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JOURNAL OF MOLECULAR RECOGNITION
J. Mol. Recognit. 2007; **20**: 418–431
Published online in Wiley InterScience
(www.interscience.wiley.com) DOI:10.1002/jmr.857

Review

Past, present and future of atomic force microscopy in life sciences and medicine

Pierre Parot[1]*, Yves F. Dufrêne[2], Peter Hinterdorfer[3], Christian Le Grimellec[4], Daniel Navajas[5], Jean-Luc Pellequer[1] and Simon Scheuring[6]

[1]CEA-Valrhô DSV/iBEB/SBTN, 30207 Bagnols sur Cèze Marcoule, France
[2]Université catholique de Louvain, Louvain-la-Neuve, Belgium
[3]Johannes Kepler Universitat Linz, Linz, Austria
[4]Institut National de la Santé et de la Recherche Médicale, Unité 554, Montpellier, France and Université de Montpellier, France
[5]Universitat de Barcelona—IDIBAPS and CIBER Enfermedades Respiratorias, Barcelona, Spain
[6]Institut Curie, Paris, France

To introduce this special issue of the *Journal of Molecular Recognition* dedicated to the applications of atomic force microscopy (AFM) in life sciences, this paper presents a short summary of the history of AFM in biology. Based on contributions from the first international conference of AFM in biological sciences and medicine (AFM BioMed Barcelona, 19–21 April 2007), we present and discuss recent progress made using AFM for studying cells and cellular interactions, probing single molecules, imaging biosurfaces at high resolution and investigating model membranes and their interactions. Future prospects in these different fields are also highlighted. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: **AFM; cell imaging; cell mechanics; cell adhesion; DFS; single molecules; high speed imaging; membrane protein imaging; high resolution imaging; protein and membrane imaging**

Received 22 August 2007; accepted 24 August 2007

INTRODUCTION

Today thousands of atomic force microscopes are used in the world and the percentage dedicated to biological or medical studies increases regularly while tens of patents relative to the use atomic force microscopy (AFM) in biology are claimed every year (WIPO, 2007). The volume of scientific publications citing AFM increases continuously (see Figure 1) and papers with a biological emphasis reach more than 21% of total publications (as in 2006). While the use of AFM in biology and medicine was initiated during the pioneering decade (1986–1996), the real take-off occurred after 1995. It should be noted that most recent scientific publications using AFM in biology do not explicitly mention the term AFM in the title anymore, indicating the maturity of the field.

The non-expert reader should be aware that this very special kind of microscopy is, in term of physiological sense, closer to touch than sight. In AFM, a nanostylus (tip) attached to a microcantilever is scanned over a sample immobilized onto an atomically flat substrate, usually mica or gold but many other kinds of surfaces can be also used. A laser beam is reflected on the back of the cantilever and the upward and downward deviations of the cantilever are read on a sensitive photo sensor.

*Correspondence to: P. Parot, DSV/iBEB/SBTN/LIRM, Service de Biochimie et Toxicologie Nucléaire/Laboratoire Interactions et Reconnaissance Moléculaires, CEA VALRHO Centre de Marcoule, BP 17171, 30207 Bagnols sur Cèze Cedex, France. E-mail: parot@cea.fr

The strength of the AFM technique relies on the possibility to operate in aqueous fluids on a wide variety of biological samples ranging from single molecules, such as nucleic acids and proteins, to macromolecular assemblies and whole cells (Radmacher *et al*., 1992; Engel *et al*., 1999; Clausen-Schaumann *et al*., 2000; Fisher *et al*., 2000a; Hinterdorfer and Dufrene, 2006; Kienberger *et al*., 2006; Li *et al*., 2006; Oesterhelt and Scheuring, 2006; Sewald *et al*., 2006; Simon and Durrieu, 2006). Importantly, AFM can be used in the force spectroscopy mode, which allows the detection and manipulation of single molecules, providing novel insight into their structure–function relationships.

However, in the early days of AFM, artefacts were sometimes published, as is frequently observed when using a emerging technique. This provided opponents with a good opportunity to discredit AFM approaches (Shao *et al*., 1996). The fallacious argument: '*what other technique did you use to be sure of what you observe*?' was often employed, even by people who were well aware that no other approach for observing native single molecules exists. For instance, we note that some of the very early papers using AFM in biology were focused on purple membranes where protein density is very high (Worcester *et al*., 1988) or on biological specimens with known structure, like porins (Lal *et al*., 1993). These early advances were underestimated and lacked recognition from the community. In addition, many laboratories have had a hard time with early commercial instruments, suggesting that the potential of AFM in biology is meagre.

The situation was quite different concerning force measurements because many other techniques, including

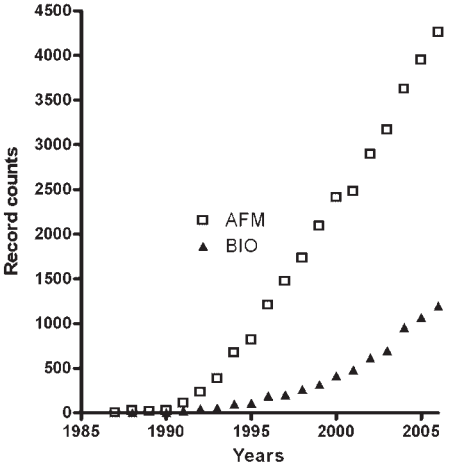

Figure 1. Evolution of the number of scientific publications regarding AFM in biology and medicine from 1986 to 2006. Analyses were performed using the Web of Science® from the Science Citation Index Expanded. Legends AFM and BIO stand respectively for the entries: '*atomic force microscopy*' (total: 32313) and '*atomic force microscopy*' *AND (medic* OR drug* OR pharmaco* OR clinic* OR health* OR disease* OR ((cell* OR membrane*) AND bio*) OR nucleic acid* OR DNA OR RNA OR protein* OR peptid* OR bio*).*

biomembrane force probe (BFP), micropipette, optical tweezers or flow chamber, have been developed with biological samples (Tha and Goldsmith, 1986; Tha *et al*., 1986; Ashkin and Dziedzic, 1987; Evans *et al*., 1991; Kaplanski *et al*., 1993). Hopefully,[1] during the past decade we have witnessed tremendous progress in sample preparation (especially in terms of time and cost), data recording and interpretation, including in pioneering groups,[2] demonstrating unambiguously the power of AFM in biology (Engel and Muller, 2000; Fisher *et al*., 2000b; Jena and Hörber, 2002; Hörber and Miles, 2003; El Kirat *et al*., 2005; Hinterdorfer and Dufrene, 2006). Fortunately things have changed and will continue to change[1].

A SHORT HISTORY OF AFM IN BIOLOGY

The history and milestones of the application of AFM in biological sciences are today well presented in books and even textbooks (Morris *et al*., 1999; Jena and Hörber, 2002, 2006; Bhushan and Fuchs, 2006; Jena and Hörber, 2006). An interesting assert of the role of 'instrumental community' has been analysed starting from the birth of the Scanning Tunneling Microscope (STM; Mody, 2006). We present here a brief survey of the past 20 years, as seen from a biophysicist/biologist perspective, in the light of the questions: *what is achieved and what is needed* raised and discussed in an essential review 10 years ago (Shao *et al*., 1996).

[1]That which does not kill AFM makes it stronger!

[2]Note that most of these groups or their former students were present at this first AFM BioMed Conference.

Pre-historical corner

With the invention of the STM in 1981,[3] the elusive goal of imaging individual atoms on surfaces was achieved (Binnig *et al*., 1982). The STM technique bypassed the diffraction limit and achieved higher sensitivity than the electron microscope while utilizing much lower energy. According to the Noble lecture, the idea to use STM in biology starts soon after its invention. '*Towards the end of 1983, we started to probe the possibilities of STM in biology together with H. Gross from the ETH, Zurich. We could follow DNA chains lying on a carbon film deposited on Ag-coated Si wafer*' (Binnig and Rohrer, 1986). But pioneers confessed that STM was a terrible method to study biological objects (Gaub, 2005).

The requirement that the surface must be conductive was overcome in March 1986 with the invention of a variation of the STM known as the Atomic Force Microscope (AFM) by Gerd Binnig, Calvin Quate and Christopher Gerber (Binnig *et al*., 1986). AFM has the ability to image conductive and non-conductive surfaces beyond the diffraction limit with molecular and atomic resolution both in air and in liquid (Marti *et al*., 1987).

Nine months later, in the same year, Gerd Binnig and Heinrich Rohrer won the Nobel Prize in physics for *their design of the* STM shared with Ernst Ruska for *his fundamental work in electron optics, and for the design of the first electron microscope*. Albeit their discoveries were separated by 50 years, it was not by chance that these two different microscopy techniques were rewarded simultaneously. Both techniques were developed for 'seeing' at the atomic scale. Hence, for nearly 10 years, many presentations comparing the respective performances of STM and AFM versus Scanning Electron Microscope (SEM) or Transmission Electron Microscopes (TEM) were published, indicating often the necessity of their simultaneous use. However, AFM has emerged as an independent technique.

Bio AFM in the literature

Excluding early papers that associated STM and AFM (Hansma *et al*., 1988), the biological field for AFM was first reviewed in 1991 by Andreas Engel (Engel, 1991). Among the nine cited references, from two or three laboratories, we find the first observation of a biological sample in action: the clotting process of fibrinogen upon activation with thrombin (Drake *et al*., 1989). Observations like: *an image of a stoma on a leaf shows that the microscope is gentle enough not to damage surfaces, even of soft biological samples* (Gould

[3]The initial results were written up in a manuscript entitled 'Tunnelling through a controllable vacuum gap', which was submitted to a leading physics journal on June 1981. However, the paper was declined by the editors based on the following referee reports: one referee said that the exponential dependence of the tunnelling current on distance was well accepted, so the experiment would not give any new insight; the other report described the work as 'extraordinary' and a 'technical jewel', but this referee said that whether such technological work should be published in this particular physics journal was an editorial decision. Eventually the results were published in another leading journal, *Applied Physics Letters*, in January 1982. (Gerber and Lang, 1982).

DOI: 10.1002/jmr

et al., 1990), seem naïve today, but were in fact fundamental in early days.

Young scientists should appreciate that in pioneering papers question like: *Does the AFM image individual molecules?* were seriously discussed (Radmacher *et al*., 1992). In 1994, the first annual review in the domain by Helen G. Hansma and Jan H. Hoh appeared in the emerging techniques section (with 131 references from three dozen laboratories) (Hansma and Hoh, 1994). By way of comparison, today this kind of review would give thousands of references coming from hundreds of labs. In the same years, the first high-resolution images of protein surfaces in aqueous solution were published by Andreas Engel (Karrasch *et al*., 1994).

