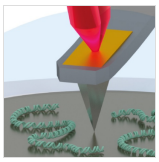

Oct 28, 2020

5 Methods for DNA-protein imaging by AFM in fluid

In 1 collection

Philip J. Haynes[1,2,3], Kavit H. S. Main[1,4], Alice L Pyne[5]

[1]London Centre for Nanotechnology, University College London, London WC1H 0AH, UK;
[2]Molecular Science Research Hub, Department of Chemistry, Imperial College London, W12 0BZ, UK;
[3]Department of Physics and Astronomy, University College London, London, WC1E 6BT, UK;
[4]UCL Cancer Institute, University College London, London, WC1E 6DD, UK;
[5]Department of Materials Science, Sir Robert Hadfield Building, University of Sheffield, S1 3JD

1 Works for me dx.doi.org/10.17504/protocols.io.bncqmavw

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

ABSTRACT

This is part 5 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.

DOI

dx.doi.org/10.17504/protocols.io.bncqmavw

PROTOCOL CITATION

Philip J. Haynes, Kavit H. S. Main, Alice L Pyne 2020. 5 Methods for DNA-protein imaging by AFM in fluid. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bncqmavw

COLLECTIONS

Atomic Force Microscopy of DNA and DNA-Protein Interactions

KEYWORDS

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

CREATED

Oct 12, 2020

LAST MODIFIED

Oct 28, 2020

OWNERSHIP HISTORY

Oct 12, 2020 Julia Rossmanith protocols.io

Oct 27, 2020 Alice Pyne Department of Materials Science, Sir Robert Hadfield Building, University of Sheffield, S1 3JD

PROTOCOL INTEGER ID

43120

PARENT PROTOCOLS

Part of collection

Atomic Force Microscopy of DNA and DNA-Protein Interactions

GUIDELINES

One of the major drawbacks in investigating DNA-protein interactions by AFM is that, at physiologically relevant protein concentrations, protein tends to bind nonspecifically to the underlying substrate. This ultimately obscurs the adsorbed DNAbound protein complexes. This is often the case when adopting adsorption methods that utilise divalent cations and poly-L-lysine (Fig. 5bi-ii). To minimise non-specific protein binding to the substrate, a PEGylated surface can be adopted which passivates the surface against protein adsorption.

