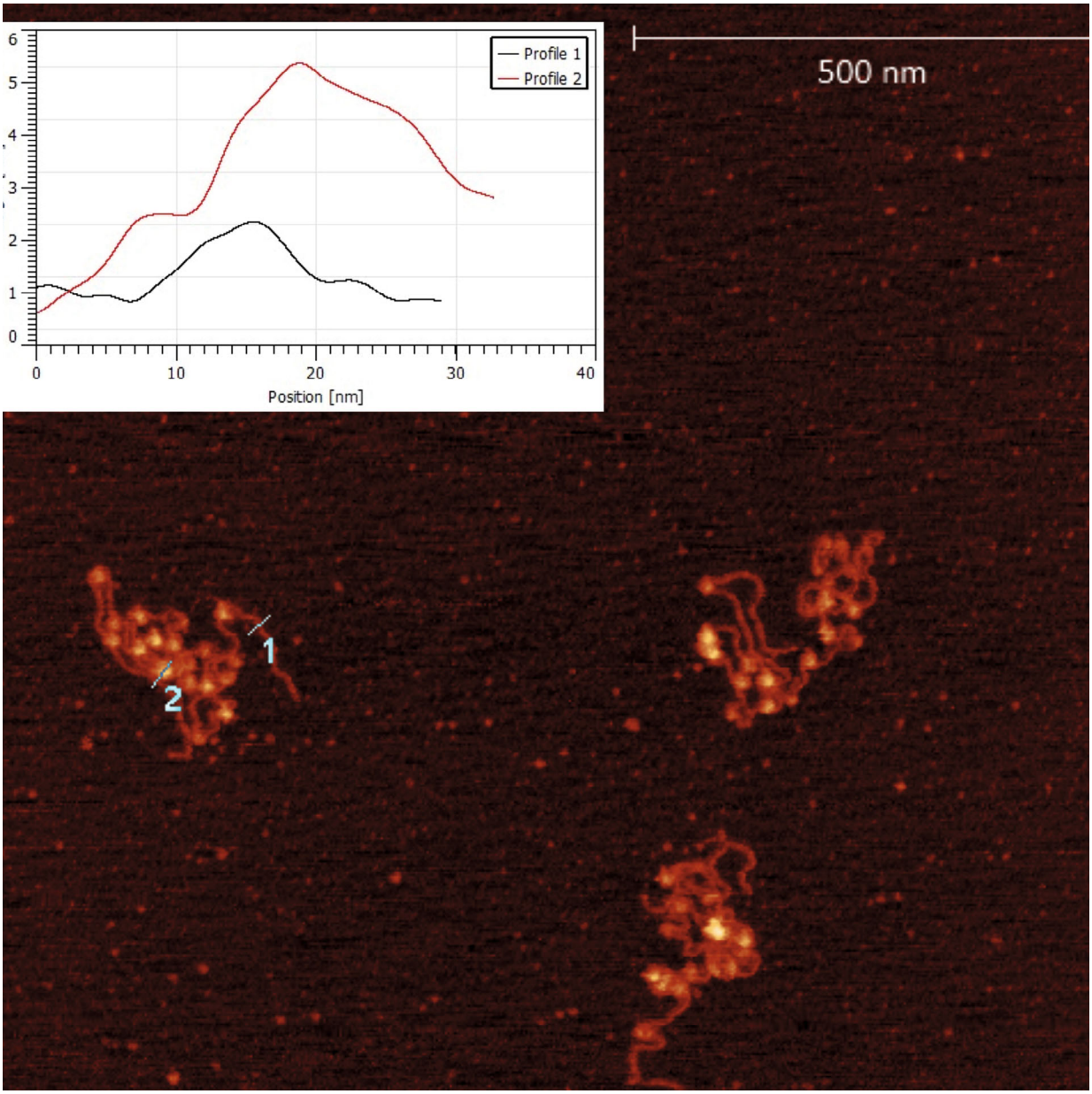

Fig. 4. A cluster of nucleosome particles [37]. Three clusters of nucleosome particles are shown in the AFM image. Two lines (1, 2) are marked. The inset in the upper left corner shows height profiles for each line (1 and 2) along the measured distance. The first profile (black line) crosses DNA and shows height and width of the double-stranded DNA. The second profile (red line) crosses the identified nucleosome.

