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Two-Component AFM Recognition Imaging of Complex Samples

H. Wang¹, R. Bash^{1,2}, and D. Lohr^{2,*}

¹ Biodesign Institute, Arizona State Univ., Tempe, AZ 85287-1604

² Department of Chemistry and Biochemistry, Arizona State Univ., Tempe, AZ 85287-1604

Abstract

Biological complexes are typically multisubunit in nature and the processes in which they participate often involve protein compositional changes, in themselves and/or their target substrates. Being able to identify more than one type of protein in complex samples and to track compositional changes during processes would thus be very useful. Toward this goal, we describe here a single molecule technique that can simultaneously identify two types of proteins in compositionally complex samples. It is an adaptation of the recently developed AFM Recognition Imaging technique but involves the tethering of two different types of antibodies to the AFM tip and sequential blocking with appropriate antigenic peptides to distinguish the recognition from each antibody. The approach is shown to be capable of simultaneously identifying in a single AFM image two specific components, BRG1 and β -actin, of the human Swi-Snf ATP-dependent nucleosome remodeling complex and two types of histones, H2A and H3 in chromatin samples.

Keywords

AFM; SPM; Recognition Imaging; Nucleosomal Arrays; hSwi-Snf; Chromatin

Introduction

Biologically relevant processes often involve protein changes in the multisubunit complexes that carry out the process and/or in the substrates upon which they act. It would therefore be very useful if it were possible to identify and track, simultaneously and at the single molecule level, more than one protein component in a complex sample.

Atomic Force Microscopy (AFM) is a single molecule technique that is uniquely suited to image complex biological materials and track events involving large biological complexes [1–3]. Imaging can be carried out in solution and, by the use of a flow-cell linked to the AFM, changes in the same set of individual molecules can be tracked during specific processes *in vitro* [4–8]. Recent developments have made it possible to identify a specific type of protein in AFM images [9] and to track its movements during biologically relevant processes [8].

The basic approach that permits the identification of a specific type of protein, Recognition Imaging, involves scanning the deposited sample with an AFM tip tethered to an antibody against the protein of interest. Scanning generates a traditional topographic AFM image and, simultaneously and in exact spatial registration, also generates a recognition image that locates

*Correspondence to: D. Lohr., Chemistry and Biochemistry Dept., Arizona State University, Tempe, AZ 85287–1604 480–965–5020 (Ph), 480–965–2747, dlohr@asu.edu (e-mail)

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the sites of antigen-antibody binding events, and thus the locations of the specific protein of interest, within the field of molecules. The two images can be electronically superimposed to obtain very accurate maps of the protein locations in the topographic image [8]. Recognition can be both efficient and specific [7–9]. The specificity of recognition can be checked in numerous ways, including the ability of recognition events in the deposited sample to be “blocked” (removed) when a peptide antigenic to the antibody on the tip is injected into the flow cell containing the deposited sample [9]. This important control can be done almost immediately after obtaining the initial recognition image and uses the same imaged sample. It provides a very specific test of recognition [9].

This work describes an extension of the basic Recognition Imaging technique. It involves tethering two different types of antibodies to the same AFM tip (Figure 1), which is then used to scan the sample. The recognition image from this scan can contain recognition signals from two types of proteins. In order to distinguish these two types of recognition events, the sample is rescanned sequentially after adding antigenic (blocking) peptides (or proteins) against first one then both antibodies on the AFM tip. This approach allows each class of recognition event to be distinguished. Two types of complex samples were tested, the human Swi-Snf (hSwi-Snf) ATP-dependent nucleosome remodeling complex, a large (> 1 M Da), multisubunit complex, and a mixture of chromatin complexes, octameric (H2A-H2B-H3-H4) nucleosomal arrays and H3-H4 tetramer/DNA arrays.

Materials and Methods

Sample Preparation

An ~ 1.9 kb Mouse Mammary Tumor Virus promoter DNA fragment [5,10] was reconstituted into nucleosomal arrays with HeLa histone octamers [11] or into H3-H4 tetramer arrays with human H3-H4 histones (Upstate Cell Signaling Solutions, Lake Placid NY) by a salt reconstitution protocol and fixed with glutaraldehyde as described previously [5,12]. The human Swi-Snf complex was a generous gift from G. Hager. It was isolated [13] as described [6].

