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AFM of self-assembled lambda DNA-histone networks

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Abstract

Atomic force microscopy (AFM) was used to investigate the self-assembly behavior of λ -DNA and histones at varying histone:DNA ratios. Without histones and at the lowest histone:DNA ratio (less than one histone per 1000 base pairs of DNA), the DNA appeared as individual (uncomplexed), double-stranded DNA molecules. At increasing histone concentrations (one histone per 500, 250 and 167 base pairs of DNA), the DNA molecules started to form extensive polygonal networks of mostly pentagons and hexagons. The observed networks might be one of the naturally occurring, stable DNA-histone structures. The condensing effects of the divalent cations Mg^{2+} and Ca^{2+} on the DNA-histone complexes were also investigated. The networks persisted at high Mg^{2+} concentration (20 mM) and the highest histone concentration. At high Ca^{2+} concentration and the highest histone concentration, the polygonal network disappeared and, instead, individual, tightly condensed aggregates were formed.

Graphical Abstract

Keywords

Lambda DNA (or λ -DNA); Self-assembly; histone; pentagon; hexagon; network

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1. Introduction

DNA condensation is the collapse of extended DNA chains into one or a few compact particles [1]. Extensive lengths (millimeters to meters) of genomic DNA are compacted by proteins, natural polyamines and other compounds. DNA compaction in eukaryotes is mediated by its interaction with nuclear proteins, initially with topoisomerases and histones to form nucleosomes, then with chromatin fibers to form chromosomes [2, 3]. *In vivo*, histone proteins neutralize approximately 57% of the negatively charged sites on DNA [2, 4]. The remaining DNA charge is neutralized by other cationic compounds, including natural polyamines, mono- and polyvalent cations; cations are known to comprise up to 1% of the cell weight [2, 5].

DNA condensation up to and including nanoparticle size is a fundamental biological phenomenon that also plays an important role during DNA packaging into virus capsids. DNA can condense into different morphologies such as toroids, spheres, and rods, and DNA condensation can be induced by various condensing agent such as, spermine, $\text{Co}(\text{NH}_3)_6$, polylysine, alcohol and hexamine cobalt, spermidine, thalidomide, cisplatin and basic proteins such as histones H1 and H5 [1, 6-11].

The inherent ability of DNA to arrange itself has motivated studies of the assembly properties of DNA molecules in recent years. Since self-assembly allows fairly large and complex structures to come together in a single step, it may also be used in constructing molecular circuits. Controlled assembly of DNA molecules into highly oriented, interlaced patterns would be a critical step in future applications, in which DNA is used as a molecular building block of nanotechnology [12]. For instance, Seeman and colleagues pioneered the growth of 2D or 3D DNA crystalline lattices by self-assembling branched DNA junctions [13-16], via DNA complementarity. The self-organization of histone-jointed three-dimensional DNA network has been studied by fluorescence microscopy [17], and patterns in a DNA-histone mixture have been observed by microscopy [18]. Adleman et al. described the self-assembly of DNA into planar structures in the form of regular hexagonal tilings [19].

Atomic force microscopy (AFM) is a useful technique for imaging DNA and DNA-protein complexes on flat surfaces [20, 21] with high spatial resolution. DNA and protein molecules were stretched by spinning methods for detection by fluorescence and AFM [22, 23]. DNA is one of the most extensively studied molecules using AFM to understand its structure and interactions with proteins and other molecules [20, 24-29].

The most popular substrate in AFM imaging is a freshly cleaved mica surface, because it provides an atomically flat, negatively charged surface. Here we report self-assembled structures of λ -DNA-histone complexes and λ -DNA-histone-cation complexes as observed by AFM on a bare, newly cleaved muscovite mica surface.

2. Experimental procedure

2.1 In vitro incubation of DNA and histone

Histones from calf thymus were purchased from Sigma-Aldrich (USA). The histones in our experiment contained H1, H2A, H2B, H3 and H4. Tris base, EDTA, HEPES, CaCl_2 , and MgCl_2 were all purchased from Sigma-Aldrich (USA). λ -DNA (48502 bp) was purchased from Sigma-Aldrich (USA). Solutions were made with 18.2 M Ω deionized water purified through the Milli-Q Water Purification System (Millipore Corporation, France).