AFM technologies, which is promising for studying biological molecules [36]. Implementation of this mode includes contact of the cantilever with the sample surface at a frequency significantly lower than the resonant one, which enables to consider such kind of contacts as a quasi-static process, and the signal is recorded as a fixed maximum contact force of the sample with the probe. This scanning mode additionally reduces the force of cantilever impact on the sample and allows topographic studies of "soft" objects to be carried out with great accuracy. Using this mode of AFM for the first time allowed to obtain nucleosome images in solution without using vacuum with resolution approaching the resolution demonstrated by the X-ray crystallography (Fig. 4) [37].

McCauley et al. [37] in their study uniquely identify nucleosomes from the height ~5.5 nm and diameter ~1 nm, which correlates with the data obtained by X-ray crystallography (height was 5.7 nm; diameter was 11 nm) [11]. Such high resolution allowed establishing that the non-histone proteins Nhp6A and Hmo1 of chromatin from the HMGB family destabilize the nucleosome structure with release of the part of DNA, and perform this in different ways. Hmo1 was shown

to relax the nucleosome structure more than Nhp6A, which is highly consistent with peculiarities of localization of these proteins on chromatin. Hmo1 is concentrated in a highly transcribed histone-depleted region enriched with the genes of translational apparatus, where high DNA compaction is not needed, while Nhp6A is localized in the rest of euchromatin.

ELECTROSTATIC AFM

Special attention should be paid to the scanning probe microscopy techniques that allow to determine electrostatic potential of a surface. They proved to be significantly more informative for studying biomolecules after the single-pass scanning options were optimized by using the resonant cantilever frequencies at two harmonics. Implementation of this approach made it possible to increase dramatically sensitivity of the method [38]. Depending on the implementation features, the Kelvin probe force microscopy (KPFM) [38] and the dual resonance frequency enhanced electrostatic microscopy (DREEM) [39] are distinguished. Fig. 2d shows principle of signal acquisition with KPFM and DREEM.

DREEM allows minute changes in the electrostatic force gradient to be detected at high resolution, making it a powerful tool for characterizing the structure of protein-nucleic complexes at the single-molecule level. DREEM enables visualization of DNA located both on the surface and inside the protein-nucleic complex, as well as DNA in the composition of large heterogeneous complexes with several proteins. This approach has a great potential in studying the structure of both single nucleosomes and nucleosomes in more complex systems.

The research of Wu et al. [39] is devoted to demonstrating capabilities of the DREEM method to study location of a DNA molecule in large multi-protein complexes. A nucleosome was chosen as the example. On the one hand, the authors showed that the overall topographic images of nucleosomes obtained by DREEM and other AFM methods are the same. On the other hand, the DREEM images demonstrate regions with reduced intensity in the nucleosome core region location of which correlates well with the trajectory of DNA location in the nucleosome according to the X-ray crystallography data (Fig. 5). This fact confirms the validity of this approach and of the results interpretation.