AFM in biology was transformed with the introduction of a vibrating mode in air (Zhong *et al*., 1993), then in liquid with the so-called tapping mode described in two papers published in the same journal, one in March (Hansma *et al*., 1994) and the other in May (Putman *et al*., 1994). This was clearly a breakthrough for the biological community since this vibrating mode minimizes sample damage by reducing contact time and lateral forces between the tip and the sample. Hence, it was possible to perform experiments in physiological conditions, even *in vivo*, including observations of biological processes at work (Shao *et al*., 1995) with continuous instrumental innovations being achieved (Poggi *et al*., 2004). A tremendous improvement of conventional instruments, yet to be achieved, concerns high speed AFM. Toshio Ando and Paul Hansma have shown fascinating films indicating how future AFM machines will enable researchers to study fast processes such as protein motion (Viani *et al*., 2000; Ando *et al*., 2001), crystal growth (Walters *et al*., 1997) and to do faster force spectroscopy (Viani *et al*., 1999). Starting in 1986, the evolution of AFM techniques for imaging purposes is presented in Table 1. See also the review of Toshio Ando *et al.* in this issue.

Twenty-seven milestones, from the day when AFM went into liquid, to present day

For obvious editorial restriction, it is hard to give all the most important advances of this short but intense story, without making the regrettable mistake of choosing among the research avenues explored with AFM. Arbitrarily we have retained only 27 breakthroughs, illustrating the huge capability and diversity of AFM. They are given here in chronological order with a concise comment.

1989. The AFM goes into liquid: key for future biological applications (Drake *et al*., 1989).

1991. First AFM topographs of membrane proteins with molecular resolution. The AFM tip is used to dissect the top-layer of the double-layered gap junction assembly (Hoh *et al*., 1991).

1992. The first 'high speed' time lapse study AFM paper showing a ~10 nm lateral resolution in living cells, with less than 10 sec for image acquisition (Häberle *et al*., 1992).

1994. One of the very first papers in which force spectroscopy is used to measure the forces between interacting biomolecules (Lee *et al*., 1994a); one of the first single molecule recognition force papers (Florin *et al*., 1994); first use of the attractive mode during high-resolution imaging using non-contact AFM in air (Anselmetti *et al*., 1994); high-resolution image of a 6.1 nm oligomer pentameric structure of the pertussis toxin simply adsorbed on mica using AFM in water (Yang *et al*., 1994).

1995. First high-resolution topographs of a membrane protein. Individual beta-turns on the porin OmpF surface are resolved (Schabert *et al*., 1995); introduction of a new mode of imaging biological macromolecules using a cryo-AFM (Han *et al*., 1995). This technique significantly reduces sample contamination and provides thermal and mechanical stabilization of samples.

1996. Pioneering work showing how AFM can detect and map single molecular recognition sites (Hinterdorfer *et al*., 1996).

1997. The first single protein unfolding paper (Rief *et al*., 1997a).

Table 1. Evolution of the AFM techniques for biological imaging since 1986 (adapted from Toshio Ando presentation at the AFM BioMed Conference Barcelona 2007)

1986	Birth of AFM	Binnig *et al*. (1986)
1987	In liquid AFM	Marti *et al*. (1987)
1987	Microfabricated cantilevers	Binnig *et al*. (1987)
1988	Optical lever method	Meyer and Amer (1988)
1989	First observation of biomolecular process	Drake *et al*. (1989)
1991	High-resolution imaging of membrane proteins	Hoh *et al*. (1991)
1991	The first paper on high-speed AFM	Barrett and Quate (1991)
1993	Tapping mode	Zhong *et al*. (1993)
1994	Revival of biomolecular-process studies	Hansma *et al*. (1994)
1996	Small cantilevers, optical detector	Walters (1996)
1999	High-speed imaging	Viani *et al*. (1999)
2001	High-speed AFM	Ando *et al*. (2001)
→Today	Various devices & control techniques for high-speed AFM	Ando *et al*. (2001); Hansma *et al*. (2006); Picco *et al*. (2007)

For perspective in high-speed AFM see also Toshio Ando's review (this issue).

DOI: 10.1002/jmr

1998. The first single molecule unfolding paper that describes conformational changes (Marszalek *et al*., 1998).

1999. Seminal paper introduces AFM in dynamic force spectroscopy (DFS) and opens the way to a powerful approach to study the energy landscape of unbinding events (Merkel *et al*., 1999).

2000. Coupling AFM imaging with single molecule force spectroscopy to extract a transmembrane protein from native membranes (Oesterhelt *et al*., 2000); implementation of a method for probing cell rheology of living cells by oscillating the AFM cantilever tip while indenting the cell surface. This study established a robust and reliable approach for measuring cell rheology taking into account tip geometry (Mahaffy *et al*., 2000); first work measuring cell–cell interactions in living cells using the AFM (Benoit *et al*., 2000). Authors measured the interaction of single receptor/ligand complexes by bringing into contact two cells, one attached to the cantilever and the other to a dish; high-resolution AFM is able to depict the number of subunits of densely packed reconstituted ATP synthase c-rings (Seelert *et al*., 2000).

2001. Ten images per second high-resolution visualization of single myosin V molecules in buffer solution: a breakthrough in one of the major limits in the AFM application to biological systems (Ando *et al*., 2001).

2003. First attempt to merge structural details of molecules into their topographic envelopes (Todd *et al*., 2003). It was shown that the lateral size of aggrecan monomers obtained from TEM data could be used to simulate a surface envelop that in turn could be used to refine topographic envelopes measured using the AFM tapping mode in air; the important contribution of the elastic properties to whole cell adhesion was demonstrated by attaching living cells to the AFM tip to measure their adhesiveness to ligand decorated surfaces under various biochemical stimuli. They also measured cell elasticity under the same conditions, showing that the rise in cell compliance is the main contribution to the increased adhesiveness (Wojcikiewicz *et al*., 2003); cell rheology with AFM over a broad frequency range probed by showing that living cells exhibit scale-free viscoelastic behaviour with a complex elastic modulus increasing with frequency as a weak power law (Alcaraz *et al*., 2003); rhodopsin, member of the G-protein coupled receptor (GPCR) membrane protein family, forms dimers in native disc membranes (Fotiadis *et al*., 2003); experimental evidence for the existence of catch bonds observed during protein–protein unbinding (Marshall *et al*., 2003).

2004. Direct observation of the native organization of the membrane protein supercomplexes involved in photosynthesis (Scheuring *et al*., 2004b); bacterial pathogenicity: In this study AFM aids the understanding of the mechanism by which water-soluble protein toxins, assembled to form oligomeric bilayer-spanning pores, penetrate the cell membrane (Czajkowsky *et al*., 2004).

2005. The first paper describing frequency modulation in liquids with atomic resolution (Fukuma *et al*., 2005); high-resolution AFM topographs of the multi-protein assembly of bacterial photosynthetic complexes-domain formation under different environmental conditions (Scheuring and Sturgis, 2005).

IMAGES AND FORCES IN LIFE SCIENCES

Today, the huge diversity of biological applications using AFM, whether focused on imaging and/or interactions, makes it quite impossible to master the entire dedicated bibliography. AFM is currently introduced in almost every domain of life sciences including studies of animal cell, bacteria, tumour cells and pit cells. Interested readers can refer to a critical review from Yang (2004), which provides useful technical analyses and some general consensus in biological applications of AFM ranging from membrane-bound bacterial toxins to condensed DNA molecules.

Morphology, topography and high-resolution imaging

In the early period, a rapid increase in the number of AFM reports was noticed in which biologically relevant molecular-resolution imaging was carried out (Weisenhorn *et al*., 1990). The first biological samples imaged with an AFM were bulk crystals of amino acids (Gould *et al*., 1988) and polymers (Drake *et al*., 1989). Soon after, individual actin filaments were imaged in solution at molecular resolution (Weisenhorn *et al*., 1990). Then, proteins in supported membranes (Egger *et al*., 1990), native purple membrane fragments (Butt *et al*., 1990a), gap junction membranes (Hoh *et al*., 1991), and DNA (Lindsay *et al*., 1992) were imaged at high resolution (Radmacher *et al*., 1992).

Some of the most impressive work has been in the area of imaging single protein complexes embedded in lipid membranes. Studies at molecular resolution on gap-junction membranes (Hoh *et al*., 1991) led to the first high-resolution topographs of porin OmpF, in which surface protruding beta-turns were reliably contoured (Schabert *et al*., 1995). High-resolution AFM reached maturity with bacteriorhodopsin, where individual surface protruding loops were imaged and manipulated (Muller *et al*., 1995b, 1999a; Scheuring *et al*., 2001). High-resolution imaging was then combined with force spectroscopy on bacteriorhodopsin, which allowed the subsequent unfolding of helices out of the membrane, highlighting interaction forces within bacteriorhodopsin (Oesterhelt *et al*., 2000). Many reports have since been published which involve some aspect of high-resolution imaging or force spectroscopy in the life sciences using AFM (Ikai, 1996). Membrane proteins ordered in a two-dimensional (2D) crystal were imaged at high resolution, revealing single components (Fotiadis *et al*., 2003; Scheuring *et al*., 2003) and multi-components (Wang and Clapham, 1999; Stolz *et al*., 2000; Scheuring and Sturgis, 2005; Buzhynskyy *et al*., 2007) of native membranes. At a larger scale, the nuclear pore complex was imaged and dynamic conformational changes of the

DOI: 10.1002/jmr

complex could be monitored using time-lapse AFM on the native nuclear envelope (Stoffler, *et al*., 1999).

Aside from imaging single molecules, the field of *in vivo* cellular imaging has also grown tremendously in the last two decades. Cells are much more difficult to image because of their softness and susceptibility to tip-induced membrane damage (Radmacher *et al*., 1992). However, high-resolution imaging has been possible for fungal (Dufrene, 2002; Touhami *et al*., 2003; Pelling *et al*., 2004), bacterial (Dufrene, 2002; Pelling *et al*., 2005) and mammalian cells (Kumar and Hoh, 2001).

AFM can be much more than a microscope → AFM-DFS

Starting with the first uses of AFM in biology, many non-topological applications have been developed; for instance, direct force measurements at molecular scale using functionalized tips have been used to examine intermolecular interactions, chemical mapping and probing of viscoelastic properties of cells and molecules (Ducker *et al*., 1991; Tsao *et al*., 1993; Florin *et al*., 1994; Moy *et al*., 1994; Lee *et al*., 1994a; Dammer *et al*., 1995; Shao *et al*., 1996). This is mainly possible because AFM has the ability to operate at multiple temperatures in air, fluid and vacuum. Furthermore, the AFM can measure and exert local forces on the order of a few pN[4] (Häberle *et al*., 1991). Such sensitivity has been utilized in the measurement of very local ($<100\,nm^2$) mechanical properties in the measurement of molecular interactions (Florin *et al*., 1994; Lee *et al*., 1994b; Hinterdorfer *et al*., 1996), bond strengths, or to stretch single molecules into novel conformations (Rief *et al*., 1997a; Fisher *et al*., 2000b), see also for a review (Zlatanova *et al*., 2000).