For DNA and histone binding, the histone solution was diluted with 18.2 M Ω deionized water. Histones dissolved or suspended in water should be stable for at least 6 months when frozen in single use aliquots. To test the effect of different histone to DNA ratios, solutions with histone to DNA molar concentration ratios of 50, 100, 200, 300, were prepared. This corresponds approximately to a 1:1000, 1:500, 1:250, 1:167 histone:base pair ratio, respectively. The molar concentration of DNA in all reaction solution was 0.5 pM. DNA and histones were incubated in Tris-HCl solution (10mM Tris, pH 7.5) for 3 hours at 37°C. The DNA-histone complexes were then diluted 5 times with loading buffer HEPES (10 mM HEPES, 10 mM NaCl, 1 mM MgCl_2) for AFM imaging.

2.2 DNA-histone complex, sample preparation and adsorption

For AFM imaging, the DNA-histone solution was diluted to an appropriate concentration (0.1 pM) with binding buffer (10 mM HEPES, pH 7.4, 10 mM NaCl, 1 mM MgCl_2). The sample (10 μl) was deposited onto a freshly cleaved mica surface. After 5 minute incubation at room temperature, the mica was rinsed thrice with 100 μl distilled water to remove salts and unbound DNA molecules, followed by drying with a flow of nitrogen. The sample was then put in the AFM for imaging (details below).

2.3 DNA–histone-cation complex, sample preparation and adsorption

To see the effect of Mg^{2+} and Ca^{2+} on the DNA-histone interaction, the sample with a molar concentration ratio of histone to DNA of 300 was used. We added MgCl_2 and CaCl_2 into the DNA-histone solution as described. The molar concentration of DNA in the reaction solution was 0.5 pM. The final concentration of MgCl_2 and CaCl_2 was 20 mM. Samples were incubated in Tris-HCl (10 mM Tris, pH 7.5) solution for 3 hours and 24 hours at 37°C respectively. The samples were then diluted 5 fold with HEPES binding buffer (see above). 10 μl of sample was deposited onto newly cleaved mica surface; incubated for 5 minutes, rinsed with 100 μl ddH $_2\text{O}$ three times, gently dried by nitrogen gas, and then put in the AFM for imaging. All experiments were repeated five to six times independently.

2.4. Atomic Force Microscopy imaging

A Nanoscope IIIa controller with a multimode AFM (Digital Instruments, Santa Barbara, CA) was used for AFM imaging. Silicon probes PPP-NCLR-W from Nanosensors (Neuchâtel, Switzerland) with a resonance frequency of 146-236 kHz were used. The scan rate was typically 2.7 Hz, and the scan size was typically between 2 μm and 4 μm . DNA tracing and measurements were done using Nanoscope IIIa Digital Instruments Version 5.31R1 software. The height of the DNA-protein complexes, the length and the width of the

pentagon or hexagon structure sides, and the volumes of the condensed structures were measured. For measuring the volume of histones and DNA-histone condensed bead

structures, we assumed the objects are spheres with volume, $V = \frac{4}{3}\pi R^3$ where R is the radius of the features, or the half the height of the features. All images shown here are height images unless noted otherwise. The images presented in this paper are free of modifications or image processing, except for flattening with a low order polynomial to remove the background curvature.

3. Results and discussion

3.1 AFM imaging of single DNA molecules on bare mica surface

Highly negatively charged DNA macromolecules, especially long DNA molecules, typically exist in a naturally compacted form, which depends strongly on its surroundings, thus, making it difficult to investigate its biological roles and biophysical functions on a molecular scale.

