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Molecular Devices and Tools for NanoTechnology
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Application Notes

Life Sciences

AFM STUDY OF MACROMOLECULES' STRUCTURE AND ORGANIZATION

Introduction

Atomic force microscopy (AFM) techniques allowed us to visualize pentameric organization of immunoglobulins IgM and domain structure of immunoglobulins IgG. Together with obtained by AFM data on conditions of ribosome-inactivating proteins' type II (RIPsII) tetramerization these results contribute to many investigations concerning RIPsII biology as well as antigen-antibody and lectin-ligand interactions.

Sample preparation

Toxins' or antibodies' solutions were diluted in phosphate-buffered saline (PBS) to concentrations in the range of 0.5-2 mg/ml. A 10-ml drop of toxin or antibody solution was deposited onto freshly cleaved mica supports and incubated for 1 min. Then each sample was washed three times in deionized water and dried by N2 gas.

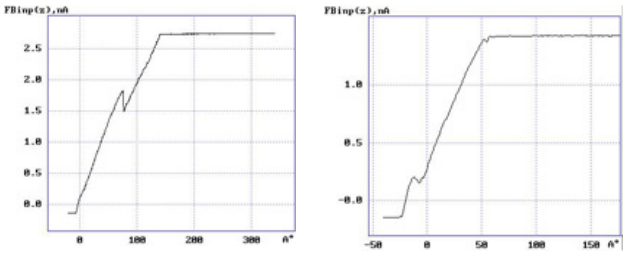
Measurements realization

Semi-contact mode of AFM was used. The advantage of semi-contact mode is decrease of lateral forces between the probe and the sample in comparison to contact mode of AFM. The normal forces can also be reduced by suitable choice of free and set-point amplitude of cantilever oscillations and by selection of force constant. Mentioned above advantages of semi-contact mode using are important when soft biological objects like adsorbed molecules weakly attached to substrate are studied.

Molecules were imaged with a scanning probe microscope Solver P47H (scanning-by-probe system configuration) equipped with attachment for atomic resolution measurements. Silicon cantilevers NSG11S with two rectangular springs with typical force constant of 5.5 N/m and 11.5 N/m were used to perform the experiments. The amplitude of free cantilever oscillations was in the range of 5-15 nm and the set-point amplitude was in the range of 1-10 % of the free amplitude. These values were defined experimentally for each cantilever from amplitude curves.
To avoid electrostatic charging of mica surface, the samples were grounded.

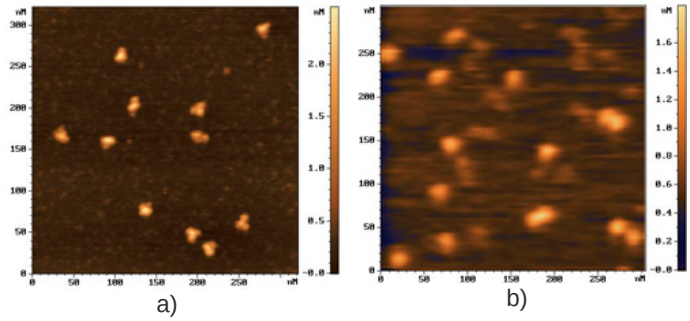
Amplitude curves

Amplitude-distance curves are the useful tool to optimize resolution and contrast in semi-contact mode of atomic force microscopy. Figure below shows amplitude curves obtained on mica region free of molecules. Left curve obtained for a cantilever with free amplitude of 15 nm and frequency of 248 kHz, right curve is for a cantilever with free amplitude of 7 nm and frequency of 181 kHz. The curves are characterized by the presence of a sudden jump in the amplitude. This step-like discontinuity marks the transition between two tip-sample interaction regimes, attractive and repulsive. Using these curves one can estimate free amplitude Ao and correctly choose set-point amplitude Asp to get best image.



Dependence on amplitude

Scanning results depend on free amplitude of cantilever oscillations. The best images can be obtained if amplitude in the range of 5-15 nm is used. Increasing of amplitude value results in resolution decrease.