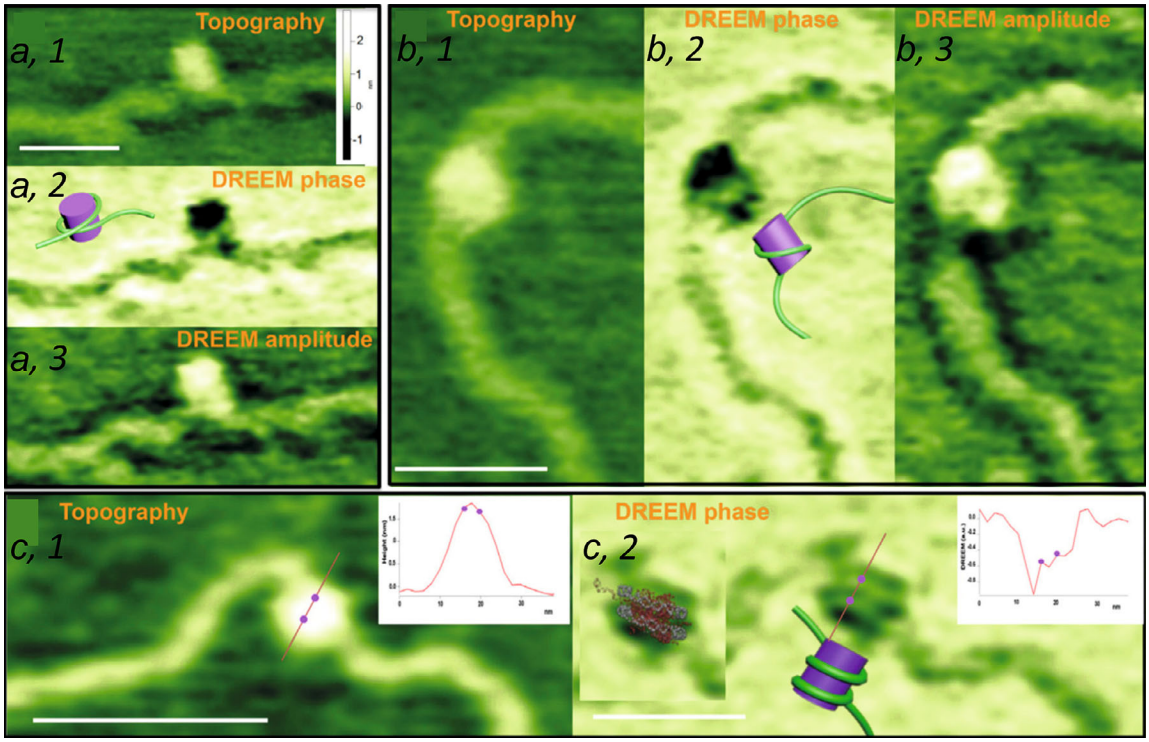

Fig. 5. Topographic images of nucleosomes obtained by AFM and DREEM [39]. Topographic (a, 1; b, 1) DREEM phase images (a, 2; b, 2) and DREEM amplitude (a, 3; b, 3) of nucleosomes showing how a DNA molecule is wrapped around histones once. c, topographic (1) and DREEM phase images of the nucleosome (2) showing that DNA is wrapped around the nucleosome twice. The insets show height profiles along the line drawn through the nucleosome in topographic (c, 1) and DREEM images (c, 2). Two dots in the graphs correspond to positions of the two dots shown on the line that mark position of the peaks corresponding to DNA in the DREEM image. Schematic models of histone-wrapping DNA are shown in each DREEM image (not-to-scale models). Crystal structure of the nucleosome superimposed on the DREEM-phase image is shown in the inset to the phase image on the panel (c, 2)

