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New frontiers in atomic force microscopy: analyzing interactions from single-molecules to cells

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Originally invented for imaging surfaces, atomic force microscopy (AFM) has evolved into a multifunctional molecular toolkit, enabling us to investigate the interactions of biological systems over scales ranging from single-molecules to whole cells. Specific highlights include the nanoscale imaging of the chemical properties of individual cells, the detection and functional analysis of cell surface receptors using single-molecule force spectroscopy and the quantitative measurement of cellular interactions using single-cell force spectroscopy. These advanced force spectroscopy modalities offer new opportunities for understanding the molecular bases of cell adhesion processes, which is a fundamental challenge in current life science and biotech research.

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Introduction

Knowledge of the molecular mechanisms of cell adhesion is of fundamental importance for understanding cellular processes such as cellular communication, tissue development, inflammation, cancer and microbial infection and for developing biotechnological applications. Besides specific interactions between receptors and ligands, non-specific interactions, such as hydrophobic and electrostatic forces, can play essential roles in mediating cell adhesion. Despite the importance of these intermolecular interaction mechanisms, their qualitative and quantitative assessment has long been challenging. During the past decades, various techniques have been developed for measuring biomolecular forces, including the osmotic stress method [1], the surface force apparatus [2], magnetic beads [3], optical tweezers [4] and the biomembrane force probe [5]. All that these methods have in common is that they can measure biological forces down to molecular range but can only hardly locate biomolecular interactions. By contrast, biological processes are linked to structures and it is of pertinent interest to structurally locate where biomolecular interactions occur. Thus, there is a growing need for methods that can measure intramolecular and intermolecular interactions of biological surfaces, both with high force sensitivity and high spatial resolution.

Within two decades, atomic force microscopy (AFM) has established as a powerful technique for probing biological systems in their native state, going from single-molecules to live cells [6–12]. While in the imaging mode AFM contours the topographs of specimens with molecular resolution and with minimal sample preparation, in the force spectroscopy mode AFM measures the intermolecular and intramolecular forces of biological systems. Within the past few years AFM-based force spectroscopy has provided unique insights into the molecular mechanisms that govern protein unfolding and folding, shape individual pathways along energy landscapes, guide ligand–receptor interactions, switch functional states of single proteins and mediate cell adhesion at molecular resolution [9]. The heart of AFM is a soft cantilever with a molecularly sharp tip that can be three-dimensionally positioned with subnanometer accuracy [7]. In the imaging mode, the AFM tip is scanned over a surface, while sensing local interactions between tip and surface. Contouring, for example, cell membranes at constant force allows us to directly generate their topography at a spatial resolution better than 1 nm and a signal-to-noise ratio being superior over that of any optical microscope [6,9–12]. Most importantly for biological applications, AFM investigates the samples in buffer solution, at ambient temperature without the need of fixing, staining or labeling. In the force spectroscopy mode, AFM positions the tip at a certain location and approaches and retracts the tip towards the sample. In these approach–retraction cycles, the cantilever deflection is recorded as a function of the vertical displacement of the tip to yield a force–distance curve. A characteristic interaction occurring between tip and sample deflects the cantilever that measures the interaction force. Such interaction forces can be used to characterize the binding strength of chemical groups, receptor–ligand interactions, interactions within and between proteins and specific adhesion events of living cells.

In the past years, detailed AFM reviews have been published, reporting images of biological systems across