In order to investigate the interactions of DNA and histones in solution, we chose AFM imaging to observe the morphology of DNA-histone complexes directly. The chemical and physical characteristics of substrates also play an important role in the preparation of DNA samples for AFM measurements. In addition to the requirement that surfaces be atomically flat, anchoring sites are needed on the substrate surface to immobilize DNA molecules. Thus, substrate surfaces are often modified with positively charged silane compounds, divalent metal ions [12, 30-32] or multiple ions [7, 8] to improve retention of DNA on the substrates. For example, mica surfaces have been treated with the polycation spermidine which can enhance DNA binding to mica. However, spermidine (in solution) can also induce DNA condensation to some degree. In our work, we selected a bare muscovite mica surface without any treatment as an imaging substrate to investigate the effect of histones on DNA conformation. The main reasons for using bare mica were i) it is easy to use, ii) it is atomically flat over a large area and no pre-treatment with positive ions is required for DNA retention, iii) it is possible to obtain images of 2D equilibrated complexes, rather than trapped complexes [29]. Also, although there is no strong evidence that ions from pretreated mica may unbind and condense DNA, we just wanted to be sure that this possible effect (of surface ions having a condensing effect on DNA) is completely eliminated. When a 10 μ l droplet of λ -DNA-histone solution was deposited on a freshly cleaved mica surface, the drop solution readily spread across the mica surface to form a thin layer, due to the high hydrophilicity of mica surface. The solution was allowed to remain on the surface for 5 min for adsorption to occur. To remove buffer salts and unbound sample, the mica chip was rinsed thrice with distilled water, followed by gently drying with a flow of nitrogen gas.

It can be seen from Fig. 1a that the unmodified λ -DNA molecules appear in an extended, curvilinear and tangled form on mica surface, as has been seen in other studies [29] of plain DNA. Many single λ -DNA molecules are approximately evenly distributed (Fig. 1a). Assuming B-form λ -DNA, a molecule with 48.5 kb corresponds to a contour length of ~ 16.5 μ m (0.34 nm/bp), and such long molecules lead to some slight natural aggregations and/or entanglements on the substrate (Fig. 1a).

3.2 AFM imaging of DNA-histone 2D self-assemble network on bare mica surface

When the molar ratio of histones to λ -DNA is 50 (one histone per 1000 base pairs), even though the λ -DNA-histone complexes were somewhat aligned by the receding water meniscus, the morphology of the λ -DNA-histone complexes shows no significant difference from that of pure DNA molecules. The alignment in Fig. 1b was presumably caused by the known molecular combing effect of DNA molecules by a receding meniscus upon drying, it was not intentional and it was observed sometimes. The flow of nitrogen gas and exact deposition conditions are difficult to control perfectly, which resulted in somewhat varying sample coverage. In addition, different DNA coverage amounts were found on the same mica surface. The DNA networks seen at the higher histone concentrations (images below) are not yet apparent at a histone to DNA ratio of 50; few histones appear to be bound to the DNA molecules (Fig. 1b).

In the case of a molar histone to DNA ratio of 100 (one histone per 500 base pairs), histones cause some DNA condensation. The DNA molecules become thicker and a DNA network is starting to form. The smooth, curvilinear shape of the DNA molecules in the pure DNA sample seems to have disappeared, and instead more linear and rigid network structures are forming. Stiffened DNA strands as well as DNA bundles have formed. These DNA fibers are linked to each other and form a network of polygons with different sizes on the mica surface (Fig.2).

When the histone concentration is further increased, DNA condensation also increases. For a molar histone to DNA ratio of 200 (one histone per 250 base pairs), the DNA fibers form many mesh networks with irregular pentagon or hexagon structures on the mica surface (Fig. 3a). Numerous beads (presumably DNA-bound histones), are now apparent on the DNA fibers, and there is significant histone-mediated DNA condensation. We can see there are many beads on the mica surface, which we interpret to be free histones that did not bind to the DNA in solution (Fig. 3b). The mean value of the volume of these single histones is 5.09 nm^3 . Some DNA-histone complexes condensed and aggregated into blocks (Fig.3b).

When the molar histone to DNA ratio is 300, histones condense DNA into thicker fibers forming a dense network on mica surface. The bundled fibers are linked to each other, forming an interconnecting network structure (Fig. 4).