Whereas AFM imaging offers a means to picture topographic surface structures at high resolution and in physiological conditions, DFS with an AFM (AFM-DFS) enables researchers to explore the energy landscape of receptor–ligand interactions and to probe for instance the unfolding pathways of single-membrane proteins, the elasticity of cell walls and surface macromolecules, and the molecular forces responsible for cell–cell and cell–solid surface interactions (Evans and Ritchie, 1997; Merkel *et al*., 1999). The AFM technique can also provide insight into the binding properties of biological components or determine the specific interaction between two kinds of molecules, the archetype being the avidin and biotin couple (Weisenhorn *et al*., 1992; Florin *et al*., 1994; Chilkoti *et al*., 1995; Merkel *et al*., 1999; Wong *et al*., 1999; Yuan *et al*., 2000; Pincet and Husson, 2005). DFS of parallel bonds between antibody and antigen (Hinterdorfer *et al*., 1996; Sulchek *et al*., 2006) or antibody and metal (Odorico *et al*., 2007) have also been studied. Combined topography and molecular recognition of interactions were recently observed between lysozyme/antibody (Stroh *et al*., 2004b), avidin/biotin (Ebner *et al*., 2005) and an S-layer-streptavidin fusion protein (Ebner *et al*., 2006).

More recently the theory on molecular interaction, derived mainly from work on cell adhesion domains and starting with the seminal paper by Bell (1978), has permitted the understanding of the importance of different experimental parameters associated with the statistical treatment of the data, such as the loading rate, the contact time and the contact energy between ligand and receptor and the energy transfered to the system. Such progress shows clearly the interest of the unbinding force measurements approach to better understand the dynamic strength of bonded surfaces. Recent results show that the application of the ergodic hypothesis—that is, for microscopic quantities, average and fluctuations over time are the same as average and fluctuations over space or in other words after a sufficiently long time a system explores all of its microscopic states—is not enough to correlate results obtained on single molecules with those coming from traditional bulk experiments, for instance when the loading rate dependence of measured forces is not taken into account in single molecule experiments (Fantner *et al*., 2006; Leckband and Prakasam, 2006). This raises the fundamental question of the nature of affinity between single molecules and commands to redefine the relevant physico-chemical parameters which describe it[5].

Combining imaging and recognition toward detection and localization

The methodology for exploring the forces and the dynamics of receptor–ligand interactions using AFM force spectroscopy is well established and should be increasingly used by biophysicists, chemical biologists, cell biologists and microbiologists. Remarkably, AFM is the only force-measuring technique that can map the nanoscale lateral distribution of single molecular recognition sites on biosurfaces. Yet, it is clear that the full potential of AFM will be best exploited when combined with other advanced microscopy and spectroscopy techniques.

Reliable protocols are available for attaching biomolecules or cells on the AFM tips and on supporting surfaces as well as established procedures to probe the forces, the dynamics and the localization of molecular recognition interactions. Nevertheless, it is fair to say that accurate data collection and interpretation remain often delicate and require a strong expertise, especially when dealing with complex specimens like living cells. The main tasks are those associated with the quality of the tip and the surface chemistry and their possible alteration during data acquisition. Thus, a detailed understanding of the principles of the different functionalities of AFM and their limitations is essential before users start their first experiment (Hinterdorfer and Dufrene, 2006).

THE FOUR SESSIONS OF THE CONFERENCE

Major biological results obtained using an AFM cover a wide range of literature (Cells, DNA, membranes, membrane proteins, pores, antibodies…). They deal with both structural and force studies and were grouped in four categories at the AFM BioMed conference. The coverage of each topic is now presented by their respective chairman.

[4]Remember, at 300 K, thermal noise limitation is $k_BT = 4.1\,pN \cdot nm$.

[5]See the review of Robert et al. (this issue).

DOI: 10.1002/jmr

Cells, cellular interactions: cell imaging, cell mechanics and cell adhesion

Structural imaging. Soon after its invention, it was quite clear that AFM would have a great potential for imaging cells (Butt *et al*., 1990b; Radmacher *et al*., 1992). A crucial issue for reliable experiments on live cells is sample preparation, that is, the way cells are immobilized on solid supports. A straightforward approach is to exploit the ability of animal cells to spread and adhere to solid surfaces (Radmacher *et al*., 1992; Matzke *et al*., 2001). Coating the substrate with adhesion proteins may be used to enhance immobilization, a method which allowed the observation of actin filament dynamics under the cell membrane of glial cells (Henderson *et al*., 1992). Another elegant approach is to image living cells fixed only by a suction pipette, using an AFM combined with an optical microscope (Hörber *et al*., 1992). In doing so, cells are kept alive for days in growth medium while being examined, making it possible to study cell activities and dynamics. In some cases, chemical fixation using cross-linking agents such as glutaraldehyde may be required either to prevent cell damage or detachment by the scanning tip or to obtain high-resolution images (Le Grimellec *et al*., 2002). Using these different protocols, various cell types were investigated, such as CV-1 kidney cells, fibroblasts, MDCK, platelets and cardiomyocytes (Jena and Hörber, 2002). Importantly, the real-time imaging capability of AFM allows the dynamic processes occurring at cell surfaces to be followed, as shown for instance for the plasma membrane of pancreatic acinar cells where depressions attributed to fusion pores were observed (Schneider *et al*., 1997).

For microbial cells (bacteria, yeast and fungi), immobilization by means of simple adsorption procedures is often inappropriate since it leads to cell detachment by the scanning tip. Non-destructive attachment may be achieved by immobilizing cells mechanically in a polymer membrane with pore size comparable to the dimensions of the cell (Kasas and Ikai, 1995). This method permitted the observation of bacterial cell surface dynamics, such as cell growth and division (Touhami *et al*., 2004), as well as structural changes resulting from cell wall–drug interactions (Verbelen *et al*., 2006).

What are the future challenges in live cell imaging? Within a few years, ultrafast AFMs should allow acquisition of high-resolution images of cell surfaces with millisecond time resolution, that is, much faster than what is currently achieved with commercial instruments (Viani *et al*., 2000; Ando *et al*., 2001; Humphris *et al*., 2003). Surely, another exciting avenue will be the imaging of intracellular structures with three-dimensional (3D) resolution using the photonic force microscope, in which the AFM cantilever is replaced by the 3D trapping potential of a laser focus (Hörber and Miles, 2003).

Nanomechanics. Mechanical forces and associated deformations play a major role in critical cell functions, including mechanotransduction, motility, infiltration, crawling, contraction and gene expression (Rico *et al*., 2005b). AFM force spectroscopy opens up exciting new possibilities for measuring cellular elasticity on a nanoscale, providing information that is complementary to that obtained with other techniques like magnetic tweezers. Elasticity measurements involve recording force curves on cells and converting them into force versus indentation curves using appropriate treatments. The curves can then be analysed with theoretical models to provide quantitative information on sample elasticity (i.e. Young's modulus). Such nanoindentation experiments have enabled the measurement of the mechanical properties of a wide variety of animal cells, including glial cells, platelets, cardiomyocytes, macrophages, endothelial cells, epithelial cells, fibroblasts and osteoblasts, in relation with dynamic processes and cellular functions (Weisenhorn *et al*., 1993; Radmacher *et al*., 1996; Rotsch *et al*., 1999; Matzke *et al*., 2001; Rico *et al*., 2005a, 2005b). One feature of particular interest for future biomedical research is the possibility to monitor elasticity changes upon incubation with pharmacological agents.

Single molecule analyses. Molecular recognition between receptors and cognate ligands plays a central role in controlling cellular behaviour. In this context, single-molecule force spectroscopy has emerged as a powerful tool for analysing and mapping individual ligands (receptors) on cellular surfaces in relation with function. During the past years, we witnessed rapid advances in developing reliable non-destructive procedures for attaching biomolecules and cells on AFM tips/cantilevers (and supporting surfaces). Much progress has also been made in optimizing data acquisition and interpretation in single-molecule force spectroscopy studies, allowing accurate determination of the interaction forces and dynamics of a variety of important cell surface proteins, including cadherins (Baumgartner *et al*., 2000), integrins (Zhang *et al*., 2002; Li *et al*., 2003), selectins (Zhang *et al*., 2004) and bacterial adhesins (Dupres *et al*., 2005). Clearly, knowledge of these cell adhesion forces contributes to refining our understanding of the molecular bases of cell adhesion, which is a critical event in biomedicine (cell-implant, cell–cell and pathogen interactions).

A unique feature of AFM is its ability to map the distribution of individual ligands (receptors) on cells with nanoscale resolution, using either adhesion force mapping or dynamic recognition force mapping. The first method is based on recording a force–volume image, that is, an array of force curves in the x, y plane on an area of given size, assessing the unbinding force values for all curves and displaying them as grey pixels. This approach has been exploited for mapping binding sites on various types of living cells, including red blood cells (Grandbois *et al*., 2000), osteoclasts (Lehenkari *et al*., 2000), endothelial cells (Almqvist *et al*., 2004), mycobacteria (Dupres *et al*., 2005) and lactic bacteria (Gilbert *et al*., 2007). While adhesion force mapping provides a quantitative analysis of unbinding forces, it is limited by its time resolution. By contrast, dynamic recognition force mapping does not provide quantitative force values but is faster and offers better lateral resolution than adhesion force mapping. Here, AFM tips carrying ligands are oscillated at very small amplitudes while being scanned along the biosurface of interest (dynamic force microscopy). Topography and recognition images are simultaneously obtained (TREC imaging) using an electronic circuit (Stroh *et al*., 2004a). Recently, this approach provided the potential to image receptor

DOI: 10.1002/jmr

distributions on vascular endothelial cells (VanVliet and Hinterdorfer, 2006).

In future single molecule studies, the use of small cantilevers should improve the force resolution, thereby allowing measurement of smaller unbinding forces on cells (Viani *et al*., 1999). Also, nanotubes functionalized with biomolecules should permit the mapping of cell surface binding sites with a resolution that would be difficult to achieve with conventional tips (Wong *et al*., 1998)

Single molecular recognition, affinity, unfolding forces: DFS, folding-unfolding, protein-ligand, DNA, single molecules and molecular recognition

A number of techniques are presently available to investigate intermolecular forces acting between single biomolecules and cellular surfaces. The most prominent tools are the AFM (Binnig *et al*., 1986), optical tweezers (Svoboda *et al*., 1993), and the bio-membrane force probe (Evans *et al*., 1995). These techniques span a measurable force window ranging from entropic forces at several femto-Newtons ($1\,\mathrm{fN} = 10^{-15}\,\mathrm{N}$) up to the rupture of covalent bonds at several nanonewtons ($1\,\mathrm{nN} = 10^{-9}\,\mathrm{N}$) (Clausen-Schaumann *et al*., 2000). Using AFM, many different types of interactions have been studied either on isolated proteins *in vitro* or on cellular surfaces *in vivo*. Intra- and intermolecular interactions were measured at the molecular level, as exemplified by detailed analysis of the binding potentials of receptor–ligand pairs involved in cell adhesion (Lehenkari and Horton, 1999; Benoit *et al*., 2000), polysaccaride elasticity (Rief *et al*., 1997b; Marszalek *et al*., 1998), DNA mechanics (Smith *et al*., 1996; Strick *et al*., 1996), and the function of molecular motors (Veigel *et al*., 1999; Tanaka *et al*., 2002).