The anti-BRG1 and anti- β -actin antibodies were purchased from Abcam (UK) and the anti-H2A and anti-H3 antibodies were purchased from Upstate. The H2A antibody was raised against the acidic patch region, on the 11 nm face of the nucleosome [14], and the H3 antibody was raised against a region from the N-terminal tail. The blocking peptides were synthesized locally in the Protein Synthesis Laboratory.

Modifying AFM Tips

Attaching two types of antibodies to AFM tips uses the same techniques described previously for attaching a single type of antibody [9]. SATP(N-Succinimidyl 3-(acetylthio)propionate)-modified antibodies are linked to APTES(Aminopropyltriethoxysilane)-modified AFM tips via a bifunctional PEG linker. Equimolar mixtures of the two types SATP-modified antibodies are used.

AFM Imaging

hSwi-Snf complexes or octameric nucleosomal arrays plus H3-H4 tetramer/DNA complexes were deposited on GD-APTES mica in 10 mM NaCl/5 mM phosphate buffer, pH 7.5, and imaged as described [7]. The AFM tips used for scanning were derivatized with antibodies as described before [9,15] but using equimolar mixtures of the SATP-modified antibodies, either anti-BRG1+anti- β -actin (images of hSwi-Snf) or anti-H3+anti-H2A (images of chromatin). Recognition signals were obtained by PicoTREC and magnetized cantilevers were driven by a MacMode dynamic-force microscope (Agilent Technologies AFM, Phoenix, Az). Images

were taken with 3-nm peak-to-peak amplitude oscillation at 8 kHz, imaging at 70% set point, and scanning at 1 Hz. For the hSwi/Snf experiments, blocking was carried out by first flowing solutions containing 30 ug/ml of actin blocking peptide into the AFM/flow-cell, then 30ug/ml of BRG1 blocking peptide. For the mixed chromatin (octamer + tetramer) experiments, recognition blocking was carried out first by flowing 30 ug/ml H3 peptide into the AFM/flow-cell, then 30 ug/ml of H2A histone.

Results and Discussion

To identify a single specific type of protein in AFM images, the deposited sample is scanned with an AFM tip tethered to an antibody against that protein [9]. Scanning simultaneously generates a topographic image and a recognition image that identifies the locations of the antibody-antigen binding events, and thus the locations of the specific protein, within the field of molecules. For technical reasons, the sites of antibody-antigen binding appear as dark spots in the recognition image (discussed in ref [9]). The recognition and topographic images can be electronically superimposed to obtain an accurate map of the locations of the specific protein within the topographic image [8]. To check that recognition is specific, a peptide (or protein) antigenic to the antibody on the tip (usually the peptide that generated the antibody) is flowed in to the flow-cell and the same field of molecules is rescanned. The presence of this antigen in the flow-cell solution will “block” the specific interaction of the tip-bound antibody with its surface-bound antigen (protein), thus causing specific recognition signals to be greatly reduced or to disappear in the recognition image from the scan done in the presence of the blocking peptide [9].

Our goal here was to test the possibility of simultaneously detecting two different types of proteins in AFM images of complex, biologically-relevant samples. We proposed to do this by scanning with AFM tips containing two different types of antibodies attached to a single tip (Figure 1). The initial scan with this dual-modified tip should generate a topographic image and a recognition image that contains signals corresponding to recognition of both types of proteins. In order to distinguish the two types of recognition signals, a peptide antigenic to one of the antibodies on the tip will be flowed in to the sample and the same field (and same set of individual molecules) rescanned. The presence of the peptide should block recognition events due to the corresponding antibody and subtract (or greatly weaken) those signals in the recognition image from that scan. Comparison of the initial and + block recognition images will identify the locations of that specific protein. Then, a peptide antigenic to the other antibody is also flowed in (both types of peptides are simultaneously present) and the same molecules rescanned. The presence of the second peptide should remove the recognition due to the second type of antibody. Again, comparison of images will provide the locations of the corresponding protein. Thus, addition of each blocking peptide should abolish the recognition due to that specific protein in the initial image. Note however that it is not possible to know whether one or both proteins in any individual molecule are actually recognized by the tip-bound antibodies in the initial scan. Incomplete recognition may occur for several reasons (see below).

