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Journal of Experimental Nanoscience
Publication details, including instructions for authors and subscription information:
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Robert D. Boyd[a]
[a] School of Water Sciences, Cranfield University, Bedfordshire, Cranfield, MK43 0AL, UK

First published on: 01 December 2010

To cite this Article Boyd, Robert D.(2007) 'Detection and localization of antibody-antigen interactions with high spatial resolution on collagen tendons', Journal of Experimental Nanoscience, 2: 1, 139 — 146, First published on: 01 December 2010 (iFirst)

To link to this Article: DOI: 10.1080/17458080600891462

URL: http://dx.doi.org/10.1080/17458080600891462

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Journal of Experimental Nanoscience,
Vol. 2, Nos. 1–2, March–June 2007, 139–146

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Detection and localization of antibody–antigen interactions with high spatial resolution on collagen tendons

ROBERT D. BOYD*

School of Water Sciences, Cranfield University,
Cranfield, Bedfordshire, MK43 0AL, UK

(*Received June 2006; in final form June 2006*)

The use of the atomic force microscope to measure antibody and antigen interactions is not new and, in theory, allows the detection of single antibody–antigen interactions with nanometre resolution. However the application of this technique has been limited to 'ideal' situations and not been applied as a general laboratory technique. Previous studies on more realistic samples have been hampered by non-specific interactions. The technique has been modified with a double attachment spacer molecule between tip and antibody in order to successfully reduce the number of non-specific events seen and improving the sensitivity of the technique. This technique has been applied to an identical sample used for immunofluorencese and electron microcopy investigations. Antibody–antigen interactions were readily detected on the sample with a resolution of 50–100 nm. The presence of specific interactions was confirmed by blocking using excess antibodies.

Keywords: Atomic force microscopy; Force measurements; Ligand-receptor interaction

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1. Introduction

The ability of an antibody to bind specifically with a particular molecule (called an antigen) and the ease of producing specific antibodies has led to interest in commercial employment of antibodies in immunoassays and biosensors [1, 2]. The atomic force microscope, invented in 1986 by Binning *et al*. [3], consists of an ultrasharp tip (typically 10–20 nm diameter) connected to a lightly sprung cantilever, allowing imaging of a non-conductive surface under ambient or fluid conditions down to sub-nanometre resolution without the need for any additional surface preparation [4].

The AFM can also be used as a force-sensing instrument where the deflection of the AFM cantilever is measured as the tip moves towards and away from the surface. Used in this way the interaction forces between the surface and the tip can be detected and measured [5]. This method has been used to measure the local mechanical properties of biological systems, including living cells [6], vesicles [7] and human platelets [8].

*Email: r.d.boyd@cranfield.ac.uk

Journal of Experimental Nanoscience
ISSN 1745–8080 print/ISSN 1745–8099 online © 2007 Taylor & Francis
http://www.tandf.co.uk/journals
DOI: 10.1080/17458080600891462

The attachment of specific particles, such as fungal spores to the cantilever rather than the tip, has been used to measure adhesive force [9]. Attaching ligands (such as antibodies) to the AFM tip allows the measurement of ligand-receptor forces, successful measurement of which normally requires the presence of a flexible spacer molecule between the tip and the ligand [1, 10, 11]. This allows the ligand to move relative to the tip and to orientate itself to the receptors on the surface and by moving the ligand away from the tip a characteristic saw-tooth shaped force–distance curve is obtained [10]. This is easily distinguished from a non-specific adhesion which produces a straight-line force curve [9].

This technique has been used extensively to detect and measure antibody–antigen interaction on ideal systems, for example single antigens adsorbed onto mica [10–20]. Studies on samples similar to those used in other immunological studies have been hampered by the presence of interactions due to the pulling of organic molecules [21]. This is in part due to the nature of the silicon nitride tip which presents an ideal surface for adsorption [22–24]. Reducing the area of silicon nitride exposed and hence available to adsorption, the number of non-specific interactions can be restricted. Here we compare the results with probes modified using two techniques, via a spacer molecule which attaches to the AFM tip at a single or double point (figure 1).