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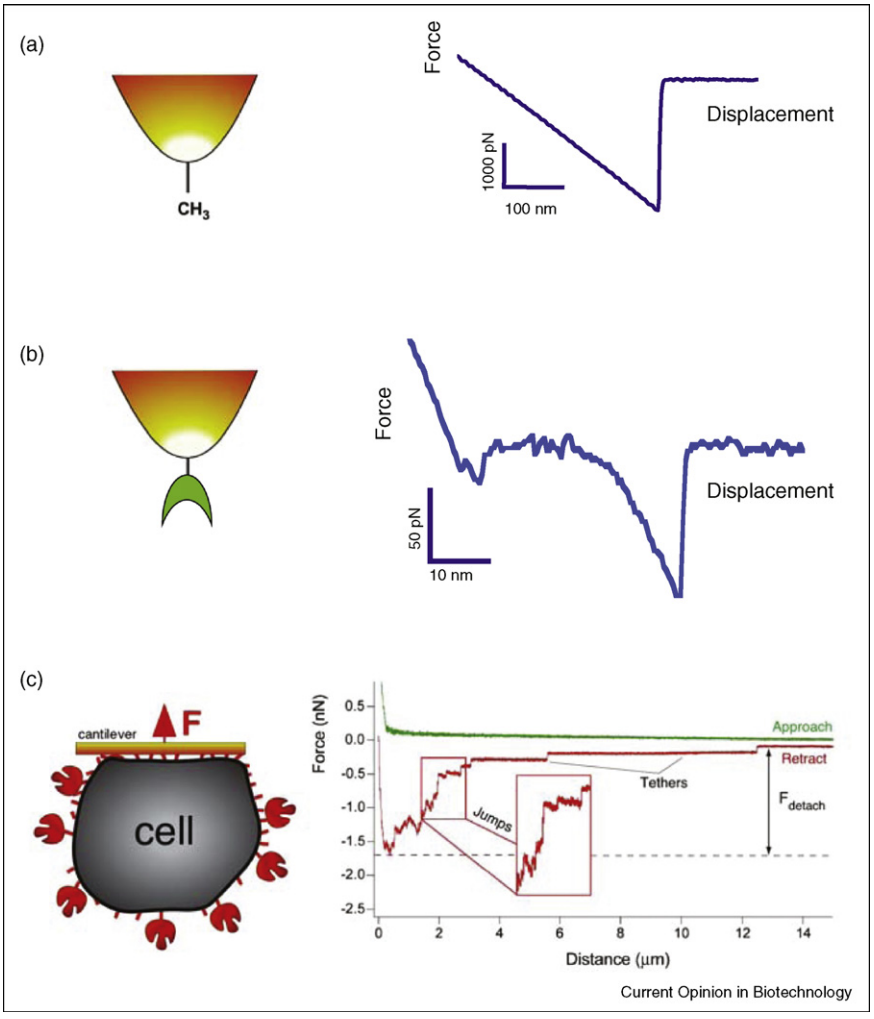
Figure 1



Analyzing interactions from single-molecules to cells using AFM: **(a)** chemical force microscopy (CFM), (**b**) single-molecule force spectroscopy (SMFS) and (**c**) single-cell force spectroscopy (SCFS). Left panels illustrate the general principle of the three techniques, while right panels present force data typically obtained.

dimensions ranging from cells to single-molecules [6–12]. Here, we extend these applications and review AFM-based methods that can be applied to characterize cellular interactions using chemical force microscopy, single-molecule force spectroscopy and single-cell force spectroscopy (Figure 1).

Chemical force microscopy

Because AFM works by sensing the local interaction between the tip and the sample, this principle may be exploited to investigate the wide range of physical, chemical and biological interactions occurring in cells, from the molecular to the cellular scale. In chemical force microscopy (CFM), AFM tips are modified with specific functional groups to probe the spatial arrangement of chemical groups and their interactions [13,14]. During the past two years, the ability of CFM to resolve the nanoscale chemical properties and interactions of live cells has been established [15,16•,17,18]. Alsteens *et al.* [15] demonstrated the feasibility of using CFM for sensing hydrophobic interactions. Monolayers of methyl-terminated and hydroxyl-terminated alkanethiols mixed in different proportions were probed using water contact angle measurements and multiple force–distance curves recorded with a methyl-terminated tip. The contact angle and adhesion force values measured on these reference surfaces increased gradually with the molar fraction of methyl-terminated alkanethiols, indicating that the measured adhesion forces reflected hydrophobic interactions. Interpretation of the CFM data in terms of interfacial thermodynamics indicated that these forces do not originate from true, direct tip-sample interactions, but rather reflect entropy changes associated with the restructuring of water near hydrophobic surfaces.