3.3 AFM imaging of DNA-histone-cation complex molecules on bare mica surface

In order to investigate the effect of divalent cations on the interaction of histone and DNA, we AFM imaged DNA-histone-cation complexes that were formed in solution. In the control sample (λ -DNA without histones), DNA molecules bind very well to the mica surface. DNA morphology shows the typical, curvilinear shape. Some DNA molecules slightly aggregate due to the high DNA density on the mica surface (Fig.5a). When the molar ratio of histones to DNA is 300, DNA-histone complexes self-assemble into a stable network structure of connected, mostly irregular pentagons or hexagons. Each side of the polygons is a histone-condensed DNA fiber. The heights and the widths of the DNA and DNA-histone complexes were also measured. The peak of the DNA height distribution was around 0.5 nm (DNA height is reduced in AFM imaging), whereas the peak value of the height distribution for the

DNA-histone complexes was around 2 nm. Comparing the height values of the DNA and DNA-histone complexes, we assume that the fibers include several DNA molecules (Fig. 5b). For the data analysis we used several different areas on a mica substrate. During the observation time (about 2 to 4 hours), the network was stable.

When adding 20 mM MgCl_2 to the sample with a molar histone to DNA ratio of 300, and incubating for 3 hours, a similar condensed network is observed as the one that was seen without Mg^{2+} (Fig. 6a). The DNA-histone- Mg^{2+} sample condensed into thicker, short, rigid fibers and formed a network of mostly irregular pentagon and hexagon shapes. Comparing with the previous experimental result it appears that Mg^{2+} may enhance the DNA and histone combination (Fig. 6a).

After a 24-hour incubation, the morphology of the DNA-histone- Mg^{2+} complexes changed drastically and as they condensed into rods. The network disappeared, the DNA-histone sample compacted into separate, condensed complexes that are distributed over the surface (Fig. 6b). This condensed rod structure is a step between the network and more compacted particles. Under certain *in vitro* conditions in Mg^{2+} solution, the nucleosome array will assembled into higher order structure such as 30 nm fibers. The structure we observed is likely not the 30 nm fibers. It may be an intermediate structure toward forming the 30 nm fiber, or it may be a different structure altogether. It could also be that the 30 nm fiber does not form under our conditions. It has been claimed that the 30 nm fiber does not form *in vivo* [33]. When divalent cations and histone are combined together, they may have a cooperative, additive effect. In previous work, the effect of divalent cations on DNA-histone interactions has been studied by molecular combing and fluorescence microscopy [34]. Due to the diffraction-based resolution limit of light microscopy, which is on the order of 500 nm, the substructure of DNA-histone-divalent cations could not be resolved. In our paper, we use AFM for which the probe-based resolution limit is typically on the order of tens of nanometers or less. The DNA-protein complexes are metastable in the presence of higher concentrations of Mg^{2+} . We believe the complexes formed in solution and not on the mica surface. These images provide new insights about DNA-histone interactions and the effect of divalent cation on these interactions.

When we incubated DNA, histones and 20 mM CaCl_2 together for 3 hours, most regions of the DNA condensed into smaller, tight beads or structures. Compared to the network of DNA-histone complex without Ca^{2+} , it appears that Ca^{2+} enhances the condensation effect of histones (Fig. 7a).

After a 24 hour incubation, the appearance of the Ca^{2+} -incubated DNA-histone sample changes dramatically as many smaller solid beads are seen (Fig. 7b). The beads have a height of about 5-8 nm, and it is not clear if they correspond to condensed DNA molecules, or not. We, therefore, determined the volume of these bead structures. The histogram of the volume distribution of the condensed structures is shown in Fig. 7c. The volume distribution is bimodal with one peak at small volumes (0 to about 60 nm^3 , peak at less than 12 nm^3), and another peak centered at around 160 nm^3 , with volumes ranging from 70 nm^3 to 270 nm^3 . The mean value of the volume histogram is 110 nm^3 ($n = 429$). The volume of a single bead is not nearly large enough to accommodate an entire λ -DNA molecule, whose volume would

be on the order of $52,000 \text{ nm}^3$. The volume distribution of a single histone, as measured by AFM is shown in the inset; it is centered around 3 nm^3 , and has a single peak. The following scenarios may explain this observation. After the Ca^{2+} incubation, the DNA did not bind well enough to the surface any longer, and only salt deposits and/or short DNA fragments are seen in the image. DNA morphology and DNA binding to mica strongly depends on the type of cation that is in solution [35, 36]. It could also be that the beads are DNA condensates, which are connected by DNA strands that were not discernable in the AFM image. These results, suggest that Ca^{2+} and Mg^{2+} have a different effect on the histone-DNA interaction (see Fig. 6, and Fig. 7).