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2. Experimental

2.1 *Preparation of collagen*

Tendons were dissected from fresh chicken legs and fixed in 2.5% paraformaldehyde prepared in 0.2 M sodium cacodylate buffer ($C_2H_6AsO_2Na$, Sigma) at 4°C for 24 hours. Individual collagen strands were teased apart, rinsed in a phosphate buffer solution (PBS) and dehydrated by immersion in aqueous ethanol solutions of 30%, 50%, 75% and 100%, then allowed to air dry. The dried strands were infiltrated with LR White Hard Grade embedding resin (London Resin Company Ltd, Berkshire, England) at 4°C with 10 changes. To achieve total infiltration of resin, samples were placed under vacuum for 24 hours, then polymerized at 56°C for an additional 24 hours. Sections (250 nm thick) were collected on gelatin-coated 8 mm circular glass disks.

2.2 *Preparation of functionized tips*

Rabbit polyclonal antibodies, raised against extracted and purified collagen type I from chicken skin, were obtained commercially (Chemicon International, Temecula, CA). These antibodies are specific for collagen I. A silicon nitride AFM tip (0.03 $N\,m^{-1}$ spring constant, Veeco Probes, CA) was first functionalized with amine groups by treatment with ethylamine [16]. Either a hetero-bifunctionalized PEG-400 molecule with amine and PDP [2-pyridyldithiopropionyl] reactive ends or a di-carboxylate PEG200 molecule (Sensopath, Bozeman, MT) was then attached to the AFM tip via amino groups [16] or hydroxyl [25] groups respectively. Finally, derivatized antibodies were attached to the PDP end of the PEG molecule.