Imaging a Multiprotein Complex

Important biological complexes often contain multiple protein components and one potential use of this technique would be to identify and perhaps track, simultaneously, two such components during a process carried out by the complex. To test the capability to image multiple proteins in large biological complexes, we deposited and imaged the human Swi-Snf ATP-dependent nucleosome remodeling complex. This $> 10^6$ dalton complex contains a number of different types of proteins [16–18] including BRG1, the major ATPase subunit, and β -actin, whose precise function in the complex is uncertain [17]. hSwi-Snf complexes are

thought generally to contain both types of subunits. We have previously imaged hSwi-Snf complexes with tips modified by anti-BRG1 [7] or by anti- β -actin (data not shown) antibodies.

To test the ability to detect both of these proteins simultaneously, samples of hSwi-Snf were deposited and scanned with AFM tips that were derivatized with both anti-BRG1 and anti- β -actin antibodies, then rescanned after addition of β -actin blocking peptide to the flow-cell (to identify β -actin recognition events) and then scanned again after addition of BRG1 peptide (to identify BRG1 recognition events). The primary goal was to test our ability to identify BRG1 or β -actin in the various complexes in a single image field but, if possible, we hoped to be able to detect both proteins within the same individual complex. Dual recognition in a single complex should produce a particular type of response; the recognition should decrease in significant steps as first β -actin blocking peptide then β -actin + BRG1 blocking peptides are added.

Figure 2 shows some typical results for such an experiment. Panel A is the topographic image from the initial scan and panels B-D are recognition images from the initial scan (panel B), the scan after blocking with β -actin peptide (panel C) and the scan after blocking with β -actin and BRG1 peptides (panel D). The topographic images from the scans made after each block are not shown. It is obvious that for some complexes (3 and 5 in panel A), recognition virtually disappears after blocking with the β -actin peptide. Thus, the recognition in those complexes in the initial scan (panel B) is likely to have involved recognition of β -actin in the hSwi-Snf complex. Those complexes are identified by dotted squares in the initial topographic and recognition images (panels A and B). For other complexes (1, 2 and 4), recognition remains strong when β -actin blocking peptide is present, in fact it seems to increase in some cases, but disappears after BRG1 blocking peptide is also added. Those examples are likely to reflect recognition of BRG1 in the initial scan and are identified by solid circles in the initial images. The small white dots in panels B-D are sometimes seen in recognition images at sites of recognition or more commonly sites where recognition has been blocked.

Quantifying the intensity of recognition for these complexes confirms the visual interpretations described above (Table 1). The number in the table reflects the strength of the recognition event; the lower the number, the stronger the recognition. For complexes 3 and 5, addition of β -actin peptide reduces the intensity of recognition basically to background levels and addition of BRG1 peptide causes no further change. However, for complexes 1, 2 and 4, recognition intensity is unaffected or even increases (complex 2) when β -actin blocking peptide is added but goes to background levels after BRG1 peptide is added. Thus, this data indicates quite clearly that we can detect both of these proteins in an image of deposited hSwi-Snf complexes and distinguish between the two types of recognition by these sequential blocking techniques. The type of all-or-none response shown in Figure 2 and Table 1 was the typical behavior throughout the entire data set. The observation of higher recognition levels in a complex after the first blocking peptide was added (cf. complex 2), sometimes very much higher (data not shown), was also fairly common. Only very rarely did we detect a stepwise decrease in recognition (as first one then the other recognition response is blocked), which would be expected for dual recognition in the same individual hSwi-Snf complex. Thus, these results show that we can detect and distinguish both BRG1 and β -actin in image fields of deposited hSwi-Snf complexes but we cannot identify both proteins within the same individual complex.

Why can both proteins be recognized in the same image but not within the same complex? One possible cause is interference effects involving the tip-bound antibodies, which could inhibit or prevent simultaneous recognition reactions of both types of antibodies with a single complex (in the initial scan). Blocking of one type of antibody-antigen recognition would be expected to at least partially relieve this interference, possibly enabling the unblocked antibody to contact its antigen more easily or more strongly. This could account for the increased recognition of a

particular complex that is sometimes seen in the singly-blocked versus unblocked images. Interference effects (in the initial scan) are likely to be especially important if BRG1 and β -actin are located very close to one another in the complex (their relative locations are unknown). Also, the two proteins in this (or any) complex may not always both be exposed to the AFM tip, depending on where they lie in the complex and exactly how the complex deposits on the surface [7]. The efficiency of BRG1 recognition in hSwi-Snf complexes is ~60–70% [7] and the recognition efficiency of β -actin is even lower (unpublished observations), due probably to such features. Lower recognition efficiencies lower the likelihood for dual recognition in individual complexes since the frequency of that recognition should depend on the product of the two individual (fractional) recognition efficiencies. Low recognition efficiencies also explain why a number of complexes show no recognition at all (Figure 2, panels A vs B). Note also that tips may contain more than one antibody of each type, which would also enhance the possibilities for interference; the relative numbers of antibodies present and where they reside on tips are unknown and uncontrollable. Thus, there are a number of reasons why these dual-modified tips appear to recognize mainly one type of protein in a hSwi-Snf complex even though both are probably present.

