OCT 28, 2020

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.bncjmaun

Protocol Citation: Philip J. Haynes, Kavit H. S. Main, Alice L Pyne 2020. 2 Methods for DNA Adsorption on a Mica Substrate for AFM Imaging in Fluid. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.bncjmaun>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
 We use this protocol and it's working

Created: Oct 12, 2020

Last Modified: Oct 28, 2020

PROTOCOL integer ID:
 43115

Keywords: Atomic force microscopy, AFM, DNA, Supercoiling, Double helix, DNA-protein binding

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

In 1 collection

Philip J. Haynes^{1,2,3}, Kavit H. S. Main^{1,4}, Alice L Pyne⁵

¹London Centre for Nanotechnology, University College London, London WC1H 0AH, UK;

²Molecular Science Research Hub, Department of Chemistry, Imperial College London, W12 0BZ, UK;

³Department of Physics and Astronomy, University College London, London, WC1E 6BT, UK;

⁴UCL Cancer Institute, University College London, London, WC1E 6DD, UK;

⁵Department of Materials Science, Sir Robert Hadfield Building, University of Sheffield, S1 3JD

Alice L Pyne

Department of Materials Science, Sir Robert Hadfield Buildin...

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 PLL₁₀₀₀₋₂₀₀₀-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].

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 1 includes a Step case.

Divalent Cation

PLL

PLL-b-PEG

step case

Divalent Cation

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 (1 $\text{ng}/\mu\text{L}$, 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.