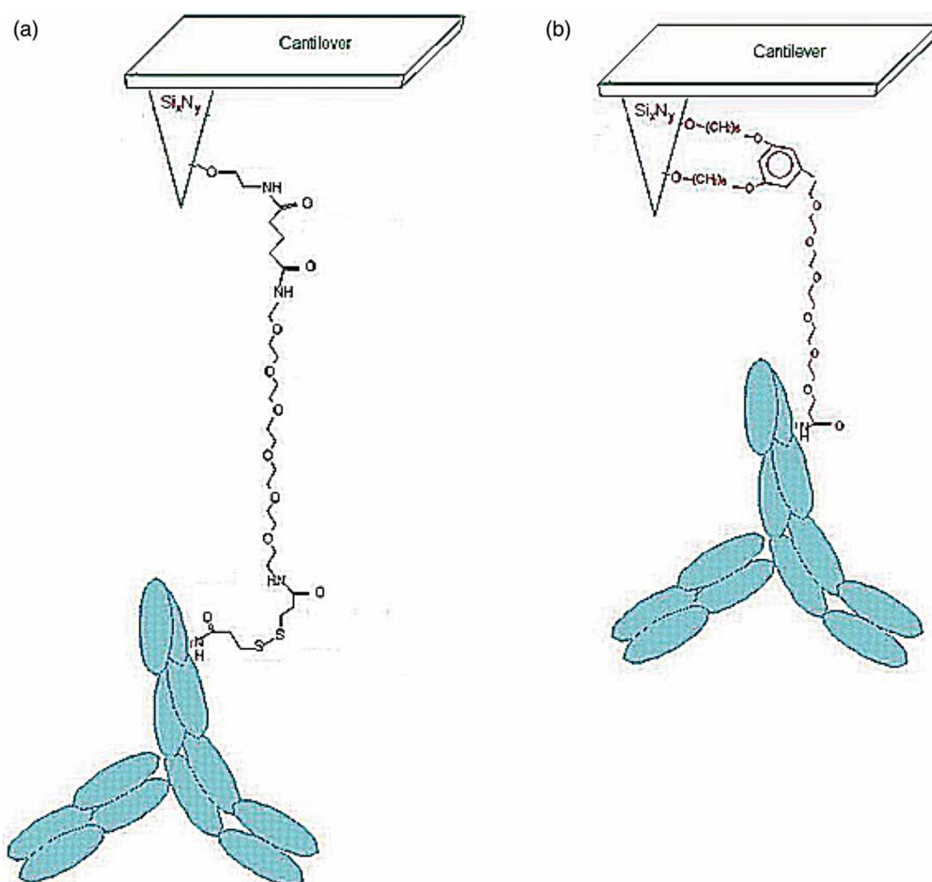

Figure 1. Schematic illustrations depicting the linkage of antibodies to the AFM tip via PEG-based spacer molecules as described in the text: (a) single attachment and (b) double attachment. Single attach.

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2.3 *AFM imaging and force measurements*

Force measures were taken using a Mulitmode instrument (Veeco, CA) in PBS solution and collected over a $4 \times 4\,\mu m^2$ area with 32×32 resolution (1024 total force curves). Each force distance curve was obtained at 1 Hz frequency, with a *z*-scan size of 200 nm and a relative deflection offset of 20 nm. After obtaining this data, 50 µl of 200 µg/ml anti-collagen primary antibodies were introduced into the AFM sample chamber and were incubated with the fibres for one hour. The excess antibodies in solution bound to the antigens and prevented the AFM-coupled antibody from interacting with the sample. Finally, after force-curve data were collected, the same functionalized tip was used to obtain a liquid tapping mode image to verify that data were collected from a fibril area on the sample as identified at the beginning of the experiment.

3. Results and discussion

The majority of AFM force curves taken from the collagen tendons using both types of modified probes show no or non-specific interactions, that is the force curve showed no peak or a straight line peak. However a significant minority (approximately 200–300 out of a total of 1024) do show the saw-tooth shaped peaks which are evidence of specific interactions (figure 2). Of these curves are seen with a single interaction that could be due to antibody–antigen interactions (figure 2a) [10]. This is complicated by the presence of curves caused by the pulling of the collagen fibrils which gives similar shaped force curves (figure 2b and c) [21]. Previous studies have shown ligand-receptor unbinding forces are in the range 50–400 pN and with a pull-off length of less than 40 nm [10]. Therefore only single event curves within these limits were chosen for analysis. In addition the analysis was repeated after the antibody–antigen interactions were blocked with excess antibodies, see figure 3.

For both the single and doubly attached tether the effect of blocking is to reduce the number of events detected. In both cases this is particularly strong between 60 and 120 pN, which agrees well with earlier analysis [10, 21]. The residue interactions are due to unblocked antibody–antigen interactions and short distance pulling of collagen fibrils. Data taken using the unmodified probe showed no change upon blocking, which supports the assertion that these events correspond to antibody–antigen interactions. The results from the doubly attached tethers (figure 3a) show a comparably larger number of events before and a lower number after blocking compared to data taken using a singly attached tether (figure 3b). The action of the doubly attached tether is then two-fold. The first is that with the stronger bonding to the tip, more antibodies are available for binding with antigens, and by covering more of the tip less bare silicon nitride is available for collagen fibrils to bond to – a phenomenon which happens just prior to collagen pulling.

An amplitude liquid tapping mode AFM image of collagen is shown in figure 4 taken with a functionized tip after the force measurements, showing the characteristic banding pattern associated with collagen [26]. The spatial distribution of events before and after blocking is also shown in figure 4. Although events are seen across the majority of the sample the distribution is not uniform. In both cases a correlation between the distribution of events and areas of the image showing the characteristic banding pattern is seen. In the cases of the single attached tether the area to the bottom left-hand side shows a lower frequency of events, likewise for the upper central area for the doubly attached tether where the binding pattern is not apparent. This suggests either that areas are either not collagen or that collagen fibres have been damaged. After blocking the distribution of events becomes more uniform across the image.