Imaging Histone-DNA Complexes

Previously, we used anti-H2A or anti-H3 histone antibodies in recognition imaging studies of MMTV nucleosomal arrays [8,9]. These antibodies are quite suitable for such studies; they recognize the histones in nucleosomal arrays with > 95% efficiency [9] and show < 10 % cross recognition (H2A antibodies recognizing H3-H4 tetramer/DNA arrays [8] and H3 antibodies recognizing H2A-H2B dimers (unpublished results)). Nucleosomes contain two copies each of H2A, H2B, H3 and H4 present in a 5 x 11 nm particle in which parts of all four types of histones are exposed to the solution [14]. At low-to medium occupation levels, arrays are generally extended and individual nucleosomes remain solvent-exposed [8,9]. The exposure and high recognition efficiency of histones in nucleosomal arrays suggested that dual recognition might be more successful with this substrate than it was with hSwi-Snf complexes.

To test this system, nucleosomal arrays and arrays of H3-H4 tetramer particles on DNA were separately reconstituted (Materials and Methods), then deposited together at an ~ 1:1 ratio and scanned with AFM tips that contained both anti-H3 and anti-H2A antibodies. The samples were then rescanned first in the presence of H3 peptide (to block H3 recognition events in the deposited sample) and then in the presence of H3 blocking peptide + H2A histone (to also block H2A recognition events). This experiment was designed to test simultaneously 1) the ability to detect two proteins (H3 and H2A) in the same image, and perhaps in the same individual (nucleosomal array) molecules, and 2) the ability to determine that one of two given types of proteins is absent in a complex (H3-H4 tetramer/DNA arrays). For nucleosomal arrays, recognition intensity should decrease at each blocking step. For the H3-H4 tetramer/DNA arrays, recognition should disappear from the image when H3 blocking peptide is added and the addition of H2A should have little effect. Nucleosomes differ significantly in height from H3-H4 tetramer/DNA particles [8], averaging 3.1 nm versus 1.9 nm in this study (data not shown). Those values are consistent with previously determined values [7,8]. Thus, height measurements can distinguish the two types of molecules in topographic images, providing an independent way to identify the two types of chromatin substrates in the image.

Some results are shown in Figure 3. Panels A and B are the topographic and recognition images from the initial scan. In the rows of smaller panels to the right of panels A and B (rows I-IV), some specific molecules from these images are shown at higher magnification. These specific examples are taken (left to right) from the initial topographic image shown in panel A (“topo”), from the initial recognition image shown in panel B (“recog”), from the recognition image after H3 blocking peptide was added (“+ bl -3”) and from the recognition image after H3 peptide

+ H2A addition (“+ bl -3/2A”). Rows I and II are nucleosomal arrays (identified in panel A by solid circles and corresponding numbers in panels A and B) and rows III and IV are H3-H4 tetramer/DNA arrays (identified in panel A by dashed squares and corresponding numbers in panels A and B).

In the nucleosomal array examples shown (rows I and II), the addition of H3 blocking peptide causes a large reduction in the initial level of recognition but recognition remains significant (+ bl -3 column). However, recognition virtually disappears when both H3 peptide and H2A are present (+ bl -3/2A column). These examples indicate that it is possible using this technique to identify simultaneously two types of proteins in an image and also within an individual array molecule. However, the results averaged for the set of nucleosomal arrays in the field show that dual recognition in a single complex is not consistently observed (Table 2). As for the hSwi-Snf results, the lower the number in the table, the stronger the recognition. The average intensity goes from 36 to 44 after H3 blocking peptide is added and to 82, background levels, after H2A is also added. Thus, although the recognition intensity does generally decrease after H3 peptide addition, the magnitude of the decrease (36 to 44) is smaller than expected if both proteins were consistently being recognized in the same individual complexes (a value of 50–60 would be expected after the first block). In some molecules, the expected stepwise decrease is observed; for example, for the array in row I, the intensity decreases from 30 to 59 (after H3 block) to 78 (after H2A +H3 block). Thus, the ability to detect and distinguish both types of proteins in the same individual complex is slightly better in nucleosomal arrays than in hSwi-Snf complexes but still not highly efficient. However, in no cases did the recognition disappear completely after the first blocking peptide was added, as was the most common occurrence in hSwi-Snf recognition (see above). As for the hSwi-Snf studies (above), recognition reaches background levels in the presence of both blocking peptides, indicating an absence of nonspecific contributions to the recognition.