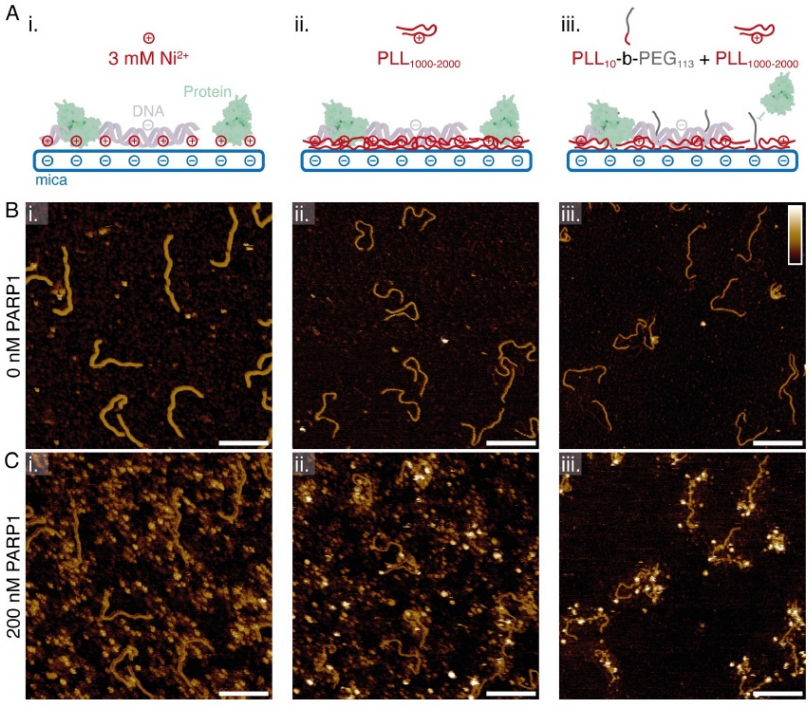

Fig. 5 Three methods used to adsorb DNA onto a mica substrate, divalent cations, poly-L-lysine, and PLL-*b*-PEG. **(a)** Illustrations of the three methods showing DNA (grey), protein (green), positive adsorption methods (red) and passivating PEG chains (grey). In PLL-*b*-PEG block copolymers **(aiii)**, the densely packed brush-like PEG chains repel proteins from the underlying substrate. **(b, c)** AFM topographic images of 496 base-pair linear DNA containing no protein **(b)** or with 200 nM PARP1 (a nuclear enzyme, seen as white blobs) **(c)**, adsorbed by **(i)** divalent cations, **(ii)** poly-L-lysine ($PLL1_{000–2000}$) and **(iii)** PLL-*b*-PEG diblock copolymers supplemented with $PLL_{1000–2000}$. Adapted from ref. 31, with permission. Scale bars: 100 nm. Color scale (scale bar inset in **biii**): 4 nm.

On a PEGylated surface, the brush-like PEG_{113} chains protrude with a vertical end-to-end distance comparable to the height of a DNA molecule (*see* Fig. 5aiii). These brushes may interact with the tip and hinder access to the surface. When imaging on such a surface, key parameters need to be adjusted when compared to the imaging conditions described in protocol 4.

SAFETY WARNINGS

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

ABSTRACT

This is part 5 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.

Citation: Philip J. Haynes, Kavit H. S. Main, Alice L Pyne (10/28/2020). 5ÃÂ Methods for DNA-protein imaging by AFM in fluid. https://dx.doi.org/10.17504/protocols.io.bncqmavw

1 Prepare a mica substrate as described in protocol 1.

2 PEGylate the substrate and adsorb DNA/DNA-protein as described in protocol 2, method 2.3. Place the sample in the AFM.

3 Setup the AFM and approach the DNA sample surface as described in protocol 3.

4 Optimise imaging parameters (protocol 4) such that the DNA and DNA-protein complexes are sufficiently tracked.

5 If inadequate DNA tracking persists due to the influence of the brush-like PEG chains, increase the *PeakForce Setpoint* (*see* **Note** 27) and *PeakForce Amplitude*. Adjust and optimise the *Lift Height* accordingly (*see* **Note** 26).

6 All other imaging parameters are synonymous to those stated in protocol 3 and protocol 4 when approaching and optimising. A non-exhaustive list of parameters when using an Fast-Scan D are outlined in Table 1.

PFT Parameters	**Typical value**
Scan Size [nm]	120 - 250
Pixel density [pixels/line]	256 - 512
Line rate [Hz]	3-5
Imaging PeakForce Setpoint [pN]	~ 70 *
PF acquisition Frequency, *see* **Note** 19 [kHz]	8
LP Deflection Bandwidth [kHz]	20
Sync Distance, *see* **Notes** 19 and 21 [µs]	70 / 20
PeakForce Amplitude, **Note** 22 [nm]	5-10 *
Lift Height, *see* **Note** 26 [nm]	5-7
Z Range (Z Limit if using MultiMode® 8) [nm]	500-1000
Deflection Limit [V]	≂12.24

Table 1: Typical parameters used for an FastScan-D cantilever on a FastScan Bio™ AFM system, operating in PeakForce Tapping mode

*Parameters may need to be adjusted when imaging on a surface passivated with PLL-*b*-PEG (protocol 2, method 2.3). The *PeakForce Setpoint* and *PeakForce Amplitude* may need to be increased to 130 pN and 20 nm respectively (protocol 5).

Citation: Philip J. Haynes, Kavit H. S. Main, Alice L Pyne (10/28/2020). 5ÃÂ Methods for DNA-protein imaging by AFM in fluid. https://dx.doi.org/10.17504/protocols.io.bncqmavw