In order to summarize the trend on the DNA-histone structure produced with the different concentration of histone, we collated an image series of ascending histone concentration (fig.8).

We will now discuss our data and propose a model to explain our experimental results. λ -DNA is a linear, double-stranded DNA molecule with 48502 base pairs; assuming B-form DNA, its contour length is about $16.5 \mu\text{m}$. λ -DNA has 12 base, single-stranded overhangs on both ends that are complementary. Their sequences are: $5'\text{-GGGCGGCGACCT-}3'$ and $5'\text{-AGGTCGCCGCCC-}3'$, which makes it possible to spontaneously link the free ends of λ -DNA molecules under appropriate conditions, forming large-scale networks without free ends of DNA molecules. However, the joined ends are not very stable at room temperature, so these networks are also not very stable.

In addition, λ -DNA is also a highly negatively charged semi-flexible polymer with one negative charge per $1.7 \text{ \AA}$, making it very sensitive to ionic surroundings. Electrostatically induced condensation by cations can, thus, be very strong. It should also be noted that slight variations in experimental conditions such as DNA concentration, histone concentration, temperature and deposition approaches can influence the network structure.

Histones have been shown to be a very efficient compacting agent, in concordance with their natural biological role. The role of electrostatic interactions is paramount in the compaction process. In the case of a low histone concentration (one histone per 1000 base pairs or less), histones bind to few positions along the DNA molecule. When the histone concentration increases, more histones bind to sites on the DNA. The binding of histones and DNA is sensitive to their molar concentration ratio. Histones contain a few lysine residues at the N terminus. Under normal cellular conditions, the side chain of lysine is positively charged, which can interact with the negatively charged phosphates of DNA backbone. In the case of high histone concentration, DNA aggregation is easily induced.

In solution, histones bind to single DNA molecules and condense the DNA molecules into more compact forms. At the same time, histones also induce different DNA molecules to bind to each other side by side to form bundles and fibers. Under the proper conditions, these DNA-histone complexes self-assemble into extended polygonal, stable network structures usually with irregular pentagons or hexagons, as seen in our experiments. The 2D DNA arrays can be several micrometers in size. Compared with non-specific DNA and histone binding, nucleosomes are formed within the assembled “beads-on-a-string”

nucleosomal array by dialysis. In our experiments, DNA and histones bind to each other via a non-specific mode without dialysis, so, we did not observe these “beads-on-a-string” structures.

AFM imaging of DNA and DNA-histone complexes reveal the actual morphology of these molecules in solution to some degree. After depositing the solution onto a mica surface, the three-dimensional DNA structures change to two-dimensional structures. However, Rivetti et al. have shown that DNA molecules and protein-DNA complexes can equilibrate in two dimensions on the surface before strongly binding to the surface [29]. Thus, the two-dimensional structures are likely close representations of the three-dimensional structures in solution. Based on these findings and on the fact that molecular assembly on the surface would be hampered by surface adhesive forces, we believe that the condensation of DNA molecules and the network formation occurred mostly in the solution, and not on the surface. Our data, thus, indicate that DNA and histones can form complex three-dimensional networks in solution. These structures were observed several times in different areas, indicating that the DNA-protein complex network forms relatively easy. During the observation time (about 2 to 4 hours), the network on mica surface was stable. We also deduce that the network and condensation occurred in solution were stable, and this condensed structure can endure the forces exerted by washing and N₂ drying step. Though, the forces of water and N₂ may somewhat effect the DNA-histone structures on the mica surface. The reported network of a DNA-histone mixture has been indirectly observed by fluorescence microscopy in a previous study [17, 18]. In our work, however, the DNA-histone network with irregular pentagons and hexagons has been observed directly by AFM.

Molecular self-assembly is a powerful approach for fabricating supramolecular architectures. Self-assembled structures on surfaces have been made from designed molecules, by templated growth, and from hydrogen-bonded systems. Semi-synthetic conjugates between DNA and protein have been used to generate self-assembled, oligomeric networks consisting of streptavidin and double-stranded DNA (dsDNA), which can be converted into well-ordered supramolecular structures [37].