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4. Conclusions

It has been shown that AFM can be used to detect antibody–antigen interactions on actual biological samples and be able to map the distribution of the interactions with tens of nanometre resolution. A doubly attached tether improves the technique by both

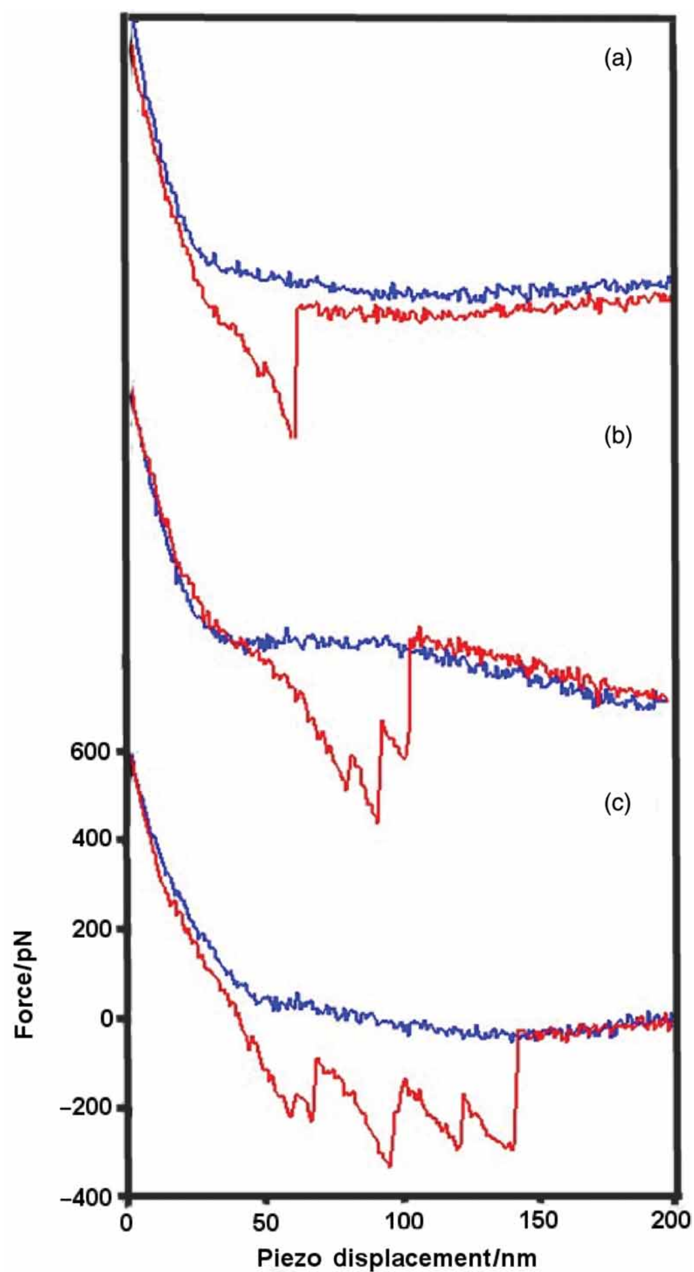

Figure 2. AFM force curves taken with antibody modified AFM tips, showing saw-toothed shaped curves: (a) single peak characteristic of antibody–antigen interactions; (b and c) multiple interactions possibly due to multiple antibody–antigen interactions, adsorption of collagen fibrils or a combination of these two effects.