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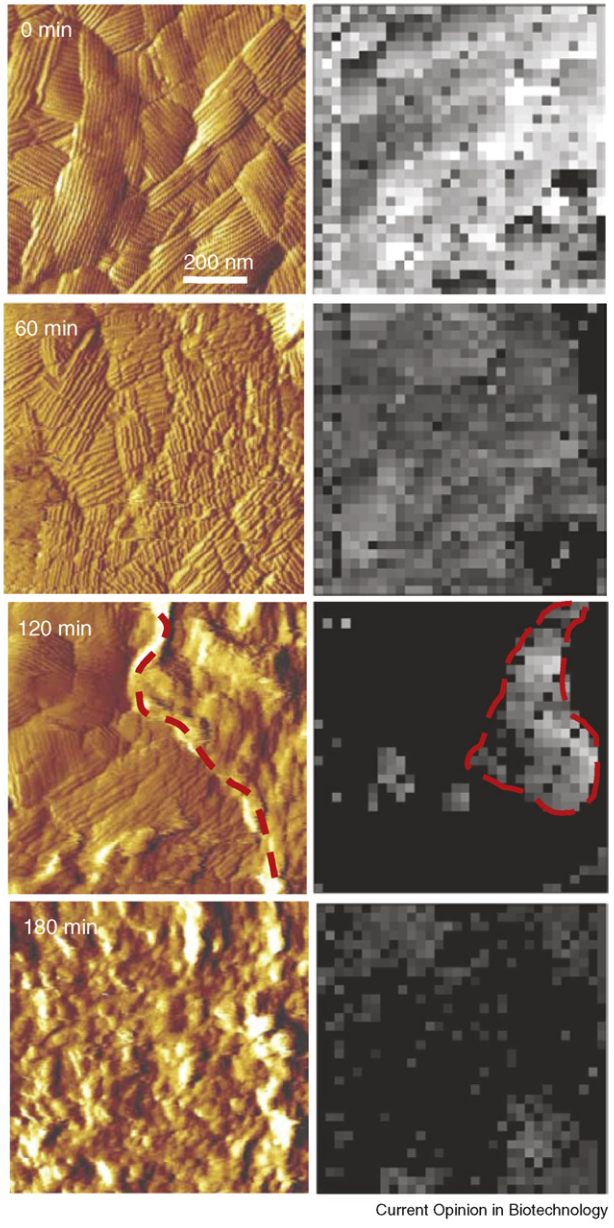
Figure 2



Measuring the nanoscale hydrophobicity of pathogens using CFM. Series of high-resolution deflection images (left) and adhesion force maps (right) recorded on a single *A. fumigatus* spore during germination. Within less than 3 h, the crystalline rodlet layer made of hydrophobic proteins (hydrophobins) changed into a layer of amorphous material, presumably reflecting inner cell wall polysaccharides (left images). After 2 h, both rodlet and amorphous regions were found to coexist (separated by dashed line). Consistent with this structural dynamics, substantial reduction of adhesion was noted with time (right images), reflecting a dramatic decrease of hydrophobicity. After 2 h, heterogeneous contrast was observed in the form of hydrophobic patches (dashed line), surrounded by a hydrophilic sea. Reprinted with permission from [17].

Notably, Dague *et al.* [16•] used the CFM method to probe the local hydrophobicity of the human opportunistic pathogen *Aspergillus fumigatus*. The surface of wild-type *A. fumigatus* spores was found to be homogeneously hydrophobic, in agreement with the presence of hydrophobic proteins (hydrophobins) in the outer rodlet layer (Figure 2, upper panels), providing direct indications as to the putative functions of hydrophobins as dispersion and adhesion structures. Surface hydrophobicity was lower in a rodletless mutant, confirming that the measured hydrophobic properties were associated with rodlets. Nanoscale variations of hydrophobicity were resolved on SDS-treated conidia, on which rodlet patches were missing in localized regions. Changes of hydrophobicity could also be tracked in real-time during spore germination [17]. Using a temperature-controlled AFM, high-resolution images of the same germinating spore were obtained (Figure 2). Significant structural alterations were observed, the rodlet layer changing into a layer of amorphous material, presumably reflecting the underlying polysaccharides. In addition, adhesion maps obtained with hydrophobic tips revealed a loss of hydrophobicity over time. After a 2-h germination, nanoscale variations of hydrophobicity were observed, reflecting the coexistence of hydrophobic rodlets and hydrophilic polysaccharides. The observed changes were suggested to be function-related. While the hydrophobic rodlets will promote spore dispersion and adhesion to surfaces, the hydrophilic nature of the germ tube cell wall will favour hyphal growth through moist environments and especially endothelia and epithelia.

Also of interest is the possibility to investigate differences in hydrophobicity following treatment of cells with drugs. Alsteens *et al.* [15,18] showed that the surface of mycobacteria has a remarkably strong hydrophobic character owing to the presence of an outermost layer of hydrophobic mycolic acids. These hydrophobic constituents are thought to represent an important permeation barrier to common antibacterial agents. Treatment of the cells with two antibiotics, isoniazid and ethambutol, lead to a decrease of cell surface hydrophobicity, attributed to the removal of the mycolic acid layer [18]. Some cells showed the coexistence of hydrophobic and hydrophilic regions, attributed to portions of unaltered mycolic acids and of underlying carbohydrates. These studies show that CFM can resolve submicron chemical heterogeneities on live cells as they grow or interact with drugs, thereby providing new insights into the cell surface architecture as well as into the action modes of drugs.

