

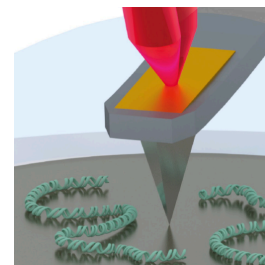
Oct 28, 2020

5 Methods for DNA-protein imaging by AFM in fluid

In 1 collection

DOI

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



Philip J. Haynes^{1,2,3}, Kavita 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...

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Protocol Citation: Philip J. Haynes, Kavita 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](https://dx.doi.org/10.17504/protocols.io.bncqmavw)



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Protocol status: Working

We use this protocol and it's working

Created: October 13, 2020

Last Modified: October 28, 2020

Protocol Integer ID: 43120

Keywords: Atomic force microscopy, AFM, DNA, Supercoiling, Double helix, DNA-protein binding, atomic force microscopy of dna, atomic force microscopy, protein imaging by afm, microscopy technique, protein imaging, methods for dna, bound dna, sample surface at nanometre resolution, protein interaction, molecular complexes in an aqueous environment, interactions with protein, molecular complex, substructure of single molecule, dna, nanometre resolution, single molecule, protein, application of afm, sample surface, tertiary structure of surface, sharp probe,

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.

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 obscures the adsorbed DNA-bound 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.

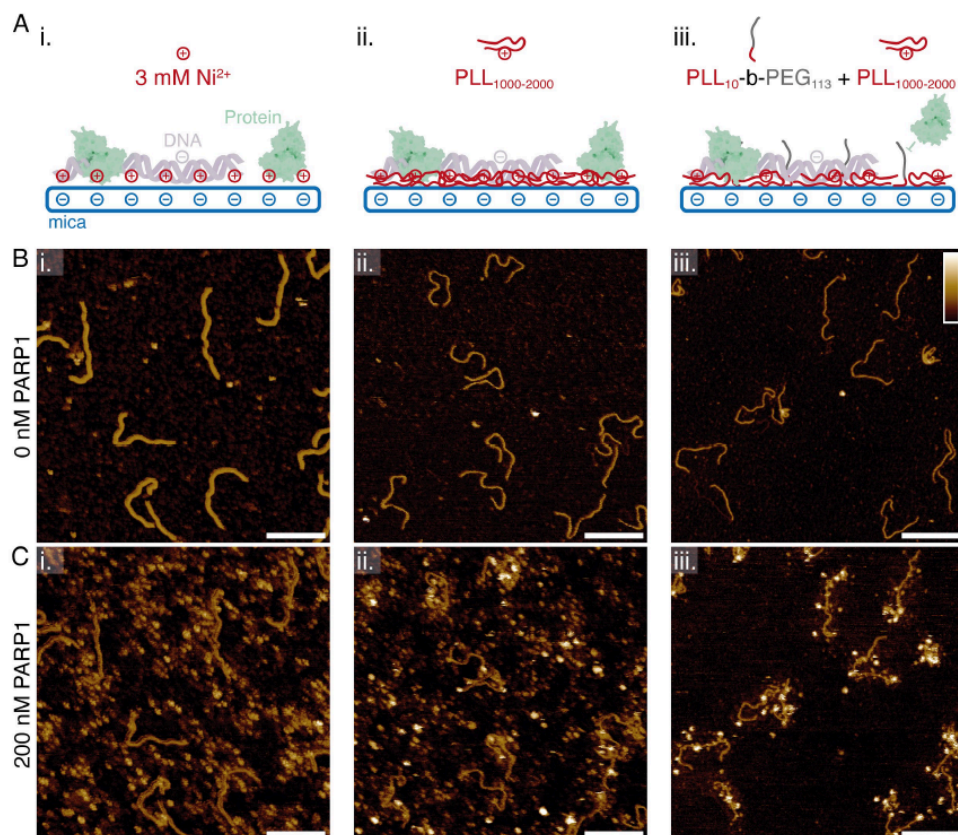



Fig. 5 Three methods used to adsorb DNA onto a mica substrate, divalent cations, poly-L-lysine, and PLL-*b*-PEG. **(a)** Illustrations of the three methods showing DNA (grey), protein (green), positive adsorption methods (red) and passivating PEG chains (grey). In PLL-*b*-PEG block copolymers **(aiii)**, 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 (PLL_{1000–2000}) 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 PEG₁₁₃ 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](#).

Troubleshooting

Safety warnings

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

Methods for DNA-protein imaging by AFM in fluid

- 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](#)).