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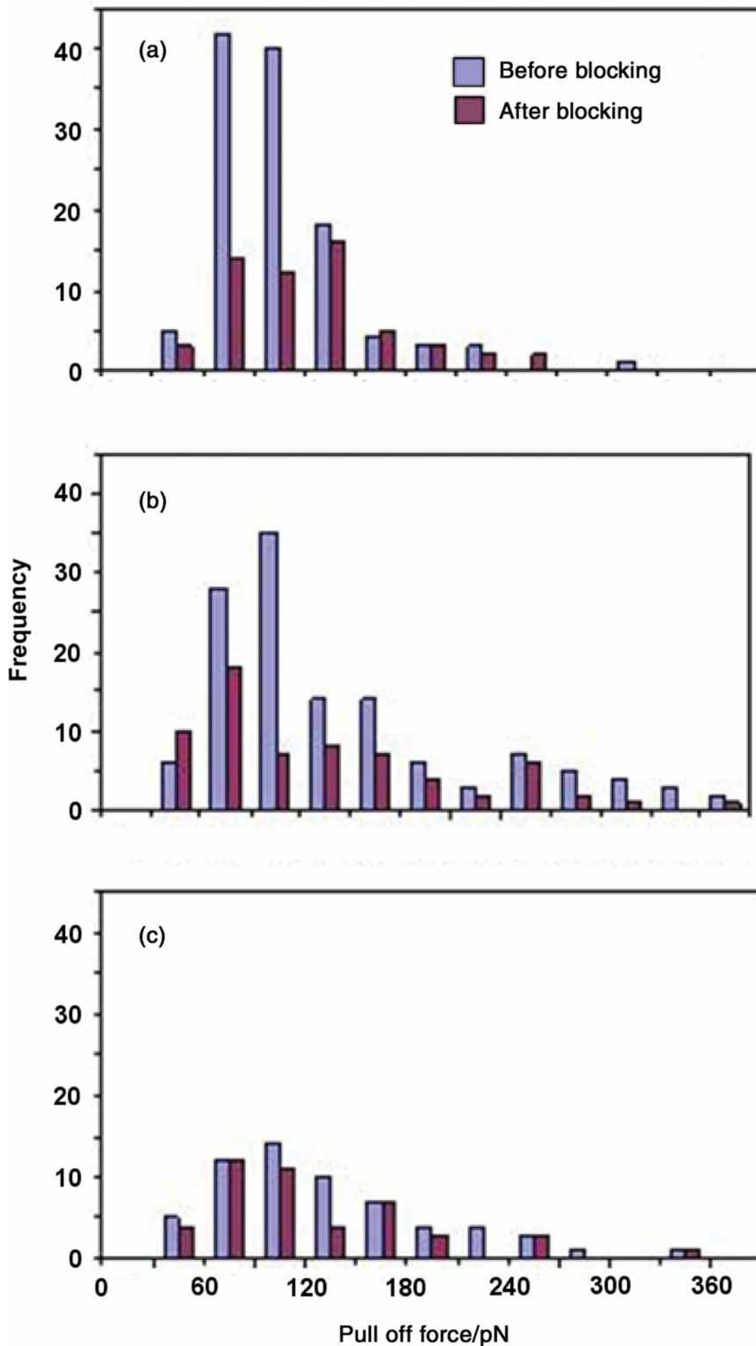

Figure 3. Distribution of unbinding events: (a) antibodies doubly attached to the AFM probe; (b) antibodies singly attached to the antibody probe; and (c) control AFM tip. Only those unbinding events that fell between 0 and 40 nm are used in the counting statistics. The blue band indicates forces measured with a functionalized tip on an uninhibited sample, while the red band corresponds to interactions between the tip and sample after antigenic sites on fibres have been blocked with excess antibodies.