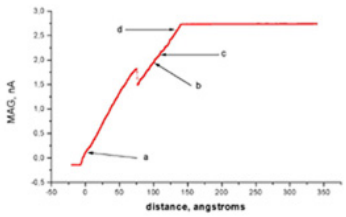
a) b)

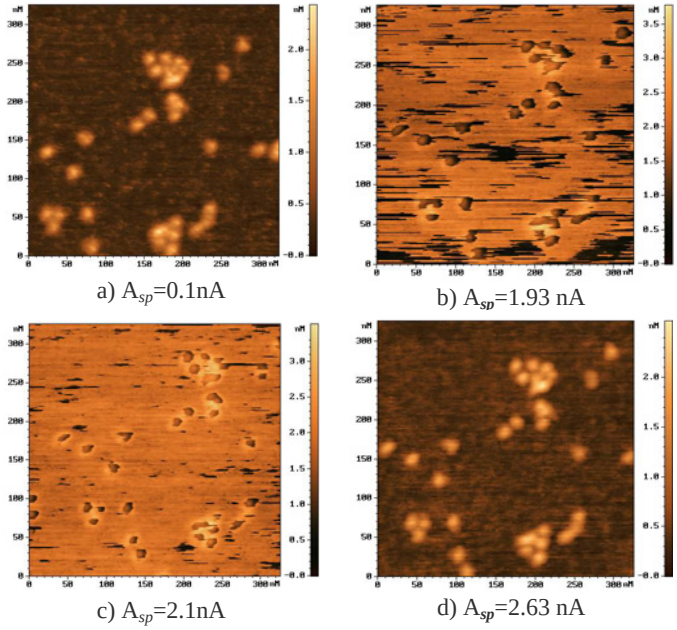
Fig. 1 Image obtained with free amplitude of a)10 nm b)34nm.

Set-point choice

The image depends on the set-point amplitude *Asp* choice. In the case of A_{sp} being close to zero (repulsive regime) as well as of A_{sp} slightly smaller than *Ao* (attractive regime) the image has good contrast and resolution. The image becomes unstable and inverse while the *Asp* is in the region that is higher than discontinuity.

In contrast with results obtained in [8] we have found the repulsive regime to be also suitable for imaging molecules. Besides, the repulsive regime is more stable than attractive one and provides better resolution. Difference between results obtained earlier [8] and our results was proposed to connect with using of smaller force constant (5 N/m and 11 N/m against 25-50 N/m) cantilevers.





a) A_{sp}=0.1nA b) A_{sp}=1.93 nA

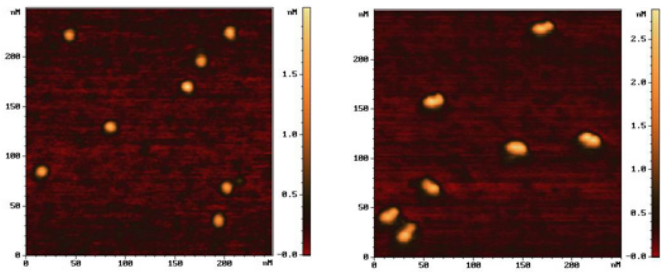
c) A_{sp}=2.1nA d) A_{sp}=2.63 nA

Fig. 2 Images of antibodies with different set-point amplitudes (a-d).

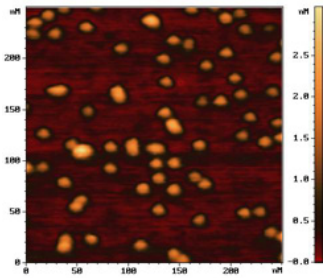
Results

- Images of toxins (ricin, ricinus agglutinin, viscumin) and IgG1 and IgM antibodies.
- Efficient size of ricin and antibodies molecules estimation.

Toxins

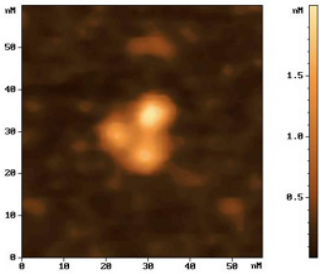


a) b)



c)

Fig. 3 Image of toxins(a-c).