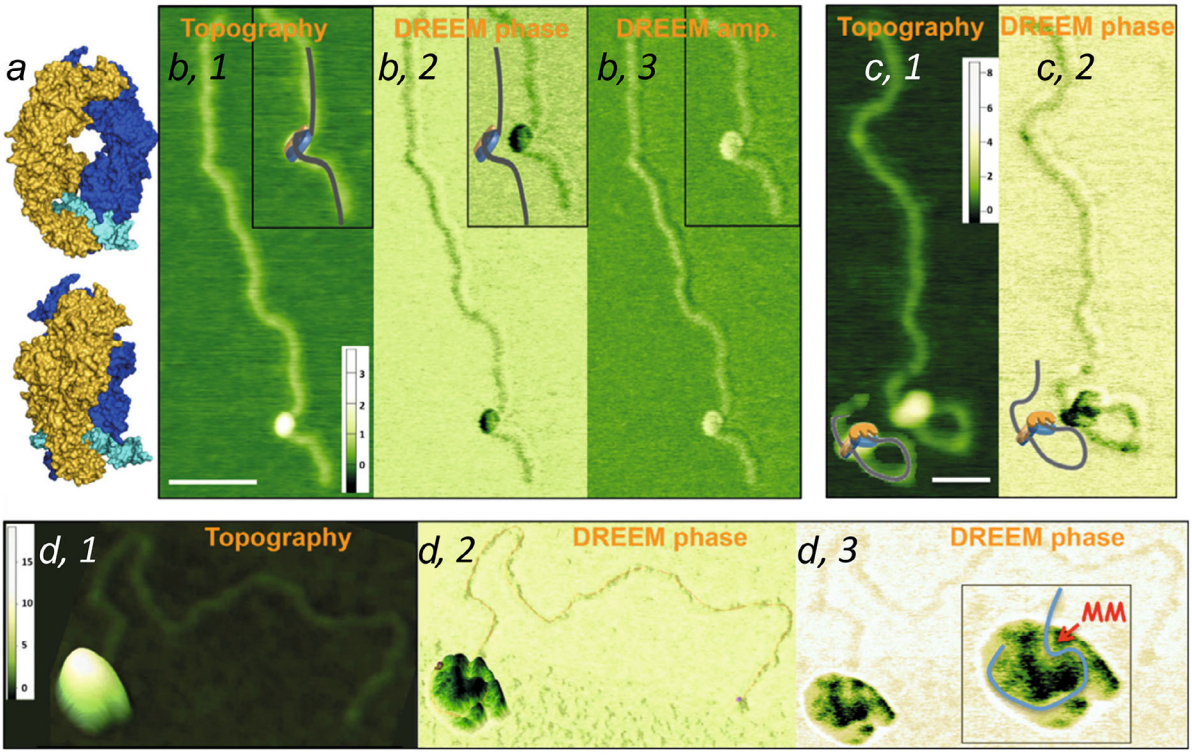

Fig. 6. Topographic AFM and DREEM images of repair complexes of mismatch repair nucleotides on 2-kb long DNA containing mismatch repair GT nucleotides [39]. a) Spatial model of the Taq MutS crystal structure (created based on PDB: 1EWQ). Subunits A, B, and DNA are colored blue, gold, and light blue, respectively. MutS bends DNA by about 60° as it passes through the DNA binding channel. b) Topographic AFM (b, 1), as well as DREEM-phase (b, 2) and amplitude (b, 3) images of the Taq MutS-DNA complex. c) Superimposing the of complex model on AFM images (c, 1) and to phase images (c, 2). d) Topographic AFM (d, 1) and DREEM phase (d, 2 and 3) images of a large complex of MutSα-MutLα-DNA containing ~10 proteins. The DNA pathway is identified as the regions with the greatest reduction in the magnitude of DREEM signals, compared to the one protein, and marked on the inset (d, 3) in blue color; MM, expected position of mismatched nucleotides.

As the second model, Wu et al. [39] used a multiprotein complex of non-complementary base pair of human DNA repair system consisting of MutSa and MutLa. Application of DREEM allowed visualization of location of the DNA containing a non-canonical pair of nucleotides in the composition of this complex (Fig. 6).

This model is designed to demonstrate applicability of the method for determining location of the DNA inside a protein complex. The obtained results were included in the work devoted to studying the MutSa and MutLa complex functioning [40].

The same approach was used by Adkins et al. [41] to confirm the structure of nucleosome-like particles reconstructed using single-stranded DNA (ssNCP). Using DREEM, it has been shown that ssNCPs have a structure and a size similar to the parameters of canonical nucleosomes, in particular, have a disk-like shape and contain the same number of turns of the single-stranded DNA on the histone octamer [41].

The authors of this research suggested that the histones released from nucleosome composition during the initial stages of the DNA double-strand break repair process can bind to the single-strand DNA region. The possibility of reconstitution of ssNCP *in vitro* has been shown. ssNCPs proved to be more dynamic than the canonical nucleosomes and capable of ATP-independent transfer to other regions of one- or double--stranded DNA. Moreover, such ATP-dependent nucleosome remodelers as RSC and Fun30 bind effectively to ssNCP and become activated. Based on these results, the authors have suggested that ssNCP could be a marker of various cellular processes accompanied by formation of single-stranded DNA regions, and emergence of these structures could be used to regulate one or another specific cellular process.

Despite the impressive capabilities of this approach, it has a number of technical limitations primarily related to high requirements for the quality of cantilevers, which must simultaneously have high sharpness and sufficient conductivity to obtain high-resolution topographic and DREEM images [39].