Recognition force spectroscopy and imaging. In molecular recognition force spectroscopy experiments, the binding of ligands immobilized on AFM tips to surface-bound receptors (or vice versa) is studied by applying a force to the receptor–ligand complex until the bond breaks at a measurable unbinding force (Hinterdorfer, 2002). Such experiments require that one or several ligand molecules are permanently tethered to the apex of the AFM tip, usually by covalent bonding via a flexible linker molecule (Hinterdorfer *et al*., 2002). In programmable force–distance cycles, defined forces are exerted on a receptor–ligand complex and the dissociation process is followed over time. Dynamic aspects of molecular recognition are addressed in force spectroscopy experiments, where distinct force–time profiles are applied to monitor changes of conformation and state during receptor–ligand dissociation. Consequently, DFS allows the detection of energy barriers not detectable by conventional near equilibrium assays and the probing of the free energy surface of proteins and molecular complexes (Evans, 2001).

In typical force-spectroscopy experiments, the loading rate dependence of the unbinding force is measured by changing the pulling velocity of the force exerted on the interaction. The lifetime of the molecular bond can then be calculated for different forces using the Boltzmann ansatz (Bell, 1978). Direct measurements of lifetimes are only possible using a force clamp, where a constant and adjustable force is applied to the complex and the time duration of bond survival is detected such that the lifetime of the interaction is directly measured at the corresponding force. For some molecular interactions, the application of force could prolong bond lifetimes by deforming the molecules such that they lock more tightly (catch bonds) (Marshall *et al*., 2003).

By combining topographical imaging with force measurements, receptor sites are localized with nanometer accuracy (Kienberger *et al*., 2006). Topography and recognition of target molecules are thereby simultaneously mapped. Thus, the AFM can identify specific components in a complex biological sample and retain its high resolution in imaging. In summary, the AFM can be used to study conformational changes of biomolecules and to analyse inter- and intramolecular interactions of molecular complexes with high resolution.

Protein unfolding forces. DFS not only allows investigation of the interaction between receptors and ligands but also permits measurement of the intra-molecular force profiles of single molecules. The application of mechanical force to biological polymers like proteins, polysaccharides and DNA produces conformations that are different from those previously investigated by chemical or thermal denaturation. The force-induced domain unfolding in proteins (Rief *et al*., 1997a), length transitions caused by conformational changes in the sugar rings (Marszalek *et al*., 1999) or in the secondary structure of polysaccharides (Li *et al*., 1998), and alterations of the secondary structure of DNA molecules were studied in great detail (Rief *et al*., 1999), elucidating the molecular determinants of mechanical stability and the role of force-induced conformational changes in the regulation of physiological function. In these experiments, the molecule is held between the tip and the support and its viscoelastic properties are studied in force–distance cycles. Similarly as in molecular recognition force-spectroscopy experiments, a detailed picture of the complex mechanical unfolding pathway through a rough energy landscape can be gained by varying the dynamics of pulling.

In a recent study, controlled unfolding and refolding of a sodium-proton antiporter has been analysed with AFM (Kedrov *et al*., 2004, 2006). Single-molecule force-spectroscopy was employed to unfold and refold single sodium-proton anti-porters (NhaA) from membrane patches. For this purpose, the AFM tip was pressed onto the membrane surface with a contact force of about 1 nN for 1 sec, and then withdrawn while recording the cantilever deflection versus tip-sample distance. The force-spectra contained detailed information on the unfolding process, each peak representing an internal potential barrier which was built up by molecular interactions within the protein. Unfolding experiments of membrane proteins were further refined to allow for controlled refolding of individual secondary structures, supporting the hypothesis that unfolding and refolding of transmembrane helices may be fully reversible, including re-insertion of the transmembrane segments into the lipid bilayer (Kedrov *et al*., 2007).

DOI: 10.1002/jmr

High-resolution imaging: high-resolution imaging, high speed imaging, coupling with other methods

Since the early 1990s AFM has recorded topographs with sufficient resolution to depict individual protein molecules in buffer solution (Drake *et al*., 1989; Hoh *et al*., 1991; Karrasch *et al*., 1994; Schabert and Engel, 1994). However, the tip, which is the key element of the AFM, is approximately three orders of magnitude larger than individual proteins that are probed. There is no doubt that the prerequisite for successful high-resolution contouring of flexible and fragile protein samples is mastery of the forces applied on the tip.

The following sections provide examples of high-resolution imaging and its contribution to the wider biological fields to provide insights into novel developments that aim at faster image acquisition or more sensible force probing.

Reproducible high-resolution imaging was established on 2D membrane protein crystals (Schabert and Engel, 1994). A superposition of long-range repulsive electrostatic, short-range attractive van der Waals, and very short-range Pauli-repulsion forces was evidenced to describe the interaction between tip and sample. Adjustment of the ionic strength and the pH in the imaging buffer solution allows balance of the forces applied by the AFM stylus to minimize the forces exerted on proteins (Muller *et al*., 1999b). When the AFM is operated in an oscillating-tip mode, much lower imaging forces can be applied (San Paulo and Garcia, 2000). Simultaneous excitation of two different flexural modes of the cantilever enables imaging under the application of weak forces ($\sim$35 pN), which are smaller than those needed to break non-covalent bonds (Tello *et al*., 2003; Garcia *et al*., 2007). Another way to circumvent the application of high loading forces is scanning at very high frequency. Shorter and more sensitive cantilevers were required (Viani *et al*., 1999) to build an AFM that is able to acquire movies of molecules in action (Ando *et al*., 2001, 2003). These developments allowed the description in real time the enzymatic cycle of GroEL-GroES action (Yokokawa *et al*., 2006). The highest resolution images to date were however acquired in contact mode on membranes. This was established on 2D crystals of membrane proteins (Muller *et al*., 1995a), and is now applied to native membranes (Fotiadis *et al*., 2003; Scheuring *et al*., 2003, 2005; Buzhynskyy *et al*., 2007). The supramolecular assembly of mammalian rhodopsin, a GPCR present in native disc membranes, was reported using AFM revealing a striking assembly of rhodopsin in rows of dimers (Fotiadis *et al*., 2003). These images were at the basis of a structural model of the rhodopsin dimer (Liang *et al*., 2003). Interestingly, this dimer, as observed in the native membrane, was found to be a perfect platform for transducin assembly (Filipek *et al*., 2004) and provided a working model for GPCR action in native membranes in general (Fotiadis *et al*., 2006). Similarly, the photosynthetic apparatus from different purple bacteria and its architectural adaptations to different light intensities were also described (Scheuring *et al*., 2003, 2004a, 2004b, 2005, 2006; Bahatyrova *et al*., 2004; Goncalves *et al*., 2005). Using cross-correlation algorithms, atomic structures of the photosynthetic membrane complexes were docked into the high-resolution AFM images to yield structural models of the entire photosynthetic unit (Scheuring *et al*., 2007). The same approach has been used to dock high-resolution AFM topographs to build models of the assembly of aquaporin 0 and connexions in junctional microdomains from the eye lens (Buzhynskyy *et al*., 2007). In conclusion, high-resolution AFM is now a complementary technique to electron microscopy and X-ray crystallography in structural biology, as it can contribute information on single molecules, their supramolecular assemblies, and their dynamics.

Model membranes and protein–membrane interactions: membrane imaging, protein–membrane interactions and possible applications

Dedicated to the study of surfaces of materials under vacuum, air or fluid, the AFM quickly attracted the interest of the biomembrane community. Heterogeneity of biological membrane lateral organization in the plasma membrane of prokaryotic (Morrisett *et al*., 1975) and eukaryotic cells (Karnovsky *et al*., 1982) explains why cell membrane surface imaging at the mesoscopic and molecular scale is a prerequisite for understanding membrane functions and structure–function relationships. These earliest studies recognized that lipids could play an important role in lateral membrane heterogeneity and were associated with the development of new membrane models, allowing the definition of miscibility properties of binary and ternary mixtures of lipids in bilayer membranes (Shimshick and McConnell, 1973; Lee, 1977). Fundamental information on lipid–lipid, lipid–peptide and lipid–protein interactions were obtained later by NMR, fluorescence, CD, and FTIR but with the exception of electron microscopy-coupled approaches (Grant *et al*., 1974.), none of these studies gave direct access to the membrane mesoscopic scale organization.

The first AFM experiments on lipid monolayers and bilayers were done in Santa Barbara, in Paul Hansma's laboratory (Weisenhorn *et al*., 1991; Zasadzinski *et al*., 1991). This very successful AFM approach in characterizing Langmuir–Blodgett films (Viswanathan *et al*., 1993) was rapidly extended to monolayers and bilayers made of biologically relevant constituents, namely lipids, peptides and proteins. AFM imaging of phosphatidylcholine bilayers under various physiological buffers demonstrated the possibility to obtain mesoscopic scale information on the organization of lipid phases (Mou *et al*., 1994), a key parameter in the understanding of membrane lateral heterogeneity. Then, AFM was used to establish directly, for the first time, the miscibility properties at the mesoscopic scale of various binary (Dufrene *et al*., 1997) and ternary (Vie *et al*., 1998) membrane lipid mixtures. In parallel, unique information on lipid–peptide and lipid–protein interactions were obtained by combining the use of model systems and AFM. Direct structural evidence for the formation of gramicidin A clusters and peptide-induced membrane reorganization (Mou *et al*., 1996) as well as the formation of filamentous supramolecular complexes in phosphatidylcholine-peptide vectors membranes (Van Mau *et al*., 1999), illustrated the essential contribution that AFM can bring in the understanding of membrane

DOI: 10.1002/jmr

structure–function relationships. Finally, in terms of lipid–protein interactions, the AFM has been successfully used to study the insertion of either GPI-anchored proteins in ordered domains (Milhiet *et al*., 2002) or transmembrane proteins in membrane under phase separation (Milhiet *et al*., 2006), and also the time course of membrane alteration by an enzyme (Grandbois *et al*., 1998).

As for the other sessions of this conference, the number of publications using AFM in the membrane model and protein–membrane interaction fields has exploded during recent years. A direct consequence of this situation has been a multiplicity of the experimental conditions used to make membrane models. Following the report of a very limited but significant shift in the gel-fluid transition temperature of phospholipid bilayers deposited on mica (Yang and Appleyard, 2000), a series of papers examined the properties of supported membranes as a function of the conditions chosen for their formation (Garcia-Manyes *et al*., 2005; Oncins *et al*., 2005; Richter *et al*., 2006). The recent coupling of fluorescence correlation spectroscopy (FCS) with AFM provided evidence for the suspected heterogeneity within single gel phase lipid domains in a fluid-gel phase separated supported bilayer (Burns *et al*., 2005).

Except for very specialized biomembrane regions, like those presented in the previous section, structure–function relationship analyses in biomembranes are still based on models of membrane molecular organization which can markedly differ (Allen *et al*., 2007; Jacobson *et al*., 2007). The use of such models is explained by the lack of molecular characterization of membrane structure, linked to the dynamic properties of membrane constituents. One has to recall that even in the ordered gel phase, phospholipid headgroup motion, and to a lesser extent lateral diffusion ($>10\,nm^2$/sec), should limit the lateral resolution accessible to commercial AFMs to the mesoscopic instead of molecular scale. Clearly, at least video rate AFM with nanometer resolution will be required for insight into the structural organization of membranes at the molecular scale. In parallel, the development of new model systems, such as the bilayers on porous supports (Steltenkamp *et al*., 2006) or double supported bilayers (Leidy *et al*., 2002; Giocondi and Le Grimellec, 2004) composed of complex lipid mixtures with different inserted proteins capable of forming supramolecular arrangements, will be necessary to better understand the molecular organization of biomembranes. In addition to providing tools for understanding membrane organization and consequently for developing drugs against the numerous diseases associated with membrane dysfunction, the model membrane/AFM coupling is now a basic tool in the development of new nanosensors in the nanobioscience and nanobiotechnology fields.