Single-molecule force spectroscopy

AFM-based single-molecule force spectroscopy (SMFS) has established as a particularly popular method to characterize molecular interactions associated with biological systems [8,9]. In fact, there are variations of how SMFS can be applied to measure such biomolecular interactions.

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In 1994, the first application of AFM to measure molecular recognition forces used an AFM tip functionalized with ligands to bring them in contact with receptors [19••,20••]. The rupture forces detected upon separating single ligand–receptor pairs can be used to provide new insights into the binding strength, binding kinetics, (un)binding energy landscape and to localize receptors on cell surfaces [8,9]. In these experiments, however, unspecific interactions can easily superimpose with specific ones. To separate unspecific from specific interactions the ligand may be attached via a molecular crosslinker (spacer) to the AFM tip [21••]. Specific interactions are then expected to occur at pulling distances that correspond to the stretched length of the linker and, thus, if the linker length is appropriately chosen, these specific interactions are well separated from unspecific interactions detected in direct proximity of tip and sample surface. In the past years, there has been much progress in developing reliable protocols for attaching biomolecules to AFM tips [8]. A powerful platform for the stable, oriented attachment of proteins uses the site-directed nitrilotriacetic acid (NTA)-polyhistidine (His) system [22••,23]. This coupling approach ensures optimal exposure of either the C-terminal or N-terminal domains of the proteins. Another generic method is to covalently anchor biomolecules on silicon tips using various amine-functionalization procedures [21••,24]. The amino-terminated surfaces are reacted with a crosslinker that provides the ligands with motional freedom and prevents their denaturation. Crosslinkers typically carry two different functional ends. This can for instance be an amine reactive N-hydroxysuccinimide (NHS) group on one end for the coupling to tip surfaces, and a 2-pyridyldithiopropionyl (PDP) or a vinyl sulfone group on the other end, which can be covalently bound to thiol groups of the ligand. Recently, Ebner et al. [24] developed a convenient method that requires only minimal amount of protein (e.g. 5 μg of protein in 50 μL of buffer) and no prederivatization, using a new heterobifunctional crosslinker having two different amino-reactive functions. The method was validated using both the biotin–avidin system and human rhinovirus particles.

Continuous improvement of the SMFS methodology (tip modification, data acquisition and interpretation) has enabled researchers to determine the interaction forces and the dynamics of a variety of cell surface proteins, including cadherins [25], integrins [26], selectins [27], growth factor receptors [28], heat shock proteins [29] and bacterial adhesins [22••,30,31]. In pharmacology, Gilbert *et al.* [32•] showed that SMFS can be used for probing the specific binding forces of antibiotics. The authors measured the average adhesion force between a vancomycin-terminated tip and D-Ala-D-Ala sites, either on model surfaces or on live bacteria, and from dynamic measurements, they assessed the association and dissociation rate constants for the complex. This study suggests that SMFS may become an important tool in medicine for investigating the action mode of drugs. SMFS has also proved useful for detecting and analyzing single polysaccharides on cells [33,34,35•]. For instance, Francius *et al.* [35•] used lectin-modified tips to reveal the coexistence of polysaccharide chains of different nature on the surface of the clinically important probiotic bacterium *Lactobacillus rhamnosus* GG (Figure 3). The measured polysaccharide properties – that is, distribution, adhesion and extension – of the wild-type bacterium were markedly different from those of a mutant strain impaired in adherence to gut epithelium, biofilm formation and exopolysaccharide production, suggesting these molecules play a role in bacterial adhesion and in promoting beneficial health effects. The above single-molecule analyses contribute to shed new light into the molecular basis of cell–cell, cell–host and cell–drug interactions.

Another remarkable feature of SMFS is its ability to map the distribution of individual receptors on cells with nanoscale resolution, a method known as 'recognition imaging'. Here, arrays of force–distance curves are recorded on the cell surface, and the unbinding force values of all curves are displayed in a grey-scale map (Figure 3c). After the pioneering work of the Gaub's group on red blood cells [36], receptors (ligands) from many different cell types have been investigated, including those from endothelial cells [28], osteoblastic cells [37], chinese hamster ovary (CHO) cells [38], mycobacteria [22••], yeast cells [33,34] and lactic bacteria [32•,35•]. In one such study, heparin-modified tips could be used to image the distribution of single adhesins on live mycobacteria, revealing that they were concentrated into nanodomains [22••]. These nanoclusters were suggested to promote adhesion to target cells by inducing the recruitment of receptors within membrane rafts. More recently, Roduit *et al.* [39] used SMFS to localize glycosylphosphatidylinositol (GPI)-anchored proteins that partition preferentially into cholesterol-rich microdomains. Using the AFM tip as an indenter to locally probe and map elasticity, it was observed that in neurons these GPI-anchored proteins concentrate into domains that were somehow stiffer compared with the surrounding membrane. The authors suggested this method should be useful for studying the relationships between membrane stiffness, molecular diffusion and signaling activation.