In this study, we have shown the assembly of predefined dsDNA molecules into surface-immobilized networks using DNA binding protein, histone. A 2-D continuous network structure was seen on the mica surface. When the histone to λ -DNA ratio was 300, a network of irregular pentagons and hexagons was observed. In order to investigate the property of the network more deeply, we focus on some data of Fig.5. Topography measurements established that the peak value of the height of the network was about 2 nm, the other peak value was about 1 nm; whereas for single DNA, the peak value was close to 0.5 nm. The mean value of single DNA was 0.62 ± 0.08 nm, and mean value for wide DNA-histone fibers was 2.00 ± 0.17 nm. We also measured the distribution of length of the sides of the network, which had a mean value of 222 nm. The mean value for the width of single DNA was about 19.4 nm, and the mean value for the width of DNA-histone fibers was about 38.0 nm. However, when considering these values, the following two caveats are important to keep in mind. First, in AFM imaging, the height values are often compressed, because of the forces exerted by the AFM probe on the sample. Second, the widths (lateral measurements) of a sample are always exaggerated by the tip-broadening effect of AFM

imaging. Nevertheless, comparing the height and width measurements, it can be deduced that these fibers very likely include several DNA strands.

Understanding and controlling dynamic, self-assembling systems is a difficult problem. A self-assembling system consists of a group of molecules or segments of a macromolecule that interact with one another. Self-assembly occurs when molecules interact with one another through a balance of attractive and repulsive interactions. These interactions are generally weak (as compared to thermal energy) and noncovalent (van der Waals and Coulomb interactions, hydrophobic interactions, and hydrogen bonds), but relatively weak covalent bonds are also increasingly recognized as appropriate for self-assembly [38].

In our previous studies, the DNA-histone complexes have been stretched and aligned by molecular combing method [39]. At higher molar concentration ratio of histone to λ -DNA (200:1), the DNA-histone complexes were condensed into many tight beads, as observed by fluorescence microscopy, and these beads could not be stretched by molecular combing. Combining the results of fluorescence microscopy and AFM, we deduce that the efficiency of non-specific binding between DNA and histones is very high. Histones may serve as a bridge between the negatively charged mica surface and the negatively charged DNA molecules, the Mg^{2+} ions in the buffer can also serve as a bridge. The network structure we describe may be the result of a two-stage self-assembly processes; first, histones binding induces discrete local DNA condensation and connections followed by network formation into irregular pentagon or hexagons. Each side has a length of about 200 to 300 nm, and the height of thick side is about 2 nm (as determined by AFM images), four times of the height of single DNA molecules (as determined in AFM images). The model proposed by the experimental result is shown in Fig. 9.

4 Conclusions

In this work, we have shown that λ -DNA-histone complexes can self-assemble into a network of irregular pentagons and hexagons. AFM images reveal that the λ -DNA-histone network structure is quite sensitive to the concentration of DNA and ratio of histone to DNA molecules. At appropriate concentration ratios of histone to DNA, DNA-histone complexes are seen as irregular pentagon and hexagon networks on a bare mica surface. The network structure may be the result of a two-stage self-assembly processes, first, DNA condensation, followed by network formation. Ca^{2+} and Mg^{2+} enhance the condensation of DNA-histone.

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Highlights

The self-assembly behavior of λ -DNA and histones was studied by AFM on a mica surface.

λ -DNA-histone complexes can self-assemble into networks with pentagon or hexagon structures.

The λ -DNA-histone network structure is sensitive to the histone:DNA ratio.

The condensing effects of Mg^{2+} , Ca^{2+} on DNA-histone complexes were also investigated.

Fig.1.

(a) AFM images of single λ -DNA molecules. (b) AFM images of single λ -DNA-histone molecules. The concentration of λ -DNA is 0.1 pM, the molar ratio of histones to λ -DNA is 50 (one histone per 1000 bp). The regions enclosed in the white squares are enlarged and shown in the middle panel. (c) Histogram representing the height distribution for the DNA molecules (n = 363) in (a). (d) Histogram representing the height distribution for DNA-histone sample (n = 426) in (b). Scale bars are 200 nm.

Fig. 2.

(a) AFM images of single λ -DNA-histone molecules, the concentration of λ -DNA is 0.1 pM, the molar ratio of histones to λ -DNA is 100 (one histone per 500 base pairs). The regions enclosed in the white squares are enlarged and shown in the middle panel. (b) Histogram representing the height distribution for the DNA molecules ($n = 324$) in (a). Scale bars are 300 nm.