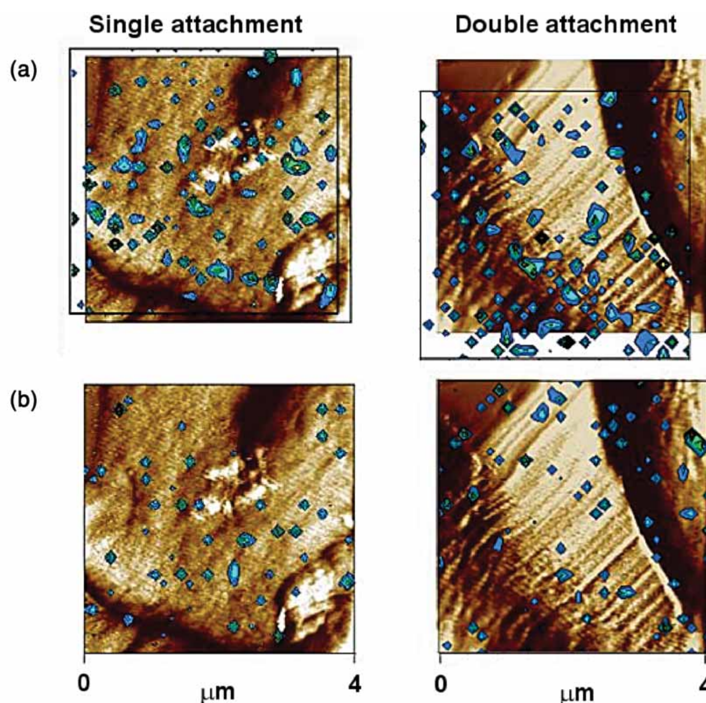

Figure 4. Liquid tapping mode AFM image of collagen fibres taken with a functionized tip after force measurements combined with the spatial distribution of unbinding events (a) before and (b) after blocking with excess antibodies. The data are offset to compensate for the drift of the piezo-scanner with time. The drift was determined from the height images obtained with the force volume data.

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increasing the number of events detected and reducing pulling of organic molecules which can complicate the analysis.

Acknowledgements

Many thanks to R. Avci, B. Spangler, K. M. Thieltges and Z.Y. Suo at Montana State University for help and the opportunity for this work, and M. Schweitzer and J. Wittmeyer for the supply of collagen samples. Funding from NASA (EPSCoR No: NCC5-579) and Montana State University is gratefully acknowledged.

References

[1] L. M. Wilde, S. Allen, M. C. Davies, S. J. B. Tendler, P. M. Williams, and C. J. Roberts. Bifunctional atomic force microscopy probes for molecular screening applications, Analyt. Chim. Acta, **479**, 77 (2003).
[2] D. H. Yu, B. Blankert, J. C. Vire, and J. M. Kauffmann. Biosensors in drug discovery and drug analysis, Analyt. Lett., **38**, 1687 (2005).
[3] G. Binnig, C. F. Quate, and C. Gerber. Atomic force microscope, Phys. Rev. Lett., **5**, 930 (1986).
[4] R. D. Boyd, A. M. Kenwright, J. P. S. Badyal, and D. Briggs. Atmospheric nonequilibrium plasma treatment of biaxially oriented polypropylene, Macromol, **30**, 5429 (1997).