While recognition imaging using SMFS provides a quantitative analysis of binding forces, it is limited by its time resolution. This problem can be solved by using dynamic recognition imaging, in which topography and recognition (abbreviated as TREC) images are acquired using tips that are magnetically oscillated during scanning and contain ligand molecules directed against the cognate receptor on the surface [8]. The first TREC studies on cells were recently performed by the Hinterdorfer group

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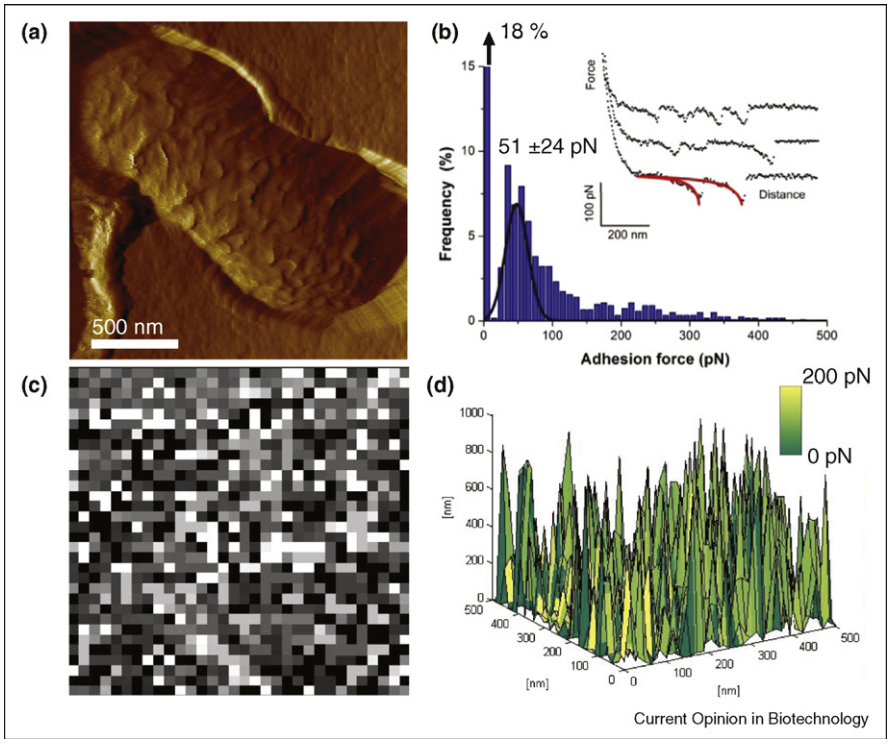
Figure 3



Detection, localization and conformational analysis of single polysaccharide molecules on probiotic bacteria using SMFS. (**a**) AFM deflection image, (**b**) adhesion force histogram ($n = 1024$) together with representative force–distance curves and (**c**) adhesion force map (500 nm × 500 nm; grey scale: 200 pN) recorded in buffered solution on the probiotic bacterium *Lactobacillus rhamnosus* GG, using an AFM tip functionalized with *Pseudomonas aeruginosa* (PA-1) lectins. Red lines on the bottom curve in panel (b) show that elongation forces were well described by an extended freely jointed chain model, as expected for polysaccharide chains. (**d**) Three-dimensional reconstructed map of polymer properties, obtained by combining adhesion force values (expressed as false colors) and rupture distances (expressed as *z* level) measured at different *x*, *y* locations. These SMFS data indicate that polysaccharide chains containing galactose were uniformly detected on the bacterial surface and stretched over distances of up to 1000 nm. These remarkable polysaccharide properties may play essential roles in mediating bacterial adhesion to intestinal tissues and interactions with specific receptors of the immune system. Reprinted with permission from [35•].

[40•]. The authors could locally identify vascular endothelial cadherin binding sites on microvascular endothelial cells from mouse myocardium and colocalize the receptor position with membrane topographical features. TREC was also used to visualize growth factor receptors on vascular endothelial cells [41], revealing that the receptors were non-uniformly distributed, with a close spatial association with the underlying cortical cytoskeleton. Hence, simultaneously revealing topography and localizing specific recognition events on cell surfaces opens a wide field of applications for investigating the structure–function relationships of receptors in their native environment. In the future, these approaches may be increasingly used to probe receptor sites on cell surfaces, in connection with medical and physiological issues.