DREEM makes it possible to visualize DNA conformation both on the surface and within individual protein–DNA complexes, as well as within the composition of large heterogeneous complexes with several proteins. Ability of DREEMs to detect minute changes in the electrostatic force gradient in combination with high resolution makes it a powerful tool for character-

izing structure of protein-DNA complexes at the single-molecule level. This approach has a great potential in studying structures of both single nucleosomes and nucleosomes in more complex systems.

HIGH-SPEED AFM

One of the key disadvantages of the above-mentioned AFM modes is long time – required to obtain individual images. This feature of the method limits its applicability for investigating dynamics of the process. The first attempts to study dynamics of the processes involved the use of sequential samples scanning. In this way, the data on the RNA polymerase moving along DNA were obtained [42]. Using the same mode, it was possible to demonstrate the process of nucleosome "unwinding" [43]. Shlyakhtenko et al. [43] showed that multiple continuous scans of the same nucleosome field allowed eight images of the same nucleosome to be obtained, demonstrating its transformations over time. As a result, gradual "unwinding" of DNA from the histone octamer culminating in the complete destruction of the complex was shown.

Further improvement of the method due to application of a more productive device allowed the Lyubchenko group to obtain more detailed data on the dynamics of nucleosomes "unwinding" with the H3 histone and its CENP-A centromeric version using high-speed atomic force microscopy (HS-AFM) [44, 45]. The data obtained in these studies revealed such relaxation mechanisms of the nucleosome structure as DNA bleaching, as well as the possibility of translocation of nucleosomes along DNA. The authors note that dynamic rearrangements of centromeric chromatin can occur in the absence of remodeling factors. At the same time, CENP-A stabilizes nucleosome particles, preventing complete histone dissociation during the DNA loosening or unwrapping and ensuring rapid nucleosome re-assembly during their core transfer [45]. Canonical nucleosome particles with H3 histone do not demonstrate such plasticity, and rearrange only in the presence of such remodeling factors as SNF2h [46]. Later some dynamics details of nucleosomes and chromatosomes were shown in the work by Onoa et al. [47] using HS-AFM that clarified the mechanism of their "unwrapping". In particular, asymmetry of the nucleosome "unwrapping", step-wise nature of the process of histone heterodimers deletion from the nucleosome core, as well as lability of position of the histone tetramer (H3-H4) × 2 in the dyad region have been demonstrated in real time.

HS-AFM scanning made it possible to analyze dynamics of nucleoproteins and other biomolecule complexes. An even greater increase in the scanning speed is required to estimate kinetic characteristics of the studied proteins. In the future, machine learning capabilities are likely be involved in achieving this goal. In the study by Kato et al. [48] an approach based on machine learning has been proposed to analyze integrated simulation dynamics data from asynchronous HS-AFM scanning. Peculiarity of the described approach is that in this case asynchronous nature of the single passes during scanning is considered. Thus, this method with appropriate resampling frequency has been shown to be a powerful tool for assessing dynamic behavior of the object based on the low spatial and time resolution HS-AFM data.

COMPARISON OF AFM CAPABILITIES WITH SOME PHYSICOCHEMICAL APPROACHES IN NUCLEOSOME STUDIES

During the research of nucleosome structure and functioning, including dynamics studies, a number of physicochemical research tools are used, such as: magnetic and optical "tweezers", "DNA curtains", nuclear magnetic resonance, X-ray crystallography analysis, and cryoEM, as well as approaches based on FRET optical methods. These methods provide a wide range of detectable parameters with high spatial and time resolution.

One of these approaches that have become widely used is based on optical tweezers that allow to apply mechanical force directly to the molecule or complex under study and simultaneously measure elongation of the object. Strength of the nucleosome complex [49] was first determined by this method. Magnetic tweezers which in addition to stretching can generate rotational movement of an object, allowed creating model DNA structures with supercoiling. In the work of Gupta et al. [50] it was shown that intramolecular stress occurring in the structure of DNA with positive supercoiling alters its conformation, compared to the DNA without supercoiling making nucleosome formation less effective.