[5] B. Cappella, and G. Dietler. Force–distance curves by atomic force microscopy, Surf. Sci. Rep., **34**, 1 (1999).
[6] E. A-Hassan, W. F. Heinz, M. D. Antonik, N. P. D'costa, S. Nageswaran, C. A. Schoenenberger, and J. H. Hoh. Relative microelastic mapping of living cells by atomic force microscopy, Biophys. J, **74**, 1564 (1998).
[7] D. E. Laney, R. A. Garcia, S. M. Parsons, and H. G. Hansma. Changes in the elastic properties of cholinergic synaptic vesicles as measured by atomic force microscopy, Biophys. J., **72**, 806 (1997).
[8] M. Walch, U. Ziegler, and P. Groscurth. Effect of streptolysin O on the microelasticity of human platelets analyzed by atomic force microscopy, Ultramicroscopy, **82**, 259 (2000).
[9] W. R. Bowen, N. Hilar, R. W. Lovitt, and C. J. Wright. Direct measurement of the force of adhesion of a single biological cell using an atomic force microscope, Coll. Surf. A – Physicochem. Engng Aspects, **136**, 231 (1998).
[10] P. Hinterdorfer, W. Baumgartner, H. J. Gruber, K. Schilcher, and H. Schindler. Detection and localization of individual antibody–antigen recognition events by atomic force microscopy, Proc. Natl. Acad. Sci. USA, **93**, 3477 (1996).
[11] S. Wielert-Badt, P. Hinterdorfer, H. J. Gruber, J. T. Lin, D. H. Badt, H. Schindler, and R. K. H. Kinne. Molecular recognition force microscopy (MRFM): A new tool for the identification and characterization of receptors in cell membranes, Biophys. J., **78**, 2247 (2000).
[12] F. Kienberger, G. Kada, H. Mueller, and P. Hinterdorfer. Single molecule studies of antibody–antigen interaction strength versus intra-molecular antigen stability, J. Mol. Biol., **347**, 597 (2005).
[13] R. Nevo, V. Brumfeld, M. Elbaum, P. Hinterdorfer, and Z. Reich. Direct discrimination between models of protein activation by single-molecule force measurements, Biophys. J., **87**, 2630 (2004).
[14] C. Stroh, H. Wang, R. Bash, B. Ashcroft, J. Nelson, H. Gruber, D. Lohr, S. M. Lindsay, and P. Hinterdorfer. Single-molecule recognition imaging-microscopy, Proc. Natl. Acad. Sci. USA, **101**, 12503 (2004).
[15] C. K. Riener, C. M. Stroh, A. Ebner, C. Klampfl, A. A. Gall, C. Romanin, Y. L. Lyubchenko, P. Hinterdorfer, and H. J. Gruber. Simple test system for single molecule recognition force microscopy, Analyt. Chim. Acta, **506**, 115 (2004).
[16] P. Hinterdorfer, H. J. Gruber, F. Kienberger, G. Kada, C. Riener, C. Borken, and H. Schindler. Surface attachment of ligands and receptors for molecular recognition force microscopy, Coll. Surf. B – Biointerfaces, **23**, 115 (2002).
[17] C. M Borken, C. Riener, H. J. Gruber, H. Schindler, S. J. Smith-Gill, and P. Hinterdorfer. Simultaneous topography and recognition imaging with dynamic force microscopy, Biophys. J., **80**, 1259 (2001).
[18] A. Raab, W. H. Han, D. Badt, S. J. Smith-Gill, S. M. Lindsay, H. Schindler, and P. Hinterdorfer. Antibody recognition imaging by force microscopy, Nature Biotech., **17**, 902 (1999).
[19] O. H. Willemsen, M. M. E. Snel, K. O. Van Der Werf, B. G. De Grooth, J. Greve, P. Hinterdorfer, H. J. Gruber, H. Schindler, Y. Van Kooyk, and C. G. Figdor. Simultaneous height and adhesion imaging of antibody–antigen interactions by atomic force microscopy, Biophys. J., **75**, 2220 (1998).
[20] F. Kienberger, G. Kada, H. Mueller, and P. Hinterdorfer. Single molecule studies of antibody–antigen interaction strength versus intra-molecular antigen stability, J. Mol. Biol., **347**, 597 (2005).
[21] R. Avci, M. Schweitzer, R. D. Boyd, J. Wittmeyer, A. Steele, J. Toporski, W. Beech, F. T. Arce, B. Spangler, K. M. Cole, and D. S. Mckay. Comparison of antibody–antigen interactions on collagen measured by conventional immunological techniques and atomic force microscopy, Langmuir, **20**, 11053 (2004).
[22] J. B. Thompson, J. H. Kindt, B. Drake, H. G. Hansma, D. E. Morse, and P. K. Hansma. Bone indentation recovery time correlates with bond reforming time, Biophys. J., **82**, 2462 (2002).
[23] R. B. Best, D. J. Brockwell, J. L. Toca-Herrera, A. W. Blake, D. A. Smith, S. E. Radford, and J. Clarke. Force mode atomic force microscopy as a tool for protein folding studies, Analyt. Chim. Acta, **479**, 87 (2003).
[24] M. Ludwig, M. Rief, L. Schmidt, H. Li, F. Oesterhelt, M. Gautel, and H. E. Gaub. AFM, a tool for single-molecule experiments, Appl. Phys. A – Mater. Sci. Process., **68**, 173 (1999).
[25] Z. Y. Suo, F. T. Arce, R. Avci, K. Thieltges, and B. Spangler.. Dendritic structures of poly(ethylene glycol) on silicon nitride and gold surfaces, Langmuir, **22**, 3844 (2006).
[26] D. F. Holmes, H. K. Graham, J. A. Trotter, and K. E. Kadler. STEM/TEM studies of collagen fibril assembly, Micron, **32**, 273 (2001).

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