Single-cell force spectroscopy

Single-cell force spectroscopy (SCFS) replaces the tip of the AFM cantilever by a living cell that is used to measure interactions towards other cells or substrates (Figure 1c) [42,43•]. Simple protocols describing how to attach a single cell to a biologically coated, tip-less cantilever are available for converting essentially any adherent cell into a cellular probe. A simple method to functionalize the cantilever is to clean it with detergent or plasma cleaner, treat it with biotinylated BSA and then further incubate with streptavidin and biotinylated concanavalin A [44,45]. These concanavalin A coated cantilevers are then ready to bind sugar residues (alpha-mannosyl groups) of cell surfaces. Moreover, modern molecular cell biological and genetic tools enable the specific adjustment of the functional state of the cell so that SCFS measurements can be performed on a reliable basis. One of the first experiments that have established SCFS to measure cell–cell interactions was introduced by the Gaub group [46••]. Cells of *Dictyostelium discoideum* were brought into contact and separated after a given contact time. It was found that the force–distance curve recorded when separating the cells from each other can detect different unbinding events of the cell (Figure 1c). The maximum detachment force (F_{detach}) of the curve

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denotes the maximum strength of cell–cell binding whereas discrete force steps can be assigned to the rupture of single cell adhesion molecules (CAMs). These discrete unbinding events can either describe the rupture force of a receptor–ligand pair, or the force that is required to form a membrane tether. Probing the receptor–ligand interactions at different loading rates (force applied versus time) allows reconstructing the energy barrier that describes features of the receptor–ligand bond [47,48••]. These include the free energy and the distance that separates the bound state from the transition state and the lifetime of the bound state at equilibrium. There are several advantages of characterizing a receptor–ligand bond in its native cellular environment. In their isolated forms receptors or ligands are often only available truncated. This is particularly the case for transmembrane receptors. To handle these receptors, their hydrophobic membrane spanning regions have been removed, to investigate the remaining hydrophilic extracellular domains in their purified form. Thus, SMFS experiments on isolated molecules can be limited by the fact that functionally important structural regions of receptor–ligand systems are missing. In addition, quite frequently cells functionally regulate receptor–ligand interactions to adapt them to their need. Thus, characterized in the absence of the cellular context, receptor–ligand interactions may show a functional state that does not necessarily resemble the native one. There is another advantage of characterizing receptor–ligand interactions in their native cellular environment. Because the functional state of a cell can be precisely controlled using molecular and cell biological tools it becomes possible to characterize how the cell, depending on its functional state, for example, modulates receptor–ligand interactions to communicate with the environment. Using these advantages, the Moy's group demonstrated that the functional activation of leukocytes induced changes in the energy landscape that describes the unbinding of LFA-1 integrin (ITGAL) from its ligand ICAM1 [26]. Other studies report that the binding dynamics of various integrins change upon activation by antibodies [27] and in the presence of magnesium [49].

In the case of specific binding, membrane tethers are expected to attach via a receptor–ligand bond at their very tip. Counter intuitively, the force describing the formation and rupture of a membrane tether do not reflect that of the receptor–ligand bond attaching the membrane tether [48••,50•]. The force required to form a membrane tether only depends on the properties of the cell membrane and, most interestingly, is constant for pulling distances extending over several microns. This natural force clamp of membrane tethers is observed as extended force plateaus, when pulling cells apart (Figure 1c). The force, however, at which a membrane tether clamps the receptor–ligand bond is intrinsic to the properties of the cell membrane and the velocity at which the tether is pulled from the cell membrane. Consequently, measuring the tether length at a given pulling speed allows us to directly determine the lifetime of the receptor–ligand bond at different forces clamped by the membrane tether [51•]. In the future, membrane tethers may be applied to characterize how the cell modulates the lifetime of CAMs in health and disease. Insights into how a living cell controls adhesion are of fundamental importance to understand, for example, tissue formation, cell migration and tumor metastasis.