Concluding sentences from the keynote speakers of the meeting.

Prof. Pierre Bongrand commented, '*Due to the combination of imaging, force application and dynamics, as well as lack of requirement for non-physiological sample processing, AFM is arguably the best suited tool to help us gain an intuitive grasp of what biomolecules look like and how they behave in the nanoscale world*'.

Prof. Paul Hansma commented, '*Back when we were developing AFM for biological applications, we were hoping that AFM would one day contribute to quality of life, like the light microscope and the electron microscope has in the past. I look forward to the day when we will see the picture of the first person who was healed because of the use of an AFM*'.

Michael Horton commented, '*This conference emphasizes the need to set up cross-disciplinary collaborations up front. Now that AFM has a relatively firm footing in the life sciences, biologists are becoming innovators in the use and applications for AFM as much as physicists have been. The next step is to connect nanoscale research to clear biomedical needs*'.

Acknowledgments

The authors as members of the organising committee thank the Commissariat à l'Energie Atomique (CEA-Valrhô), Universitat de Barcelona, Institut de Bioenginyeria de Catalunya (IBEC) for their support; the CosmoCaixa Science Museum for a warm welcome and perfect organization; the *Journal of Molecular Recognition* for opening up to the field of AFM and finally Leica, Telstar and Veeco who generously supported the AFM BioMed 2007 Conference. Authors acknowledged the helpful editorial assistance of Dr Julie Tubbs.

REFERENCES

Alcaraz J, Buscemi L, Grabulosa M, Trepat X, Fabry B, Farre R, Navajas D. 2003. Microrheology of human lung epithelial cells measured by atomic force microscopy. *Biophys. J.* **84**: 2071–2079.

Allen JA, Halverson-Tamboli RA, Rasenick MM. 2007. Lipid raft microdomains and neurotransmitter signalling. *Nat. Rev. Neurosci.* **8**: 128–140.

Almqvist N, Bhatia R, Primbs G, Desai N, Banerjee S, Lal R. 2004. Elasticity and adhesion force mapping reveals real-time clustering of growth factor receptors and associated changes in local cellular rheological properties. *Biophys. J.* **86**: 1753–1762.

Ando T, Kodera N, Takai E, Maruyama D, Saito K, Toda A. 2001. A high-speed atomic force microscope for studying biological macromolecules. *Proc. Natl. Acad. Sci. U.S.A.* **98**: 12468–12472.

Ando T, Kodera N, Naito Y, Kinoshita T, Furuta K, Toyoshima YY. 2003. A high-speed atomic force microscope for studying biological macromolecules in action. *Chem. Phys. Chem.* **4**: 1196–1202.

Anselmetti D, Lüthi R, Meyer E, Richmond T, Dreier M, Frommer JE, Güntherodt H-J. 1994. Attractive-mode imaging of biological materials with dynamic force microscopy. *Nanotechnology* **5**: 87–94.

Ashkin A, Dziedzic JM. 1987. Optical trapping and manipulation of viruses and bacteria. *Science* **235**: 1517–1520.

Bahatyrova S, Frese RN, Siebert CA, Olsen JD, Van Der Werf KO, Van Grondelle R, Niederman RA, Bullough PA, Otto C, Hunter

DOI: 10.1002/jmr

CN. 2004. The native architecture of a photosynthetic membrane. *Nature* **430**: 1058–1062.
Barrett RC, Quate CF. 1991. High-speed, large-scale imaging with the atomic force microscope. *J. Vac. Sci. Tech. B.* **9**: 302–306.
Baumgartner W, Hinterdorfer P, Ness W, Raab A, Vestweber D, Schindler H, Drenckhahn D. 2000. Cadherin interaction probed by atomic force microscopy. *Proc. Natl. Acad. Sci. U.S.A.* **97**: 4005–4010.
Bell GI. 1978. Models for the specific adhesion of cells to cells. *Science* **200**: 618–627.
Benoit M, Gabriel D, Gerisch G, Gaub HE. 2000. Discrete interactions in cell adhesion measured by single-molecule force spectroscopy. *Nat. Cell. Biol.* **2**: 313–317.
Bhushan B, Fuchs H. 2006. Applied Scanning Probe Methods, III Characterization, Springer: Heidelberg.
Binnig G, Rohrer H. 1986. In Nobel Lecture.
Binnig G, Rohrer H, Gerber C, Weibel E. 1982. Surface studies by scanning tunnelling microscopy. *Phys. Rev. Lett.* **49**: 57–61.
Binnig G, Quate CF, Gerber C. 1986. Atomic force microscope. *Phys. Rev. Lett.* **56**: 930–933.
Binnig G, Gerber C, Stoll E, Albrecht T, Quate CF. 1987. Atomic resolution with atomic force microscope. *Europhys. Lett.* 1281–1286.
Burns AR, Frankel DJ, Buranda T. 2005. Local mobility in lipid domains of supported bilayers characterized by atomic force microscopy and fluorescence correlation spectroscopy. *Biophys. J.* **89**: 1081–1093.
Butt HJ, Downing KH, Hansma PK. 1990a. Imaging the membrane protein bacteriorhodopsin with the atomic force microscope. *Biophys. J.* **58**: 1473–1480.
Butt HJ, Wolff EK, Gould SA, Dixon Northern B, Peterson CM, Hansma PK. 1990b. Imaging cells with the atomic force microscope. *J. Struct. Biol.* **105**: 54–61.
Buzhynskyy N, Hite RK, Walz T, Scheuring S. 2007. The supramolecular architecture of junctional microdomains in native lens membranes. *EMBO Rep.* **8**: 51–55.
Chilkoti A, Boland T, Ratner BD, Stayton PS. 1995. The relationship between ligand-binding thermodynamics and protein–ligand interaction forces measured by atomic force microscopy. *Biophys. J.* **69**: 2125–2130.
Clausen-Schaumann H, Seitz M, Krautbauer R, Gaub HE. 2000. Force spectroscopy with single bio-molecules. *Curr. Opin. Chem. Biol.* **4**: 524–530.
Czajkowsky DM, Hotze EM, Shao Z, Tweten RK. 2004. Vertical collapse of a cytolysin prepore moves its transmembrane beta-hairpins to the membrane. *EMBO J.* **23**: 3206–3215.
Dammer U, Popescu O, Wagner P, Anselmetti D, Guntherodt HJ, Misevic GN. 1995. Binding strength between cell adhesion proteoglycans measured by atomic force microscopy. *Science* **267**: 1173–1175.
Drake B, Prater CB, Weisenhorn AL, Gould SA, Albrecht TR, Quate CF, Cannell DS, Hansma HG, Hansma PK. 1989. Imaging crystals, polymers, and processes in water with the atomic force microscope. *Science* **243**: 1586–1589.
Ducker WA, Senden TJ, Pashley RM. 1991. Direct measurement of colloidal forces using an atomic force microscope. *Nature* **353**: 239–241.
Dufrene YF. 2002. Atomic force microscopy, a powerful tool in microbiology. *J. Bacteriol.* **184**: 5205–5213.
Dufrene YF, Barger WR, Green J-B, Lee GU. 1997. Nanometer scale surface properties of mixed phospholipid monolayers and bilayers. *Langmuir* **13**: 4779–4784.
Dupres V, Menozzi FD, Locht C, Clare BH, Abbott NL, Cuenot S, Bompard C, Roze D, Dufrene YF. 2005. Nanoscale mapping and functional analysis of individual adhesins on living bacteria. *Nat. Methods.* **2**: 515–520.
Ebner A, Kienberger F, Kada G, Stroh CM, Geretschlager M, Kamruzzahan AS, Wildling L, Gruber HJ, Sleytr UB, Sara M, Hinterdorfer P. 2005. Localization of single avidin–biotin interactions using simultaneous topography and molecular recognition imaging. *Chem. Phys. Chem.* **6**: 897–900.
Ebner A, Kienberger F, Huber C, Kamruzzahan AS, Pastushenko VP, Tang J, Kada G, *et al*. 2006. Atomic-force-microscopy imaging and molecular-recognition-force microscopy of recrystallized heterotetramers comprising an S-layer-streptavidin fusion protein. *Chembiochem* **7**: 588–591.
Egger M, Ohnesorge F, Weisenhorn AL, Heyn SP, Drake B, Prater CB, Gould SAC, Hansma PK, Gaub HE. 1990. Wet lipid–protein membranes imaged at submolecular resolution by atomic force microscopy. *J. Struct. Biol.* **103**: 89–94.
El Kirat K, Burton I, Dupres V, Dufrene YF. 2005. Sample preparation procedures for biological atomic force microscopy. *J. Microsc.* **218**: 199–207.
Engel A. 1991. Biological applications of scanning probe microscopes. *Annu. Rev. Biophys. Biophys. Chem.* **20**: 79–108.
Engel A, Muller DJ. 2000. Observing single biomolecules at work with the atomic force microscope. *Nat. Struct. Biol.* **7**: 715–718.
Engel A, Gaub HE, Muller DJ. 1999. Atomic force microscopy: a forceful way with single molecules. *Curr. Biol.* **9**: R133–R136.
Evans E. 2001. Probing the relation between force—lifetime—and chemistry in single molecular bonds. *Annu. Rev. Biophys. Biomol. Struct.* **30**: 105–128.
Evans E, Ritchie K. 1997. Dynamic strength of molecular adhesion bonds. *Biophys. J.* **72**: 1541–1555.
Evans E, Berk D, Leung A. 1991. Detachment of agglutinin-bonded red blood cells. I. Forces to rupture molecular-point attachments. *Biophys. J.* **59**: 838–848.
Evans E, Ritchie K, Merkel R. 1995. Sensitive force technique to probe molecular adhesion and structural linkages at biological interfaces. *Biophys. J.* **68**: 2580–2587.
Fantner GE, Oroudjev E, Schitter G, Golde LS, Thurner P, Finch MM, Turner P, Gutsmann T, Morse DE, Hansma H, Hansma PK. 2006. Sacrificial bonds and hidden length: unraveling molecular mesostructures in tough materials. *Biophys. J.* **90**: 1411–1418.
Filipek S, Krzysko KA, Fotiadis D, Liang Y, Saperstein DA, Engel A, Palczewski K. 2004. A concept for G protein activation by G protein-coupled receptor dimers: the transducin/rhodopsin interface. *Photochem. Photobiol. Sci.* **3**: 628–638.
Fisher TE, Carrion-Vazquez M, Oberhauser AF, Li H, Marszalek PE, Fernandez JM. 2000a. Single molecule force spectroscopy of modular proteins in the nervous system. *Neuron* **27**: 435–446.
Fisher TE, Marszalek PE, Fernandez JM. 2000b. Stretching single molecules into novel conformations using the atomic force microscope. *Nat. Struct. Biol.* **7**: 719–724.
Florin EL, Moy VT, Gaub HE. 1994. Adhesion forces between individual ligand-receptor pairs. *Science* **264**: 415–417.
Fotiadis D, Liang Y, Filipek S, Saperstein DA, Engel A, Palczewski K. 2003. Atomic-force microscopy: Rhodopsin dimers in native disc membranes. *Nature* **421**: 127–128.
Fotiadis D, Jastrzebska B, Philippsen A, Muller DJ, Palczewski K, Engel A. 2006. Structure of the rhodopsin dimer: a working model for G-protein-coupled receptors. *Curr. Opin. Struct. Biol.* **16**: 252–259.
Fukuma T, Kobayashi K, Matsushige K, Yamada H. 2005. True atomic resolution in liquid by frequency-modulation atomic force microscopy. *Appl. Phys. Lett.* **87**: 034101.
Garcia R, Magerle R, Perez R. 2007. Nanoscale compositional mapping with gentle forces. *Nat. Mater.* **6**: 405–411.
Garcia-Manyes S, Oncins G, Sanz F. 2005. Effect of temperature on the nanomechanics of lipid bilayers studied by force spectroscopy. *Biophys. J.* **89**: 4261–4274.
Gaub H. 2005. *Introduction to AFM in single molecule*. Accessed http://www.diffusion.ens.fr/index.php?res=conf&idconf=778
Gerber C, Lang HP. 2006. How the doors to the nanoworld were opened. *Nature Nanotech.* **1**: 3–5.
Gilbert Y, Deghorain M, Wang L, Xu B, Pollheimer PD, Gruber HJ, Errington J, Hallet B, Haulot X, Verbelen C, Hols P, Dufrene YF. 2007. Single-molecule force spectroscopy and imaging of the vancomycin/D-Ala-D-Ala interaction. *Nano. Lett.* **7**: 796–801.
Giocondi MC, Le Grimellec C. 2004. Temperature dependence of the surface topography in dimyristoylphosphatidylcholine/distearoylphosphatidylcholine multibilayers. *Biophys. J.* **86**: 2218–2230.
Goncalves RP, Bernadac A, Sturgis JN, Scheuring S. 2005. Architecture of the native photosynthetic apparatus of