The results for H3-H4 tetramer/DNA arrays differ from the nucleosomal array results, although again they are not as quantitatively compelling as hoped. In some molecules (cf. row IV), the level of recognition was virtually abolished when H3 blocking peptide was added but more often recognition was strongly reduced by addition of H3 blocking peptide but only fell completely to background levels after the addition of H2A (row III). Quantitative results (Table 2) show that, on average for the set of H3-H4/DNA tetramer arrays in the field, the intensity value starts out at 38, rises to 66 after H3 peptide is flowed in and goes to background (82) after adding H2A. Thus, the addition of H3 blocking peptide causes a much larger decrease in recognition than the change produced in nucleosomal arrays (38 to 66 vs 36 to 44) but it does not completely abolish the recognition of H3-H4 tetramer/DNA particles by these dual-modified tips. The very substantial decrease in recognition observed in H3-H4 tetramer/DNA arrays after H3 blocking peptide addition does clearly demonstrate that H3 is being recognized in these images (a possible explanation for the nucleosomal array results is that H3 recognition was not working well). The source of the residual recognition in the H3-H4 tetramer arrays after H3 peptide addition is unclear. Blocking of recognition in H3-H4 tetramer/DNA particles by H3 peptide is fairly efficient (70 %, Wang et al., unpublished results) and this H2A antibody does not recognize H3-H4 tetramer/DNA particles alone [8]. The chromatin is fixed so H2A/H2B exchange is unlikely.

The results in this section again show that it is possible to detect two types of proteins (H3/H2A) in an AFM image of complex particles and show, in addition, that there is a significant difference in the quantitative responses of complexes containing two versus only one of the targeted proteins, i.e. recognition decreases much more strongly for H3-H4 tetramer/DNA particles than for nucleosomes after H3 blocking peptide is added (Table 2). However, we could not consistently achieve dual recognition in the same individual nucleosomal arrays. Arrays are relatively extended molecules and the histone epitopes to these particular antibodies,

the tails (H3) and the 11 nm face (H2A), should be exposed, so the kinds of masking effects suggested for hSwi-Snf (due to target protein location in the complex or to the surface) are less likely to occur in nucleosomal arrays. This suggests that antibody interference in the initial scan and/or enhanced recognition by the unblocked antibody (H2A) when the other antibody (H3) is blocked may be the major problems. In agreement with the suggestion of interference effects, the recognition efficiencies of nucleosomal arrays by singly-modified tips (H3 or H2A) are higher than by tips containing both anti-H3 and anti-H2A antibodies (Table 2).

Conclusion

We have developed a technique that uses an AFM tip containing two tethered antibodies and sequential blocking techniques to identify two types of proteins in single AFM images of compositionally complex molecules. Two very different types of complexes, human Swi-Snf, an ATP-dependent nucleosome remodeling complex, and chromatin arrays (of two types) were analyzed. The technique was able 1) to detect two components of human Swi-Snf, BRG1 and β -actin, in the same image 2) to detect the histones H3 and H2A in an image and 3) to detect significant differences between complexes containing one protein antigen (H3-H4 tetramer-DNA complexes) and complexes containing two protein antigens (nucleosomes) in samples containing both. This technique can thus be used for qualitative compositional analyses of complexes and tracking of the fates of two components of a complex or a substrate upon which the complex is acting during a process. We could not identify the two types proteins within the same individual complex with the consistency required for quantitative analyses, due probably to interference effects involving the presence of two types of antibodies on the tips. However, this technique might work better for analyses of processes that involve protein release from complexes or substrates, of either one or both proteins of interest, because interference effects should be minimized in such a situation. Released (free) proteins can be recognition imaged; free H2A histone was directly and quantitatively detected in a recent Recognition Imaging study of nucleosome remodeling [8].