Fig.3.

(a-b) AFM images of single λ -DNA-histone molecules, the concentration of λ -DNA is 0.1 pM, the molar ratio of histones to λ -DNA is 200 (one histone per 250 base pairs). The regions enclosed in the white squares are enlarged and shown in the middle panel. (c) Histogram representing the height distribution for DNA molecules (n = 438) in (a). (d) Histogram representing the height distribution for DNA-histone (n = 475) in (b). Scale bars are 200 nm.

Fig.4.

AFM images of single λ -DNA-histone molecules, the concentration of λ -DNA is 0.1 pM, the molar ratio of histones to λ -DNA is 300 (one histone per 167 base pairs). The regions enclosed in the white squares are enlarged and shown in the middle panel. (b) Histogram representing the height distribution for DNA (n = 476) in (a). Scale bars are 300 nm.

Fig.5.

(a) AFM images of single λ -DNA molecules. (b) AFM images of single λ -DNA-histone complexes molecules, the concentration of λ -DNA is 0.1 pM. The molar ratio of histones to DNA is 300 (one histone per 167 base pairs). The following numerical values are given as mean $\pm$ standard deviation. (c) Histogram representing the height distribution of the histone-free DNA in (a); $n = 402$; $h = 0.62 \pm 0.08$ nm. (d) Histogram representing the height distribution for DNA-histone fibers in (b); $n = 422$; $h = 2.00 \pm 0.17$ nm. (e) Histogram representing the length distribution of DNA segments in (b); $n = 335$; $L = 222 \pm 62$ nm. (f)

Histogram representing the width distribution of DNA molecules in (a); $n = 368$; $W = 19.4 \pm 3.5$ nm. (g) Histogram representing the width distribution of DNA segments in (b); $n = 444$; $W = 38.0 \pm 4.3$ nm. Scale bars are 300 nm.

Fig. 6.

(a) AFM images of single λ -DNA-histone - Mg^{2+} complexes molecules. Incubation time: 3 hours. (b) AFM images of single λ -DNA-histone- Mg^{2+} complexes molecules. Incubation time: 24 hours. The concentration of λ -DNA is 0.1 pM. The molar ratio of concentration of histone to that of DNA is 300 (one histone per 167 base pairs). Scale bars are 300 nm.

Fig. 7.

(a) AFM images of single λ -DNA-histone- Ca^{2+} complexes molecules, incubation time: 3 hours. (b) AFM images of single λ -DNA-histone- Ca^{2+} complexes molecules, incubation time: 24 hours. The concentration of λ -DNA is 0.1 pM. The molar ratio of concentration of histone to that of DNA is 300 (one histone per 167 base pairs). (c) The zoomed-in images of figure (b) are shown in the upper panel. The large histogram represents the volume distribution of the condensed DNA-histone- Ca^{2+} complexes in (b) ($n = 429$). The distribution is bimodal with one peak at small volumes (0 to about 60 nm^3 , peak at less than 12 nm^3), and another peak centered at around 160 nm^3 . The mean value of the volume histogram is 110 nm^3 . The inset histogram represents the volume distribution of individual histones (from image 3b); it is a distribution with unimodal distribution with an average of 3 nm^3 ($n = 146$); it partially overlaps with the smaller volume peak of the features in image (7b). Scale bars are 300 nm.

Fig. 8.

(a) AFM images of single λ -DNA molecules. The concentration of λ -DNA is 0.1 pM. (b-d) AFM images of single λ -DNA-histone complexes at different molar histone to λ -DNA ratios. (b) Histone to λ -DNA ratio of 50 (one histone per 1000 bp). (c) Histone to λ -DNA ratio of 100 (one histone per 500 base pairs). (d) Histone to λ -DNA ratio of 300 (one histone per 167 base pairs). Scale bars are 300 nm.

a

Fig. 9.

Schematic for λ -DNA and histone binding. (a) Initial binding of histones to λ -DNA induces local condensation of DNA fragments into a pentagon and hexagon structures. (b) A network of λ -DNA-histone structures is formed by further linkages of local pentagon and hexagon structures, which are distributed along DNA molecules.