In contrast to SMFS experiments using isolated single-molecules, it can be much more complex to probe a particular specific interaction using living cells. The reason for this is the multitude of possible specific and unspecific cell-surface interactions that naturally occur on the cell surface [43•]. Therefore, special attention must be paid to ensure that the interactions detected occur predominately, if not exclusively, between the receptor and ligand of interest. Rigorous control experiments that demonstrate the specificity of the measured interactions must be performed. To this end, purified substrates, blocking antibodies or biologically passivated surfaces are used. Recent SCFS studies [52] observed that leukaemic cells expressing the characteristic myeloid leukaemia fusion protein BCR/ABL show a significantly increased adhesion to bone-marrow stromal cells (Figure 4). Characterizing this adhesion in the presence and absence of the β1 integrin blocking antibody Ha2/5, SCFS could further demonstrate that this integrin makes up the increased adhesion of leukaemic cells expressing the tyrosin kinase BCR/ABL. Cells can also be genetically modified to functionally modulate the receptor of interest. Alon *et al.* [53•] for example inserted a mutation into α_4 integrin that impaired the talin association to the $\alpha_4\beta_1$ heterodimer and used SCFS to demonstrate that this α_4 integrin mutation suppressed the $\alpha_4\beta_1$ integrin dependent capture and adhesion strengthening of leukocytes. Alternatively one may limit the number of possible receptors that are expressed. Friedrichs *et al.* [54], for example, used knockdown MDCK cell lines to quantify the individual contributions of galectin-3 and galectin-9 to epithelical cell adhesion. Such examples demonstrate that by comparing differences introduced by genetic modifications SCFS can qualitatively and quantitatively identify the contribution of individual receptors to adhesive cellular interactions.

Allowing short contact times of the cell surface to the substrate or to another cell (<60 s) enables us to characterize the binding behaviour of single-molecules, whereas at extended contact times cellular processes become evident. At such extended contact times, the adhesion between cells and either cells or substrate generally increases. However, significant deviations can be observed depending on the cell type investigated. For example, the high early adhesion forces that occur

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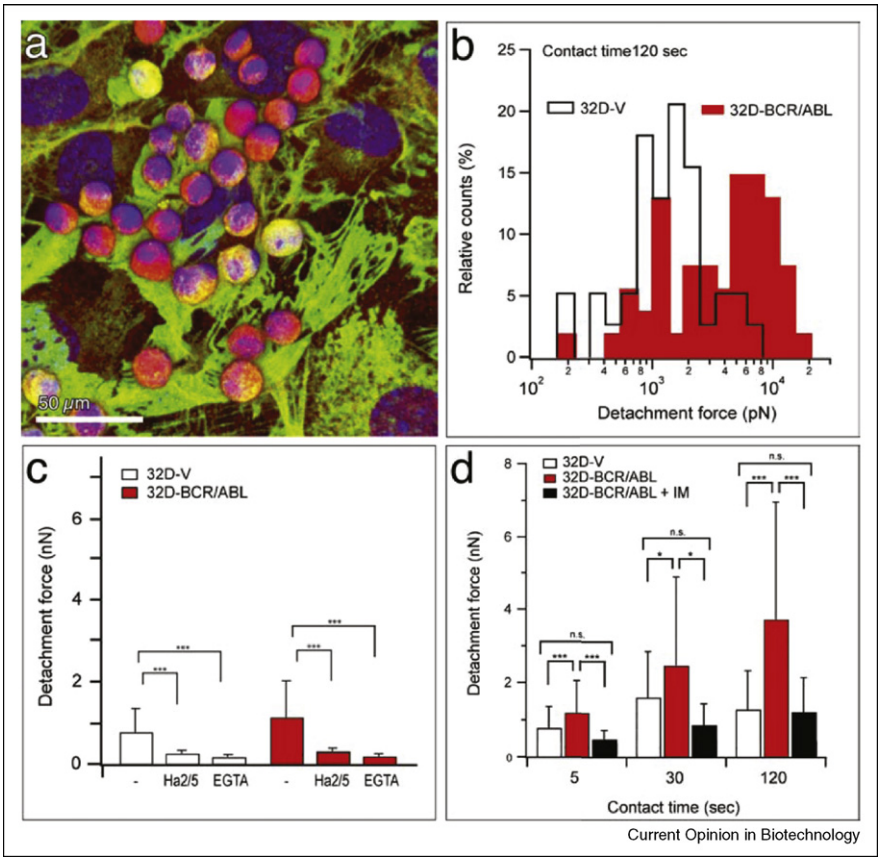
Figure 4