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References

1. Lindsay, S. Scanning Probe Microscopy, Techniques and Applications. 2. John Wiley; 2000. The scanning Probe Microscope in Biology.
2. Frederix P, Akiyama T, Staufer U, Gerber C, Fotiadis D, Müller DJ, Engel A. Atomic Force Bioanalytics. *Curr Opin Chem Biol* 2003;7:641–647. [PubMed: 14580570]
3. Hansma H, Kasuya K, Oroudjev E. Atomic force microscopy imaging and pulling of nucleic acids. *Curr Opin Sturc Biol* 2004;14:380–385.
4. Wang H, Bash R, Yodh J, Hager G, Lohr D, Lindsay S. Glutaraldehyde Modified Mica: A New Surface for Atomic Force Microscopy of Chromatin. *Biophys J* 2002;83:3619–3625. [PubMed: 12496129]
5. Bash R, Wang H, Yodh J, Hager G, Lindsay SM, Lohr D. Nucleosomal Array Can be Salt Reconstituted on a Single Copy MMTV Promoter DNA Templates; Their Properties Differ in Several Ways from those of Comparable 5s Concatemeric Arrays. *Biochemistry* 2003;42:4681–4690. [PubMed: 12705831]
6. Wang H, Bash R, Yodh JG, Hager G, Lohr D, Lindsay SM. Using AFM To Track Nucleosome Remodeling on Individual Nucleosomal Arrays In Situ. *Biophys J* 2004;87:1964–1971. [PubMed: 15345572]
7. Wang H, Bash R, Lindsay SM, Lohr D. Solution AFM Studies of Human Swi-Snf and Its Interactions With MMTV DNA and Chromatin. *Biophys J* 2005;89:3386–3398. [PubMed: 16100261]

8. Bash R, Wang H, Anderson C, Yodh J, Hager G, Lindsay SM, Lohr D. AFM Imaging of Protein Movements: Histone H2A-H2B Release During Nucleosome Remodeling. *FEBS Lett* 2006;580:4757–4761. [PubMed: 16876789]
9. Stroh C, Wang H, Bash R, Ashcroft B, Nelson J, Gruber H, Lohr D, Lindsay SM, Hinterdorfer P. Single Molecule Recognition Imaging Microscopy. *Proc Natl Acad Sci USA* 2004;101:12503–07. [PubMed: 15314231]
10. Fletcher T, Ryu B, Baumann C, Warren B, Fragoso G, John S, Hager G. Structure and dynamic properties of a glucocorticoid receptor-induced chromatin transition. *Mol Cell Biol* 2000;20:6466–6475. [PubMed: 10938123]
11. Yodh JG, Lyubchenko YL, Shlyakhtenko LS, Woodbury N, Lohr D. Evidence for nonrandom behavior in 208–12 subsaturated nucleosomal array populations analyzed by AFM. *Biochemistry* 1999;38:15756–63. [PubMed: 10625441]
12. Bash R, Yodh J, Lyubchenko Y, Woodbury N, Lohr D. Population analysis of subsaturated 172–12 nucleosomal arrays by atomic force microscopy detects nonrandom behavior that is favored by histone acetylation and short repeat length. *J Biol Chem* 2001;276:48362–48370. [PubMed: 11583994]
13. Sif S, Stukenberg P, Kirschner M, Kingston R. Mitotic Inactivation of a HumanSwi-Snf Chromatin Remodeling Complex. *Genes Dev* 1998;12:2842–54. [PubMed: 9744861]
14. Luger K, Mader A, Richmond R, Sargent D, Richmond T. Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature* 1997;389:251–260. [PubMed: 9305837]
15. Lohr D, Bash R, Wang H, Lindsay SM. Using Atomic Force Microscopy to Study Chromatin and Nucleosome Remodeling. *Meth Mol Biol*. 2006
16. Kingston RE, Narlikar GJ. ATP-dependent Remodeling and Acetylation as Regulators of Chromatin Fluidity. *Genes Dev* 1999;13:2339–2352. [PubMed: 10500090]
17. Olave IA, Reck-Peterson SL, Crabtree GR. Nuclear Actin and Actin-Related Proteins in Chromatin Remodeling. *Annu Rev Biochem* 2002;71:755–781. [PubMed: 12045110]
18. Sif S. ATP-dependent Nucleosome Remodeling Complexes: Enzymes Tailored to Deal With Chromatin. *Curr Opin Struc Biol* 2004;91:1087–1098.

Figure 1.

