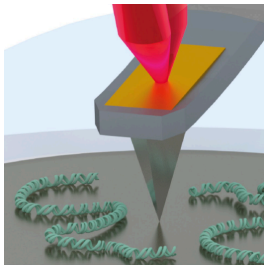
Oct 28, 2020

2 Methods for DNA Adsorption on a Mica Substrate for AFM Imaging in Fluid



In 1 collection

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We use this protocol and it's working

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Abstract

This is part 2 of the "Atomic Force Microscopy of DNA and DNA-Protein Interactions" collection of protocols.

Collection Abstract: Atomic force microscopy (AFM) is a microscopy technique that uses a sharp probe to trace a sample surface at nanometre resolution. For biological applications, one of its key advantages is its ability to visualize substructure of single molecules and molecular complexes in an aqueous environment. Here, we describe the application of AFM to determine the secondary and tertiary structure of surface-bound DNA, and it’s interactions with proteins.

Guidelines

Figure 2 shows DNA plasmids adsorbed on a mica substrate by both the divalent cation (Fig. 2a) and poly-L-lysine (Fig. 2c) methods. Both methods yield stable DNA adsorption on the substrate for imaging by AFM.

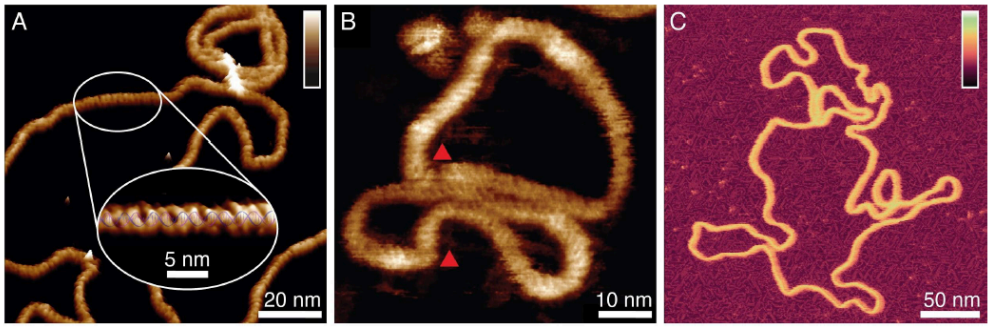

Fig. 2 High-resolution topographic images of DNA acquired by PeakForce Tapping mode (**protocol 4.**). The divalent cation method (protocol 2., method 2.1) is used to adsorb **(a)** DNA plasmids and **(b)** 339 base-pair DNA minicircles. In a, the two strands of the DNA double-helix are captured. *Inset*: a higher resolution image digitally straightened and overlaid with a cartoon representation of the B-DNA crystal structure. Color scales: 2.5 nm (main), 1.2 nm (*inset*). In **b**, defects and disruptions in the canonical B-form DNA are observed (red triangles), as a step-change in the angle of the helix. Color scale (scale bar in **a**): 2.5 nm [ref. 11, with permission]. **(c)** A DNA plasmid adsorbed onto $PLL_{1000–2000}$-functionalized mica (protocol 2., method 2.3) where the chains of poly-L-lysine making up the underlying substrate are resolved. Colour scale: 8 nm [adapted from ref. 31, with permission].

Troubleshooting

Safety warnings

For hazard information and safety warnings, please refer to the SDS (Safety Data Sheet).

1 The three Methods for DNA Adsorption on a Mica Substrate for AFM Imaging in Fluid are outlined below:

STEP CASE

2.1 DNA Adsorption Using Divalent Cations 11 steps

2.1 DNA Adsorption Using Divalent Cations 30m

2

Note

Divalent cations (in this case Ni^{2+}) can be used to overcome the electrostatic repulsion between DNA and mica, thus facilitating DNA adhesion to the mica, which can also be tuned via the cationic concentration in the solution as outlined below.

3 Immediately before DNA adsorption, cleave a 6 mm mica disc that has been prepared as described in **protocol 1**.

4 Cover the freshly cleaved mica with 20 µL nickel adsorption buffer (*see* **Note** 10).

5 Add 4 µL DNA ([M] 1 ng/µl , *see* **Note** 11) and distribute evenly in the meniscus by gently purging.

6 Adsorb for 00:30:00 . Then gently exchange the buffer to the nickel imaging buffer *four times* to remove any unbound DNA. 30m

6.1 Gently exchange the buffer to the nickel imaging buffer to remove any unbound DNA. (1/4)

6.2 Gently exchange the buffer to the nickel imaging buffer to remove any unbound DNA. (2/4)

6.3 Gently exchange the buffer to the nickel imaging buffer to remove any unbound DNA. (3/4)

6.4 Gently exchange the buffer to the nickel imaging buffer to remove any unbound DNA. (4/4)

7 Add sufficient nickel imaging buffer to form a droplet covering the sample (dependent on the AFM system, *see* **Note** 12).

8 Mount sample on AFM.