Phaeospirillum molischianum. *J. Struct. Biol.* **152**: 221–228.
Gould S, Marti O, Drake B, Hellemans L, Bracker CE, Hansma PK, Keder NL, Eddy MM, Stucky GD. 1988. Molecular resolution images of amino acid crystals with the atomic force microscope. *Nature* **332**: 332.
Gould SAC, Drake B, Prater CB, Weisenhorn AL, Manne S, Kelderman GL, Butt H-J, Hansma H, Hansma PK, Magonov S, Contow HJ. 1990. The atomic force microscope: A tool for science and industry. *Ultramicroscopy* **33**: 93–998.
Grandbois M, Clausen-Schaumann H, Gaub H. 1998. Atomic force microscope imaging of phospholipid bilayer degradation by phospholipase A2. *Biophys. J.* **74**: 2398–2404.
Grandbois M, Dettmann W, Benoit M, Gaub HE. 2000. Affinity imaging of red blood cells using an atomic force microscope. *J. Histochem. Cytochem.* **48**: 719–724.
Grant CW, Wu SH, McConnell HM. 1974. Lateral phase separations in binary lipid mixtures: correlation between spin label and freeze-fracture electron microscopic studies. *Biochim. Biophys. Acta.* **363**: 151–158.
Häberle W, Hörber JKH, Binnig G. 1991. Force microscopy on living cells. *J. Vac. Sci. Tech. B.* **9**: 1210–1213.
Häberle W, Horber JK, Ohnesorge F, Smith DP, Binnig G. 1992. In situ investigations of single living cells infected by viruses. *Ultramicroscopy* **42–44**: 1161–1167.
Han W, Mou J, Sheng J, Yang J, Shao Z. 1995. Cryo atomic force microscopy: A new approach for biological imaging at high resolution. *Biochemistry* **34**: 8215–8220.
Hansma HG, Hoh JH. 1994. Biomolecular imaging with the atomic force microscope. *Annu. Rev. Biophys. Biomol. Struct.* **23**: 115–139.
Hansma PK, Elings VB, Marti O, Bracker CE. 1988. Scanning tunneling microscopy and atomic force microscopy: application to biology and technology. *Science* **242**: 209–216.
Hansma PK, Cleveland JP, Radmacher M, Walters DA, Hilner PE, Bezanilla M, Prater CB, Massie J, Fukugona L, Gurley J, Elings V. 1994. Tapping mode atomic force microscopy in liquids. *Appl. Phys. Lett.* **64**: 1738–1740.
Hansma PK, Schitter G, Fantner GE, Prater C. 2006. Applied physics. High-speed atomic force microscopy. *Science* **314**: 601–602.
Henderson E, Haydon PG, Sakaguchi DS. 1992. Actin filament dynamics in living glial cells imaged by atomic force microscopy. *Science* **257**: 1944–1946.
Hinterdorfer P. 2002. Molecular recognition studies using the atomic force microscope. *Methods. Cell. Biol.* **68**: 115–139.
Hinterdorfer P, Dufrene YF. 2006. Detection and localization of single molecular recognition events using atomic force microscopy. *Nat. Methods* **3**: 347–355.
Hinterdorfer P, Baumgartner W, Gruber HJ, Schilcher K, Schindler H. 1996. Detection and localization of individual antibody–antigen recognition events by atomic force microscopy. *Proc. Natl. Acad. Sci. U.S.A.* **93**: 3477–3481.
Hinterdorfer P, Gruber HJ, Kienberger F, Kada G, Riener C, Borken C, Schindler H. 2002. Surface attachment of ligands and receptors for molecular recognition force microscopy. *Coll. Surf. B* **23**: 115–123.
Hoh JH, Lal R, John SA, Revel JP, Arnsdorf MF. 1991. Atomic force microscopy and dissection of gap junctions. *Science* **253**: 1405–1408.
Hörber JKH, Miles MJ. 2003. Scanning Probe Evolution in Biology. *Science* **302**: 1002–1005.
Hörber JKH, Häberle W, Ohnesorge F, Binnig G, Liebich HG, Czerny CP, Mahnel H, Mayr, A. 1992. Investigation of living cells in the nanometer regime with the scanning force microscope. *Scanning Microsc.* **6**: 919–930.
Humphris ADL, Hobbs JK, Miles MJ. 2003. Ultrahigh-speed scanning near-field optical microscopy capable of over 100 frames per second. *Appl. Phys. Lett.* **83**: 6–8.
Ikai A. 1996. STM and AFM of bio/organic molecules and structures. *Surf. Sci. Reports.* **26**: 261–332.
Jacobson K, Mouritsen OG, Anderson RG. 2007. Lipid rafts: at a crossroad between cell biology and physics. *Nat. Cell. Biol.* **9**: 7–14.
Jena BP, Hörber JKH. 2002. Atomic Force Microscopy in Cell Biology. Methods in Cell Biology, Vol. 68, Academic Press: San Diego.
Jena BP, Hörber JKH. 2006. Force Microscopy Applications in Biology and Medicine. John Wiley & Sons, Inc.: Hoboken, New Jersey.
Kaplanski G, Farnarier C, Tissot O, Pierres A, Benoliel AM, Alessi MC, Kaplanski S, Bongrand P. 1993. Granulocyte-endothelium initial adhesion. Analysis of transient binding events mediated by E-selectin in a laminar shear flow. *Biophys. J.* **64**: 1922–1933.
Karnovsky MJ, Kleinfeld AM, Hoover RL, Klausner RD. 1982. The concept of lipid domains in membranes. *J. Cell. Biol.* **94**: 1–6.
Karrasch S, Hegerl R, Hoh JH, Baumeister W, Engel A. 1994. Atomic force microscopy produces faithful high-resolution images of protein surfaces in an aqueous environment. *Proc. Natl. Acad. Sci. U.S.A.* **91**: 836–838.
Kasas S, Ikai A. 1995. A method for anchoring round shaped cells for atomic force microscope imaging. *Biophys. J.* **68**: 1678–1680.
Kedrov A, Ziegler C, Janovjak H, Kuhlbrandt W, Muller DJ. 2004. Controlled unfolding and refolding of a single sodium-proton antiporter using atomic force microscopy. *J. Mol. Biol.* **340**: 1143–1152.
Kedrov A, Janovjak H, Ziegler C, Kuhlbrandt W, Muller DJ. 2006. Observing folding pathways and kinetics of a single sodium-proton antiporter from Escherichia coli. *J. Mol. Biol.* **355**: 2–8.
Kedrov A, Janovjak H, Sapra KT, Muller DJ. 2007. Deciphering molecular interactions of native membrane proteins by single-molecule force spectroscopy. *Annu. Rev. Biophys. Biomol. Struct.* **36**: 233–260.
Kienberger F, Ebner A, Gruber HJ, Hinterdorfer P. 2006. Molecular recognition imaging and force spectroscopy of single biomolecules. *Acc. Chem. Res.* **39**: 29–36.
Kumar S, Hoh JH. 2001. Probing the machinery of intracellular trafficking with the atomic force microscope. *Traffic* **2**: 746–756.
Lal R, Kim H, Garavito RM, Arnsdorf MF. 1993. Imaging of reconstituted biological channels at molecular resolution by atomic force microscopy. *Am. J. Physiol.* **265**: C851–C856.
Le Grimellec C, Giocondi MC, Lenoir M, Vater M, Sposito G, Pujol R. 2002. High-resolution three-dimensional imaging of the lateral plasma membrane of cochlear outer hair cells by atomic force microscopy. *J. Comp. Neurol.* **451**: 62–69.
Leckband D, Prakasam A. 2006. Mechanism and dynamics of cadherin adhesion. *Annu. Rev. Biomed. Eng.* **8**: 259–287.
Lee AG. 1977. Lipid phase transitions and phase diagrams. II. Mictures involving lipids. *Biochim. Biophys. Acta.* **472**: 285–344.
Lee GU, Chrisey LA, Colton RJ. 1994a. Direct measurement of the forces between complementary strands of DNA. *Science* **266**: 771–773.
Lee GU, Kidwell DA, Colton RJ. 1994b. Sensing discrete S t reptavidin-bio tin interactions with atomic force microscopy. *Langmuir* **10**: 354–357.
Lehenkari PP, Horton MA. 1999. Single Integrin molecule adhesion forces in intact cells measured by atomic force microscopy. *Biochem. Biophys. Res. Commun.* **259**: 645–650.
Lehenkari PP, Charras GT, Nykanen A, Horton MA. 2000. Adapting atomic force microscopy for cell biology. *Ultramicroscopy* **82**: 289–295.
Leidy C, Kaasgaard T, Crowe JH, Mouritsen OG, Jorgensen K. 2002. Ripples and the formation of anisotropic lipid domains: imaging two-component supported double bilayers by atomic force microscopy. *Biophys. J.* **83**: 2625–2633.
Li H, Rief M, Oesterhelt F, Gaub H. 1998. Single-molecule force spectroscopy on xanthan by AFM. *Adv. Mater.* **3**: 316–319.
Li F, Redick SD, Erickson HP, Moy VT. 2003. Force measurements of the alpha5beta1 integrin–fibronectin interaction. *Biophys. J.* **84**: 1252–1262.
Li G, Xi N, Wang DH. 2006. Probing membrane proteins using atomic force microscopy. *J. Cell. Biochem.* **97**: 1191–1197.