'Two-Component' Recognition Imaging. An AFM tip is functionalized with an equimolar mixture of two types of antibodies (closed versus open ovals) and used to scan a compositionally complex sample. The precise numbers of antibodies that attach to a given tip during the modification process are impossible to determine and can probably vary from tip to tip and from process to process. Thus, the relative numbers of the two types of antibodies or even that both types are present cannot be known.

Figure 2.

Testing the Ability to Identify Different Subunits in a Multiprotein Complex. Human Swi-Snf ATP-dependent nucleosome remodeling complexes (hSwi-Snf) were deposited and scanned with an AFM tip containing both anti-BRG1 and anti- β -actin antibodies, then rescanned in the presence of β -actin blocking peptide, then rescanned in the presence of BRG1 and β -actin blocking peptides. Panel A is the topographic image from the initial scan, panel B is the corresponding recognition image (no blocking), panel C is the recognition image obtained after blocking with β -actin peptide and panel D is the recognition image obtained when both BRG1 and β -actin blocking peptides are present. Dashed squares identify complexes whose recognition disappears after blocking with actin peptide and solid circles identify complexes whose recognition disappears only when BRG1 blocking peptide is also present. The squares and circles are shown so long as the recognition is present, i.e. in panels B and C. The numbers in panel A correspond to the complexes whose recognition intensity is listed in Table 1. Note that the topographic images obtained from scans made after blocking (not shown) help insure that the same complexes are analyzed in every recognition image. This is especially important when both blocking peptides are present and recognition is very weak. The scale bar is 200 nm.

Figure 3.

Testing H3 and H2A Recognition in Mixed Chromatin Samples. H3-H4 tetramer/DNA arrays and octameric nucleosomal arrays reconstituted separately on MMTV promoter DNA were deposited together at $\sim 1:1$ stoichiometry and scanned with an AFM tip containing anti-H3 and anti-H2A antibodies, then rescanned in the presence of H3 blocking peptide and then rescanned in the presence of H3 blocking peptide plus H2A histone. Panels A and B show topographic and recognition images from the initial scan of this deposited sample. In the rows of smaller panels to the right of panels A and B (rows I-V), several specific molecules are shown at higher magnification. These examples are taken from (left to right) the initial topographic image (“topo”, panel A), the initial recognition image (“recog”, panel B), the recognition image after H3 blocking peptide was added (“+ bl -3”) and the recognition image after H3 blocking peptide + H2A histone addition (“+ bl -3/2A”). Note that these latter two recognition images are not shown here. The examples are marked in panel A, by solid circles for nucleosomal arrays and by dashed squares for H3/H4 tetramer-DNA arrays, and identified by numbers in panels A and B. The determinations of whether a molecule is an octameric array or an H3/H4 tetramer-DNA complex are based on particle heights. The topographic images taken after each blocking peptide are not also shown but again are useful to insure that the same chromatin complexes are consistently analyzed. The scale bars are 500 nm in panel A and 100 nm in panel 1.

Table 1
Recognition Intensity Values for hSwi-Snf Complexes^a

hSwi-Snf Complex ^b	Intensity(no block)	Intensity(β -actin block)	Intensity (actin + BRG1 block)
1	42	43	74
2	50	36	69
3	28	67	69
4	39	45	65
5	38	67	72

^aThe intensity of recognition events is measured in traces across the recognition "spot". The *lower* the number, the stronger the recognition intensity. The average background value measured before blocking was ~ 80.

^bThe numbers correspond to complexes identified in Figure 2.

Table 2
Recognition Intensity Values for Chromatin Samples^a

Chromatin Particle	Intensity(no blocking)	Intensity(H3 block)	Intensity H3+H2A block)
Nucleosomes ^b	36 ± 10	44 ± 12	82 ± 3
5H3-H4 Tetramer/DNA ^c	38 ± 8	66 ± 8	82 ± 3

^aThe intensity of recognition is measured in traces across a recognition “spot” The *lower* the number, the stronger the recognition. The average background value measured before blocking was ~ 80. The values shown are averages over all the examples of each type of molecule in the field.

^bOnly nucleosomal array molecules that showed good recognition in the initial recognition image (some arrays were very poorly-recognized) were included. Recognition efficiency of nucleosomes with these doubly-labeled tips is lower (< 80 %) than that obtained with singly- modified tips (> 95%).

^cThe recognition efficiency was > 90 %, in contrast to results with singly-modified tips (~70 %).