Nevertheless, applicability of both approaches is limited to measuring changes of the object linear characteristics and amount of the applied force. Combining these methods with fluorescence microscopy enhances greatly the possibilities for the researchers, but still prevents investigation of conformational changes within the complex. Employment of fluorescence microscopy in the FRET mode, a non-radiative mode of transferring energy between fluorophores, partially release this limitation, however, conformational changes recorded by this method could not be always interpreted clearly. Despite this fact, use of FRET technique is a powerful tool for studying the structure and conformational dynamics of protein-nucleic complexes. For example, use of FRET allowed Andreeva et al. [51]

to record the effect of buffer components on nucleosome structure. In particular, the stabilizing effect of potassium ions has been shown, and this stabilization of the nucleosome structure has also been demonstrated to create a barrier for such ATP-independent proteins/complexes as FACT and PARP1.

Another approach facilitating examination of the protein complexes with nucleic acids is based on formation of a nanobarrier on the substrate surface, which ensures anchoring of DNA molecule with formation of DNA curtains. Nucleosome DNA curtains are a unique system allowing to track behavior of thousands of individual nucleosome particles containing a fluorescent label in real time. The use of this approach made it possible to monitor dynamics of DNA compaction by condensins I and II, as well as to clarify mechanistic model of chromatin loop formation by yeast condensin and human cohesin [52-56]. Visnapuu and Greene [57] were able to determine energy landscape of the nucleosome localization on DNA and confirm theoretical predictions based on the model designed using this approach. In addition, in the same work, positions of the nucleosome assembly sites were shown to correlate with the regulatory transcription regions. Unfortunately, this approach does not allow investigating explicitly structural changes within the nucleosomes themselves induced by various factors.

One of the most powerful tools in studying the structure of biopolymers is the X-ray crystallography. Atomic structure of nucleosome has been determined using X-ray crystallography a long time ago [58]. Despite the high resolution, applicability of this method is severely limited. Limitations are largely due to the need to grow a crystal, which does not enable studying dynamics of structural changes. Moreover, obtaining a crystal is on itself not a trivial task, while the image quality directly depends on the crystal quality. The task is further complicated in the case of investigation of the structure of protein complexes with nucleic acids, especially of such complexity as nucleosome, and even more so chromatin. Obtaining a X-ray-suitable crystal is also difficult if the studied polymer contains unordered regions in its composition. In addition, possible difference between the protein structure in the crystal and that in the solution should be considered while interpreting the X-ray crystallography data. The difficulties of decoding the obtained primary data should be also mentioned, it introduces additional limitations on the size of the object selected for research.

At present, cryoEM allows obtaining structures with resolution approaching the X-ray crystallography data. For example, in the study by Markert et al. [59] an image of nucleosome associated with complete histone deacetylation complex Rpd3S was obtained using cryoEM with final resolution of 2.8 Å. This method is deprived of some limitations specific to X-ray crystallography. For example, cryoEM allows studying proteins with unordered structures, while they are difficult objects to investigate with X-ray crystallography. Nevertheless, the use of this method has some limitations. CryoEM works best for studying large objects over 200 kDa. Like X-ray crystallography, cryoEM is poorly adapted to study conformational dynamics. The achievements of recent years have provided a possibility of time-resolution analysis in the range of milliseconds to seconds, which allows studying conformational dynamics of the structure, but is hardly applicable to studying such large-scale changes in the structure as nucleosome translocation or its core transfer.

Analysis of the biomolecule conformational dynamics including of their complexes such as a nucleosome could be performed by NMR spectroscopy. Advances in solution NMR spectroscopy have allowed monitoring conformational dynamics of both the bounded core of histones and their free N- and C-tails [60]. Solid-state NMR techniques represent additional tools of nucleosome research [61]. For example, secondary structure of the H2B histone in nucleosome was obtained using solid-state NMR, which was consistent with the structure obtained by X-ray crystallography. The authors were able to show dynamic interactions of H2B histone with H4 histone, as well as with DNA [62]. Applicability of this method is mainly limited by the need to introduce isotopes of different elements into the sample for unambiguous interpretation of the spectra. Thus, in the work of Shi et al. [62] the histone containing ^{13}S and ^{15}N isotopes was used.