DOI: 10.1002/jmr

Liang Y, Fotiadis D, Filipek S, Saperstein DA, Palczewski K, Engel A. 2003. Organization of the G protein-coupled receptors rhodopsin and opsin in native membranes. *J. Biol. Chem.* **278**: 21655–21662.
Lindsay SM, Tao NJ, DeRose JA, Oden PI, Lyubchenko Yu L, Harrington RE, Shlyakhtenko L. 1992. Potentiostatic deposition of DNA for scanning probe microscopy. *Biophys. J.* **61**: 1570–1584.
Mahaffy R, Shih C, MacKintosh F, Kas J. 2000. Scanning probe-based frequency-dependent microrheology of polymer gels and biological cells. *Phys. Rev. Lett.* **85**: 880–883.
Marshall BT, Long M, Piper JW, Yago T, McEver RP, Zhu C. 2003. Direct observation of catch bonds involving cell-adhesion molecules. *Nature* **423**: 190–193.
Marszalek PE, Oberhauser AF, Pang YP, Fernandez JM. 1998. Polysaccharide elasticity governed by chair-boat transitions of the glucopyranose ring. *Nature* **396**: 661–664.
Marszalek PE, Pang YP, Li H, El Yazal J, Oberhauser AF, Fernandez JM. 1999. Atomic levers control pyranose ring conformations. *Proc. Natl. Acad. Sci. U.S.A.* **96**: 7894–7898.
Marti O, Drake B, Hansma PK. 1987. Atomic force microscopy of liquid-covered surfaces: atomic resolution images. *Appl. Phys. Lett.* **51**: 484–486.
Matzke R, Jacobson K, Radmacher M. 2001. Direct, high-resolution measurement of furrow stiffening during division of adherent cells. *Nat. Cell. Biol.* **3**: 607–610.
Merkel R, Nassoy P, Leung A, Ritchie K, Evans E. 1999. Energy landscapes of receptor–ligand bonds explored with dynamic force spectroscopy. *Nature* **397**: 50–53.
Meyer G, Amer NM. 1988. Novel optical approach to atomic force microscopy. *Appl. Phys. Lett.* **53**: 1045.
Milhiet PE, Giocondi MC, Baghdadi O, Ronzon F, Roux B, Le Grimellec C. 2002. Spontaneous insertion and partitioning of alkaline phosphatase into model lipid rafts. *EMBO Rep.* **3**: 485–4490.
Milhiet PE, Gubellini F, Berquand A, Dosset P, Rigaud JL, Le Grimellec C, Levy D. 2006. High resolution AFM of membrane proteins directly incorporated at high density in planar lipid bilayer. *Biophys. J.* **91**: 3268–3275.
Mody C. 2006. Corporations, Universities, and Instrumental Communities. *Technol. Cult.* **47**: 56–80.
Morris VJ, Gunning AP, Kirby AR. 1999. Atomic force Microscopy for Biologists. Imperial college Press: London.
Morrisett JD, Pownall HJ, Plumlee RT, Smith LC, Zehner ZE. 1975. Multiple thermotropic phase transitions in Escherichia coli membranes and membrane lipids. A comparison of results obtained by nitroxyl stearate paramagnetic resonance, pyrene excimer fluorescence, and enzyme activity measurements. *J. Biol. Chem.* **250**: 6969–6976.
Mou J, Yang J, Shao Z. 1994. Tris(hydroxymethyl)aminomethane (C4H11NO3) induced a ripple phase in supported unilamellar phospholipid bilayers. *Biochemistry* **33**: 4439–4443.
Mou J, Czajkowsky DM, Shao Z. 1996. Gramicidin A aggregation in supported gel state phosphatidylcholine bilayers. *Biochemistry* **35**: 3222–3226.
Moy VT, Florin EL, Gaub HE. 1994. Intermolecular forces and energies between ligands and receptors. *Science* **266**: 257–259.
Muller DJ, Buldt G, Engel A. 1995a. Force-induced conformational change of bacteriorhodopsin. *J. Mol. Biol.* **249**: 239–243.
Muller DJ, Schabert FA, Buldt G, Engel A. 1995b. Imaging purple membranes in aqueous solutions at sub-nanometer resolution by atomic force microscopy. *Biophys. J.* **68**: 1681–1686.
Muller DJ, Baumeister W, Engel A. 1999a. Controlled unzipping of a bacterial surface layer with atomic force microscopy. *Proc. Natl. Acad. Sci. U.S.A* **96**: 13170–13174.
Muller DJ, Fotiadis D, Scheuring S, Muller SA, Engel A. 1999b. Electrostatically balanced subnanometer imaging of biological specimens by atomic force microscope. *Biophys. J.* **76**: 1101–1111.
Odorico M, Teulon J-M, Bellanger L, Vidaud C, Bessou T, Chen S-wW, Quéméneur E, Parot P, Pellequer JL. 2007. Energy landscape of chelated uranyl—antibody interactions by dynamic force spectroscopy. *Biophys. J.* **93**: 645–654.
Oesterhelt F, Scheuring S. 2006. High-resolution imaging and force measurement of individual membrane proteins by AFM. *Curr. Nanosci.* **2**: 329–335.
Oesterhelt F, Oesterhelt D, Pfeiffer M, Engel A, Gaub HE, Muller DJ. 2000. Unfolding pathways of individual bacteriorhodopsins. *Science* **288**: 143–146.
Oncins G, Garcia-Manyes S, Sanz F. 2005. Study of frictional properties of a phospholipid bilayer in a liquid environment with lateral force microscopy as a function of NaCl concentration. *Langmuir* **21**: 7373–7379.
Pelling AE, Sehati S, Gralla EB, Valentine JS, Gimzewski JK. 2004. Local nanomechanical motion of the cell wall of Saccharomyces cerevisiae. *Science* **305**: 1147–1150.
Pelling AE, Li Y, Shi W, Gimzewski JK. 2005. Nanoscale visualization and characterization of Myxococcus xanthus cells with atomic force microscopy. *Proc. Natl. Acad. Sci. U.S.A.* **102**: 6484–6489.
Picco L, Bozec L, Ulcinas A, Engledew D, Antognozzi M, Horton M, Miles M. 2007. Breaking the speed limit with atomic force microscopy. *Nanotechnology* **18**: 1–4.
Pincet F, Husson J. 2005. The solution to the streptavidin–biotin paradox: the influence of history on the strength of single molecular bonds. *Biophys. J.* **89**: 4374–4381.
Poggi MA, Gadsby ED, Bottomley LA, King WP, Oroudjev E, Hansma H. 2004. Scanning probe microscopy. *Anal. Chem.* **76**: 3429–3443.
Putman CAJ, Wetf KVd, Grooth BGD, Hulst NFV, Greve J. 1994. Tapping mode atomic force microscopy in liquid. *Appl. Phys. Lett.* 64.
Radmacher M, Tillamnn RW, Fritz M, Gaub HE. 1992. From molecules to cells: imaging soft samples with the atomic force microscope. *Science* **257**: 1900–1905.
Radmacher M, Fritz M, Kacher CM, Cleveland JP, Hansma PK. 1996. Measuring the viscoelastic properties of human platelets with the atomic force microscope. *Biophys. J.* **70**: 556–567.
Richter RP, Berat R, Brisson AR. 2006. Formation of solid-supported lipid bilayers: an integrated view. *Langmuir* **22**: 3497–3505.
Rico F, Alcaraz J, Fredberg JJ, Navajas D. 2005a. Nanomechanics of lung epithelial cells. *Int. J. Nanotech.* **2**: 180–194.
Rico F, Roca-Cusachs P, Gavara N, Farre R, Rotger M, Navajas D. 2005b. Probing mechanical properties of living cells by atomic force microscopy with blunted pyramidal cantilever tips. *Phys. Rev. E* **72**: 021914.
Rief M, Gautel M, Oesterhelt F, Fernandez JM, Gaub HE. 1997a. Reversible unfolding of individual titin immunoglobulin domains by AFM. *Science* **276**: 1109–1112.
Rief M, Oesterhelt F, Heymann B, Gaub HE. 1997b. Single molecule force spectroscopy on polysaccharides by atomic force microscopy. *Science* **275**: 1295–1297.
Rief M, Clausen-Schaumann H, Gaub HE. 1999. Sequence-dependent mechanics of single DNA molecules. *Nat. Struct. Biol.* **6**: 346–349.
Rotsch C, Jacobson K, Radmacher M. 1999. Dimensional and mechanical dynamics of active and stable edges in motile fibroblasts investigated by using atomic force microscopy. *Proc. Natl. Acad. Sci. U.S.A.* **96**: 921–926.
San Paulo A, Garcia R. 2000. High-resolution imaging of antibodies by tapping-mode atomic force microscopy: attractive and repulsive tip-sample interaction regimes. *Biophys. J.* **78**: 1599–1605.
Schabert FA, Engel A. 1994. Reproducible acquisition of Escherichia coli porin surface topographs by atomic force microscopy. *Biophys. J.* **67**: 2394–2403.
Schabert FA, Henn C, Engel A. 1995. Native Escherichia coli OmpF porin surfaces probed by atomic force microscopy. *Science* **268**: 92–94.
Scheuring S, Sturgis JN. 2005. Chromatic adaptation of photosynthetic membranes. *Science* **309**: 484–487.
Scheuring S, Fotiadis D, Moller C, Muller SA, Engel A, Muller DJ. 2001. Single proteins observed by atomic force microscopy. *Single. Mol.* **2**: 59–67.
Scheuring S, Seguin J, Marco S, Levy D, Robert B, Rigaud JL. 2003. Nanodissection and high-resolution imaging of the