Characterizing the molecular mechanisms that increase the adhesion of leukaemia cells to bone marrow stroma cells (BMSC) using SCFS. (**a**) Confocal image of murine myeloid progenitor cells attached to BMSC. Progenitor cells (32D) were retrovirally transformed to express the fusion protein BCR/ABL, a hallmark of chronic myeloid leukaemia. Samples were stained for β_1 integrin (red), fibronectin (green) and nuclei (blue). The 3D image was constructed from acquired *Z* stacks and projected onto an *X*-*Y* plane. (**b**–**d**) In their paper, Fierro *et al.* [52] characterized the adhesion of 32D cells to BMSC using SCFS and observed an increased adhesion of the BCR/ABL expressing cells (**b**). 32D-V, are 3D cells expressing an empty vector, 32D-BCR/ABL are 32D cells expressing the BCR/ABL fusion protein. (**c**) Identifying the adhesion molecule β_1 integrin to increase the adhesion of BCR/ABL expressing cells. In the presence of the β_1 integrin blocking antibody the adhesion of 32D cells is significantly reduced. Many ligand-binding mediated interactions of the cell surface depend on divalent ions. Consequently, in the presence of the divalent ion chelator EGTA this β_1 integrin mediated interaction could be blocked as well. (**d**) Upon addition of imatinib mesylate (IM), the specific inhibitor of the tyrosine kinase BCR/ABL, this β_1 integrin dominated adhesion to stromal cells could be significantly reduced. Reprinted with permission from [52].

between cells that express the surface receptor Notch and its ligand Delta diminish as the receptors are cleaved and internalized as part of the signaling pathway [55]. By contrast, Chinese ovary hamster (CHO) cells that express $\alpha_2\beta_1$ integrin switch to an activated adhesion state to enforce their attachment to a collagen type I matrix [56]. It is assumed that the controlled assembly of individual integrins into clusters increase the adhesive force of these cells. In another example, SCFS was applied to examine how Wnt signaling stimulates the adhesion of cells that have been isolated in the early development from zebrafish embryos [45,57]. The specific adhesion of different types of primary cells was tested to functionalized substrates and to other cells. One of the most fundamental questions in developmental biology is to understand the factors that direct tissue organization during development. Various hypotheses suggested that cell sorting may be governed by adhesive and mechanical properties. SCFS has been applied to characterize the specific contributions of cell adhesion versus cell-cortex tension to cell sorting in zebrafish gastrulation [58••]. It could be shown that signaling processes control cell-cortex tension that directs progenitor-cell sorting.

We have exemplified a number of studies that have used living cells to probe interactions of cellular surfaces down to molecular resolution. These investigations, as well as other excellent examples reviewed earlier [10,42,43•], impressively show that SCFS can be applied to characterize dynamic cellular adhesion events. The relative experimental ease of SCFS is being made more complex by the need to show the specificity of the measured

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adhesion events and, thus, to make the experimental results interpretable. First SCFS standards have been established that show the importance of controlling the cellular function, introduce solid statistical analysis procedures and open ways to reveal cellular interactions energies and kinetics [48••]. We anticipate that the continuous improvement of the SCFS methodology will soon establish this approach as a powerful tool in cell biology, biotechnology and medicine.

Conclusions

With its ability to analyze interactions over a large range of scales – that is, from single-molecules (∼pN) to whole cells (∼nN) – AFM imaging and force spectroscopy techniques have recently enabled a major paradigm shift in cell biology and biomedicine. Although single-molecule AFM imaging at a resolution of 1 nm, and even better, is still confined to membrane patches extracted from cells [12,59], current AFM images of living cells can approach a resolution of ∼10 nm [60]. Thus, one of the great challenges in AFM will be to image single-molecules of living cells. We are optimistic that with the new technological achievements of AFM, this will be possible in the near future [7,9]. As we have shown, AFM is a multifunctional tool that can not only image cellular surfaces at high resolution but also probe fundamental interactions that give cells their characteristic structure–function relationship. Currently, several advanced AFM modalities are available for probing the physical, chemical and biological nature of these interactions. One key example discussed here is CFM that helps us to understand how the unique properties of cell surfaces are chemically determined. In addition, cell surfaces communicate with their environment through all kinds of biological interactions. In this context, SMFS and SCFS are two very promising approaches for characterizing the specific interactions between cell surface receptors and their cognate ligands, for mapping the distribution of individual receptors on cells and for quantifying cell–cell and cell–substrate interactions. The unique strength of these approaches lies in the fact that they provide qualitative and quantitative insights into cellular interactions and energies down to the contribution of single-molecules. A further unique advantage is the possibility to combine force spectroscopy and imaging. Thus, AFM may be viewed as a cell or molecular biological lab mounted onto a tip, enabling us to address a wide range of pertinent questions in biology, medicine and biotechnology.

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- •• of outstanding interest

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