The use of AFM largely complements the data obtained using the above-described approaches. In particular, AFM facilitates determination of geometric parameters of nucleosomes, as well as their changes due to nucleosome interactions with various protein factors or histone replacements with their variants, as has been shown in a case of H3 replacement with CENP-A. The possibility of scanning in a liquid medium allows simulating conditions closer to physiological ones, and development of the high-speed scanning made it possible to observe relaxation dynamics of the nucleosome structure. Using the capabilities of electrostatic force microscopy facilitated observation of the trajectory of DNA folding in the composition of nucleosome.

One of the considered AFM advantages is the possibility of using this technique for investigation of complex macromolecular structures, such as chromatin fragments or model polynucleosomal fragments [15, 63]. Investigation of such objects using FRET or tweezers could be limited by the need to add fluorescent labels or microspheres into the tested sample structure.

CONCLUSION

Rapid development of AFM as a method of studying complex protein-nucleic systems allowed to deepen significantly understanding of the nucleosome structure. Importance of the scanning environment during the AFM application should be emphasized. It is well known that implementation of AFM under vacuum conditions has a positive effect on image quality. However, in order to simplify interpretation of the results, it is preferable to carry out scanning in an aqueous environment when studying biomolecules. Drying of biomolecules like DNA, proteins, or their complexes leads to disruption of their spatial structure [64]. In addition, scanning in an aqueous environment is necessary to study dynamics of the processes. At the same time, implementation of AFM in a liquid is accompanied by a number of technical difficulties, in particular it limits of the range of used substrates and methods for immobilization of scanned objects, as well as it increases requirements for cantilevers preparation.

AFM capabilities are likely to be enhanced by the development and implementation of machine learning for primary data processing. This approach would reduce the level of "noise" when scanning at the software level and increase accuracy of the measurements provided AFM. In addition, performance improvement at the software level would increase the scan speed.

Some approaches based on the AFM technique were not widely used so far for studying nucleosomes should be noted. For example, combination of fluorescence microscopy and AFM could be used for studying interactions of protein molecules or their complexes with the substrates in the form of nucleosomes or chromatin. At the same time, the AFM-derived data on the sample topology could allow to localize interaction area in the protein complex with the substrate, and the fluorescence data could facilitate identification of the labeled molecule. A similar approach was used by the Wyman group to investigate homologous recombination [65].

Wide use of the combination of AFM and fluorescence techniques is primarily limited by the need to use a substrate which would be atomically smooth while being optically transparent. At present, preparation of such substrates is a non-trivial task; one of these solutions has been suggested by Rahman et al. [66]. This problem is likely to be solved in the future, opening up opportunities to an arsenal of fluorescence detection-based approaches, including FRET and fluorescence polarization combined with AFM.

Implementation of electrostatic force microscopy in a liquid medium could significantly contribute to studying dynamics of conformational DNA changes in the protein-nucleic complexes including nucleosome or chromatin. At present, measuring electrostatic potential of biological molecules in liquids is limited by high dielectric constant of the medium, by the possibility of a solvate shell forming on the sample surface, as well as by shielding the probe and sample surfaces with solution ions. Nevertheless, some studies show the possibility of using electrostatic force microscopy of solid materials at low ionic strength [67, 68]. Scanning electrostatic potential in liquid increases demands primarily on cantilever preparation. It should not only meet all the cantilever requirements for electrostatic force microscopy in DREEM mode, but also have a dielectric coating on the probe surface, excluding the area that is in direct contact with the sample. Apparently, further development of the method associated with increase in the device sensitivity would facilitate measurements of electrostatic potential of biomolecules in liquid.

In conclusion, it should be added that studies of the last decade have dramatically changed the views on the structure of chromatin and its basic unit, nucleosome. The static image is now complemented by the dynamic one, and methods providing the ability to study single molecules have played an important role in characterizing dynamic properties of nucleosomes. The use of AFM offers an opportunity to characterize a complex supramolecular system at the nanoscale, allowing direct visualization of the structural features of nucleosomes and changes within it such as, for example, the process of their unwrapping. In addition, the possibility of using small amounts of biopolymer preparations is a favorable feature of AFM measurements in comparison with some other methods.

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