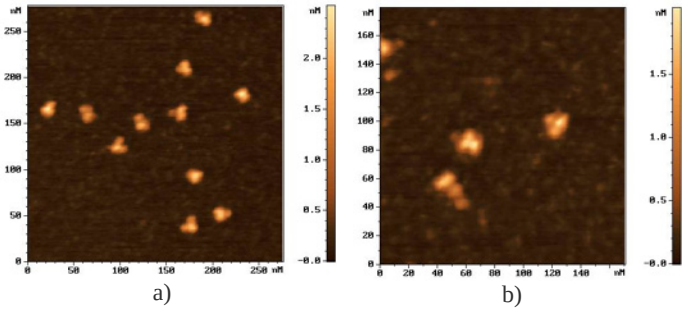


c)

Fig. 4 Images of IgG1 isotype antibodies(a-c).

a) Ricin is a plant ribosome-inactivating protein type II (RIPII). The toxin is present in the seeds of Ricinus communis. It consists of two subunits (A and B) joined by disulfide bond. Molecular mass of each subunit is about 30 kDa. Toxically active A-subunit can modify ribosomal RNA and thereby inhibits protein synthesis. Binding B-subunit is responsible for both toxin binding to the cellular membrane and toxin delivery to its intracellular target - ribosome. Toxin binding to the cell surface requires interaction between B-subunit lectin centres and carbohydrate part of cellular receptors. b)Image of ricinus agglutinin molecules. Ricinus agglutinin is another RIPII from the seeds of Ricinus communis. In contrast to ricin this protein consists of two B- and two A-subunits. Thus, ricinus agglutinin has tetrameric structure formed by covalent interaction between two A-subunits. c)Image of viscumin molecules. Viscumin is another RIPII being produced in Viscum album leaves. Viscumin structure and properties are similar to those of ricin. The differences between vicumin and ricin lie in both specificity of their B-subunits to bind to different carbohydrates and ability of viscumin to form tetramer.

Antibodies



a) b)

Different monoclonal antibodies of isotype IgG1 with molecular mass of 150 kDa were used: a) MNA9 is monoclonal antibodies interacting with both viscumin and its A-subunit; b)1RK2 is monoclonal antibodies interacting with isolated ricin A-subunit; c)3F12 is glycosilated monoclonal antibodies. Carbohydrate part of these antibodies contains terminal galactose residue. Antigen-binding centres of the glycosilated monoclonal antibodies do not interact with toxins. And binding of toxins with such antibodies is interaction of lectin with its ligand.

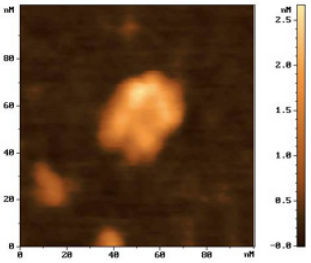
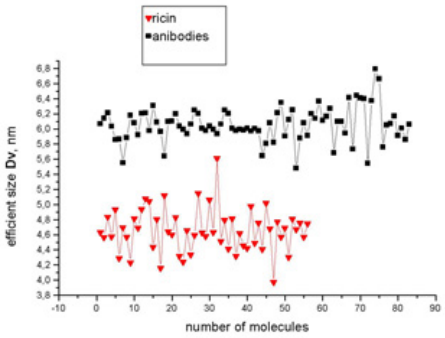


Fig. 5 Image of IgM isotype antibodies.

IgM antibodies from human serum with molecular mass of about 900 kDa were used. Estimation of ricin and antibodies molecules' efficient size

Software for the probe microscopes produced by NT-MDT company includes "Grain analysis" tool which is designed for statistical processing of the image of smooth plane containing projecting objects (for example pits of CD/DVD matrixes, microparticles and nanoparticles, biological objects etc.).

We used this program to define the geometrical sizes of ricin and 1RK2 antibodies' molecules. Dv, the efficient size of the object above cutting plane level in the XYZ volume ($V^{1/3}$), was taken as estimated value. Calculations based on the number of images which were scanned with step of 10 angstroms and contained 50 ricin molecules and 85 antibodies' molecules were done.



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