DOI: 10.1002/jmr

Rhodopseudomonas viridis photosynthetic core complex in native membranes by AFM. Atomic force microscopy. *Proc. Natl. Acad. Sci. U.S.A.* **100**: 1690–1693.
Scheuring S, Rigaud JL, Sturgis JN. 2004a. Variable LH2 stoichiometry and core clustering in native membranes of Rhodospirillum photometricum. *EMBO J.* **23**: 4127–4133.
Scheuring S, Sturgis JN, Prima V, Bernadac A, Levy D, Rigaud JL. 2004b. Watching the photosynthetic apparatus in native membranes. *Proc. Natl. Acad. Sci. U.S.A* **101**: 11293–11297.
Scheuring S, Busselez J, Levy D. 2005. Structure of the dimeric PufX-containing core complex of Rhodobacter blasticus by in situ atomic force microscopy. *J. Biol. Chem.* **280**: 1426–1431.
Scheuring S, Goncalves RP, Prima V, Sturgis JN. 2006. The photosynthetic apparatus of Rhodopseudomonas palustris: structures and organization. *J. Mol. Biol.* **358**: 83–96.
Scheuring S, Boudier T, Sturgis JN. 2007. From high-resolution AFM topographs to atomic models of supramolecular assemblies. *J. Struct. Biol.* **159**: 268–276.
Schneider S, Sritharan KC, Geibel JP, Oberleithner H, Jena BP. 1997. Surface dynamics in living acinar cells imaged by atomic force microscopy: identification of plasma membrane structures involved in exocytosis. *Proc. Natl. Acad. Sci. U.S.A.* **94**: 316–321.
Seelert H, Poetsch A, Dencher NA, Engel A, Stahlberg H, Muller DJ. 2000. Structural biology. proton-powered turbine of a plant motor. *Nature* **405**: 418–419.
Sewald N, Wilking SD, Eckel R, Albu S, Wollschlager K, Gaus K, Becker A, Bartels FW, Ros R, Anselmetti D. 2006. Probing DNA-peptide interaction forces at the single-molecule level. *J. Pept. Sci.* **12**: 836–842.
Shao Z, Yang J, Somlyo AP. 1995. Biological atomic force microscopy: from microns to nanometers and beyond. *Annu. Rev. Cell. Dev. Biol.* **11**: 241–265.
Shao Z, Mou J, Czajkowsky DM, Yang J, Yuan J-Y. 1996. Biological atomic force microscopy: what is achieved and what is needed. *Adv. Phys.* **45**: 1–86.
Shimshick EJ, McConnell HM. 1973. Lateral phase separation in phospholipid membranes. *Biochemistry* **12**: 2351–2360.
Simon A, Durrieu MC. 2006. Strategies and results of atomic force microscopy in the study of cellular adhesion. *Micron* **37**: 1–13.
Smith SB, Cui Y, Bustamante C. 1996. Overstretching B-DNA: the elastic response of individual double-stranded and single-stranded DNA molecules. *Science* **271**: 795–799.
Steltenkamp S, Muller MM, Deserno M, Hennesthal C, Steinem C, Janshoff A. 2006. Mechanical properties of pore-spanning lipid bilayers probed by atomic force microscopy. *Biophys. J.* **91**: 217–226.
Stoffler D, Fahrenkrog B, Aebi U. 1999. The nuclear pore complex: from molecular architecture to functional dynamics. *Curr. Opin. Cell Biol.* **11**: 391–401.
Stolz M, Stoffler D, Aebi U, Goldsbury C. 2000. Monitoring biomolecular interactions by time-lapse atomic force microscopy. *J. Struct. Biol.* **131**: 171–180.
Strick TR, Allemand JF, Bensimon D, Bensimon A, Croquette V. 1996. The elasticity of a single supercoiled DNA molecule. *Science* **271**: 1835–1837.
Stroh C, Wang H, Bash R, Ashcroft B, Nelson J, Gruber H, Lohr D, Lindsay SM, Hinterdorfer P. 2004a. Single-molecule recognition imaging microscopy. *Proc. Natl. Acad. Sci. U.S.A.* **101**: 12503–12507.
Stroh CM, Ebner A, Geretschlager M, Freudenthaler G, Kienberger F, Kamruzzahan AS, Smith-Gill SJ, Gruber HJ, Hinterdorfer P. 2004b. Simultaneous topography and recognition imaging using force microscopy. *Biophys. J.* **87**: 1981–1990.
Sulchek T, Friddle RW, Noy A. 2006. Strength of multiple parallel biological bonds. *Biophys. J.* **90**: 4686–4691.
Svoboda K, Schmidt CF, Schnapp BJ, Block SM. 1993. Direct observation of kinesin stepping by optical trapping interferometry. *Nature* **365**: 721–727.
Tanaka H, Homma K, Iwane AH, Katayama E, Ikebe R, Saito J, Yanagida T, Ikebe M. 2002. The motor domain determines the large step of myosin-V. *Nature* **415**: 192–195.
Tello M, San Paulo A, Rodriguez TR, Blanco MC, Garcia R. 2003. Imaging cobalt nanoparticles by amplitude modulation atomic force microscopy: comparison between low and high amplitude solutions. *Ultramicroscopy* **97**: 171–175.
Tha SP, Goldsmith HL. 1986. Interaction forces between red cells agglutinated by antibody. I. Theoretical. *Biophys. J.* **50**: 1109–1116.
Tha SP, Shuster J, Goldsmith HL. 1986. Interaction forces between red cells agglutinated by antibody. II. Measurement of hydrodynamic force of breakup. *Biophys. J.* **50**: 1117–1126.
Todd BA, Rammohan J, Eppell SJ. 2003. Connecting nanoscale images of proteins with their genetic sequences. *Biophys. J.* **84**: 3982–3991.
Touhami A, Jericho MH, Beveridge TJ. 2004. Atomic force microscopy of cell growth and division in Staphylococcus aureus. *J. Bacteriol.* **186**: 3286–3295.
Tsao YH, Evans DF, Wennerstrom H. 1993. Long-range attractive force between hydrophobic surfaces observed by atomic force microscopy. *Science* **262**: 547–550.
Van Mau N, Vie V, Chaloin L, Lesniewska E, Heitz F, Le Grimellec C. 1999. Lipid-induced organization of a primary amphipathic peptide: a coupled AFM-monolayer study. *J. Membr. Biol.* **167**: 241–249.
VanVliet KJ, Hinterdorfer P. 2006. Probing drug–cell interactions. *Nanotoday* **1**: 18–25.
Veigel C, Coluccio LM, Jontes JD, Sparrow JC, Milligan RA, Molloy JE. 1999. The motor protein myosin-I produces its working stroke in two steps. *Nature* **398**: 530–533.
Verbelen C, Dupres V, Menozzi FD, Raze D, Baulard AR, Hols P, Dufrene YF. 2006. Ethambutol-induced alterations in Mycobacterium bovis BCG imaged by atomic force microscopy. *FEMS Microbiol. Lett.* **264**: 192–197.
Viani MB, Schäffer TE, Chand A, Rief M, Gaub HE, Hansma PK. 1999. Small cantilevers for force spectroscopy of single molecules. *J. Appl. Phys.* **86**: 2258–2262.
Viani MB, Pietrasanta LI, Thompson JB, Chand A, Gebeshuber IC, Kindt JH, Richter M, Hansma HG, Hanjma PK. 2000. Probing protein–protein interactions in real time. *Nat. Struct. Biol.* **7**: 644–647.
Vie V, Van Mau N, Lesniewska E, Goudonnet JP, Heitz F, Le Grimellec C. 1998. Distribution of ganglioside GM1 between two-component, two-phase phosphatidylcholine monolayers. *Langmuir* **14**: 4574–4583.
Viswanathan R, Zasadzinski JA, Schwartz DK. 1993. Strained-layer van der Waals epitaxy in a Langmuir–Blodgett film. *Science* **261**: 449–452.
Walters DA. 1996. Short cantilevers for atomic force microscopy. *Rev. Sci. Instrum.* **67**: 3583.
Walters DA, Smith BL, Belcher AM, Paloczi GT, Stucky GD, Morse DE, Hansma PK. 1997. Modification of calcite crystal growth by abalone shell proteins: an atomic force microscope study. *Biophys. J.* **72**: 1425–1433.
Wang H, Clapham DE. 1999. Conformational changes of the in situ nuclear pore complex. *Biophys. J.* **77**: 241–247.
Weisenhorn AL, Drake B, Prater CB, Gould SA, Hansma PK, Ohnesorge F, Egger M, Heyn SP, Gaub HE. 1990. Immobilized proteins in buffer imaged at molecular resolution by atomic force microscopy. *Biophys. J.* **58**: 1251–1258.
Weisenhorn AL, Egger M, Ohnesorge F, Gould C, Heyn S-P, Hansma HG, Sinsheimer RL, Gaub HE, Hansma PK. 1991. Molecular resolution images of Langmuir–Blodgett films and DNA by atomic force microscopy. *Langmuir* **7**: 8–12.
Weisenhorn AL, Schmitt FJ, Knoll W, Hansma PK. 1992. Streptavidin binding observed with an atomic force microscope. *Ultramicroscopy* **42-44**: 1125–1132.
Weisenhorn AL, Khorsandi M, Kasas S, Gotzos V, Butt H-J. 1993. Deformation and height anomaly of soft surfaces studied with an AFM. *Nanotechnology* **4**: 106–113.
WIPO. 2007 http://www.wipo.int/pctdb/en/ Accessed 2007.
Wojcikiewicz EP, Zhang X, Chen A, Moy VT. 2003. Contributions of molecular binding events and cellular compliance to the modulation of leukocyte adhesion. *J. Cell. Sci.* **116**: 2531–2539.

DOI: 10.1002/jmr

Wong SS, Joselevich E, Woolley AT, Cheung CL, Lieber CM. 1998. Covalently functionalized nanotubes as nanometer-sized probes in chemistry and biology. *Nature* **394**: 52–55.
Wong J, Chilkoti A, Moy VT. 1999. Direct force measurements of the streptavidin–biotin interaction. *Biomol. Eng.* **16**: 45–55.
Worcester DL, Miller RG, Bryant PJ. 1988. Atomic force microscopy of purple membranes. *J. Microsc.* **152**: 817–821.
Yang J. 2004. AFM as a high-resolution imaging tool and a molecular bond force probe. *Cell. Biochem. Biophys.* **41**: 435–450.
Yang J, Appleyard J. 2000. The main phase transition of mica-supported phosphatidylcholine membranes. *J. Phys. Chem. B* **104**: 8097–8100.
Yang J, Mou J, Shao Z. 1994. Structure and stability of pertussis toxin studied by in situ atomic force microscopy. *FEBS Lett.* **338**: 89–92.
Yokokawa M, Wada C, Ando T, Sakai N, Yagi A, Yoshimura SH, Takeyasu K. 2006. Fast-scanning atomic force microscopy reveals the ATP/ADP-dependent conformational changes of GroEL. *EMBO J.* **25**: 4567–4576.
Yuan C, Chen A, Kolb P, Moy VT. 2000. Energy landscape of streptavidin–biotin complexes measured by atomic force microscopy. *Biochemistry* **39**: 10219–10223.
Zasadzinski JA, Helm CA, Longo ML, Weisenhorn AL, Gould SA, Hansma PK. 1991. Atomic force microscopy of hydrated phosphatidylethanolamine bilayers. *Biophys. J.* **59**: 755–760.
Zhang X, Wojcikiewicz E, Moy VT. 2002. Force spectroscopy of the leukocyte function-associated antigen-1/intercellular adhesion molecule-1 interaction. *Biophys. J.* **83**: 2270–2279.
Zhang X, Craig SE, Kirby H, Humphries MJ, Moy VT. 2004. Molecular basis for the dynamic strength of the integrin alpha4beta1/VCAM-1 interaction. *Biophys. J.* **87**: 3470–3478.
Zhong Q, Inniss D, Kjoller K, Elings V. 1993. Fractured polymer/silica fiber surface studied by tapping mode atomic force microscopy. *Surf. Sci. Lett.* **290**: L688–L692.
Zlatanova J, Lindsay SM, Leuba SH. 2000. Single molecule force spectroscopy in biology using the atomic force microscope. *Prog. Biophys. Mol. Biol.* **74**: 37–61.

DOI: 10.1002/jmr